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Original article

A single-dose, randomized, cross-over, two-way, open-label study for comparing the absorption of boswellic acids and its lecithin formulation

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ABSTRACT

Background: The oral administration of the gum resin extracts of Indian frankincense (*Boswellia serrata* Roxb. ex Colebr) results in very low plasma concentrations of boswellic acids (BAs), being far below the pharmacologically active concentrations required *in vitro* for anti-inflammatory activity. For that reason the use of Indian frankincense in clinical practice and pharmaceutical development has substantially lagged behind. Recently the application of new formulation technologies resulted in a formulation of frankincense extract with lecithin, which revealed improved absorption and tissue penetration of BAs in a rodent study, leading for the first time to plasma concentrations of BAs in the range of their anti-inflammatory activity.

Purpose: In order to verify these encouraging results in humans, the absorption of a standardized *Boswellia serrata* extract (BE) and its lecithin formulation (CSP) was comparatively investigated in healthy volunteers.

Study design: According to a randomized cross-over design with two treatments, two sequences and two periods, 12 volunteers alternatively received the lecithin-formulated *Boswellia* extract (CSP) or the non-formulated *Boswellia* extract (BE) at a dosage of 2×250 mg capsules.

Methods: The plasma concentrations of the six major BAs (KBA, AKBA, βBA, αBA, AβBA, AαBA) were determined using LC/MS.

Results: With the exception of KBA, a significantly higher (both in terms of weight-to-weight and molar comparison) and quicker absorption of BAs from the lecithin formulation was observed, leading to C_{max} in the range required for the interaction with their molecular targets.

Conclusion: These findings pave the way to further studies evaluating the clinical potential of BAs, and verify the beneficial effect of lecithin formulation to improve the absorption of poorly soluble phytochemicals.

Introduction

The belief that natural medicines are safer than synthetic drugs led to a tremendous growth of phytopharmaceuticals, reaching a

Abbreviations: αBA, Alpha-boswellic acid; AαBA, Acetyl- alpha-boswellic acid; AβBA, Acetyl- beta-boswellic acid; AKBA, Acetyl-11-keto-boswellic acid; βBA, Beta-boswellic acid; BAs, Boswellic acids; BE, *Boswellia* extract; catG, Cathepsin G; CI, Confidence interval; COX-1, Cyclooxygenase-1; ECG, Electrocardiogram; ESI, Electro Spray Ionization; HBSAg, hepatitis B surface antigen; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HLE, Human leuokocyte elastase; IL, Interleukin; KBA, 11-keto-boswellic acid; LSM, Least squares means; 5-LO, 5-lipoxygenase; mPGES-1, microsomal prostaglandin E synthase-1; NF-κB, nuclear factor-kappaB; S.A.S., Société Anonyme Simplifiée (form of organization for companies); SIM, single ion mode; SAS®, Statistical Analysis System; THF, tetrahydrofurane; TNFα, tumor necrosis factor alpha.

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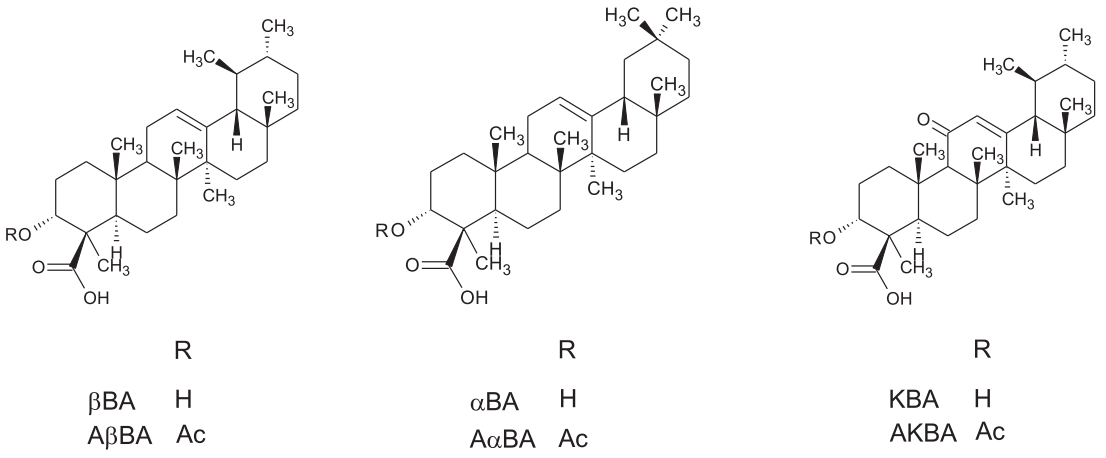


Fig. 1. Chemical structures of the six characteristic boswellic acids of *Boswellia serrata*.

worldwide 25% share of the pharmaceutical arsenal (Bhattaram et al., 2002). Especially the interest in alternative, well-tolerated anti-inflammatory herbal remedies has re-emerged in the last decades, as all efforts aiming to develop safe synthetic anti-inflammatory drugs are still far from achieving a real breakthrough. Gum resin extracts of *Boswellia serrata* Roxb. ex. Colebr. (Indian frankincense) have been found to represent a promising anti-inflammatory herbal remedy. Numerous experimental data from *in vitro* studies and animal models support the potential of boswellic acids (BAs) (Fig. 1), a class of pentacyclic triterpenoids representing the major active principles of *B. serrata*, for the treatment of various diseases. Hence it was shown that a number of pivotal enzymes in inflammation like 5-lipoxygenase (5-LO), cyclooxygenase-1 (COX-1), human leuokocyte elastase (HLE), cathepsin G (catG) and the microsomal prostaglandin E synthase-1 (mPGES-1) as well as the nuclear factor-kappaB (NF-κB) and several cytokines like TNFα, IL-1β and IL-6 are inhibited by BAs with IC_{50} values in the range of 0.6 µm to 24 µm (Abdel-Tawab et al., 2011; Poeckel and Werz, 2006). Modern research has highlighted the relevance of the whole fraction of triterpenoid acids for the anti-inflammatory activity of *Boswellia* extracts, since all BAs show significant *in vivo* activity in animal models of inflammation (Bannoa et al., 2006). This represents a dramatic change compared to the early studies, that emphasized the relevance of a single constituent (AKBA, a minor constituent of the BAs bouquet) and a single target (5-lipoxygenase, 5-LO) (Safayhi et al., 1992).

However, various pharmacokinetic studies in human and animals revealed very low plasma concentrations of BAs, far below the pharmacologically active concentrations determined *in vitro*, in spite of administering very high doses of *Boswellia* extract reaching up to 3000 mg/day (Abdel-Tawab et el., 2011; Du et al., 2015). This limited the use of *B. serrata* in clinical practice and pharmaceutical development. Consequently the anti-inflammatory potential of BAs could as yet not been translated into an approved clinical application, (Ernst, 2008). The dismally low oral absorption of BAs is not surprising, since these compounds are very poorly soluble in water, suggesting a strong tendency to self-aggregation. This hypothesis is supported by the marked increase of absorption observed when *B. serrata* extracts are administered with food, as expected from the disruption of self-aggregates by biliary salts (Skarke et al., 2012). These considerations provided a rationale for the development of a lecithin formulation of BAs, which revealed a significantly improved absorption of BAs accompanied with enhanced tissue penetration in rats, leading for the first time to plasma concentrations of BAs in the range of their anti-inflammatory activity (Huesch et al., 2013).

In the wake of these promising animal studies, we have carried out a comparative pharmacokinetic study in healthy volunteers on weight-equivalent (500 mg) dosages of the lecithin-formulated *Boswellia* extract (CSP) and the non-formulated *Boswellia* extract (BE) used for its preparation. Since CSP contains only 33% *Boswellia* extract on molar basis, this involved comparison between 150 mg of extract formulated with lecithin and 500 mg of non-formulated extract.

Materials and methods

Products

Gum resin extracts of *Boswellia serrata* Roxb. ex. Colebr. (plant name checked with http://www.theplantlist.org) (Batch N.: 11239, Code # 36BW60090) (BE) and its lecithin formulation Casperome® (Batch N.: 12146, Code # 36BWP0090) (CSP) used in the study were produced by Indena S.A.S. (Tours, France). Both were assigned voucher numbers and representative voucher specimen has been deposited in the Central Laboratory of German Pharmacists, Eschborn, Germany. CSP is composed of *B. serrata* extract and soy lecithin in a 1:1 ratio, with about half part of microcrystalline cellulose being 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 this study, 250 mg of BE or CSP were formulated by Indena S.p.A. (Milan, Italy) into Swedish orange size "0" hard gelatin capsules containing: 54.0 mg of corn starch and pregelatinized starch (StarCap 1500®, Colorcon, UK), 50.0 mg of Citric Acid Anhydrous, 9.0 mg of Croscarmellose Sodium (Solutab®, Blanver, San Paolo, Brazil), 4.0 mg of Silicon Dioxide (Syloid® 244 FP, W. R. Grace & Co., Conn., Columbia, USA), 9.0 mg of magnesium stearate, and 4.0 mg of talc. Before releasing the hard gelatin capsules containing BE (Batch # 89035) or CSP (Batch # 89036), the appearance, average mass, uniformity of mass (Eur. Pharm. 2.9.1.), HPLC content of BAs, disintegration time (< 10 min according to Eur. Pharm 2.9.1) and microbiological quality were tested. For the detailed quantification of the six major BAs contained in the capsules see Table 1.

Subjects

In order to standardize all variables that may affect the pharmacokinetics of BAs, twelve healthy non-smoking subjects aged between 20 to 51 years of Caucasian race were recruited and considered eligible for enrolment as per protocol inclusion and exclusion criteria. The screening procedures included collection of anamnesis and demographic data (gender, age, race, body weight [kg], height

Table 1
Contents of individual boswellic acids (BAs) in a single capsule of non-formulated *Boswellia* extract (BE) and lecithin-formulated *Boswellia* extract (CSP).

Boswellic Acid (BA)	BE (mg per capsule)	CSP (mg per capsule)
αBA	20.71	9.19
AαBA	40.10	13.76
βBA	47.28	20.63
AβBA	40.01	13.76
KBA	16.44	5.81
AKBA	12.40	4.11
Total	**176.94**	**67.26**

Table 2
Overview on the demographic data of the subjects included in the study.

	Male	Female
Gender	6	6
Age in years (mean ± S.D.)	33.5 ± 9.03	33.0 ± 12.02
Weight in Kg (mean ± S.D.)	75.67 ± 7.47	57.00 ± 9.08
Height in m (mean ± S.D.)	1.72 ± 0.06	1.60 ± 0.05
BMI (mean ± S.D.)	25.66 ± 2.72	22.30 ± 3.11

[cm] and BMI), a physical examination, a resting 12-lead electrocardiogram (ECG), blood pressure, heart rate, and temperature. The laboratory test performed included a standard urine test and clinical laboratory tests (haematology, biochemistry, urinalysis, human immunodeficiency virus [HIV], hepatitis C [HCV] antibodies and hepatitis B surface antigen [HBSAg]). An overview on the demographic data of the subjects included in the study is presented in Table 2. The volunteers received a full explanation of the nature and purpose of the study from the investigator in which was stated that they were free to withdraw from the study at any time without prejudice. Each volunteer was then required to give written informed consent for participation in the study. All the volunteers completed the study and none of them referred adverse events or significant alteration of the hematochemical parameters, so with reference to the safety conclusions, it is possible to assert that both formulations were well tolerated.

The study was carried out in accordance with the relevant guidelines of the Declaration of Helsinki (1964) and its amendments and the general principles of ICH Harmonised Tripartite Guidelines for Good Clinical Practice (ICH Topic E6, CPMP/ICH/135/95, Note for Guidance on the Investigation of Bioavailability and Bioequivalence: CPMP/EWP/QWP/1401/98 Rev. 1) and Bulgaria's legislation (LMPHM and regulation 31). The conduction of the clinical trial was approved by the Ethics Committee of MHAT "Lyulin" (Sofia - Bulgaria) with the outgoing No. 108 dated August 28th, 2014.

Study design

An open-label, randomized, crossover study with two treatments, two sequences and two periods was conducted, in which the volunteers alternatively received CSP or BE according to a randomization schedule. The dosage for CSP (500 mg) was selected on the basis of a pilot human pharmacokinetic study and was in compliance with the rodent toxicological profile of the product (not published Indena S.p.A. proprietary information). It was also backed up by preliminary evidence of clinical activities (Lazzaro and Loiero, 2014a, 2014b, 2015) and previous human absorption studies generally using similar dosages (Abdel-Tawab, 2011). As the weight of CSP and BE contained in the two-capsule administration was the same for both treatments, namely 500 mg, this study involved comparison of the formulated extract with a three-fold molar excess of the corresponding native extract. In line with the dilution factor associated with the presence of lecithin and microcrystalline cellulose in CSP the total content of the six major BAs (Table 1) amounted 67.26 mg in the CSP capsule compared to 176.94 mg in the BE capsule.

The check-in procedure was carried out at around 19:00 in the clinical center on study day -1 (the day prior to the scheduled dosing of each period). After that, by 19:30 the volunteers received a dinner and were then maintained in a fasting condition until the study formulations (2 × 250 mg capsules) were administered as single-dose with 200 ml of water between 8.00 a.m. and 9.00 a.m. of the following day.

Blood samples were collected before dosing (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h post dose. The duration of each hospitalization was about 24 h (approximately 12 h before the product administration and 12 h thereafter) after which the volunteer was discharged. They came back to the clinical center at 08:00 a.m. for the blood sampling of the 24, 48 and 72 h. The wash-out between treatment periods was 21 days. The volunteers had specialized attendance and cares during all treatment periods including a summary verification of their conditions.

Plasma samples collection and analysis

A total amount of about 330 ml of blood was withdrawn from each volunteer during the whole study. This amount includes 34 blood samples of 9 ml during the two treatment periods, and about 24 ml (2 samples of 12 ml) for laboratory tests during screening and follow-up procedures. Each sample volume was collected into tubes containing Li-heparine and was rapidly centrifuged at 3000 rpm for 10 min. Immediately after the centrifugation ± 2.5 ml of plasma from each sample was transferred to polypropylene tubes and frozen at – 20 °C. For the analytical evaluation the samples were transported in a frozen state to the Central Laboratory of German Pharmacists, Carl-Mannich-Str. 20 D-65760 Eschborn. The packaging of the samples met the Biosafety rules and IATA recommendations related to the documentation and package.

The BAs content in plasma samples was determined using a sensitive previously developed and validated LC-MS method (Gerbeth et al., 2011, 2013).

For that purpose, 1 ml of human plasma samples were spiked with 25 µl of the internal standard solution consisting of fluoxymesterone in methanol at a concentration of 4 µg/ml and 40 µl of pure methanol (to correct for the volume in accordance with the calibration samples). Afterwards the samples were extracted with THF:ethyl acetic acid: n-hexan:2-propanol (480:480:480:45) and evaporated to dryness followed by reconstitution in 100 µl of the mobile phase. After vortexing, ultrasonic treatment, and centrifugation LC-MS 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 SecurityGuard 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 40°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

Table 3
Overview on the pharmacokinetic parameters of the individual BAs following the oral administration of non-formulated *B. serrata* extract (BE) and lecithin-formulated *B. serrata* extract (CSP) at equiweight dosage and corrected dosage (normalized pharmacokinetic parameters).

Parameters		BE (not formulated)	Lecithin-formulated BE (equiweight dosage)		Lecithin-formulated BE (corrected dosage)	
		Mean ± SD	Mean ± SD	Ratio[g] (90% CI)[h]	Mean ± SD	Ratio (90% CI)
KBA						
	C_{max} [ng/ml][a]	71.12 ± 39.41	120.60 ± 57.15	1.90 (1.26 – 2.86)**	341.3 ± 161.73	5.38 (3.57 – 8.10)***
	T_{max} [h][b]	3.25 ± 1.20	2.75 ± 1.14			
	AUC_{last} [ng/nL*h][c]	980.60 ± 1376.45	1033.54 ± 1234.81		2924.9 ± 3494.5	4.31 (2.42 – 7.68)***
	AUC_{∞} [ng/nL*h][d]	1395.20 ± 1973.75	2050.10 ± 4237.95		5801.8 ± 11,993	3.77 (2.07 – 6.88)**
	$T_{1/2}$ [h][e]	15.4 ± 13.49	22.84 ± 42.83			
	Ke [h^{-1}][f]	0.08 ± 0.06	0.17 ± 0.16			
AKBA						
	C_{max} [ng/ml][a]	6.27 ± 6.83	14.30 ± 5.78	4.01 (1.65 – 9.78)**	43.18 ± 17.45	11.06 (4.28–28.55)***
	T_{max} [h][b]	2.5 ± 0.91	1.27 ± 0.34	−1.20 (−1.85 to −0.56)**		
	AUC_{last} [ng/nL*h][c]	19.35 ± 31.49	27.20 ± 17.79	3.84 (1.28 – 11.55)*	82.14 ± 53.73	10.58 (3.35–33.41)**
	AUC_{∞} [ng/nL*h][d]	38.10 ± 48.48	39.78 ± 23.68	3.69 (1.05 – 12.94)*	120.13 ± 71.52	10.17 (2.79–37.09)**
	$T_{1/2}$ [h][e]	5.51 ± 3.11	1.78 ± 1.05	−4.08 (−7.00 to −1.17)**		
	Ke [h^{-1}][f]	0.15 ± 0.05	0.59 ± 0.47			
βBA						
	C_{max} [ng/ml][a]	174.94 ± 163.25	338.87 ± 140.56	2.23 (1.67 – 2.98)***	776.01 ± 321.8	5.12 (3.83 – 6.83)***
	T_{max} [h][b]	6.25 ± 2.59	4.08 ± 1.51	−2.17 (−3.77 to −0.56)**		
	AUC_{last} [ng/nL*h][c]	3687.13 ± 3723.72	5244.78 ± 2148.64	1.70 (1.38 – 2.10)***	12,011 ± 4920.4	3.90 (3.15 – 4.82)***
	AUC_{∞} [ng/nL*h][d]	4438.90 ± 4398.75	6309.22 ± 2706.17	1.68 (1.37 – 2.05)***	14,448 ± 6197.2	3.84 (3.13 – 4.70***
	$T_{1/2}$ [h][e]	26.12 ± 7.35	27.01 ± 8.65			
	Ke [h^{-1}][f]	0.03 ± 0.01	0.03 ± 0.01			
αBA						
	C_{max} [ng/ml][a]	60.42 ± 45.60	120.24 ± 53.10	2.16 (1.64- 2.85)***	270.54 ± 119.48	4.86 (3.68 – 6.41)***
	T_{max} [h][b]	5.92 ± 2.64	3.96 ± 1.64	−1.96 (−3.65 to −0.27)*		
	AUC_{last} [ng/nL*h][c]	1369.31 ± 994.62	1946.86 ± 520.72	1.61 (1.31 – 1.97)**	4380.4 ± 1171.6	3.61 (2.94 – 4.44)***
	AUC_{∞} [ng/nL*h][d]	1703.62 ± 1179.32	2610.84 ± 961.01	1.66 (1.33– 2.08)**	5874.4 ± 2162.3	3.75 (2.99 – 4.69)***
	$T_{1/2}$ [h][e]	31.76 ± 8.57	38.58 ± 13.35			
	Ke [h^{-1}][f]	0.02 ± 0.01	0.02 ± 0.01			
AαBA						
	C_{max} [ng/ml][a]	119.94 ± 322.96	66.79 ± 30.12	1.80 (1.07 – 3.03)*	201.04 ± 90.66	5.41 (3.21 – 9.13)***
	T_{max} [h][b]	6.92 ± 2.02	4.96 ± 1.78	−1.96 (−3.00 to −0.92)**		
	AUC_{last} [ng/nL*h][c]	2022.55 ± 5228.92	1202.17 ± 521.45	1.68 (1.05 – 2.68)*	3618.5 ± 1569.5	5.04 (3.16 – 8.06)***
	AUC_{∞} [ng/nL*h][d]	2339.48 ± 6022.60	1413.35 ± 562.18	1.70 (1.07 – 2.69)*	4254.2 ± 1692.1	5.11 (3.23 – 8.09)***
	$T_{1/2}$ [h][e]	23.55 ± 6.55	28.06 ± 10.84			
	Ke [h^{-1}][f]	0.03 ± 0.01	0.03 ± 0.01			
AβBA						
	C_{max} [ng/ml][a]	100.44 ± 133.61	173.75 ± 68.44	2.29 (1.61 – 3.24)*∗	505.62 ± 199.16	6.66 (4.69 – 9.44)***
	T_{max} [h][b]	5.92 ± 1.68	4.29 ± 1.50	−1.62 (−2.76 to −0.49)**		
	AUC_{last} [ng/nL*h][c]	1722.25 ± 2555.58	2465.55 ± 895.85	1.99 (1.44 – 2.76)**	7174.8 ± 2606.9	5.80 (4.20 – 8.03)***
	AUC_{∞} [ng/nL*h][d]	2013.22 ± 3099.16	2743.74 ± 975.76	1.94 (1.38 – 2.72)**	7984.3 ± 2839.4	5.65 (4.03 – 7.93)***
	$T_{1/2}$ [h][e]	23.57 ± 7.65	22.09 ± 6.10			
	Ke [h^{-1}][f]	0.03 ± 0.01	0.03 ± 0.01			

[a] C_{max}: maximal plasma concentration calculated as mean of the individual maximal plasma concentrations of each subject; [b] T_{max}: time required to C_{max}; [c]AUC_{last}: area under the plasma concentration–time curve calculated by trapezoidal rule from time zero to the time of the last quantifiable concentration; [d] AUC_{∞}: area under the plasma concentration–time curve calculated by trapezoidal rule from time zero extrapolated to infinite time; [e]$T_{1/2}$: the mean time taken for the plasma concentration to fall to half of its original value; [f]Ke: Elimination rate constant from the central compartment; [g]Ratio: geometric mean ratio of lecithin-formulated BE (equiweight dosage) to non-formulated BE; 90% [h]CI: 90% confidence interval; statistically significant differences: * means $P < 0.1$; ** means $P \leq 0.05$; *** means $P \leq 0.001$. Note that the maximal plasma concentrations in the table may differ from the maximal plasma concentration in Fig. 2 due to interindividual variability in T_{max}.

(Agilent Technologies, Waldbronn, Germany) equipped with an Electro Spray Ionization source (ESI). Dwell time was chosen to be 200 ms. The detected ions in single ion mode were m/z 511.5 for AKBA, m/z 469.3 for KBA, m/z 497.4 for AαBA and AβBA, respectively, m/z 455.5 for αBA and βBA, respectively, and m/z 381.2 for the internal standard fluoxymesterone. Quantification of plasma samples was carried out with the internal standard method using peak area ratios. A chromatographic run consisted of the calibration samples, the quality control samples and the respective volunteer samples. The MassHunter® software was used for data acquisition and processing.

Pharmacokinetic analysis

All subjects completed the two treatment periods without any major protocol deviation and were therefore included into the statistical analysis. The pharmacokinetic parameters have been calculated using the software SAS® (Statistical Analysis System) version 93 and EquivTest/Pk.

Statistical analysis

All plasma data for the individual BAs were expressed as mean ± S.D. For statistical analysis the pharmacokinetic parameters (AUC_{last}, AUC_{∞} and C_{max}) derived from the plasma concentrations of the individual BAs have been log-transformed and analyzed by mixed-effects ANOVA according to a two-treatment, two-period, two-sequence randomized crossover design. The ANOVA model included Treatment, Period and Sequence as fixed factors and Volunteers nested in sequences as random factor. The least squares means (LSM) of CSP and BE and the difference (CSP-BE) between LSM with the 90% confidence interval (90% CI) have been calcu-

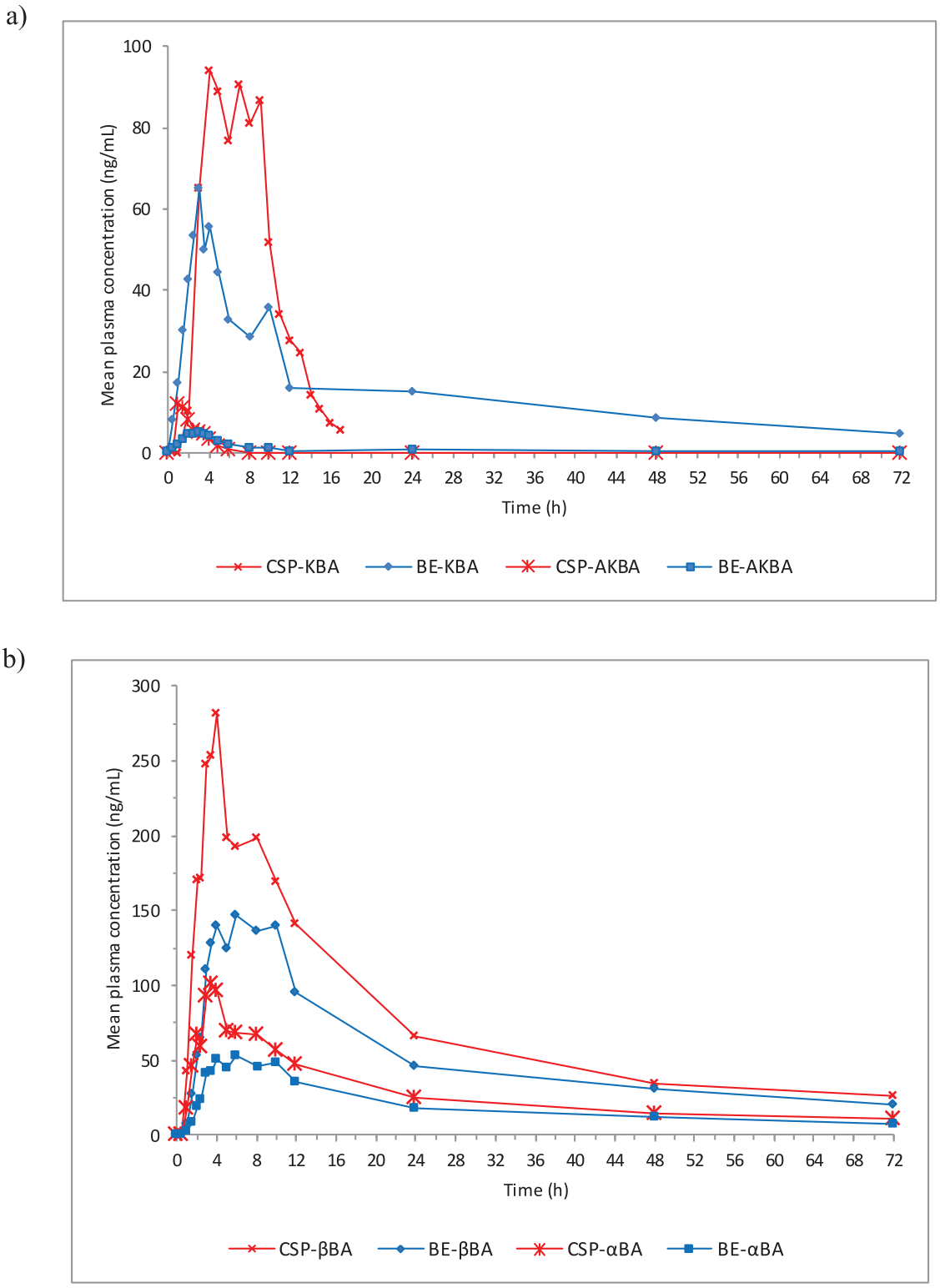


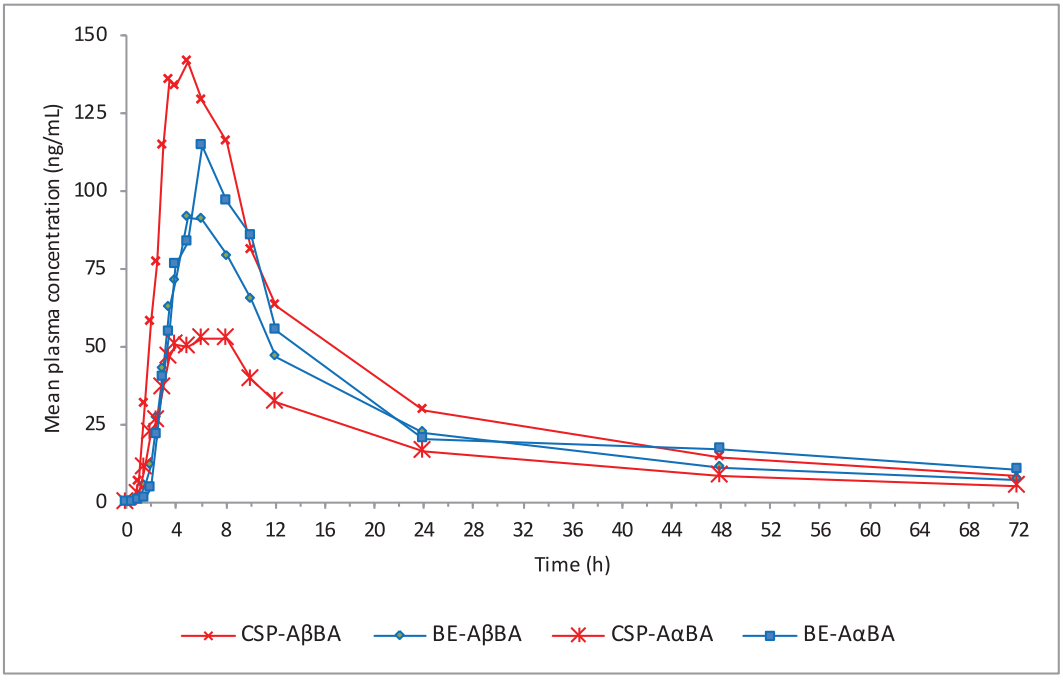
Fig. 2. Plasma concentrations of boswellic acids (BAs) after single oral administration of the non-formulated extract (BE-series) and their corresponding lecithin formulation (CSP-series). a: KBA and AKBA. b: αBa and βBA. c: AαBa and AβBA. d: Total boswellic acids.

lated. The CSP/BE ratio was found by calculating the anti-log of the difference between LSM. ANOVA was also performed on the untransformed data of the other pharmacokinetic parameters: T_{max}, $T_{1/2}$ and Ke. The difference between LSM and the 90% CI have been reported.

Results and discussion

The mean plasma concentrations and pharmacokinetic parameters of each individual BA are summarized in Table 3. Notably, the administration of the non-formulated BE was asso-

c)



d)

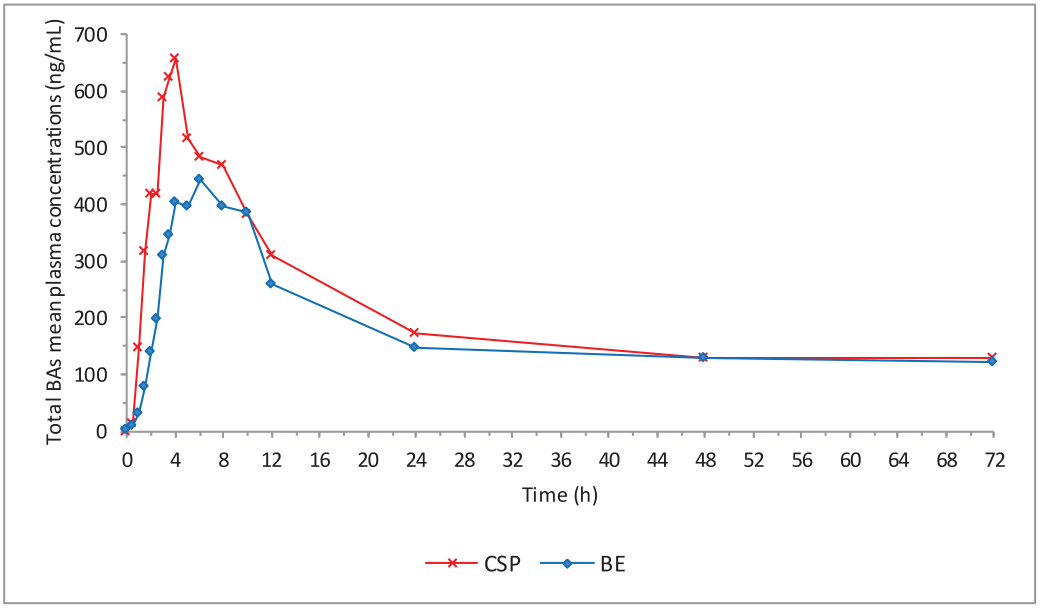


Fig. 2. Continued

ciated with a higher variability compared to the administration of the lecithinized CSP formulation. This may be attributed to the high interindividual variability often noted in pharmacokinetic studies upon administration of sparingly soluble and poorly bioavailable compounds. Nevertheless, in spite of this great variability, a clear conclusion could be made upon statistical analysis. As reflected by the geometric mean ratio of CSP to BE, found by calculating the anti-log of the difference between LSM, the administration of CSP at equivalent weight dosage resulted in statistically higher C_{max} and AUC for AKBA (4-fold), and AβBA, βBA, αBA, and AαBA (2-fold), while only a modest increase for the C_{max} (10%) and no significant change in the AUC was observed for KBA. These results clearly demonstrate an enhanced absorption of BAs from the lecithinized CSP formulation compared to the non-formulated BE even though the administered amount of *B. serrata* extract in CSP corresponded only to around one third of the amount of *B. serrata* extract in the BE formulation. In addition the absorption was about 1.5–2 h faster in case of the lecithin formulation compared to the non-formulated extract (Table 3) for all BAs that showed an improved absorption.

The increased absorption of BAs from CSP manifests itself also in the higher plasma concentration profiles presented in Fig. 2 a-d. Only the C_{max} of AαBA (Fig. 2c), in contrast to the other BAs, was higher in the BE formulation. This may be simply attributed to a particularly high value determined for the plasma concentration of AαBA in one subject of the BA group, resulting

in an overall increased mean C_{max} for AαBA in the BE group. Since however, this aberrant value could not be ascribed to an analytical mistake, it wasn't eliminated. Notably, the plasma concentration profiles of the non-acetylated BAs KBA, βBA, and αBA reveal multiple peakings in the plasma concentration over time. This phenomenon is often encountered in case of enterohepatic circulation. In this case extensively metabolized drugs are transported from the liver to the small intestine via the bile duct, where they are subsequently reabsorbed back through the lumen of the gastrointestinal tract into the portal blood circulation (Davies et al., 2010). In fact, it could be shown that the non-acetylated BAs KBA, βBA, and αBA undergo extensive metabolism, whereas the acetylated BAs AKBA, AβBA, and AαBA are rather metabolically stable (Gerbeth et al., 2013). In order to get an approximate idea of the pharmacokinetics of BAs in case an equimolar amount of the BAs in the non-formulated extract would have been administered as lecithin formulation, the pharmacokinetic data (C_{max}, AUC_{last}, and AUC_{∞}) were normalized taking into consideration the dilution factor for the respective BA in the lecithin formulation. Also the extrapolated data revealed an improved BA absorption (Table 3 corrected dosages), being highest for AKBA (almost 10-to 11-fold increase in Cmax and AUC), and significant for AαBA and AβBA (5-fold improvement). Although not so marked, also the increase for αBA, βBA and KBA could compensate the almost 1:3 dilution effect of lecithin addition. This significant improvement in the absorption of acetylated BAs is unique to the present study and could not be observed before, even when BAs were administered with a fatty meal (Skarke et al., 2012). Obviously the acetylation of the 3-hydroxyl group in BAs exerts a remarkable effect on the enhanced lecithin-associated absorption of BAs. Of course, a precise evaluation of the improved absorption associated to the lecithin formulation would have involved an equimolar comparison between the amounts of BAs administered as formulated and non-formulated extract. But as further clinical studies are planned, we had opted for a more practical weight-to-weight comparison when designing the present study. Nevertheless, the present study revealed for the first time in human studies, plasma levels in the range of the active concentrations of BAs after a single administration of a *Boswellia* extract. Thus, the C_{max} values of βBA, and AβBA were found to fall in the range of their IC_{50} values (0.8 μM, 1.2 μM, respectively) for the inhibition of cathepsin G, a major target of *Boswellia* triterpenoids (Tausch et al, 2009). The C_{max} value of βBA was also in the range of the IC_{50} (1.8 μM) value for the inhibition of LPS activity in a cell-free LAL assay (Henkel et al., 2012). On the other hand, even though AKBA mostly benefited in terms of absorption from the lecithin formulation, its plasma concentration (C_{max} 0.03 μM) remained below its pharmacological activity range. Based on the fact that the pharmacological activity of *Boswellia* extract cannot be assigned to a single BA, our results underlie the relevance of lecithin formulation to improve the absorption of the whole multicomponent botanical extract and not just of specific constituents. This is reflected in the enhanced absorption of the whole BA bouquet in the present study as well as in the improved absorption of a lecithin formulation of curcuminoids in a former study (Cuomo et al., 2011). However excipient enhancers should not be evaluated only in terms of improved molar absorption of an active, but also in terms of their added weight to the final formulation, as the overall weight of a formulation (tablets, capsules, soft gels) represents a critical parameter in formulation development and administration. Also in this regard the lecithin formulation provides a great advantage. Hence the weight of the adsorption (lecithin)- and formulation (cellulose) adjuvants is kept within reasonable limits (about 2/3 of the final product) in the lecithin-formulated *Boswellia* extract, allowing thus the extract's use in a finished solid product form. This remarkable technological achievement is of special relevance for herbal extracts that are often sticky resinous materials requiring normally a high load of additives to be formulated into solid forms (capsules, tablets, soft gels).

Conclusion

In summary, the present study revealed an enhanced absorption of BAs from the lecithin formulation, resulting for the first time in plasma concentrations in the range of their pharmacologically active concentrations. These promising results pave the way to more clinical studies in the future evaluating the clinical potential of BAs. Last but not least, the present study supports the beneficial effect of emulsification with lecithin on the absorption of strongly self-aggregating and poorly soluble phytochemicals, by simply mimicking the natural absorption process of a fatty meal.

Conflict of interest

PM and AR are employed by Indena, the manufacturer of the lecithin-formulated BE (Casperome®). CA was employed by Indena at the time of the study. GA consults regularly for Indena. The other authors have no conflict of interest.

Supporting information

Graphical comparative representation of C_{max}, t_{max} and AUC for the six major boswellic acids of the *Boswellia* extract (BE) and its corresponding lecithin formulation.

Analytical method validation

Acknowledgment

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2016.07.009.

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