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Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis — An open pilot study

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Abstract

Background and aims: A significant fraction of patients with ulcerative colitis (UC) is not sufficiently controlled with conventional therapy or suffers from therapy related side effects. Anthocyanins, highly abundant in bilberries (*Vaccinium myrtillus*), were shown to have antioxidative and anti-inflammatory effects. We aimed to explore the therapeutic potential of bilberries in active UC.
Methods: In an open pilot trial with a total follow-up of 9 weeks the effect of a daily standardized anthocyanin-rich bilberry preparation was tested in 13 patients with mild to moderate UC. Clinical, biochemical, endoscopic and histologic parameters were assessed.
Results: At the end of the 6 week treatment interval 63.4% of patients achieved remission, the primary endpoint, while 90.9% of patients showed a response. In all patients a decrease in total Mayo score was detected (mean: 6.5 and 3.6 at screening and week 7, respectively; $p<0.001$). Fecal calprotectin levels significantly decreased during the treatment phase (baseline: mean 778 μg/g, range 192–1790 μg/g; end of treatment: mean 305 μg/g, range <30–1586 μg/g; $p=0.049$), including 4 patients achieving undetectable levels at end of treatment. A decrease in

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endoscopic Mayo score and histologic Riley index confirmed the beneficial effect. However, an increase of calprotectin levels and disease activity was observed after cessation of bilberry intake. No serious adverse events were observed.
Conclusions: This is the first report on the promising therapeutic potential of a standardized anthocyanin-rich bilberry preparation in UC in humans. These results clearly indicate a therapeutic potential of bilberries in UC. Further studies on mechanisms and randomized clinical trials are warranted.

1. Introduction

There is a worldwide increase in the prevalence and incidence of ulcerative colitis especially in countries that recently adopted a "westernized lifestyle" such as India or China.[1] Around 30–50%[2] and 15–20%[3]of patients with ulcerative colitis (UC) are not sufficiently controlled with conventional anti-inflammatory treatment with 5-ASA and after corticosteroid therapy, respectively, or suffer from therapy related side effects, so that about 15–20% needs more advanced therapy, typically an immunosuppressive agent such as a purine analog. About half of these,[4,5] around 5–10% of all UC patients, again are non-responders in need of more intensive therapeutic strategies, such as cyclosporine, anti-TNF therapy (still with a number needed to treat of four to five for steroid-free remissions), or colectomy.[6] New therapeutic strategies are intensively investigated. However, only few new "small molecule drugs" are studied at present, despite the obvious need to introduce new therapeutic options.

In recent years the interest in polyphenols has risen due to accumulating evidence for beneficial effects in human health.[7,8] There are several thousand different polyphenol molecules known, which are classified according to their phenol ring structure into phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids, which are abundant in regular diet,[9,10] can further be sub-classified into flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols.[8] Anthocyanins (glycosides of anthocyanidins) are especially abundant in red, blue and black berries and other fruits, but also occur in red wine, cereals and certain vegetables.[8,11] Daily dietary intake is highly variable. Anthocyanins constitute the largest fraction of food flavonoids and the intake may exceed 100 mg per day in a diet rich in vegetables (such as aubergines), dark grapes and berries.[12,13]

Bilberries (*Vaccinium myrtillus*), also referred to as "European blueberries", and other members of the *Vaccinium* family possess a naturally high content of polyphenols, of which anthocyanins constitute the biggest fraction with 50–80%.[14,15] An anthocyanin rich bilberry extract was shown to alter the expression of various genes implicated in the pathogenesis of atherosclerosis in an apo E-deficient mouse model.[16] In a randomized human study including individuals at increased risk for cardiovascular disease significant decreases in plasma concentrations of C-reactive protein (CRP), interleukin (IL)-6, IL-15, and monokine induced by INF-c (MIG) were observed in the group receiving bilberry juice.[17] Moreover, anthocyanins inhibited the adhesion of *Neisseria meningitidis* to cultured human epithelial cells.[18] In rats, bilberry extract was protective against doxorubicin induced oxidative cardiotoxicity.[19] In humans, a decrease in postprandial insulin demand after the ingestion of a bilberry containing drink was shown recently.[20] In addition, a potential chemopreventive effect of anthocyanins in colorectal cancer (CRC) was suggested in a pilot trial of human subjects with CRC, where bilberry intake induced a significant decrease of proliferative markers in tumor tissue.[21]

In UC (similar to CD), excessive amounts of reactive oxygen species (ROS) are generated by activated macrophages and neutrophils in the inflamed intestine. In addition, an imbalance of oxidative stress and the limited capacity of the intestinal antioxidant defense system have been described.[22–24] As anthocyanins have antioxidative as well as anti-inflammatory properties, they are potential therapeutic agents in IBD.

Blueberries and black raspberries, both containing high concentrations of anthocyanins, have shown to attenuate the course of colitis in animal models.[25–28] The effect of anthocyanins on the course of IBD in humans has not yet been studied. Here we report the results of an open prospective interventional trial of a six week high-dose intake of a standardized bilberry preparation on the clinical, endoscopic and biochemical disease course in patients with mild to moderate UC. Furthermore safety, tolerability, side effects and patient satisfaction were assessed.

2. Materials and methods

2.1. Study design and study population

We conducted an open, prospective, non-blinded and non-controlled pilot trial on an anthocyanin-rich bilberry preparation in well characterized patients with mild to moderate UC. Between March 2010 and April 2011 13 patients were enrolled in our ambulatory IBD unit at the Division of Gastroenterology & Hepatology of the University Hospital Zurich. The study was approved by the local ethics committee (EK-1733). Written informed consent was obtained from all patients and the study was performed according to the declaration of Helsinki. Inclusion criteria comprised an established diagnosis of UC for at least 6 month, current mild to moderate disease activity (clinical activity index (CAI): 4–8), age between 18 and 65 years, and a stable use of medication with stable doses of 5-ASA, thiopurines, therapeutic antibodies and of corticosteroids for at least 3 months and 4 weeks prior to inclusion, respectively. Exclusion criteria comprised an intake of drugs or natural products including bilberries, grapefruit and quinine containing drinks with known interference with

CYP3A or CYP2D6 in the last two weeks prior to inclusion, HIV, Hepatitis B and C infection, abscesses, elevation of CRP over 100 mg/l, known intolerance to anthocyanin or related substances, or a simultaneous participation in another clinical trial within the last 30 days prior to inclusion. The study comprised of 6 study visits (Table 1).

At the screening visit (7 days prior to baseline = week 1), a full medical history, physical examination with vital signs, a lab screen, urine analyses and a sigmoidoscopy were obtained. Furthermore, the CAI and the complete Mayo Score were determined, the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) was completed, and a patient diary – including a quantification of daily liquid or very soft bowel movements, abdominal pain, general well-being, temperature and intake of loperamide or similar medication – was delivered to the patients. The second study visit 7 days after screening (baseline = week 1) was pre-specified as the beginning of bilberry intake. The intake period was 6 weeks and ended at the week-7-visit. At the week-1-visit and the following study visits at week 3, 5, 7 and follow-up (final study visit between week 9 and 11) a laboratory screen and control of vital signs were performed. The patients' diaries were collected and CAI as wells as SIBDQ were assessed. Patients underwent a second sigmoidoscopy at the end of bilberry intake during the week-7-visit.

2.2. Outcome measures

The primary endpoint was defined as achievement of remission (CAI <4) at end of treatment. Response (reduction of CAI ≥ 3 or remission), 2 point improvement of endoscopic Mayo Score, improvement of SIBDQ and improvement of CAI were pre-specified secondary endpoints. Clinical response was evaluated on the basis of the CAI and SIBDQ outcome as well as on additional specifications collected in the diaries, such as the state of general well-being or the rate and intensity of abdominal pain. Analyses of routine clinical inflammatory parameters (leukocytes, CRP and thrombocytes) and a safety laboratory were performed at every study visit. Fecal calprotectin (Bühlmann® Laboratories AG, Schönenbuch, Switzerland) was determined at every study visit with the exception of screening (sample at week 1 was obtained prior to the first bilberry intake). Sigmoidoscopy with biopsy taking and assessment of endoscopic Mayo Score was performed twice after standard preparation of the left-sided colon with one to two preparation enemas, once prior to and once at the last day of bilberry intake (Screening and week 7, respectively). Biopsies were obtained from all patients from the rectum and sigmoid colon in both sigmoidoscopies. Histologic specimen were graded by a fully blinded expert GI-pathologist (A.W.) according to the Riley index using the three variables representative of acute inflammatory activity (polymorphonuclear cells in the lamina propria, crypt abscesses and mucin depletion), which were shown to correlate with risk of future disease relapse.[29] For each variable a score of 0–3 was assigned (with 3 representing most severe changes), total score was calculated as the sum of these 3 acute inflammatory variables (range 0–9).

2.3. Study medication

The bilberry (*Vaccinium myrtillus*) preparation was specifically manufactured for the purpose of this study by Symrise *(*Symrise GmbH & Co, Holzminden, Germany*)* under highly standardized conditions with dried, sieved bilberries (59.63%) and concentrated bilberry juice (25.90%) as the main components. The preparation was packed in small aluminum trays, each containing 40 g. Patients received a daily bilberry preparation dose of 160 g (4 trays per day), corresponding to 95 g dry weight (corresponding to an amount of around 600 g of fresh fruit, assuming a water content in fresh bilberries of 80–85%) for a total of six weeks. The anthocyanin content of the preparation was determined by repetitive high performance liquid chromatography (HPLC) scans, revealing highly reproducible values with a mean content of 210 mg anthocyanins per tray, corresponding to an average anthocyanin dose of 840 mg per day. Patients were asked to refrain from food or

Table 1 Study design.

	Screening (day-7)	Treatment period				Follow-up (week 9–11)
		Week1 = baseline	Week 3	Week 5	Week 7	
Medical history, update	●	●	●	●	●	●
Physical examination	●					
Vital signs, body mass index	●	●	●	●	●	●
Lab screen	●	●	●	●	●	●
HIV and hepatitis serology	●					
Urine analysis	●					●
Fecal sample for calprotectin		●	●	●	●	
Sigmoidoscopy	●				●	
Bilberrry intake (4 aluminum cups daily)		●	●	●	●	
CAI assessment	●	●	●	●	●	●
Adverse events		●	●	●	●	●
Patient diary (issue/retraction)		●	●	●	●	●
SIBDQ	●	●	●	●	●	●

HIV, Human Immunodeficiency Virus; CAI, Clinical Activity Index; SIBDQ, Short Inflammatory Bowel Disease Questionnaire.

liquid intake within one hour prior to and after bilberry ingestion. Time and daily dose of ingestion had to be recorded in the patient's diary.

2.4. Safety evaluation, additional analyses

Vital signs, a short physical examination, a safety lab (including blood count, renal function and electrolytes) and prior medical history were obtained at screening. During the following study visits patients were asked about any changes in clinical symptoms and definite or potential side effects related to bilberry intake. Vital signs and a safety lab were controlled at each visit. At the end of the study a questionnaire to survey overall patient satisfaction with bilberry intake with regard to taste, applicability, side effects and clinical efficacy had to be completed.

2.5. Statistical analysis

All statistical analyses were performed using SPSS 19 (IBM, Armonk, NY, USA). A level of $p<0.05$ was considered as significant. Continuous variables are presented as median (interquartile range [IQR]) or mean ± standard deviation and compared using the Wilcoxon rank-sum test and the Friedman-test or the paired *t*-test, respectively.

3. Results

3.1. Patient characteristics

In total, 14 patients were screened. One individual had to be excluded due to a too low CAI. Hence, 13 individuals could be included in the study. Eleven individuals completed the study. Two patients dropped out of the study due to an unforeseen longer travel of one patient and a disease flare requiring treatment with systemic steroids during the bilberry intake phase in the second, respectively. According to the predefined inclusion criteria all patients had a mild to moderate active disease (mean CAI at Screening: 6.1; 6.2 intention to treat (ITT)). Mean age of the 13 patients (9 male, 4 female) was 38 years. Most patients suffered from long-standing disease (mean disease duration 91 months), including a high proportion with extensive disease (61.5%; no cases with ulcerative proctitis). In addition, all patients had previously received systemic 5-ASA and corticosteroids, whereas a significant fraction of patients was experienced to first- and second-line immunosuppressive agents, such as thiopurines or tacrolimus, as well as anti-TNF-therapy and alternative treatment strategies, indicative of a rather difficult-to-treat patient population (Table 2).

Table 2 Demographic data, disease characteristics, medical treatment.

	ITT population	PP population
	n = 13	n = 11
Demographic data		
Males	76.90%	81.80%
Age (y), (median (range))	38 (19–61)	41 (19–61)
Localization of UC		
Extensive	8 (61.5%)	7 (63.6%)
Left sided	5 (38.5%)	4 (36.4%)
Proctitis	0	0
Duration of disease in month mean (range)	91 (16–282)	98 (16–282)
Current medical treatment		
5-ASA topical/systemic	6 (46.1%)/ 9 (69.2%)	5 (45.5%)/ 8 (72.7%)
Steroids topical/systemic	7 (53.8%)/ 7 (53.8%)	6 (54.5%)/ 6 (54.5%)
Tacrolimus	3 (23.1%)	2 (18.2%)
Probiotics	3 (23.1%)	2 (18.2%)
Previous and current medical treatment		
5-ASA topical/systemic	10 (76.9%)/ 13 (100%)	10 (90.9%)/ 11 (100%)
Steroids topical/systemic	9 (69.2%)/ 13 (100%)	9 (81.8%)/ 11 (100%)
Thiopurine	7 (53.8%)	6 (54.5%)
Tacrolimus	3 (23.1%)	2 (18.2%)
Infliximab	5 (38.5%)	5 (45.5%)
Cyclosporine	3 (23.1%)	3 (27.3%)
Antibiotics	4 (30.1%)	4 (36.3%)
Probiotics	4 (30.1%)	4 (36.3%)
Photodynamic therapy	1 (7.7%)	1 (9.9%)
Leucocyte apheresis	1 (7.7%)	1 (9.9%)

ITT, intention to treat population; PP, per protocol; UC, ulcerative colitis; 5-ASA, 5 aminosalicylates.

3.2. Clinical study endpoints

Remission, the primary endpoint, defined as CAI<4 was achieved in 63.4% (53.8% ITT) of patients at end of treatment, week 7 (mean CAI 2.6; 2.8 (ITT)). Two of the patients, who were in remission at the end of the treatment phase showed a relapse of clinical symptoms during the follow-up phase that resulted in an increase of CAI from 3 to 4 and 1 to 7, respectively. Hence, the remission rate at the end of the follow-up phase (end of study) was 54.5% (46.2% ITT). Mean CAI at weeks 1, 3, 5, 7 and follow-up revealed to be significantly different to the mean CAI at screening ($p=0.041$; 0.007; 0.005; 0.004 and 0.025 (PP) as well as 0.041; 0.004; 0.005; 0.004 and 0.025 (ITT), Fig. 1.).

The response rate (defined as CAI value drop ≥3 or remission), was 90.9% (76.9% ITT) at end of treatment and 72.7% (61.5% ITT) at end of study.

We further found a significant reduction in the complete Mayo Score of at least two points in all patients (100% PP; 84.6% ITT; ≥3 points in 54.5%, 46.2 ITT; $p<0.001$; Fig. 2.) with a mean complete Mayo Score of 6.5 and 3.6 at screening and week 7 respectively (for both ITT and PP).

In 81.8% of patients, the SIBDQ score was significantly higher at end of treatment as compared to screening (mean SIBDQ scores 48.1 and 54 at baseline and week 7 respectively; ITT: 47.8 and 53, higher score in 69.2%, Supplementary Table 1). However, after the stop of bilberry intake SIBDQ showed a decline in most but not all patients, so that the mean value at follow-up (50.5; ITT: 49.7) was only slightly above the mean SIBDQ score at screening. An improvement

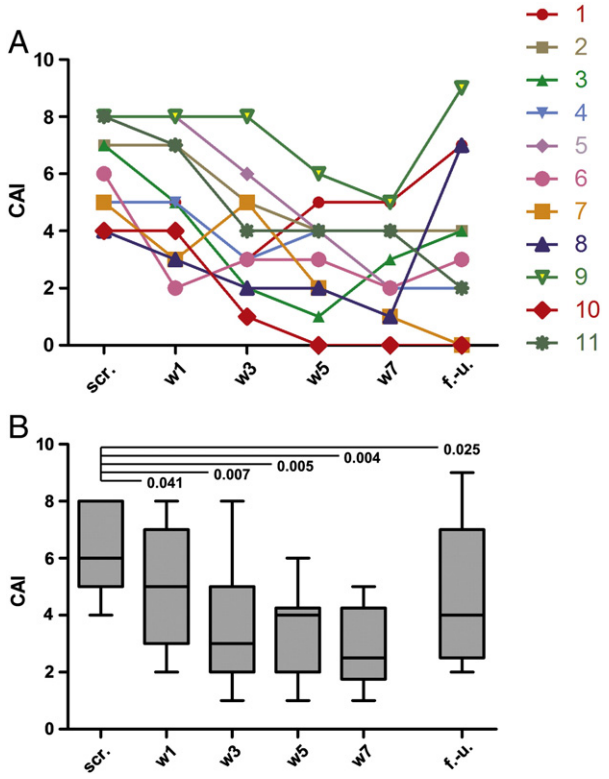

Figure 1 Evolution of CAI during the bilberry intake phase and follow-up. Two of the patients, who were in remission at the end of the treatment phase showed a relapse of clinical symptoms during the follow-up phase that resulted in an increase of CAI from 3 to 4 and 1 to 7, respectively. Hence, the remission rate at the end of the follow-up phase (end of study) was lower than at end of treatment (A, individual CAI values). Boxplots (B) depicting median (solid black line within the box), interquartile (IQ) range (box represents the middle 50% of CAI values) and values within 1.5 times the IQ range (according to Tukey, indicated by whiskers; scr.: screening, w: week, f.-u.: follow-up; p-values are depicted above the error bars).

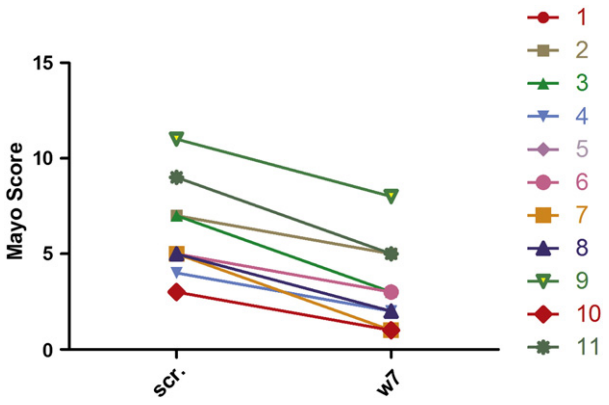

Figure 2 Individual evolution of the complete Mayo Score during the study. All patients (PP) showed a reduction of at least two points (a secondary endpoint).

of SIBDQ between screening and follow-up was detected in 63.6% of patients (ITT: 53%).

3.3. Sigmoidoscopy and histology

Although there was a significant decrease in the endoscopic Mayo Score (mean at inclusion: 1.5 points; mean at week 7: 1.2; p=0.038, Fig. 3., Supplementary Table 1), none of the patients showed a drop of ≥2 points, which was our ambitious secondary endpoint. Subsequently this secondary endpoint of the study could not be achieved. Fully blinded examination of biopsy specimen from the rectum and sigmoid colon in random order revealed a decline in acute inflammatory changes (summation of three histologic parameters from the Riley Index, representative of acute inflammation: 2.9 and 2.2 at screening and week 7, respectively). However, these changes did not reach statistical significance (p=0.17).

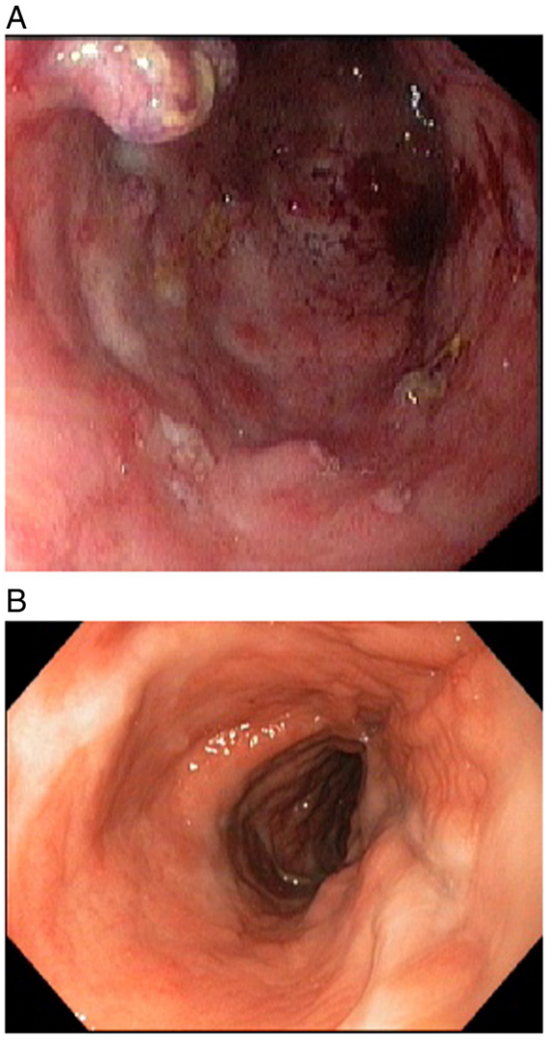

Figure 3 Endoscopic view of the sigmoid colon prior to bilberry intake at screening (A) and at the end of the 6-week intake phase at week 7 (B) showing an almost complete resolution of acute inflammatory mucosal changes. However, mucosal atrophy and scar formation indicative of longstanding prior inflammatory activity can be seen.

3.4. Biomarkers

We did not observe relevant changes in serum markers associated with inflammation (CRP, leukocytes, neutrophil granulocytes, and thrombocytes), or in the parameters included in the safety laboratory (electrolytes, markers of renal and liver function).

In contrast, striking differences in the levels of fecal calprotectin were detected further indicating a significant therapeutic benefit due to the bilberry intake. The lower limit of detection of the assay was 30 μg per g stool, levels below 50 μg/g were considered as normal. Initially, all patients showed significant elevations of baseline fecal calprotectin (mean level at baseline (PP): 778 μg/g stool (SD±484.5), range: 192 μg/g–1790 μg/g). In concordance with clinical activity profile over the treatment period a 2.9 fold (range 1.4–44.3 fold) decrease in calprotectin levels was observed already after two weeks of bilberry intake in the 11 patients, who completed the study (mean calprotectin level: 214 μg/g (SD±179; range: <30–542 μg/g; p=0.005; levels <30 μg/g) were considered 30 μg/g for calculation) at week 3. A further decrease in fecal calprotectin was observed after 4 weeks of bilberry intake again in every patient completing the study (mean level: 134 μg/g SD± 125.4; range: <30–324 μg/g; p=0.002). However, after the end of the 6 week bilberry intake period 7 patients started to rise in calprotectin levels again (mean: 305 μg/g; SD± 465.8; range <30–1586 μg/g; p=0.049), while 4 patients continued to have undetectable fecal calprotectin levels (<30 μg/g)(mean calprotectin level at follow-up: 995 μg/g; SD±1132.2; range <30–3027 μg/g; p=0.552; increase from 0.4 to 80 fold; Fig. 4.). Of note, the solitary patient, who dropped out due to lack of response and a disease flare during the treatment period resulted in the only observed increase of fecal calprotectin levels after initiation of bilberry intake (baseline: 1570 μg/g, week 3: 1709 μg/g).

3.5. Adherence to bilberry intake, patient satisfaction

Overall adherence to daily intake of the four portions of the bilberry preparation assessed by questionnaire and counting of residual trays was high. Patients consumed 91.2% of all bilberry trays in the first week of the intake phase. This amount revealed to be stable up to week 5 (89.3%), with a modest decrease in the last week of the intake phase (82.8% in week 6). In the questionnaire at the end of the intake phase, where patients were asked to evaluate tolerability of intake as well as the subjective clinical effect, the preparation was described as tasting naturally (58.3% of patients), intense (25%) but also sourly (41.6%). 33% of patients judged the preparation as being easy to ingest, while 50% evaluated the consistency as both too dry and too compact. The total daily amount was considered to be acceptable but at the upper limit feasible by the majority of patients (58.3%), while 16.7% reported that the ingested amount could easily be higher. The rest (25%) judged the amount as being too high. Similar results were obtained concerning the duration of the intake phase (16.7% longer phase possible, acceptable (50%), costing quite an effort in the end of the intake phase (8.3%) or already at the beginning (25%)). With regard to overall efficacy in amelioration of disease activity, a majority of patients reported a marked (66.7%) or moderate (16.7%) improvement of their symptoms, while one patient reported no effect on disease activity and one noticed a marked deterioration. Continuing the intake phase for a longer time with the same preparation would be feasible for 16.7% and not at all considerable for 8.3% of patients. Most of the patients (75%) reported that they would principally continue with the intake phase given that a more convenient mode of delivery of the therapeutic principle would be provided.

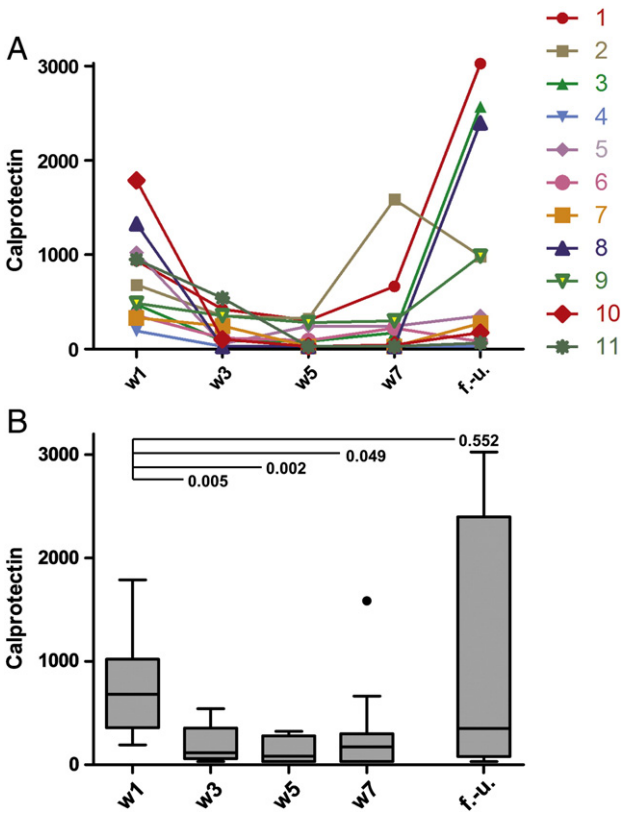

Figure 4 Fecal calprotectin before, during and after bilberry intake. Significant decreases in individual calprotectin levels were observed already after two weeks of bilberry intake in all patients, who completed the study. This decrease was sustained in most patients until end of treatment. However, a de novo rise in fecal calprotectin levels was observed in the follow-up period in 7 patients while 4 patients sustained undetectable fecal calprotectin levels (A, individual levels). Boxplots (B) depicting median (solid black line within the box), interquartile (IQ) range (box represents the middle 50% of CAI values) and values within 1.5 times the IQ range (according to Tukey, indicated by whiskers; scr.: screening, w: week, f.-u.: follow-up; p-values are depicted above the error bars; an extreme outliner (value more than 3 times the IQ range) at week 7 is depicted separately with a dot).

3.6. Side effects

In all patients a dark-bluish to black discoloration of both the feces and the tongue were observed (one patient furthermore reported slight discoloration of the teeth). One patient

reported the occurrence of mild dyspeptic symptoms (heartburn).

In addition, 33% of patients reported mild to moderate flatulence. We did neither observe any serious clinical adverse events nor any alterations in the safety laboratory parameters.

4. Discussion

In this open label human pilot study, investigating the administration of an oral anthocyanin-rich bilberry preparation in patients with UC high rates of clinical remission and response as indicated by significant decreases in CAI and SIBDQ could be achieved. Moreover, a parallel reduction of mucosal inflammation with significant decline in fecal calprotectin levels, a trend to lower histological activity score as well as attenuated inflammatory alterations in sigmoidoscopy were observed. These positive effects could be obtained despite the fact that 53.8% and 38.5% of the patients had shown clinical activity on previous treatment with an immune modulator (thiopurines) and anti-TNF therapy (infliximab), respectively, and 23.1% of patients currently receiving second-line immunosuppressive therapy (tacrolimus). Moreover, 76.9% and 69.2% of patients had previously received topical therapy with 5-ASA (mostly in conjunction with systemic 5-ASA) and/or steroids, the first-line therapeutic strategy for mild to moderate left sided as well as extensive UC according to consensus guidelines.[30] The persisting disease activity with failure to achieve sustained clinical remission despite the aforementioned therapeutic options indicates that the study participants belong to a difficult-to-treat UC patient population. Thus, the observed high rates of remission and response observed in this pilot trial are intriguing and encouraging, particularly in view of previous clinical trials in UC patients including various different agents, such as for instance azathioprine (remission: 53% vs. 5-ASA 21% (58% and 21% PP),[31]) tacrolimus (53% achieving complete response or remission of in total 83 patients treated,[32]) methotrexate (remission: 46.7%, yet with no significant difference to placebo,[33]) the probiotic VSL#3 (remission: 42.9% vs. 15.7% placebo[34]) or an antibody to the $\alpha 4\beta 7$-integrin (remission: 33% vs. 14% placebo[35]).

Moreover, in contrast to most of these other therapeutic strategies, bilberry intake is neither associated with any observed moderate to severe side effects nor potential theoretical concerns with regard to long-term intake. Hence, our findings implicate a highly favorable risk–benefit ratio.

According to the questionnaires obtained patient satisfaction was high. So was overall adherence, despite the rather high amount of the daily bilberry preparation (160 g) and the relatively inconvenient mode of administration. This is especially remarkable and indicative of a true net clinical benefit of bilberry ingestion in UC in view of the fact, that a high dietary intake of nutritional fibers in fruits and vegetables often is not well tolerated in patients with active UC.

The main limitation of our study is the lack of a placebo group. There is a well-known high placebo response rate in clinical studies of UC. The average placebo rates of remission and response in UC are 13% (95% confidence interval 9–18%) and 28% (95% confidence interval 23–33%), respectively, as described in a meta-analysis including 40 clinical studies in UC patients[36]. Due to practical aspects such as the large volume, the taste of the bilberry preparation, as well as the discoloration of the tongue and feces after intake, parallel investigation of a double blinded control group was not possible. For a double-blind study on the effects of anthocyanins in UC patients, a color- and odorless preparation with pure anthocyanins should be used, which is currently not available for the use in humans. We therefore chose the solid bilberry extract. However, the percentage of patients achieving response (91%) and remission (63%) in our study is considerably higher compared to these well-established placebo rates.[36] In addition, the parallel improvement of objective (fecal calprotectin, histologic score) disease parameters strongly argues against a mere placebo effect of bilberry administration. Likewise, the correlating decrease of clinical, endoscopic and histologic disease activity observed under bilberry intake argues against the assumption that the impressively rapid and profound decrease in fecal calprotectin levels happened by chance. A further argument is the increase in calprotectin and disease activity observed in seven patients after treatment stop.

Use of pure anthocyanins, the presumed substance for therapeutic efficacy, was not possible in this human pilot trial according to assignations of the local ethical committee, referring to our recent study in a colitis model in mice, where dried bilberries were fed and a reduction of INT-y and TNF from mesenteric lymph node was found.

We did not study a dose–response effect. The current daily bilberry dose (95 g dry weight per day) represents an extrapolation of our findings in the mouse model of colitis,[28] although it remains doubtful, whether the metabolism of flavonoids in humans is comparable to the metabolism in rodents.[37] With respect to the several potential beneficial effects of flavonoids observed in animal studies it is important to consider, that in the vast majority of these studies, the applied dosages by far exceed the average daily human intake in a normal diet.[37] Hence, a recommendation for UC patients to simply increase intake of flavonoid-rich food will most likely not translate to comparable clinical efficacy and reproduction of the significant clinical benefits observed in our study.

There is considerable variation in estimated mean daily nutritional intake of anthocyanins ranging from 82.5 to 170 mg/d, strongly depending on the study and variation of diet.[38,12] The bilberry dose chosen in our trial resulted in a total daily anthocyanin-intake of around 840 mg/d (in addition to normal dietary ingestion), exceeding the average intake in a typical western diet by a factor 5 to 10. Thus, our study can neither answer the question, whether a further increase of the ingested daily dose would translate in an increase in clinical benefit, nor whether a comparable beneficial effect on disease activity could be achieved with smaller dosages. In addition, it is not possible to estimate the potential of a long-term bilberry intake as a maintenance therapy. And although no long-term follow-up was performed in our study, the substantial fraction of patients with relapsing increase in both disease activity and fecal calprotectin levels soon after the end of the treatment

phase argues against a sustained clinical benefit of a timely delimited oral intake of the therapeutic principle, such as the 6-week interval used in this study.

Our study was not designed to elucidate the mechanisms of action underlying the beneficial effects of the bilberry preparation. However, in vitro and in vivo data suggest both direct anti-inflammatory effects on the intestinal mucosa[39,40,27,28,25,26,17] and beneficial influences on UC-associated dysbiosis. A two-way phenolic–microbiota interaction[41] with reciprocal modulation may be assumed (high contents of fibers and polyphenols constitute a substrate for the intestinal microbiota[37] and presumably induce a beneficial modification of the colonic bacterial composition[42–44] as well as microbial metabolic products, that are important for the homeostasis of colonic epithelial cells,[45,46] while in turn the amount and composition of phenolic metabolites is modulated by the microbiota).

In conclusion, we report the first human trial investigating the clinical efficacy of an oral anthocyanin-rich bilberry preparation in patients with UC. Natural anthocyanins occurring in bilberries had a significant beneficial effect on the inflammatory activity in UC. To our knowledge this is the first ever reported clinical investigation in humans on the disease modifying effect of bilberries in UC. In the future, larger, double-blind controlled trials with anthocyanin preparations are desirable to confirm the promising results of this pilot trial.

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Conflict of interest

Simone Peschke and Gerhard Krammer are employees of Symrise AG, Holzminden Germany, the manufacturer of the dried bilberry extract.

Authors' contribution

GR developed the principal study hypothesis together with HUH and GK and designed the study together with AJ, GAKU, JZ, HUH and MF. LB and JM carried out the studies and data analyses, LB drafted the manuscript with the help of PF, JM, MS and SRV; MF, GR, SP, AJ, PF, SRV, HUH and GAKU gave critical input. SP and GK composed and produced the bilberry extract and were responsible for safety analysis. AW performed blinded histological scoring of biopsy specimen. JZ and MS wrote the protocol of the study. LB and JM performed the statistical analysis together with AJ. LB, JZ, PF, MS, SRV and GR recruited the study patients and performed the study visits including sigmoidoscopy. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2012.07.010.

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