Regulation of faecal biomarkers in Inflammatory Bowel Disease patients treated with oral Mastiha (Pistacia lentiscus) supplement: a double-blind and placebo controlled randomised trial

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Short title: Regulation of faecal biomarkers in active IBD; a RCT

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Abstract

There is keen research upon the effects of nutraceuticals on Inflammatory Bowel Disease. The purpose of this study was to explore the effect of Mastiha supplement, rich in bioactive nutraceuticals, in active Inflammatory Bowel Disease. This is a randomised, double-blind, placebo-controlled clinical trial. A total of 60 Inflammatory Bowel Disease patients were enrolled and randomly allocated Mastiha (2.8g/day) or placebo groups for 3 months adjunct to stable medical treatment. Medical and dietary history, Inflammatory Bowel Disease Questionnaire, Harvey-Bradshaw Index, Partial Mayo Score, biochemical indices, faecal and blood inflammatory markers were assessed. A clinically important difference between groups in IBDQ was defined as primary outcome.

Inflammatory Bowel Disease Questionnaire score significantly improved in verum compared with baseline (p=0.004). There was a significant decrease in faecal lysozyme in Mastiha patients (p=0.018) with the mean change being significant (p=0.021) and significant increases of faecal lactoferrin (p=0.001) and calprotectin (p=0.029) in the placebo group. Fibrinogen reduced significantly (p=0.006) with a significant mean change (p=0.018), whereas iron increased (p=0.032) in Mastiha arm.

Our results show regulation of faecal lysozyme by Mastiha supplement adjunctive to pharmacological treatments in active inflammatory bowel disease. An effect secondary to a prebiotic potency is proposed.

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Keywords: nutraceuticals; lysozyme; Inflammatory Bowel Disease; Crohn's disease; Ulcerative Colitis; Mastiha (Pistacia lentiscus)

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic gastrointestinal disease including Crohn's Disease (CD) and Ulcerative Colitis (UC), which has turned into a global disease (Ng et al., 2017). Lack of adherence to therapeutic regimens, suboptimal treatment and serious side effects, increased the research interest in natural nutritional supplements rich in phytochemicals with antioxidant and anti-inflammatory properties that could be used for maintenance and recovery of intestinal functions.

Many natural products might be ineffective, since the necessity for the manufacturer to prove efficacy, safety and quality of a marketed product is less strongly enforced than in the pharmaceutical sector due to lack of rigorous regulation (Andrew & Izzo, 2017; Izzo et al., 2016; Hunter and Hegele, 2017). Therefore, the research upon the efficacy of natural products must be based on mechanistic investigation and formal clinical research studies (Minuz et al., 2017).

Mastic or Mastiha is the dried resinous exudate from stems and branches of Pistacia lentiscus (Pistacia lentiscus L. var latifolius Coss or Pistacia lentiscus var. Chia) according to Eur. Ph. Monograph (01/2008:1876). It is rich in terpenic acids with its main triterpenic acids, mastihadienonic and isomastihadienonic, being absorbed and bioavailable in humans (Assimopoulou & Papageorgiou, 2005; Paraschos et al., 2007; Papada et al., 2018). Additionally, it contains arabino-galactanes proteins (AGPs) (Kottakis et al., 2009), and some simple phenols have also been detected, however in low concentrations or even in traces (Kaliora et al., 2004). Ancient Greek physicians (Hippocrates, Dioscorides, Galenos) reported for first time the properties of Mastiha and recommended its use for its therapeutic properties. Medical practitioners and botanists used Mastiha for more than 2500 years mainly for the treatment of stomach and

intestine disorders like gastralgia, dyspepsia and peptic ulcer. Previous studies have documented its antioxidant (Dedoussis et al., 2004) and anti-inflammatory properties (Heo et al., 2006), in addition to its beneficial effects on the gastrointestinal system. More specifically, in patients with gastric ulcers, who were supplemented only with Mastiha at a dose of 2 g/day, symptoms resolved, while the treatment was confirmed endoscopically (Huwez et al, 1986). Furthermore, Dabos and colleagues, (2010), showed that Mastiha is effective in improving symptoms of functional dyspepsia. A pilot study of safety on patients with established mild to moderately active CD suggested that a 4-week treatment with Mastiha not only was safe, but also significantly decreased the activity index, IL-6 and CRP plasma levels. Additionally, Mastiha might act as an immunomodulator on peripheral blood mononuclear cells, by inhibiting tumor necrosis factor-alpha and stimulating macrophage migration inhibitory factor (Kaliora et al., 2007a; 2007b).

The European Medicines Agency has recognised Mastiha as a herbal medicinal product with the following indications, a) mild dyspeptic disorders, and b) symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds (EMA, 2015).

This study is based on the necessity for novel treatments for IBD without serious side effects, the increasing interest in natural products with favorable effects on health and disease, and on previous research in Mastiha. Thus, we aimed at demonstrating the effectiveness of this supplement in quality of life, clinical course and inflammatory biomarkers of patients in mild to moderate relapse adjunct to current pharmacological IBD treatments.

METHODS

Ethics and trial registration

The study protocol was reviewed and approved by the Harokopio University Ethics Committee (49/29-10-2015) and it was conducted according to the principles of the Declaration of Helsinki of 1975 as reflected in the approval by the Harokopio University Ethics Committee. Laboratory techniques were standardised and the staff of the study was trained according to Good Clinical Practice. The trial was registered with ClinicalTrials.gov where the full trial protocol can be accessed (Identifier: **NCT02796339**).

Participants

Enrolment was stimulated through announcement to the *Hellenic Society of CD and UC patients*. Confirmed IBD patients, with endoscopy proven UC or CD were enrolled based on certain inclusion and exclusion criteria (Table 1). They were provided with detailed information regarding the aims, methods, anticipated benefits and potential hazards of the study and all patients received the Patient Information Leaflet. Each patient agreeing to participate provided written Informed Consent and a copy of the signed Informed Consent form was given to the participant. Patients were recruited between May 2016 and June 2017. Follow-up visits were completed on September 2017. The study took place in Athens, Greece.

Study design

This is a randomised, double-blind, placebo-controlled, parallel group clinical trial (Figure 1). IBD patients were diagnosed in relapse (moderate disease) as scored by Harvey Bradshaw Index (HBI) ≥5 and ≤16, and Partial Mayo Score (PMS) ≥2 and ≤6 by experienced gastroenterologists. Since access to the patients full list was unavailable due to privacy policy of the Society, simple randomisation was applied. After obtaining the patient's consent, the contact who was independent of the recruitment process would allocate participants to intervention (verum or placebo). Randomisation was by a computer generated random number list prepared by an investigator with no clinical involvement in the trial. Blinding was strictly maintained to intervention staff and participants. Except for the intervention staff, investigators and analysts were kept blind to intervention assignment of the participants. Staffs who obtained outcome measurements were not informed of the intervention group assignment. The Mastiha group received natural Mastiha supplement at a dose of 2.8 g daily (4 tabs x 700mg Mastiha), while the placebo group received identical placebo tablets. This dose was chosen based on a recently published bioavailability study on healthy men, where an acute dose of 10g of Mastiha was well tolerated and no side effects were observed (Papada et al, 2018). Preclinical data in TNBS colitic mice showed effectiveness in a daily dose of 100mg/Kg body weight (Gioxari et al., 2011). Most importantly, a pilot study of Kaliora et al (2007a 2007b) on patients with mild to moderate activity Crohn's disease has served to identify and address any health and safety issues in Mastiha supplementation in a daily dose above 2 g. No side effects or any discomfort was reported. The intervention lasted 3 months. Both groups continued their usual medical treatment, which had to remain unaltered throughout the trial. Additionally, all patients received standard nutritional advice from dieticians and were encouraged to report any

adverse effects experienced during the intervention. The verum tablets weighed 0.98 g/tablet and consisted of 70% Mastiha, 14% microcrystalline cellulose, 14% dibasic calcium phosphate anhydrous and 2% magnesium stearate. The resin was powdered prior to inclusion in tablet and the powder obtained was off-white to yellowish. Similarly, the placebo tablets weighed 0.99 g/tablet and consisted of 49% microcrystalline cellulose with a characteristic off-white to yellowish colour for similarity to verum, 49% dibasic calcium phosphate anhydrous, and 2% magnesium stearate. The verum and placebo tablets shared identical appearance and organoleptic characteristics. Standardisation of the tablets was successful by using crude Mastiha from the crop of a particular year. Chios Mastiha Growers Association is operating under a GMP process in its facilities, and has been granted a manufacturer's license by the National Organization for Medicines (EOF). Additionally, it is certified under the standards of ISO 9001:2008 for the elaboration, packaging and trade of Mastiha, and ISO 22000:2005, for the elaboration and packaging of Mastiha. In studies using the present formulation, no natural impurities and related substances have been found in Mastiha above 0.5 %.

Baseline assessment

Quality of life assessment*:* Quality of life was assessed with the validated Inflammatory Bowel Disease Questionnaire (IBDQ). The IBDQ consists of 32 questions about bowel, social, systemic and emotional performance and is scored from 32 to 224 points. Higher scoring indicates a better quality of life (Guyatt et al., 1989).

Medical history*:* A medical history was recorded by a gastroenterologist, including general information (i.e. allergic reactions, smoking habits, etc.) as well as specific data

regarding IBD (i.e. brief history of IBD, age of diagnosis, complications, treatment). HBI was calculated for CD patients (Harvey & Bradshaw, 1980) and PMS for UC patients (Lewis et al., 2008).

Blood and stool sample collection*:* Standard Blood sampling (20mL) was performed. After collection, blood samples were centrifuged at 3000rpm for 10 minutes at 4°C for plasma and serum isolation. All samples were stored at -80°C until further analysis. Additionally, patients provided stool samples using a stool preparation system filled with extraction buffer IDK Extract® (Immundiagnostik, AG). Stool extracts were kept for a maximum of 9 days at -20°C until further analysis.

Anthropometric assessment: Body weight was measured to the nearest 0.1 kg. Height was measured with a standard stadiometer to the nearest millimeter. Both measurements were performed twice. Body Mass Index was also calculated.

Follow-up assessment

There was a biweekly telephone contact with the patients to check upon compliance and side effects. Compliance to treatments was evaluated by asking the patients to return a monthly diary completing the daily consumption of tablets. At the end of the intervention, all parameters of baseline assessment were re-evaluated.

Laboratory analyses

Evaluation of inflammation*:* IL-6 (R&D Systems, Inc.) and IL-10 (OriGene Technologies, Inc.) were assessed applying sandwich ELISA at baseline and at follow-up. Additionally C-Reactive Protein (CRP) was measured in serum. Calprotectin, lysozyme and lactoferrin were quantified in stool samples (Immundiagnostik, AG).

***Biochemical analyses**:* Iron (Fe), Albumin, and plasma Fibrinogen were quantified with an automatic biochemical analyzer at baseline and at follow-up. Routine laboratory tests included Lactate Dehydrogenase (LDH), serum urea measured to assess kidney safety, Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic-Pyruvic Transaminase (SGPT), γ-Glutamyl Transferase (γ-GT), Alkaline Phospatase (ALP) and Total & Direct Bilirubin measured to assess liver safety. Serum amylase was measured to predict any pancreatic disorders.

Sample size determination and statistical analysis

Sample size calculation was based on the findings of Irvine et al (2000). A sample size of 58 subjects, 29 per arm, is sufficient to detect a clinically important difference of 28.3 between groups in IBDQ score assuming a standard deviation of 37.3 using a two-tailed t-test of difference between means with 80% power and a 5% level of significance. Considering a dropout rate of 10% the sample size required is 64 (32 per group).

Our primary outcome was a clinically significant improvement (namely an increase), in quality of life as assessed with IBDQ at baseline and after intervention in both arms. Secondary outcomes included improvement in faecal and serum inflammatory markers as assessed with the measurement of faecal lysozyme, calprotectin and lactoferrin and serum IL-6, IL-10 and CRP at baseline and follow-up. Additionally, an improvement of disease severity reflected by a reduction in HBI and PMS was another secondary outcome. Improvement in biochemical indices associated with nutritional state (i.e.

serum Fe, albumin) or acute phase reactants (i.e. plasma fibrinogen) were set as secondary outcomes.

Continuous variables are presented with mean and standard deviation (SD). Quantitative variables are presented with absolute and relative frequencies. All analyses were conducted on an intention-to-treat basis. For the comparison of proportions, chi-squared and Fisher's exact tests were used. For the comparison of means between the control and intervention group the Student's t-test was computed. To reduce the bias implicit in utilizing only complete cases, multiple imputation procedures for the all data were implemented. Differences in changes of study variables during the follow up period between the two study groups were evaluated using repeated measurements analysis of variance (ANOVA). Variables with skewed distribution were log-transformed for the analysis of variance. All p values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS statistical software (version 22.0).

RESULTS

Sixty IBD patients (N=60) met our criteria for recruitment. Out of the 60 patients, 27 (45.0%) were randomised to the placebo group and 33 (55.0%) to the verum group, while 40 (66.7%) of them were diagnosed with CD and 20 (33.3%) with UC. Clinical, anthropometric and demographic characteristics for all groups are presented in Tables S1 and S2 (Supplementary material). No significant differences were reported between groups at baseline regarding the demographics, anthropometrics, clinical, biochemical and inflammatory markers. No adverse events were reported in either treatment arm.

Table 2 presents quality of life and disease activity indices for the two groups. IBDQ score improved significantly at follow-up only in the intervention arm (Table 3) although the mean change was not statistically different between the two groups. Concerning HBI values, a significant decrease was found only in the Mastiha arm, no significant difference was reported in mean changes between groups.

Table 3 describes routine biochemical data at baseline and at follow-up for Mastiha and placebo. Also serum Fe, albumin and fibrinogen are presented. There were no significant differences at baseline between the two groups. At follow-up, only serum Fe was found to be significantly different between verum and placebo, with the verum experiencing increase. The mean change in plasma fibrinogen differed significantly between the two groups. More specifically, plasma fibrinogen decreased significantly only in the Mastiha group at follow-up. Additionally, serum Fe increased significantly only in the verum group at follow-up, while serum glucose decreased significantly.

Table 4 illustrates levels of inflammation markers in serum and in stools for the Mastiha and placebo groups. The change in faecal lysozyme was significantly different between the two groups. Even when excluding patients who deviated from the protocol,

lysozyme levels were found to be significantly different in the Mastiha group after treatment ($p=0.027$), the change between the two groups being significant after treatment ($p=0.036$). Particularly in UC patients, a significant difference in faecal lysozyme was reported at follow up between these enrolled into the placebo and verum groups ($p=0.048$). More specifically, there was a significant decrease only in the Mastiha group at follow-up. IL-6 increased in both study groups. Faecal calprotectin and lactoferrin increased significantly in the placebo group at follow-up, while in the verum group they remained similar to baseline.

DISCUSSION

Herein, we investigated the effect of a natural supplement prepared with Mastiha to influence the clinical course of IBD. There are pre-clinical and clinical data supporting its administration in IBD (Kaliora et al., 2007a; 2007b; Gioxari et al., 2011), but this is the first study, to our knowledge, evaluating the effectiveness of Mastiha on the basis of a Phase-II randomised placebo-controlled clinical trial of both CD and UC patients in relapse. It is important to mention that no adverse effects were reported strengthening the safety profile of this natural product and coming into agreement with the results of other clinical studies that evaluated the effectiveness of Mastiha in duodenal ulcer, dyspepsia and hypercholesterolaemia with no adverse effects and excellent tolerability (Al-Habbal et al., 1984; Dabos et ., 2010; Kartalis et al., 2016).

In order to assess our primary outcome, namely improvement in quality of life, we used the IBDQ, the most widely used and validated tool (Alrubaiy et al., 2015), which is fully validated in the Greek language as well in the original English (Pallis et al., 2001). Despite a lack of significant difference in mean changes between the study groups, the IBDQ improved significantly in the Mastiha group but not in the placebo group. Although the mean score at follow-up in the verum group did not reach the usual range of patients in remission (170-190 points), this increase shows that Mastiha supplementation may improve quality of life in IBD patients in relapse when given as an adjunct to conventional medical therapy.

When evaluating disease severity, we observed a significant decrease in HBI at follow-up compared with baseline only in the Mastiha group. However, the detected decrease should be interpreted with caution due to the subjective variables comprising the score (e.g. intensity of abdominal pain and general well-being). A longer period of treatment

283 could possibly induce a further reduction in HBI and a significant mean change between
284 groups, as well as a reduction in PMS for UC patients. However, the nature of active
285 IBD with frequent changes in medical treatments with fluctuations in dosages would
286 jeopardise the compliance with our inclusion and exclusion criteria.
287 To avoid patient discomfort as well as increased drop-out rate and public health cost
288 due to repeated endoscopies, we evaluated serum and faecal biomarkers of
289 inflammation. No significant changes in CRP have been identified. Although, CRP is
290 widely used in clinical practice in order to assess and monitor disease activity and
291 response to therapy, however it has a low specificity. Therefore, we evaluated also IL-6,
292 a cytokine with pro-inflammatory effects, and IL-10, a cytokine with anti-inflammatory
293 activity. Even if we detected no significant changes in the mean changes between the
294 two groups, further exploration of the effects of Mastiha on other molecules of the
295 inflammatory cascade is necessary. However we detected noteworthy regulation in
296 lysozyme, lactoferrin and calprotectin levels. Previous research suggested that faecal
297 lysozyme is increased in IBD patients and correlates with disease activity, especially in
298 colonic IBD (van der Sluys Veer et al., 1998). Our study showed a significant decrease
299 in the lysozyme levels of the Mastiha group, most importantly it showed a significant
300 difference in mean change between the two groups. When analyzing lysozyme in UC or
301 CD individually, significantly lower levels at follow-up for the Mastiha group in UC
302 patients were reported. Lysozyme, an antimicrobial protein that regulates innate
303 immune response, is expressed in both entities, CD and UC, mainly in small intestine,
304 but markedly also in the colon, as shown in both experimental animals (Coulombe et al.,
305 2016) and humans (Rubio, 2011; Fahlgren et al., 2003). Since increased lysozyme
306 expression is correlated with dysbiosis and with inflammation, herein we could

hypothesise that the effect of Mastiha is rather a prebiotic one, and possibly stronger in UC patients, related to the contained phenolic compounds and arabinogalactanes, both reported to have prebiotic activities (Williamson et al., 2017; Dion et al., 2016). In addition, faecal calprotectin, a protein present in granulocytes and neutrophils, is released into the intestinal lumen after leucocyte epithelial migration into the inflamed gut mucosa and has good diagnostic accuracy in assessing mucosal healing in IBD patients. Furthermore, faecal lactoferrin is usually elevated in patients with active IBD detecting inflammation and correlates well with mucosal healing and histological improvement (Kochhar et al., 2017). In our study, levels of calprotectin and lactoferrin significantly increased in the placebo but not in the Mastiha group. Significantly lower levels of lactoferrin at follow-up were found in UC patients under Mastiha treatment.

Regarding biochemical markers, fibrinogen is an acute phase reactant participating in thrombi formation and its levels increase in inflammatory conditions. Increased fibrinogen levels are related with higher plasma viscosity and platelet activation, additionally to the microcirculation of the inflamed intestine (Hudson et al., 1996). Higher levels of fibrinogen have been detected previously in IBD patients and increasing levels were associated with disease activity (Dolapcioglu et al., 2014). In our study, plasma fibrinogen levels decreased significantly in the Mastiha group compared with baseline, notably the change in mean values between the two groups remained significant as well. A significant decrease of fibrinogen in the Mastiha group at follow up (250.2±57.4 mg/dl) compared with baseline (288.3±65.5 mg/dl, p= 0.022) was reported in CD but not in UC patients. These findings come into agreement with a study investigating the antidiabetic effects of two pentacyclic triterpenic acids, madecassid acid and rotundic acid, on a mouse model. Apart from beneficial effects on oxidative

and inflammatory stress, madecassic acid induced a significant reduction in fibrinogen levels and plasminogen activator inhibitor (Hsu et al., 2015). Furthermore, another protocol on a mouse model of diabetes showed that the triterpenoids asiatic and maslinic acids, act as anti-coagulants, by lowering fibrinogen levels among others (Hung et al., 2015). Even though these results refer to mouse models and should not be directly extrapolated to humans, we could hypothesise that terpenes in Mastiha possibly exhibit favorable effects on homeostatic imbalance of IBD patients.

Serum Fe levels significantly increased in the Mastiha group compared with baseline and compared with the follow-up levels in the placebo group. Additionally, serum Fe levels increased significantly in the Mastiha group at follow up (75.3±32.2 μg/dl) compared with baseline (61.3±30.7 μg/dl, p=0.034) in CD but not in UC patients. Even though this increase could be partially attributed to increased food intake due to symptoms improvement, better nutrient absorption or less intestinal losses, this finding should be interpreted with caution, since several nutrients may fluctuate in serum as positive or negative acute phase reactants as a part of inflammatory response (Forbes et al., 2017).

The above findings could also point toward anti-inflammatory properties of the Mastiha terpenes which have been shown to be absorbed and bioavailable in the systemic circulation in healthy humans (Papada et al., 2018).

While this study reports interesting findings, it has some limitations, such as the absence of endoscopy at follow-up, precluding our ability to comment on any histological alterations. On the other hand, this limitation was compensated by the very tight control of the verum and placebo groups to ensure compliance of patients with the protocol, as well as the adequate number of participants that allowed for a power of 0.90

for the between-subjects main effect at an effect size of 0.37; a power of 0.95 for the within-subjects main effect at an effect size of 0.25; and a power of 0.95 for the interaction effect at an effect size of 0.25. Nevertheless, larger-scale clinical trials are essential to determine the effect of terpene-rich Mastiha in the health of patients with IBD and its potential underlying mechanisms.

Conclusive remarks

Since there is an increasing usage of natural products among IBD patients, our study aimed at investigating the effects of Mastiha in quality of life, disease activity, and biochemical and faecal inflammatory markers. Our results showed for the first time that a natural supplement prepared with Mastiha could serve as an innovative treatment approach as an adjunct to conventional medical therapy. Mechanistically, regulation of faecal lysozyme secondary to a prebiotic effect is proposed.

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Statement of Authorship

ACK and AF designed research (project conception, development of overall research plan, and study oversight). EP conducted research (hands-on conduct of the experiments and data collection) and analysed data. AG and CA conducted part of the research. . CT performed statistical analysis. IS supervised the design and distribution of verum and placebo supplements. IS also contributed to manuscript preparation. EP wrote paper finally edited and approved by AF and ACK. ACK supervised the study. All authors approved the final version of the manuscript.

Conflict of Interest Statement. The authors declare that they have no conflict of interest.

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513 microbiota in determining the metabolic fate of polyphenols. *Biochemical*
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515 **TABLE 1.** Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Sex: Male and Female	Positive stool culture for enteric pathogens or Clostridium difficile toxin
Age: 18-67 years old	Antibiotic treatment during and 2 months prior to screening
IBD established by endoscopy, with consistent histology and clinical course	Bowel surgery ≤3 months prior to screening; a planned elective surgery or hospitalisation during the study; clinically significant short bowel syndrome; presence of an intra-abdominal abscess or a fistula with clinical or radiological evidence of an associated abscess; ileostomy; colostomy
Active disease (moderate) - defined by $5 \leq HBI \leq 16$ in CD, - defined by $2 \leq PMS \leq 6$ in UC	Enteral or Parenteral Nutrition; alcohol or drug abuse, vitamin or inorganic supplements, vegan or macrobiotic diet before and during the trial
Stable treatment with steroids for at least 2 weeks before the start of the trial, mesalamine and mesalamine analogues for 4 weeks and immunosuppressants for 8 weeks	Any malignancy in the year prior to screening; cardiovascular disease; peptic ulcer
Stable medication during the trial	Pregnancy, lactation

516

517 **TABLE 2.** Quality of life and disease activity indices at baseline and follow up.

	Baseline	**Follow-up**	**Change**		
	Mean (SD)	Mean (SD)	Mean (SD)	P^1	P^2
IBDQ score					
Placebo	144.9 (29.0)	155.1 (33.3)	10.2 (43.6)	0.137	0.380
Mastiha	145.2 (27.3)	163.4 (30.6)	18.3 (26.4)	**0.004**	
P^3	0.975	0.319			
HBI$^+$					
Placebo	6.1 (1.8)	4.7 (2.6)	-1.4 (2.6)	0.055‡	0.635‡
Mastiha	7.8 (2.3)	4.7 (3.8)	-3.1 (4.1)	**<0.001**‡	
P^3	0.134‡	0.691‡			
PMS^{++}					
Placebo	3.2 (2.0)	2.2 (1.6)	-1.0 (2.1)	0.055	0.324
Mastiha	2.8 (1.8)	2.0 (1.3)	-0.9 (2.0)	0.481	
P^3	0.114	0.355			

518 [1]p-value for time effect; [2]Effects reported include differences between the groups in
519 mean changes (repeated measurements ANOVA); [3]p-value for group effect; ‡based on
520 logarithmic transformations; $^+$ only in patients with CD; $^{++}$only in patients with UC

521 **TABLE 3**. Biochemical data at baseline and at follow up.

	Baseline	**Follow-up**	**Change**		
	Mean (SD)	Mean (SD)	Mean (SD)	P[1]	P[2]
Plasma fibrinogen (mg/dL)					
Placebo	288.1 (66.9)	276.7 (83.2)	-11.4 (83.7)	0.441	**0.018**
Mastiha	281.5 (58.1)	243.3 (54.4)	-38.3 (69.9)	**0.006**	
P[3]	0.687	0.067			
Serum Fe (μg/dL)					
Placebo	53.9 (27.0)	56.9 (24.0)	3.0 (24.7)	0.614	0.278
Mastiha	61.1 (30.5)	72.8 (29.2)	11.7 (34.9)	**0.032**	
P[3]	0.343	**0.027**			
Serum albumin (g/dL)					
Placebo	4.3 (0.3)	4.2 (0.6)	-0.1 (0.5)	0.364	0.427
Mastiha	4.3 (0.4)	4.3 (0.5)	0.0 (0.6)	0.858	
P[3]	0.474	0.764			
Serum amylase (IU/L)					
Placebo	68.2 (23.0)	68.8 (23.5)	0.6 (21.1)	0.871	0.577
Mastiha	67.6 (20.3)	65.3 (20.7)	-2.2 (17.8)	0.514	
P[3]	0.909	0.546			
Serum urea (mg/dL)					
Placebo	29.9 (7.7)	28.2 (6.9)	-1.8 (6.3)	0.238‡	0.797‡
Mastiha	31.9 (10.1)	29.0 (7.2)	-2.9 (8.7)	0.094‡	
P[3]	0.504‡	0.621‡			
Serum LDH (U/L)					

Placebo	154.8 (41.1)	188 (66)	33.2 (65.6)	**0.006**‡	0.975‡
Mastiha	143.9 (30.9)	183.5 (119.7)	39.5 (117.9)	**0.003**‡	
P[3]	0.284‡	0.421‡			
Serum total bilirubin (mg/dL)					
Placebo	0.32 (0.24)	0.48 (0.45)	0.15 (0.52)	0.275‡	0.361‡
Mastiha	0.43 (0.41)	0.55 (0.71)	0.12 (0.71)	0.877‡	
P[3]	0.221‡	0.899‡			
Serum direct bilirubin (mg/dL)					
Placebo	0.15 (0.12)	0.19 (0.13)	0.04 (0.15)	0.050‡	**0.001**‡
Mastiha	0.23 (0.17)	0.15 (0.15)	-0.08 (0.21)	**0.003**‡	
P[3]	0.281‡	0.251‡			
Serum SGOT (IU/L)					
Placebo	17.5 (9.3)	18.5 (8.6)	0.9 (12.8)	0.381‡	0.095‡
Mastiha	14.8 (4.8)	19.3 (7.0)	4.5 (6.7)	**0.001**‡	
P[3]	0.182‡	0.414‡			
Serum SGPT (IU/L)					
Placebo	18.3 (12.5)	15.3 (7.7)	-3.1 (9.4)	0.208‡	0.810‡
Mastiha	18.9 (10.3)	17.4 (8.6)	-1.6 (7.6)	0.299‡	
P[3]	0.519‡	0.317‡			
Serum γ-GT (IU/L)					
Placebo	19.2 (11.6)	18.2 (8.9)	-0.9 (9.9)	0.942‡	0.221‡
Mastiha	21.4 (13.3)	17.1 (6.2)	-4.3 (10.5)	0.059‡	
P[3]	0.330‡	0.921‡			
Serum ALP (IU/L)					

Placebo	64 (17.8)	65.6 (15.8)	1.6 (9.4)	0.550	0.107
Mastiha	68.3 (21.4)	64 (16.9)	-4.2 (16.4)	0.081	
P[3]	0.408	0.720			

[1]p-value for time effect; [2]Effects reported include differences between the groups in mean changes (repeated measurements ANOVA); [3]p-value for group effect; ‡based on logarithmic transformations

525 **TABLE 4**. Serum and faecal inflammatory markers at baseline and at follow up.

	Baseline	**Follow-up**	**Change**		
	Mean (SD)	Mean (SD)	Mean (SD)	P[1]	P[2]
Serum CRP (mg/L)					
Placebo	6.4 (7.6)	5.3 (4.8)	-1.1 (8.0)	0.767‡	0.791‡
Mastiha	6.9 (8.2)	5.7 (5.9)	-1.2 (8.3)	0.946‡	
P[3]	0.616‡	0.788‡			
Serum IL-6 (pg/mL)					
Placebo	14.4 (16.8)	24.3 (43.8)	9.9 (33.6)	**0.030**	0.955
Mastiha	11.5 (12.3)	15.7 (13.3)	4.2 (9.7)	**0.021**	
P[3]	0.552	0.502			
Serum IL-10 (pg/mL)					
Placebo	8.8 (18.9)	9.5 (20.1)	0.6 (3.4)	0.454	0.607
Mastiha	6.1 (2.7)	6.1 (2.7)	0.0 (3.6)	0.951	
P[3]	0.920	0.713			
Faecal lysozyme (μg/g)					
Placebo	11.7 (10.6)	15.6 (15.8)	3.9 (18.2)	0.326	**0.021**
Mastiha	18.8 (21.2)	10.3 (5.2)	-8.4 (21.8)	**0.018**	
P[3]	0.075	0.208			
Faecal calprotectin (μg/g)					
Placebo	2170.6 (4444.4)	3598.5 (3620.4)	1427.9 (5606.1)	**0.029**	0.348
Mastiha	1688.6 (1712.4)	2744 (4910.6)	1055.4 (5043.1)	0.289	
P[3]	0.825	0.357			
Faecal lactoferrin (μg/g)					

Placebo	102.4 (128.7)	306.3 (373.7)	203.9 (376.4)	**0.001**	0.130
Mastiha	130.4 (140.2)	165.6 (150.8)	35.2 (179.9)	0.109	
P[3]	0.479	0.240			

526 [1]p-value for time effect (based on logarithmic transformations); [2]Effects reported
527 include differences between the groups in mean changes (repeated measurements
528 ANOVA) (based on logarithmic transformations); [3]p-value for group effect (based on
529 logarithmic transformation).

530 **Figure legends**

531 **Figure 1.** Study design flowchart