

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

4

5 Efstathia Papada¹, Aristeia Gioxari¹, Charalampia Amerikanou¹, Alastair Forbes², Chara
6 Tzavara¹, Ilias Smyrnioudis³, Andriana C. Kaliora^{1*}

7

8 ¹Department of Dietetics and Nutritional Science, School of Health Science and
9 Education, Harokopio University, Athens, Greece

10 ²Norwich Medical School, University of East Anglia, Bob Champion Building, James
11 Watson Road, Norwich, NR4 7UQ, United Kingdom

12 ³Chios Mastic Gum Growers Association, 1 K. Monomachou St., Chios, 82100, Greece.

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

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16 **Address for correspondence.** Dr Andriana C. Kaliora, 70 El. Venizelou Ave, 17671,
17 Athens, Greece. Tel: +30 210 9549226, fax: +30 210 9577050, email: akaliora@hua.gr ;
18 andrianakaliora@gmail.com

19

20 **Funding Sources.** This work was supported by ENOSI MASTIHOPARAGWGWN
21 CHIOU that covered all consumables. The funder had no role in the design of the study
22 or the analysis of data.

23

24 **Abstract**

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

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

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

44 **ClinicalTrials.gov Identifier:** NCT02796339

45

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

48

49 INTRODUCTION

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

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

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

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

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

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

96 **METHODS**

97

98 **Ethics and trial registration**

99 The study protocol was reviewed and approved by the Harokopio University Ethics
100 Committee (49/29-10-2015) and it was conducted according to the principles of the
101 Declaration of Helsinki of 1975 as reflected in the approval by the Harokopio
102 University Ethics Committee. Laboratory techniques were standardised and the staff of
103 the study was trained according to Good Clinical Practice. The trial was registered with
104 ClinicalTrials.gov where the full trial protocol can be accessed (Identifier:
105 [NCT02796339](https://clinicaltrials.gov/ct2/show/study/NCT02796339)).

106

107 **Participants**

108 Enrolment was stimulated through announcement to the *Hellenic Society of CD and UC*
109 *patients*. Confirmed IBD patients, with endoscopy proven UC or CD were enrolled
110 based on certain inclusion and exclusion criteria (Table 1). They were provided with
111 detailed information regarding the aims, methods, anticipated benefits and potential
112 hazards of the study and all patients received the Patient Information Leaflet. Each
113 patient agreeing to participate provided written Informed Consent and a copy of the
114 signed Informed Consent form was given to the participant. Patients were recruited
115 between May 2016 and June 2017. Follow-up visits were completed on September
116 2017. The study took place in Athens, Greece.

117

118 **Study design**

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

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

159

160 **Baseline assessment**

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

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

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

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

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

179

180 **Follow-up assessment**

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

185

186 **Laboratory analyses**

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

191

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

199

200 **Sample size determination and statistical analysis**

201 Sample size calculation was based on the findings of Irvine et al (2000). A sample size
202 of 58 subjects, 29 per arm, is sufficient to detect a clinically important difference of
203 28.3 between groups in IBDQ score assuming a standard deviation of 37.3 using a two-
204 tailed t-test of difference between means with 80% power and a 5% level of
205 significance. Considering a dropout rate of 10% the sample size required is 64 (32 per
206 group).

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

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

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

227 **RESULTS**

228 Sixty IBD patients (N=60) met our criteria for recruitment. Out of the 60 patients, 27
229 (45.0%) were randomised to the placebo group and 33 (55.0%) to the verum group,
230 while 40 (66.7%) of them were diagnosed with CD and 20 (33.3%) with UC. Clinical,
231 anthropometric and demographic characteristics for all groups are presented in Tables
232 S1 and S2 (Supplementary material). No significant differences were reported between
233 groups at baseline regarding the demographics, anthropometrics, clinical, biochemical
234 and inflammatory markers. No adverse events were reported in either treatment arm.
235 Table 2 presents quality of life and disease activity indices for the two groups. IBDQ
236 score improved significantly at follow-up only in the intervention arm (Table 3)
237 although the mean change was not statistically different between the two groups.
238 Concerning HBI values, a significant decrease was found only in the Mastiha arm, no
239 significant difference was reported in mean changes between groups.
240 Table 3 describes routine biochemical data at baseline and at follow-up for Mastiha and
241 placebo. Also serum Fe, albumin and fibrinogen are presented. There were no
242 significant differences at baseline between the two groups. At follow-up, only serum Fe
243 was found to be significantly different between verum and placebo, with the verum
244 experiencing increase. The mean change in plasma fibrinogen differed significantly
245 between the two groups. More specifically, plasma fibrinogen decreased significantly
246 only in the Mastiha group at follow-up. Additionally, serum Fe increased significantly
247 only in the verum group at follow-up, while serum glucose decreased significantly.
248 Table 4 illustrates levels of inflammation markers in serum and in stools for the Mastiha
249 and placebo groups. The change in faecal lysozyme was significantly different between
250 the two groups. Even when excluding patients who deviated from the protocol,

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

259 **DISCUSSION**

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

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

279 When evaluating disease severity, we observed a significant decrease in HBI at follow-
280 up compared with baseline only in the Mastiha group. However, the detected decrease
281 should be interpreted with caution due to the subjective variables comprising the score
282 (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

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

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

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

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

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

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

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

360

361 **Conclusive remarks**

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

368

369

370 **Acknowledgments**

371 We are grateful to the patients for participating in this study and for the funder for
372 financial support.

373

374 **Statement of Authorship**

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

382

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

385

386

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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 $5 \leq \text{HBI} \leq 16$ in CD, - defined by $2 \leq \text{PMS} \leq 6$ 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 |

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

| | Baseline | Follow-up | Change | P ¹ | P ² |
|-------------------------|--------------------|--------------------|---------------|------------------------------|--------------------|
| | Mean (SD) | Mean (SD) | Mean (SD) | | |
| 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 ¹p-value for time effect; ²Effects reported include differences between the groups in
519 mean changes (repeated measurements ANOVA); ³p-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 | P ¹ | P ² |
|----------------------------------|--------------------|--------------------|---------------|--------------------|--------------------|
| | Mean (SD) | Mean (SD) | Mean (SD) | | |
| 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 | | | |

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

530 **Figure legends**

531 **Figure 1.** Study design flowchart