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Plasma free amino acid profile in quiescent Inflammatory Bowel Disease patients orally administered with Mastiha (Pistacia lentiscus); a randomised clinical trial.

Efstathia Papada[a], Charalampia Amerikanou[a], Ljilja Torović[b], Nick Kalogeropoulos[a], Chara Tzavara[a], Alastair Forbes[c] and Andriana C. Kaliora[a,*]

[a]Department of Dietetics and Nutritional Science, School of Health Science and Education, Harokopio University, Athens, Greece

[b]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

[c]Norwich Medical School, University of East Anglia, Bob Champion Building, James Watson Road, Norwich, NR4 7UQ, United Kingdom

[*]*Corresponding author*

Dr Andriana C. Kaliora, Department of Dietetics and Nutritional Science, School of Health Science and Education, Harokopio University, 70 El. Venizelou Ave, 17671, Athens, Greece. Tel: +30 210 9549226, fax: +30 210 9577050,

E-Mail address: akaliora@hua.gr; andrianakaliora@gmail.com

ABSTRACT

Background: Natural products have been studied regarding their effectiveness on Inflammatory Bowel Disease (IBD).

Hypothesis/Purpose: To examine the effects of Mastiha (Pistacia lentiscus var. Chia) on clinical course and amino acid (AA) profile of patients in remission.

Study design: This is a randomised, double-blind, placebo-controlled clinical trial.

Methods: Patients (N = 68) were randomLy allocated to Mastiha (2.8g/day) or placebo adjunct to sTable medication. Free AAs were identified applying Gas Chromatography-Mass Spectrometry in plasma. Medical-dietary history, Inflammatory Bowel Disease Questionnaire, Harvey-Bradshaw Index, Partial Mayo Score, biochemical, faecal and blood inflammatory markers were assessed. Primary endpoint was the clinical relapse rate at 6 months. Secondary

endpoints included variations in free AAs, inflammatory biomarkers and quality of life. Statistical significance was set at 0.05.

Results: Concerning AAs and biochemical data, alanine ($p = 0.006$), valine ($p = 0.047$), proline ($p = 0.022$), glutamine ($p < 0.001$) and tyrosine ($p = 0.043$) along with total cholesterol ($p = 0.032$) and LDL cholesterol ($p = 0.045$) increased only in placebo group compared with baseline and the change between the study groups was significantly different. Inflammatory markers had not a significantly different change between the two groups, even serum IL-6, faecal calprotectin and faecal lactoferrin increased only in the placebo group. Although Mastiha was not proven superior to placebo in remission rate (17.6% vs. 23.5%, $p = 0.549$), attenuation in increase of free AAs levels in verum group is reported.

Conclusion: Mastiha inhibited an increase in plasma free AAs seen in patients with quiescent IBD. Since change of AAs is considered an early prognostic marker of disease activity, this indicates a potential role of Mastiha in remission maintenance.

(ClinicalTrials.gov Identifier: NCT02796339)

Keywords: Free amino acids, Inflammatory Bowel Disease, Pistacia lentiscus L., crude Mastiha, Remission

Abbreviations: AA, amino acid; ALP, alkaline phosphatase; ANOVA, analysis of variance; CD, Crohn's Disease; CRP, C-reactive protein; GC, gas chromatography; HBI, Harvey Bradshaw Index; IBD, Inflammatory Bowel Disease; IBDQ, Inflammatory Bowel Disease Questionnaire; IL, interleukin; LDH, lactate dehydrogenase; MedDiet, Mediterranean Diet; MS, mass spectrometry; PMS, Partial Mayo Score; SD, standard deviation; SGOT, glutamic-oxaloacetic transaminase; SGPT, glutamic-pyruvic transaminase; SIM, selective ion monitoring; UC, Ulcerative Colitis; γ-GT, γ-glutamyl transferase.

1. Introduction

Inflammatory Bowel Disease (IBD), including Crohn's Disease (CD) and Ulcerative Colitis (UC) is a chronic ailment of the gastrointestinal tract with relapse and remission periods. Therapeutic strategies aim at induction and maintenance of remission, and although they have improved remission rates, long-term maintenance of remission without adverse effects remains challenging. (Neurath, 2017).

Therefore, there is an increasing interest upon the role of plant-derived dietary supplements for IBD prevention and treatment, since they exhibit anti-inflammatory, immunoregulatory and antioxidant properties. Amongst others (i.e. curcumin, broccoli sprouts), there is clinical evidence showing promising effects on IBD for Mastiha (Farzaei et al, 2016).

According to Eur. Ph. Monograph (01/2008:1876), 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). Since 2015 Mastiha is considered a traditional herbal medicinal product for mild dyspeptic disorders, symptomatic treatment of minor skin inflammations and healing of minor wounds (EMA, 2015). It is rich in terpenic acids (Assimopoulou and Papageorgiou, 2005), with antioxidant (Dedoussis et al, 2004), anti-inflammatory (Heo et al, 2006) and cytotoxic (Balan et al, 2007) properties. Mastiha has been shown to be safe and effective in active CD (Kaliora et al, 2007a; 2007b). As an oleoresin, phenolic compounds have been detected in leaves and fruits of Pistacia lentiscus (Amel et al, 2016), In general, phenolic compounds possess protective and therapeutic role in IBD through various mechanisms, such as down-regulation of inflammatory cytokines and enzymes (Farzaei et al, 2015). However, in this resin only simple phenols have been detected, however in low concentrations or even in traces (Kaliora et al, 2004). We have recently reported the absorption and bioavailability of the main triterpenic acids mastihadienonic and isomastihadienonic acids that have been identified in the highly active acidic fraction (Assimopoulou and Papageorgiou 2005; Papada et al, 2018; Paraschos et al, 2007; Xynos et al., 2018). Concentrations of these triterpenic compounds have been determined by Paraschos

and coworkers (2007); namely mastihadienonic acid 20.9mg/g mastiha and isomastihadienonic acid 19mg/g mastiha.

Amino acids (AAs) play a key role in pathways regulating intestinal health, participating in protein synthesis, gene expression, intracellular protein turnover, oxidative stress and in stimulation of lymphocyte proliferation, inflammatory cytokines production and T cell-mediated immunity (Li et al, 2007; Nakaya et al, 2014). AA profile differentiates in IBD and healthy pointing towards a link between AA profile and IBD. AAs are used as therapeutic options in order to maintain intestinal integrity in IBD (Liu et al, 2017).

Based on the need for effective maintenance treatment without serious side effects and on the key role of AAs in remission maintenance, we aimed to investigate the effects of Mastiha on clinical course and plasma free AAs in quiescent IBD.

2. Materials and methods

2.1 Ethics and trial registration

Harokopio University Ethics Committee reviewed and approved the protocol (49/29-10-2015) conducted according to the principles of Declaration of Helsinki and Tokyo for humans and Good Clinical Practice. The trial was registered with ClinicalTrials.gov (Identifier: NCT02796339).

2.2 Participants

Enrolment was initiated through the Hellenic Society of IBD patients. Confirmed patients, with biopsy proven UC or CD were enrolled based on certain criteria (Table 1). Detailed information about the study was provided and each patient agreeing to participate provided written Informed Consent. Recruitment lasted between May 2016 and March 2017 in Athens, Greece. Follow-up visits were completed by September 2017.

2.3 Study design

This is a randomised, double-blind, placebo-controlled, parallel group clinical trial (Fig. 1). IBD patients were designated to be in remission from a Harvey Bradshaw Index (HBI) ≤ 4 (CD) and Partial Mayo Score (PMS) ≤ 1 (UC) scored by experienced gastroenterologists. Since access to the patients full list was unavailable due to privacy policy of the Society, simple randomization was applied. After obtaining informed consent, the independent of the recruitment process contact would allocate participants to intervention. Randomisation was applied by a computer generated random number list prepared by an independent investigator. Blinding of all other staff, analysts and participants was strictly maintained. The Mastiha group received natural Mastiha at a dose of 2.8g daily (4 tabs x 700 mg Mastiha), while the placebo group received identical placebo Tablets for 6 months, as an adjunct to conventional medical treatment. The choice of this dose was based on a previous pilot study in CD patients experiencing no side effects and a bioavailability study in healthy humans (Kaliora et al. 2007a, 2007b, Papada et al. 2018). Additionally, standard nutritional advice was provided and patients were encouraged to report adverse effects. The verum Tablets weighed 0.98g/Tablet and consisted of 70% Mastiha resin from the stems and brunches of Pistacia lentiscus var. Chia, 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 barely yellowish. The placebo Tablets weighed 0.99g/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. In terms of odour similarity, the cap in placebo box was sprayed with the mastiha water obtained by steam distillation. The verum and placebo shared identical appearance and organoleptic characteristics. Production of Mastiha is conducted under a GMP process, 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%.

2.4 Baseline assessment

2.4.1. Medical history: Medical history was recorded by a gastroenterologist, including general data as well as IBD-specific information. HBI was calculated for CD patients (Harvey and Bradshaw, 1980) and PMS for UC patients (Lewis et al, 2008).

2.4.2. Anthropometric and dietary assessment: Body weight was measured to the nearest 0.1kg and height was measured to the nearest millimeter twice. Body Mass Index was calculated. Compliance with Mediterranean diet was evaluated with MedDiet Score (Panagiotakos et al, 2006).

2.4.3. Blood and stool sample collection: Standard blood sampling (20mL) was performed and stool samples were collected with a preparation system with extraction buffer IDK Extract® (Immundiagnostik, AG).

2.4.4. Quality of life assessment: Quality of life was assessed with the validated Greek version of Inflammatory Bowel Disease Questionnaire (IBDQ) with higher scores indicating better quality of life (Guyatt et al, 1989; Pallis et al, 2001).

2.5. Follow-up assessment

There was a biweekly telephone contact with patients to check upon compliance and side effects. At the end of intervention, the full baseline assessment was repeated.

2.6. Laboratory analyses

2.6.1. *Biochemical analyses:* Iron (Fe), albumin, and plasma fibrinogen were quantified with an automatic biochemical analyzer at baseline and follow-up. Routine laboratory tests included lactate dehydrogenase (LDH), urea, glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), total and direct bilirubin, amylase and a lipid profile.

2.6.2. *Amino acid analysis:* Free (physiological) plasma AAs were determined by gas chromatography-mass spectrometry (GC-MS), employing Phenomenex® EZ:faast kit. The procedure involves a solid phase extraction followed by derivatisation and subsequent liquid/liquid extraction. The derivatised AAs are then analyzed by GC-MS.

An Agilent (Wallborn, Germany) series GC 6890 N gas chromatograph, coupled with an HP5973 Mass Spectrometer detector (EI, 70eV), split-splitess injector and an HP7683 autosampler was used for analysis. An aliquot (2 µl) of derivatized samples was injected into GC at a split ratio of 1:15. AA separation was achieved using Phenomenex Zebron ZB-A AA analysis-dedicated column (length = 10m, internal diameter = 0.25mm, film thickness = 25µm). The carrier gas was helium at a constant flow of 1.1 ml/min. The injector and transfer line temperatures were 250 and 340 °C, respectively. The initial oven temperature was 110 °C, increased to 320 °C at 30 °C/min and held at 320 °C for 3min. A selective ion monitoring (SIM) GC-MS method was applied for detection of 26 AAs, based on the ±0.05 retention time presence of target and qualifier ions at the predetermined ratios. The retention times, target and qualifier ions of AAs, are shown in Table S1. Quantification was carried out employing norvaline as internal standard and constructing reference curves for each AAs by standard solutions.

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

2.7 Sample size determination and statistical analysis

Sample size to detect the effect of verum over placebo was calculated on the assumption that 10% on verum versus 40% on placebo would experience clinical relapse (in CD scoring HBI ≥ 5 and in UC scoring PMS ≥ 2). As such, a sample size of 64 patients was required for a 80% chance of detecting significance at 5% level ($\alpha = 0.05$) (power 80%) (Pocock, 1983). To allow for a drop-out rate of 10%, the number was increased to 70 patients. The primary

endpoint was clinical relapse rate over 6 months. Secondary endpoints were plasma free AAs profile, serum and faecal inflammatory biomarkers and quality of life.

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

3. Results

Sixty-eight IBD patients (N = 68) met our recruitment criteria. A total of 34 patients were randomised to the placebo group and 34 patients to the Mastiha group; 46 were diagnosed with CD and 22 with UC. Clinical, anthropometric and demographic characteristics are presented in Table S2. No adverse events affecting gastrointestinal, renal and hepatic systems were reported.

We detected no significant differences in relapse rates between the groups at follow-up (Table 2). Additionally, disease activity indices and IBDQ score did not alter significantly at follow-up in either group. MedDiet score decreased significantly only in placebo group at follow-up, although the mean change was not different between the groups (Table 3). All biochemical markers assessed were within normal range. Serum albumin increased significantly in Mastiha, and the mean change differed significantly between the groups. Total and LDL cholesterol increased significantly in placebo but not in Mastiha arm, resulting in a significant difference of mean changes between the arms. Serum glucose decreased significantly, while LDH increased significantly in placebo (Table 4).

Alanine, glycine, alpha-aminobutyric acid, valine, leucine, allo-isoleucine, isoleucine, threonine, serine, proline, asparagine, phenylalanine, ornithine, tyrosine, tryptophane and cystine increased significantly in placebo, while in Mastiha changes were insignificant (Table 5). The mean changes in alanine, valine, proline and tyrosine differed significantly between the groups. Glutamine increased significantly in placebo and decreased significantly in Mastiha, the mean change between the two arms being significant. Glutamic acid increased significantly in Mastiha.

Levels of serum and faecal inflammatory markers are given in Table 6. Serum IL-6 increased significantly in placebo. Also, faecal calprotectin and lactoferrin increased significantly in placebo, while they remained unaffected in Mastiha. No significant difference in mean changes between the groups was identified for serum or faecal biomarkers.

4. Discussion

A plethora of natural supplements have been studied on regulating IBD activity and several mechanisms have been proposed for their beneficial properties such as anti-inflammatory, immunomodulatory and antioxidant effects, as well as enhancement of gut microbiota and modulation of intracellular transcription and transduction signaling pathways (Farzaei et al, 2016). For example, curcumin and pomegranate extract have been investigated as potential treatment approach of intestinal inflammation (Camacho-Barquero et al, 2007; Larrosa et al, 2010). However this is the first study on the effect of a natural product in modulation of AAs profile in IBD patients, to the best of our knowledge.

Currently there are preclinical and clinical data supporting Mastiha's beneficial effects on IBD (Gioxari et al, 2011; Kaliora et al, 2007a; 2007b; Papalois et al, 2012). However, for first, our study evaluates Mastiha in preventing IBD relapse on the basis of a Phase-II randomised placebo-controlled clinical trial. The relapse rates at 6 months (23.5% versus 17.6%) with no significant differences between groups did not show that Mastiha was superior to placebo, but interesting results in free plasma AAs response were observed. AAs act as substrates and regulators in various metabolic paths. Different circulating AA profiles

have been reported in IBD reflecting nutritional state, but also inflammatory status and disease activity. For example, proline has been found upregulated in IBD patients (Hisamatsu et al, 2012) and valine has been found significantly increased in DSS-treated mice (Shiomi et al, 2011).

We detected significant changes in valine and proline, and also in alanine, glutamine and tyrosine between the groups at follow up, significantly increasing in placebo arm, while remaining unchanged in Mastiha. Glutamine increased significantly in placebo and decreased significantly in Mastiha, the mean change between the arms being significant. Data on circulating AAs in inactive IBD are very limited, most deriving from studies in active disease or from experimental animals (Hisamatsu et al, 2012; Shiomi et al, 2011; Gupta et al, 2012; Zhang et al, 2013). However, Williams et al. (2012) found a significant decrease in serum alanine in inactive IBD patients, and although not investigated in their cohort, this was attributed to increased protein catabolism and intestinal permeability. A different AA profile was detected in IBD patients by Ooi et al. (2011), but presented data are on both active and inactive patients. Increase in circulating AAs of our placebo patients is of considerable potential importance, as it may demonstrate the need for de novo AA synthesis in patients with increasing inflammation depicted by increased IL-6, faecal calprotectin and lactoferrin. Alanine and glutamine account for high proportion of total AAs in muscles and their efflux during catabolism might be reflected in plasma free AAs, proposed as a sensitive marker for early prognosis of catabolism and inflammation (Storr et al, 2013). Although no significant differences were detected in precursors of alanine (i.e. aspartic acid), it could be hypothesized that increased catabolism provides the nitrogen source and contributes to the carbon pool necessary for AAs synthesis. Mastiha treatment resulted in attenuation of the above-mentioned increase in plasma AAs, indicative of a protective role in relapse onset. Additionally, unpublished data on plasma free AAs in healthy humans showed that plasma AAs decrease after Mastiha administration, and this correlates strongly with enhanced human antioxidant capacity (data not shown).

A significant increase was reported in albumin in verum group. Although albumin is a poor marker of nutritional status, its undoubted prognostic importance indicates advantage for Mastiha-treated patients. Additionally, total and LDL cholesterol significantly increased in the placebo compared to the verum group. Previous research showed that Mastiha lowers total cholesterol (Kartalis et al, 2016), which could be beneficial for IBD patients characterized by dyslipidemia and altered lipoprotein profile (Sappati Biyyani et al, 2010)

While our study has interesting results and the quality of being fully double-blinded, it has limitations, including absence of colonoscopy at follow-up, precluding our ability to comment on histological alterations and the absence of the full metabolic profile identification. The study was however very tightly controlled to ensure compliance with the protocol, and had a final power of 0.90 for the between-subjects main effect at an effect size of 0.37.

Conclusively, although superiority of Mastiha versus placebo in remission maintenance in IBD was not confirmed, the amelioration in increased plasma AAs indicates a role in limiting disease activity. Future research upon the mechanism underlying the effects of Mastiha on modulation of amino acid profile in IBD is of interest. Identification of changes in the metabolome of IBD patients supplemented with Mastiha may indicate a plethora of metabolites (i.e. lipids, TCA intermediate metabolites etc) regulated by this natural product. We are confident that these results are of potential clinical significance and fully justify further exploration of Mastiha use in IBD.

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Author contributions

ACK and AF designed the study. EP collected, analysed and interpreted data. LT and NK set up the chromatographic analysis protocol and were in charge of the analysis. CA contributed in laboratory work, data analysis and interpretation. CT undertook statistical analysis. EP wrote the manuscript finally edited and approved by ACK and AF. ACK supervised the study. All authors approved the final version of manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Figure captions

Fig. 1. Study design flowchart

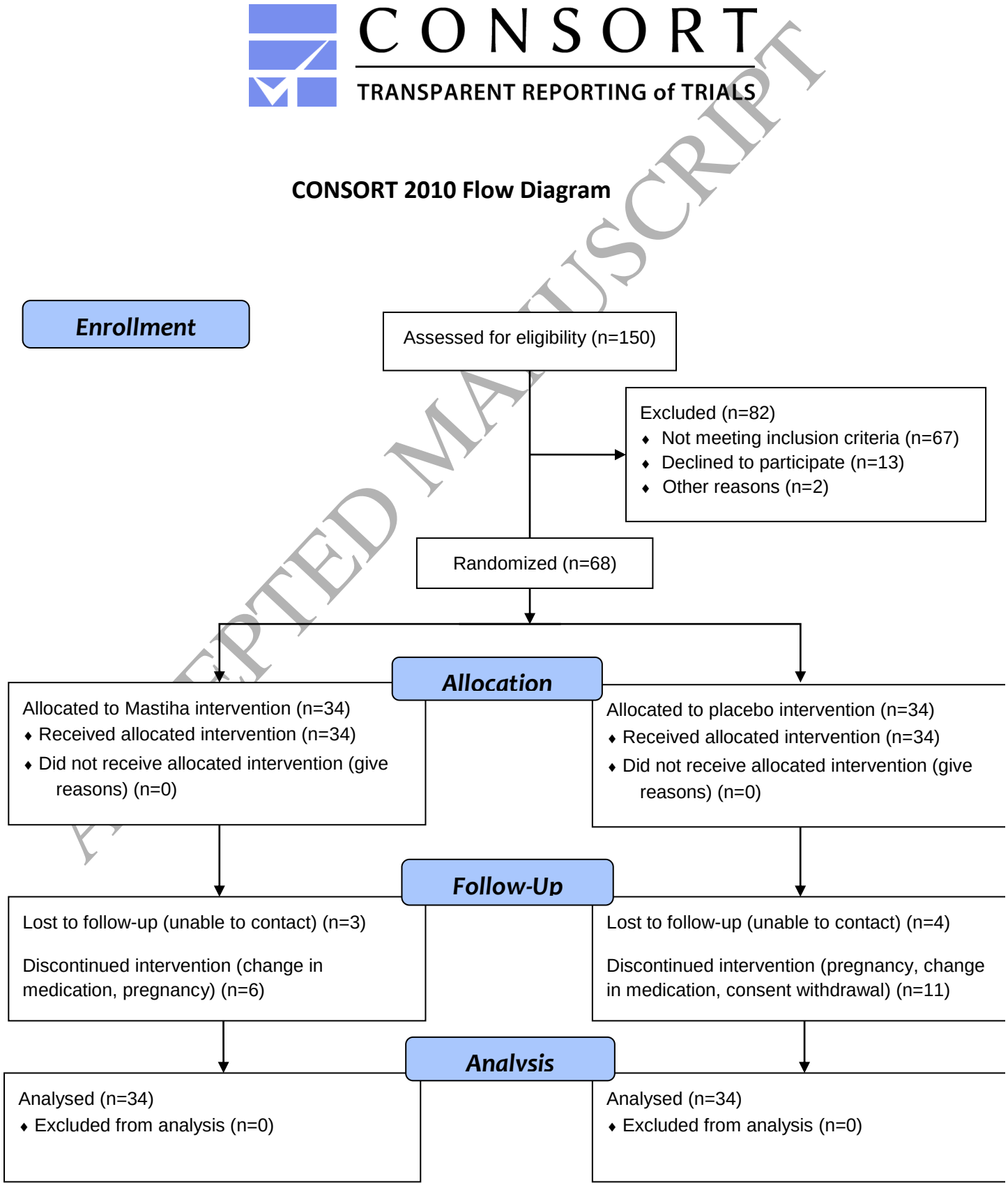

TABLE 1. Inclusion and exclusion criteria

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
Inactive disease (>3 months) • Defined by HBI ≤ 4 in CD • Defined by PMS ≤ 1in UC	Bowel surgery ≤ 3 months prior to screening; a planned elective surgery or hospitalization 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
Childbearing age with a negative pregnancy test at eligibility and baseline assessment	Enteral or Parenteral Nutrition; Alcohol or drug abuse, Vitamin or inorganic supplements, vegan or macrobiotic diet before and during the trial
STable treatment with azathioprine or mesalamine and mesalamine analogues during the trial	Any malignancy in the year prior to screening; cardiovascular disease; peptic ulcer Pregnancy, lactation

TABLE 2. Remission rate in placebo and verum group. Results are given as N(%) of total number.

	Baseline	**Follow-up**	P
	Placebo (34)	Mastiha (34)	
Baseline	0 (0.0)	0 (0.0)	-

Follow-up	8 (23.5)	6 (17.6)	0.549

TABLE 3. Quality of life, disease activity indices and MedDiet scores at baseline and follow up.

	Baseline	**Follow-up**	**Change**		
	Mean (SD)	Mean (SD)	Mean (SD)	P[1]	P[2]
IBDQ score					
Placebo	173.2 (24.8)	178.4 (23.5)	5.2 (22.0)	0.249	0.572
Mastiha	180.1 (28.6)	181.7 (16.0)	1.6 (26.5)	0.720	
P[3]	0.290	0.496			
HBI[+‡]					
Placebo	2.24 (1.48)	2.70 (1.82)	0.46 (1.81)	0.355	0.436
Mastiha	2.36 (1.25)	2.67 (2.14)	0.31 (1.88)	0.887	
P[3]	0.567	0.805			
PMS[++‡]					
Placebo	0.77 (0.44)	1.44 (1.37)	0.67 (1.16)	0.234	0.366
Mastiha	0.67 (0.50)	0.76 (1.04)	0.10 (1.19)	0.857	
P[3]	0.616	0.269			
MedDiet Score					
Placebo	31.4 (6.2)	29.6 (6.2)	-1.7 (4.6)	**0.036**	0.426
Mastiha	30.3 (5.5)	29.6 (4.9)	-0.7 (5.6)	0.438	
P[3]					

[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; [+] in CD patients; [++]in UC patients.

TABLE 4. 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	247.5 (62.8)	225.3 (51.0)	-22.2 (70.1)	0.109	0.879
Mastiha	264 (70.6)	242.8 (57.3)	-21.2 (72.1)	0.080	
P[3]	0.295	0.191			
Serum Fe (μg/dL)‡					
Placebo	72.4 (34.6)	73.7 (25.3)	1.3 (36.9)	0.534	0.912
Mastiha	73 (63.8)	71.2 (30.5)	-1.8 (67.7)	0.437	
P[3]	0.618	0.621			
Serum albumin (g/dL)‡					
Placebo	4.38 (0.35)	4.36 (0.54)	-0.02 (0.48)	0.639	**0.016**
Mastiha	4.32 (0.3)	4.61 (0.49)	0.28 (0.56)	**0.004**	
P[3]	0.482	**0.049**			
Serum glucose (mg/dL)					
Placebo	87.1 (18.1)	77.9 (15.2)	-9.2 (21.4)	**0.004**	0.204
Mastiha	85.7 (13.5)	82.1 (10.4)	-3.6 (13.5)	0.246	
P[3]	0.728	0.186			
Total cholesterol (mg/dL)‡					
Placebo	171.7 (43.7)	188.8 (46.9)	17 (39.9)	**0.004**	**0.032**
Mastiha	178.5 (42.6)	175.9 (32.9)	-2.6 (29.6)	0.916	

P[3]	0.453	0.262			
LDL cholesterol (mg/dL)‡					
Placebo	96.4 (38.4)	111.4 (39.5)	15.1 (33.3)	**0.002**	**0.045**
Mastiha	106.4 (36.6)	105.7 (27.3)	-0.7 (25.9)	0.693	
P[3]	0.173	0.744			
Serum amylase (IU/L)‡					
Placebo	68 (24.2)	69.3 (16.1)	1.3 (24)	0.351	0.124
Mastiha	71.1 (24.7)	64.4 (11.4)	-6.7 (21.3)	0.211	
P[3]	0.562	0.221			
Serum urea (mg/dL)					
Placebo	30.7 (7.7)	31.2 (6.3)	0.5 (8.3)	0.703	0.242
Mastiha	32.5 (7.2)	30.8 (4.7)	-1.7 (6.7)	0.202	
P[3]	0.326	0.777			
Serum LDH (U/L)‡					
Placebo	149.7 (33.7)	165.1 (36.2)	15.4 (35.4)	**0.015**	0.463
Mastiha	146.8 (27.8)	154.7 (26)	7.9 (31.1)	0.150	
P[3]	0.765	0.292			
Serum total bilirubin (mg/dL)‡					
Placebo	0.40 (0.31)	0.42 (0.59)	0.20 (0.63)	0.100	0.112
Mastiha	0.39 (0.22)	1.24 (3.25)	0.85 (3.25)	0.217	
P[3]	0.663	0.077			
Serum direct bilirubin (mg/dL)‡					
Placebo	0.18 (0.1)	0.18 (0.17)	0 (0.16)	0.106	0.965
Mastiha	0.18 (0.1)	0.2 (0.21)	0.02 (0.2)	0.119	
P[3]	0.707	0.870			

Serum SGOT (IU/L)‡					
Placebo	16.8 (4.5)	18.1 (4.5)	1.3 (6.1)	0.166	0.744
Mastiha	18.2 (9.6)	19.2 (5.7)	1 (8.4)	0.067	
P[3]	0.813	0.410			
Serum SGPT (IU/L)‡					
Placebo	19.6 (10.5)	16.9 (6.9)	-2.7 (9.7)	0.190	0.114
Mastiha	17.1 (9.1)	17.1 (5.7)	0.1 (7)	0.350	
P[3]	0.249	0.675			
Serum γ-GT (IU/L)‡					
Placebo	18.3 (10.3)	19.2 (8.4)	0.9 (11.4)	0.356	0.955
Mastiha	29.3 (72.4)	26.7 (49.6)	-2.6 (25.4)	0.317	
P[3]	0.799	0.723			
Serum ALP (IU/L)‡					
Placebo	65.4 (19)	69.2 (16.5)	3.8 (20.2)	0.217	0.642
Mastiha	67.8 (41)	73 (39.3)	5.2 (19.1)	0.061	
P[3]	0.780	0.870			

[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

TABLE 5. Amino acids levels (nmol/ mL) at baseline and at follow-up.

	Baseline	**Follow-up**	**Change**		
	Mean (SD)	Mean (SD)	Mean (SD)	P[1]	P[2]
Alanine‡					
Placebo	278.9 (77.9)	329.1 (86.4)	50.2 (101)	**0.002**	**0.006**

Mastiha	343.9 (80.4)	324.5 (40.2)	-19.4 (91.5)	0.418	
P[3]	**< 0.001**	0.767			
Glycine[‡]					
Placebo	212 (65.4)	237.4 (54.5)	25.4 (75.1)	**0.008**	0.115
Mastiha	238.9 (55)	239.9 (25.5)	1 (52.7)	0.229	
P[3]	**0.045**	0.499			
α-Aminobutyric acid[‡]					
Placebo	13.9 (5.4)	16.4 (5.8)	2.5 (7.3)	**0.018**	0.401
Mastiha	16 (6.5)	17.2 (6.6)	1.2 (7.1)	0.220	
P[3]	0.157	0.561			
Valine[‡]					
Placebo	311.7 (93.8)	363.4 (88)	51.7 (120.7)	**0.001**	**0.047**
Mastiha	346.9 (75.7)	354.1 (39.6)	7.2 (80.7)	0.454	
P[3]	**0.045**	0.848			
β-Aminobutyric acid[‡]					
Placebo	97.8 (2.3)	98.6 (2.6)	0.8 (3.5)	0.722	0.249
Mastiha	98.1 (2.3)	104.6 (29)	6.5 (32.1)	0.157	
P[3]	0.607	0.192			
Leucine[‡]					
Placebo	118.5 (44.2)	143.1 (41.7)	24.6 (60.7)	**< 0.001**	0.118
Mastiha	127.5 (32)	135.9 (15.8)	8.4 (35.2)	0.137	
P[3]	0.146	0.632			
Allo-isoleucine[‡]					
Placebo	50 (24.8)	58.6 (19.3)	8.6 (31.9)	**0.004**	0.122
Mastiha	54.8 (18.9)	55.4 (6.9)	0.6 (21)	0.438	

P[3]	0.146	0.698			
Isoleucine‡					
Placebo	58.8 (28.5)	67.3 (22.1)	8.5 (36.8)	**0.011**	0.159
Mastiha	64.4 (21.6)	64.5 (8.2)	0.1 (24)	0.547	
P[3]	0.139	0.831			
Threonine‡					
Placebo	117.4 (41)	139 (46.1)	21.6 (56.6)	**0.004**	0.210
Mastiha	124.9 (34.8)	131.2 (25.3)	6.3 (32.1)	0.238	
P[3]	0.321	0.591			
Serine‡					
Placebo	101.5 (29.6)	117.1 (32.6)	15.5 (34.4)	**0.003**	0.105
Mastiha	110 (31)	111.1 (16.9)	1.2 (25.8)	0.458	
P[3]	0.238	0.550			
Proline‡					
Placebo	215.2 (65.5)	277.2 (73.4)	62 (85.6)	< **0.001**	**0.022**
Mastiha	269 (104.1)	277.8 (71.8)	8.8 (88.6)	0.241	
P[3]	**0.018**	0.922			
Asparagine‡					
Placebo	42.2 (12.4)	47.7 (11.8)	5.5 (15.3)	**0.005**	0.062
Mastiha	47.7 (12.8)	47.1 (6.3)	-0.7 (11.2)	0.844	
P[3]	**0.050**	0.918			
Thioproline‡					
Placebo	16.3 (4)	13.8 (2.9)	-2.5 (5)	0.146	0.831
Mastiha	17.8 (6.4)	14.6 (3.8)	-3.2 (7.3)	0.081	
P[3]	0.856	0.406			

Aspartic acid‡					
Placebo	7.7 (4.2)	7.4 (4.4)	-0.3 (5.8)	0.547	0.964
Mastiha	9.2 (5.9)	8.1 (4.4)	-1.2 (8.6)	0.591	
P[3]	0.723	0.730			
Methionine					
Placebo	20.7 (8.7)	21.8 (7.8)	1.1 (11.2)	0.437	0.879
Mastiha	20.9 (6)	21.7 (4.1)	0.8 (5)	0.576	
P[3]	0.928	0.923			
Hydroxyproline‡					
Placebo	17 (5.4)	18.1 (4.8)	1.1 (7.8)	0.207	0.074
Mastiha	19.7 (5.7)	18.4 (6.8)	-1.3 (6.6)	0.201	
P[3]	**0.028**	0.295			
Glutamic acid‡					
Placebo	18.2 (19.6)	22.2 (14.1)	4 (22.7)	0.143	0.688
Mastiha	18.5 (11.5)	24.7 (12.3)	6.2 (15.8)	**0.044**	
P[3]	0.319	0.120			
Phenylalanine‡					
Placebo	62.4 (18.6)	74.1 (15.3)	11.7 (25.6)	**0.001**	0.188
Mastiha	65.8 (13.2)	71.4 (8.1)	5.7 (16.6)	0.082	
P[3]	0.206	0.544			
Glutamine‡					
Placebo	355.9 (76.3)	405.5 (68)	49.6 (91.3)	**0.003**	< **0.001**
Mastiha	422 (90.1)	365.9 (59.7)	-56 (102.3)	**0.005**	
P[3]	**0.003**	**0.014**			
Ornithine‡					

Placebo	78.3 (27.1)	89.2 (27.3)	10.9 (38.7)	**0.032**	0.085
Mastiha	89.8 (44)	82.3 (10.6)	-7.4 (47.9)	0.778	
P[3]	0.163	0.279			
Lysine[‡]					
Placebo	177 (80.5)	187.2 (60.4)	10.2 (91.6)	0.100	0.098
Mastiha	193.7 (68.4)	177.6 (21.8)	-16.1 (73.2)	0.483	
P[3]	0.150	0.672			
Histidine[‡]					
Placebo	78.2 (23.4)	83.2 (17.7)	5 (27.5)	0.140	0.336
Mastiha	78.2 (21.7)	76.8 (12.6)	-1.4 (23.6)	0.903	
P[3]	0.894	0.135			
Tyrosine[‡]					
Placebo	50.6 (17.8)	65.9 (18.5)	15.4 (26.8)	**< 0.001**	**0.043**
Mastiha	54.9 (15.3)	59.2 (10.5)	4.3 (20.2)	0.190	
P[3]	0.180	0.100			
Tryptophane[‡]					
Placebo	57.3 (16.1)	66.3 (16.5)	9 (26.2)	**0.006**	0.278
Mastiha	59.1 (15.2)	62.1 (9.2)	3 (15.7)	0.207	
P[3]	0.575	0.275			
Cysteine[‡]					
Placebo	27 (2.6)	28.5 (3.1)	1.5 (3.9)	**0.023**	0.338
Mastiha	27.1 (2.9)	27.6 (2.4)	0.6 (3.8)	0.340	
P[3]	0.972	0.203			

[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.

TABLE 6. 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	4.4 (8.3)	5.2 (9.8)	0.8 (5.6)	0.161	0.859
Mastiha	5.4 (9.1)	4.9 (5.5)	-0.5 (8.6)	0.099	
P[3]	0.510	0.357			
Serum IL-6 (pg/ mL)‡					
Placebo	6.8 (16.6)	11.3 (12.7)	4.5 (12.4)	**0.005**	0.360
Mastiha	6.5 (7.7)	10.3 (12.2)	3.8 (14)	0.093	
P[3]	0.279	0.916			
Serum IL-10 (pg/ mL)‡					
Placebo	7 (3.6)	6.2 (3)	-0.8 (2.5)	0.222	0.770
Mastiha	7.2 (10.1)	5.8 (3)	-1.4 (9.3)	0.416	
P[3]	0.334	0.465			
Faecal lysozyme (μg/g)‡					
Placebo	12.2 (9.8)	9.1 (6.1)	-3.1 (12)	0.155	0.614
Mastiha	15.7 (23.6)	9.4 (3.3)	-6.3 (25.6)	0.472	
P[3]	0.973	0.359			
Faecal calprotectin (μg/g)‡					
Placebo	940.9 (988.8)	5221.3 (18634.4)	4280.3 (18705.7)	**0.002**	0.117
Mastiha	1396.6 (4547.6)	977.63 (960.3)	-419.0(4756.2)	0.145	
P[3]	0.817	**0.026**			
Faecal lactoferrin (μg/g)‡					

Placebo	112.4 (210.2)	141.4 (155.5)	29 (267.5)	**0.035**	0.419
Mastiha	65.7 (98.3)	86.12 (143.7)	-20.4(155.5)	0.060	
P[3]	0.715	0.156			

[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.

Graphical-Abstract.