Research Protocol

Full title: A pilot randomized, double-blind, placebo-controlled trial to investigate the anti-inflammatory effects of Frondanol in adults with inflammatory bowel disease

Short title: Frondanol in IBD

Protocol Number: 1

Protocol Date: 04 December 2021

Version Number: 3.2

Version Control Table:

Version	Date	Author	Rationale
1.0	14/03/21	Reem Jan	First Protocol Version submitted to MOHAP
2.0	28/06/21	Reem Jan	Resubmission to MOHAP following initial review and requested changes – approved 01/09/21
3.0	19/10/21	Reem Jan	Resubmission to MOHAP to correct typing error in Frodnanol strength and with other amendments such as minor changes in inclusion/exclusion criteria and in placebo content from cellulose to corn starch and addition of post-doctoral fellow
3.1	30/11/21	Reem Jan	Addition of coinvestigator Dr Mazin Aljabiri
3.2	04/12/21	Reem Jan	Addition of coinvestigators Dr Usama Warshow and Dr Hossam Al-Hilou

Confidentiality Statement:
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Ethics Statement:
This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

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STUDY FUNDER (S)	
	Institution: MBRU City, Country: Dubai, UAE Tel: 800-MBRU (6278) Email: research.com@mbru.ac.ae Role: MBRU Internal Grant and MBRU Post-doctoral Fellowship salary provision

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Principal Investigator Agreement

I, the undersigned, have reviewed this protocol, and I agree to conduct this study in accordance with the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), the ethical principles set forth in the Declaration of Helsinki and other applicable local regulatory requirements. The protocol may not be modified without written approval of the Sponsor/ Funder. All changes to the protocol must be submitted to the applicable regulatory authorities and Research Ethics Committee (REC) and must be approved by the REC prior to their implementation, except where necessary to prevent immediate danger to the subject.

Principal Investigator Signature: _____ _28/06/2021___
Date: DD/MM/YY

The signatory agrees to the content of the final study protocol as presented.

The signatory agrees to the content of the final study protocol as presented.

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1. PROTOCOL SUMMARY

Study Title	A pilot randomized, double-blind, placebo-controlled trial to investigate the anti-inflammatory effects of Frondanol in adults with inflammatory bowel disease
Internal ref. no. (or short title)	Frondanol in IBD
Study Design	This is a pilot, prospective, double-blinded, two-arm, randomized controlled trial of the efficacy of Frondanol in comparison to placebo in decreasing bowel inflammation in patients with a clinical diagnosis of IBD who are in remission and on standard of care treatment.
Study Participants	Inclusion criteria: A confirmed clinical diagnosis of IBD of any duration, age 18 years or older, with mild to moderate disease and on standard therapy. The diagnostic criteria for IBD include the presence of chronic diarrhoea for more than four weeks, and evidence of active inflammation on endoscopy and chronic changes on biopsy. Patients with stable mild to moderate IBD will be eligible for the study. Stable IBD is defined as having stable symptoms over a period of several weeks, diagnostic evaluation has been completed and the patient has been on consistent medication. Mild to moderate IBD is indicated by a Partial Mayo score (Mayo Clinic Score/Disease Activity Index for Colitis) of between 1-6, and a total of Mayo score of 1-10 (Pabla and Schwartz, 2020). For patients with Crohn's disease, only those with Crohn's colitis will be included (patients with small bowel disease are eligible to enter the trial as long as they also have large bowel inflammation). Exclusion criteria: Pregnancy, breastfeeding, allergy to seafood or marine products, severe medical illness such as uncontrolled diabetes (HbA1C>10), significant or unstable cardiovascular or pulmonary disease, impaired renal function (Cr>2.0mg/dL), current or recent (<1 year) malignancy, or other significant medical illness that in the view of the investigators may impair participation in the study. Patients with severe IBD (defined by a Partial Mayo score of 7-9 and a total Mayo score of 11-12 (Pabla and Schwartz, 2020), with active symptoms) will not be eligible to participate in the study. Reference:

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	Pabla BS, Schwartz DA. Assessing Severity of Disease in Patients with Ulcerative Colitis. Gastroenterol Clin North Am. 2020; 49(4):671–688
Planned Size of Sample (if applicable)	100 patients (50 per arm) with stable IBD will be recruited into this pilot study.
Follow up duration (if applicable)	Six months
Planned Study Period	Two years
Research Question/Aim(s)	1) To compare plasma levels, biopsy mRNA (using low density expression arrays) and protein (using either Luminex or Western blotting) levels of the following parameters between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment: a) Cytokines: TNFα, IL1β, IL6, IL17A, IL21, IL22, IL23, IFNγ, IFNα, as well as vascular endothelial growth factor (VEGF-A), MIP-1, MIP-2; b) Markers of inflammation: Myeloperoxidase, calprotectin, S100A12, β2-microglobulin, amyloid A (A-SAA), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), MIP-2α (CXCL2), MCP-1 (CCL2). Leukotriene B4 (LTB4), 5-hydroxyeicosatetraenoic acid (5-HETE) and prostaglandin E2 (PGE2) will be measured by specific ELISA assays. 2) To compare the following routine clinical parameters between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment: a) Patient symptoms; b) Blood: Complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin; c) Stool: Fecal calprotectin level; d) Endoscopic and histologic features. 3) To compare in-vitro concentration-dependent responses to Frondanol and placebo on inflamed organoids by measurement of cytokines and other inflammatory markers, in particular 5-HETE, LTB4 and PGE2.

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2. ABBREVIATIONS

5-HETE	5-hydroxyeicosatetraenoic acid
CBC	Complete blood count
CRP	C-reactive protein
DCF	Data Collection Form
DHCR	Dubai Healthcare City Authority-Regulation
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IL	Interleukin
LOX	Lipoxygenase
LTB4	Leukotriene B4
MCH	Mediclinic City Hospital
MCME	Mediclinic Middle East
PGE2	Prostaglandin E2
REC	Research & Ethics Committee
TNF	Tumor necrosis factor
VCAM-1	Vascular cell adhesion molecule 1

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Introduction

3. BACKGROUND

Inflammatory bowel disease (IBD), consisting of Crohn's disease and ulcerative colitis, is a debilitating condition, particularly during active periods (flares) of the disease and can sometimes lead to life-threatening complications (1). The prevalence of IBD has rapidly increased worldwide; it affects between one in 200 and one in 300 individuals in high-income countries, and despite its incidence being lower in low-middle income countries, this has also been rapidly increasing (2, 3). The highest reported prevalence in the Middle East is one in 1,800 for Crohn's disease and one in 1,000 for ulcerative colitis (3).

IBD is characterized by chronic gut inflammation resulting in symptoms such as severe diarrhea, abdominal pain, blood in stool, fatigue and unintended weight loss, which significantly affect the quality of life of patients (1). Although the exact mechanism underlying the chronic gut inflammation is not fully understood, several cytokine networks are thought to be involved (4-6). The cytokine networks underlying the progression of IBD are depicted below in Figure 1 (4).

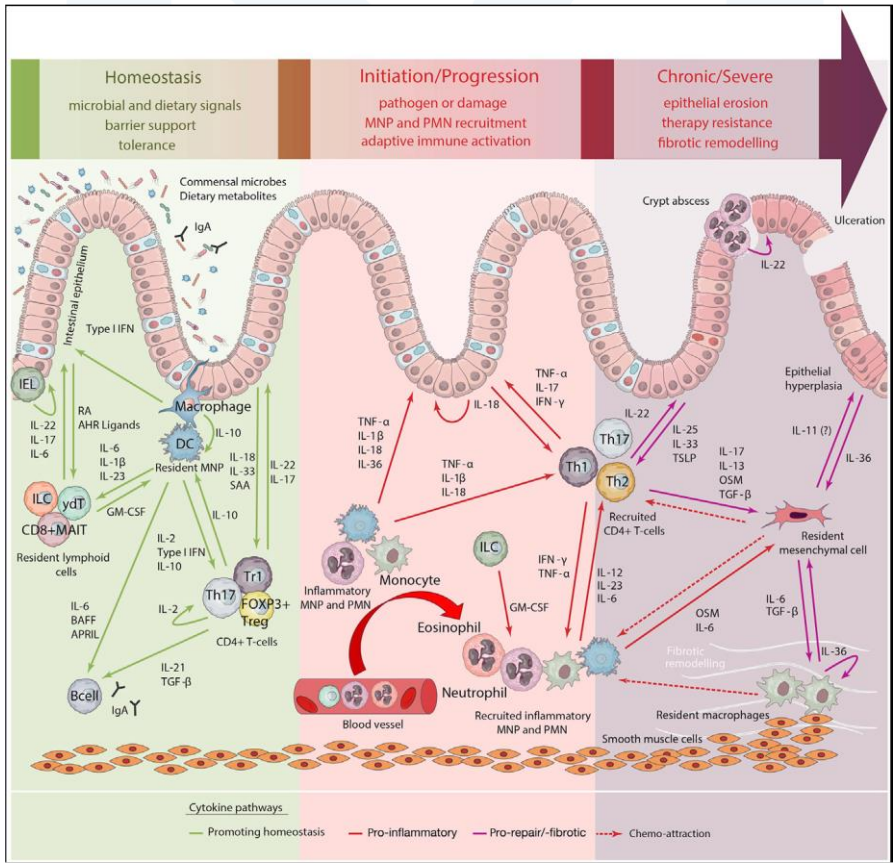

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Figure 1: Cytokine networks underlying the progression of IBD (4)

Currently, treatment of IBD relies on minimizing symptoms and improving quality of life through the control of disease progression and complications; however, these drugs have significant systemic side effects that reduce their tolerability (7). Moreover, up to 40% of patients still exhibit non-response to therapy, and these treatment-refractory patients would require alternative therapeutic approaches (8, 9).

Sea cucumbers and their extracts have been reported to have high nutritional value and several potential health benefits including anti-inflammatory effects, and used for centuries as folk medicine or traditional foods in countries such as Japan, Indonesia, Korea and China (10, 11). Frondanol, a widely available neutraceutical extract of the edible sea cucumber, *Cucumaria frondosa*, has been reported to possess potent chemopreventive and anti-inflammatory effects in both animals and humans (12-15), whilst showing no signs of toxicity (12, 16). The potent anti-inflammatory effects of Frondanol in a mouse model of IBD provide encouragement for investigating its effects in human IBD patients (15).

Over the past 25 years, it is estimated that more than three million Frondanol capsules have been consumed on the human market with no reported side effects. An even larger amount has been consumed on the veterinary market without a single reported incident. If proven beneficial, Frondanol, will be a useful supplement in treating the underlying chronic gut inflammation in IBD patients, increasing the likelihood of patients remaining in remission and potentially providing an effective, natural and safe treatment for treatment naive patients in the future.

The proposed study is a pilot, double-blinded, placebo-controlled trial of Frondanol in patients with IBD (Crohn's disease or ulcerative colitis) who are currently in remission and are on standard therapy. One hundred patients will be randomized (1:1) to receive Frondanol or placebo as an adjunct to their standard therapy for the period of six months. Blood and tissue samples from colon biopsies obtained during routine visits and endoscopies at baseline and six months later will be collected. The levels of inflammatory markers such as myeloperoxidase, tumor necrosis factor (TNF)-α, interleukin (IL)1β, IL6, IL17A, IL22, interferon gamma (IFN-γ) and several other inflammatory markers will be compared between patients treated with Frondanol and those treated with placebo, and the findings will be correlated with clinical and histological parameters. In addition, we plan to develop a stem cell-derived gut organoid model to investigate the mechanism of action of Frondanol.

4. RATIONALE

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The overall aim of this project is to investigate the anti-inflammatory effects of Frondanol on bowel inflammation in patients with IBD.

5. RESEARCH OBJECTIVES

1) To compare plasma levels, biopsy mRNA (using low density expression arrays) and protein (using either Luminex or Western blotting) levels of the following parameters between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment:
a) Cytokines: TNFα, IL1β, IL6, IL17A, IL21, IL22, IL23, IFNγ, IFNα, as well as vascular endothelial growth factor (VEGF-A), MIP-1, MIP-2;
b) Markers of inflammation: Myeloperoxidase, calprotectin, S100A12, β2-microglobulin, amyloid A (A-SAA), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), MIP-2α (CXCL2), MCP-1 (CCL2). Leukotriene B4 (LTB4), 5-hydroxyeicosatetraenoic acid (5-HETE) and prostaglandin E2 (PGE2) will be measured by specific ELISA assays.

2) To compare the following routine clinical parameters between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment:
a) Patient symptoms;
b) Blood: Complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin;
c) Stool: Fecal calprotectin level;
d) Endoscopic and histologic features.

3) To compare in-vitro concentration-dependent responses to Frondanol and placebo on inflamed organoids by measurement of cytokines and other inflammatory markers, in particular 5-HETE, LTB4 and PGE2.

6. STUDY DESIGN/METHODOLOGY

5.1 Design overview

This is a pilot, prospective, double-blinded, two-arm, randomized controlled trial of the efficacy of Frondanol in comparison to placebo in decreasing bowel inflammation in patients with a clinical diagnosis of IBD who are in remission and on standard of care treatment.

6.2 Study setting

Patients will be seen in-person at the Gastroenterology Department of any of the study centres that will participate in patient recruitment (Mediclinic City Hospital, Mediclinic Parkview Hospital and Rashid hospital) by one of the coninvestigator consultant gastroenterologists at screening and randomization (T0), at a follow-up visit at 3 months (T1), and at a second follow-up visit at 6 months (T2). Routine blood tests as well as an extra sample for research

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purposes will be obtained at these timepoints. Colonoscopies will be performed as part of the routine clinical practice and these include acquisition of routine biopsies, typically 10-15 from each patient. A few additional biopsies (2-4) will be needed for our studies, to be snap frozen and analyzed in MBRU research laboratory facilities. IRB approval for these additional biopsies and serological testing is being sought from both Mediclinic City Hospital's REC, MBRU-IRB and DHA-REC. It is hoped that the routine colonoscopies can be scheduled prior to starting the study treatment and 6 months later.

6.3 Sample and recruitment

All eligible IBD patients visiting Gastroenterology Department of any of the study centres that will participate in patient recruitment (Mediclinic City Hospital, Mediclinic Parkview Hospital and Rashid hospital) under the care of any of the coinvestigator consultant gastroenterologists during the period of recruitment (12 months from the start date of the study – to be decided following ethical approval) will be offered to take part in the study.

Block randomization over four blocks of 25 patients will be used to ensure an equal number of patients in each treatment group, even if the trial was terminated early for any reason. Two blocks will be reserved for patients with quiescent/minimal disease and two blocks for patients with mild disease, in order to ensure an equal number of these patients in each treatment group. Patients will be randomised (1:1) to receive Frondanol (one 1000 mg capsule twice daily) or placebo (in a similar capsule) as an adjunct to their standard therapy using a randomization number sequence generator. This will be conducted by the study statistician using the Microsoft Excel random number generator and will only be shared with the study coordinator.

All patients and investigators, excluding the study coordinator, will be blinded to treatment allocation. Frondanol and placebo capsules will be placed in identical-looking vials which will be labelled with the study name and number, and no identifying information. The labelling of the vials will be done with the open randomization sheet by the study coordinator. When a patient agrees to participate, and signs the informed consent form, the physician will assign to the patient the next available randomization number from the randomization sheet (supplied by the study coordinator) with the corresponding labelled medication vial. Patients will be instructed to take one capsule of their assigned medication twice daily. Hence, capsules will be given in a double-blind manner where patient and physician are not aware of treatment allocation as the treatment and placebo vials will only indicate the pilot study name. Investigators involved in the laboratory analysis of samples will also be blinded, they will only know if a patient belongs to group 1 or 2 but will not know which group is taking Frondanol and which is taking placebo.

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6.4 Study population

One hundred patients (50 per arm) with stable IBD (Crohn's disease or ulcerative colitis, in remission) will be recruited in one of the participating study centres (Mediclinic City Hospital, Mediclinic Parkview Hospital, Rashid Hospital) by one of the coinvestigator consultant gastroenterologists.

6.5 Eligibility criteria

- **Inclusion criteria:**

A confirmed clinical diagnosis of IBD of any duration, age 18 years or older, with mild to moderate disease and on standard therapy. The diagnostic criteria for IBD include the presence of chronic diarrhoea for more than four weeks, and evidence of active inflammation on endoscopy and chronic changes on biopsy. Patients with stable mild to moderate IBD will be eligible for the study. Stable IBD is defined as having stable symptoms over a period of several weeks, diagnostic evaluation has been completed and the patient has been on consistent medication. Mild to moderate IBD is indicated by a Partial Mayo score (Mayo Clinic Score/Disease Activity Index for Colitis) of between 1-6, and a total of Mayo score of 1-10 (Pabla and Schwartz, 2020). For patients with Crohn's disease, only those with Crohn's colitis will be included (patients with small bowel disease are eligible to enter the trial as long as they also have large bowel inflammation).

- **Exclusion criteria:**

Pregnancy, breastfeeding, allergy to seafood or marine products, severe medical illness such as uncontrolled diabetes (HbA1C>10), significant or unstable cardiovascular or pulmonary disease, impaired renal function (Cr>2.0mg/dL), current or recent (<1 year) malignancy, or other significant medical illness that in the view of the investigators may impair participation in the study. Patients with severe IBD (defined by a Partial Mayo score of 7-9 and a total Mayo score of 11-12 (Pabla and Schwartz, 2020), with active symptoms) will not be eligible to participate in the study.

Reference:
Pabla BS, Schwartz DA. Assessing Severity of Disease in Patients with Ulcerative Colitis. Gastroenterol Clin North Am. 2020; 49(4):671–688

6.6 Sampling technique and recruitment

All eligible IBD patients visiting the Gastroenterology Department of any of the study centres that will participate in patient recruitment (Mediclinic City Hospital, Mediclinic Parkview Hospital and Rashid hospital) under the care of any of the coninvestigator consultant gastroenterologists during the period of recruitment (12 months from the start date of the study – to be decided following ethical approval) will be offered to take part in the study.

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Patients will be randomised (1:1) to receive Frondanol (one 1000 mg capsule twice daily) or placebo (in a similar capsule) as an adjunct to their standard therapy using a randomization number sequence generator. This will be conducted by the study statistician using the Microsoft Excel random number generator and will only be shared with the study coordinator.
All patients and investigators, excluding the study coordinator, will be blinded to treatment allocation.

6.7 Intervention

Patients will be randomized (1:1) to receive Frondanol (one 1000 mg capsule twice daily) or placebo (in a similar capsule) as an adjunct to their standard therapy by a designated study coordinator. Both Frondanol and placebo capsules will be supplied by Coastside Bio Resources, Stonington, ME, USA. The placebo capsules will be red/brown-coloured gelatin capsules (similar in texture and colour to the Frondanol capsules), but will contain an inert (inactive) substance called food-grade corn starch, which will not interact with the immune system. Patients will be instructed to take one capsule of their assigned medication twice daily.

6.8 Consent

Informed consent will be obtained following consultation with one of the coinvestigator consultant gastroenterologists to assess eligibility for the study. The study will be explained to the patient by the consultant gastroenterologist and informed consent will be obtained prior to treatment. Patients will be requested to inform the study coordinator or the consultant gastroenterologist of any adverse events following the start of treatment. A direct phone number will be given to patients so that they are able to call the study coordinator in case of any adverse events. The study coordinator, PI and co-investigator gastroenterologists will have thorough understanding of the protocol and patient population. Retention of patients will be encouraged through discussion at the time of consent and throughout the duration of the study regarding the importance of adherence with the study procedures. The study will be conducted in accordance with the IRB-approved protocol and with the guidelines of the Good Clinical Practices (GCP). Patients may withdraw from the study at any time, and no further data will be collected from him/her or analyzed for the purpose of this research.

6.9 Voluntary participation

The following paragraph is included in the consent form regarding voluntary participation:
“Your participation in the study is entirely voluntary and you may refuse to participate or discontinue participation at any time without penalty or loss of

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benefits to which you would normally be entitled. Your decision about whether or not to participate in the study will not affect your relationship with MBRU or medical treatment at the hospital/clinic, if applicable."

6.10 Criteria for subject withdrawal

Patients may withdraw from the study at any time, and no further data will be collected from him/her or analyzed for the purpose of this research.

6.11 Withdrawal from the study

The following paragraph is included in the consent form regarding withdrawal from the study:

"You are at liberty to withdraw from the research study at any stage, without prejudice. Should any new information become available that may affect your willingness in participation, you will be informed in a timely manner. However, the principal investigator or designee may take you out of the study at any time with or without your agreement. This may happen for example if it is in your best medical interest to stop your participation, or if the study is canceled. If you wish to withdraw from the study, any collected data will be safely stored for a minimum of three years following the completion of the study then discarded in a confidential manner that does not risk identifying your personal information."

6.12 Premature discontinuation of the study

The whole study may be discontinued prematurely in the event of the following:

- Mediclinic decision that continuation of the study is unjustifiable for medical or ethical reasons
- Poor enrolment of subjects making completion of the study within an acceptable time frame unlikely

DHCR and the MBRU-IRB and MCME-REC will be informed about the discontinuation of the study in accordance with applicable regulations. The whole study may be terminated or suspended upon the request of DHCR.

6.13 Subject Identification

All patient data and specimen will be de-identified and coded with the unique study number of the patient. The code linking the study number with the patient will be accessed by the study coordinator only and maintained at MBRU in a database and will not be provided to the PI or her research team. Patient data will be collected using protocol-specific case report forms (CRFs), which will be used to create electronic CRFs (e-CRFs). Clinical records for all patients, including CRFs, will be retained by the PI in a secure storage facility for ten years after the completion of the study. Paper data will be stored in a locked

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cabinet and electronic data will be stored on a password-protected computer in the custody of the PI. The data linking patient identifying information and their de-identified data will be in the custody of the study coordinator who will be unblinded. All patients will complete the following study visits: Screening (medical history, physical examination, vital signs and standard of care tests including blood and stool tests), baseline endoscopy (day 1 of treatment, date to be agreed between the gastroenterologist and patient), 3 months (routine monitoring with standard of care tests including blood and stool tests), 6 months (second endoscopy and standard of care tests including blood and stool tests).

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6.14 Study schedule

Table 1: Variables to be collected from participants

Method of data collection	Variables
Questionnaires	Mayo Score, IBDQ
Medical record	Symptoms
Blood samples	Routine blood tests as well as an extra sample (10 mL) for research purposes will be obtained at T0 (start of study), T1 (3 months) and T2 (6 months).
Stool samples	Routine stool sample will be collected for measurement of fetal calprotectin levels at the recruiting hospital at T0, T1 and T2.
Tissue samples	Colonoscopies will be performed as part of the routine clinical practice and these include acquisition of routine biopsies, typically 10-15 from each patient. A few additional biopsies (2-4) will be collected for our studies during routine colonoscopies when these are performed, then they will be snap frozen and analyzed in MBRU research laboratory facilities.

Table 2: Schedule of Activities

Variable	Visit # 1 (T0)	Visit # 2 (T1 – 3months)	Visit # 3 (T2 – 6 months)
Questionnaires	Mayo Score, IBDQ	Mayo Score, IBDQ	Mayo Score, IBDQ
Medical record	Symptoms	Symptoms	Symptoms
Blood samples	Routine blood tests as well as an extra sample (10 mL) will be obtained, stored and analyzed in MBRU research laboratory facilities.	Routine blood tests as well as an extra sample (10 mL) will be obtained, stored and analyzed in MBRU research laboratory facilities.	Routine blood tests as well as an extra sample (10 mL) will be obtained, stored and analyzed in MBRU research laboratory facilities.
Stool sample	Routine stool sample will be collected for measurement of fetal calprotectin levels at MCH	Routine stool sample will be collected for measurement of fetal calprotectin levels at MCH	Routine stool sample will be collected for measurement of fetal calprotectin levels at MCH
Tissue samples	If colonoscopy is performed for routine clinical practice at the start of the study, a few additional biopsies (2-4) will be collected for our studies, snap frozen and analyzed in	No tissue samples collected	If colonoscopy is performed for routine clinical practice at the end of the study, a few additional biopsies (2-4) will be collected for our studies, snap frozen and analyzed in

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	MBRU research laboratory facilities.		MBRU research laboratory facilities.

6.15 Definition of End of Study

The study ends after six months of treatment with either Frondanol or placebo.

7. SAFETY CONSIDERATIONS

The recommended dose on the marketed neutraceutical product of Frondanol is 1000 mg twice daily. Over the past 25 years more than 3 million 1000 mg capsules have been consumed in the human market, with no reported side effects. Hence, we based the dosing for our study on the marketed recommended dose, as we do not anticipate any side effects. We will be excluding patients with allergy to seafood or marine products to avoid the risk of allergic reactions.

Colonoscopies performed in this study are considered part of routine care and not for research purposes. The overall expected rate of serious complications (bleeding or perforation) from routine colonoscopies is 2-3 per 1000 procedures (Lee and Salzman, 2020). The only additional procedure that is done for the research is to take several additional biopsies after all routine biopsies are taken. The expected increase in risk from taking these several additional biopsies is considered to be very small and likely insignificant. The consent form will state that the “increased risk of taking additional biopsies, for the purposes of this research, after all routine biopsies are taken is considered to be very small.”

In the unlikely event that side effects occur, patients will be requested to immediately inform the study coordinator/research nurse, PI or consultant gastroenterologist. A direct phone number will be given to patients so that they are able to call the study coordinator or assigned nurse in case of any adverse events. Depending on the severity of the event, the participant will be instructed whether to report to the Gastroenterology unit at the study site or to head to the emergency department of one of the hospitals covered by the trial’s liability insurance, for further investigation and possible treatment.

Additional risks to the study subjects would be embarrassment or discomfort in answering items on questionnaires to be completed. Participants will be instructed not to answer any questions that they feel uncomfortable with.

8. STUDY OUTCOME EVALUATIONS/STATISTICAL ANALYSIS

8.1 Sample size/Power calculation

All eligible IBD patients visiting the Gastroenterology Department of any of the study centres that will participate in patient recruitment (Mediclinic City Hospital, Mediclinic Parkview Hospital and Rashid hospital) under the care of

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any of the coninvestigator consultant gastroenterologists during the period of recruitment (12 months from the start date of the study – to be decided following ethical approval) will be offered to take part in the study.
Patients will be randomised (1:1) to receive Frondanol (one 1000 mg capsule twice daily) or placebo (in a similar capsule) as an adjunct to their standard therapy using a randomization number sequence generator. This will be conducted by the study statistician using the Microsoft Excel random number generator and will only be shared with the study coordinator.
All patients and investigators, excluding the study coordinator, will be blinded to treatment allocation.

8.2 Statistical methods

All statistical analyses will be carried out using SPSS Software (Armonk, New York, USA). Potential confounders such as age and sex will be controlled for during the analysis phase. A per-protocol analysis including only participants who have a full data set will be used to assess the efficacy of Frondanol treatment in lowering inflammatory markers. An intention-to-treat analysis will also be conducted including patients who drop out before the study ends, for whom we will use last observation carried forward (LOCF) i.e. we will impute the last observed data for all subsequent (missing) observation points. For quantitative data, within-group and between-group comparisons will be analyzed using the General linear Repeated Model (MANOVA), which would allow for adjustment of potential confounders such as age and sex. Data will be plotted as mean ± SEM and p values <0.05 will be considered statistically significant.

9. DATA MANAGEMENT

9.1 Subject Identification and Privacy

A unique subject number will be assigned to each subject at inclusion, immediately after informed consent has been obtained. This number will serve as the subject's identifier in the study.
The subject's data collected in the study will be stored under this number only. Only the study coordinator will be able to link the subject's study data to the subject via an identification list kept at MBRU and/or MCH sites.
Data protection and privacy regulations will be observed in capturing, forwarding, processing and storing subject data. Subjects will be informed accordingly, and will be requested to give their consent on data handling procedures in accordance with MCH and DHCR policies and guidelines.

9.2 Data handling and record-keeping

A data collection form (DCF) will be completed for each subject enrolled into the study. The investigator will review, approve and sign/date each completed

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DCF; the investigator's signature serving as attestation of the investigator's responsibility for ensuring that all data entered on the DCF are complete, accurate and authentic. All study data will be stored at MCH according to ICH-GCP standard for a period of 10 years, or until all regulatory and funder requirements are met.

9.3 Record maintenance and retention

The investigator will maintain and retain the investigator site file (ISF). The file will contain all documents necessary for the conduct of the study and will be updated and completed throughout the study. The investigator will permit study-related monitoring, audits, REC review, and regulatory inspections, providing direct access to source data.

9.4 Monitoring, QUALITY CONTROL AND QUALITY ASSURANCE

This study will be monitored in accordance with the ICH Note for Guidance on Clinical Practice (ICH Topic E6, 1996). The assigned site monitor will monitor data collected at regular intervals. The quality control and quality assurance of the study will be performed by MCH.

Representatives of DHCR will be permitted to inspect all study related documents and other materials at the site, including the ISF, completed DCFs and the subject's medical records/files.

10. ETHICAL and REGULATORY CONSIDERATIONS

10.1 Responsibilities of the Investigator

The Investigator is responsible for the conduct of the study and will ensure that the study is performed in accordance with the *study* protocol and with the ethical principles that have their origin in the Declaration of Helsinki, as well as with the ICH Note for Guidance on Good Clinical Practice (ICH Topic E6, 1996) and applicable regulatory requirements. In particular, the Investigator must ensure that only subjects who have given their informed consent are included into the study.

10.2 Benefits to participants

Currently, treatment of IBD relies on minimizing symptoms and improving quality of life through the control of disease progression and complications. Treatments for IBD include aminosalicylates, high-dose oral steroids, azathioprine, cyclosporine or methotrexate. Alternatively for severe refractory disease, anti-TNF drugs such as infliximab, adalimumab and golimumab can be used. More recently, integrin antagonists such as vedolizumab and etrolizumab have shown some promising results. However, these drugs have significant systemic side effects that reduce their tolerability. Moreover, up to 40% of patients still exhibit non-response to therapy, and these treatment-refractory patients would require alternative therapeutic approaches. Frondanol is a widely available neutraceutical extract from the edible sea cucumber which has been shown to have potent anti-inflammatory (as well as anti-cancer) effects in rats, in particular in the colon and in humans. Therefore,

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we decided to investigate the anti-inflammatory effects of Frondanol in human IBD patients. Over the past 25 years, it is estimated that more than 3 million Frondanol capsules have been consumed on the human market with no reported side effects. An even larger amount has been consumed on the veterinary market without a single reported incident. LD50 for Frondanol has not be ascertained because no deaths were observed following the administration of large amounts to rats, to the point that no more could be given due to gastric distention (unpublished observations by our group). All patients in the proposed study will continue to receive standard therapy. If proven beneficial, Frondanol, will be a useful supplement in treating the underlying chronic gut inflammation in IBD patients, increasing the likelihood of patients remaining in remission and potentially providing an effective, natural and safe treatment for treatment naive patients in the future.

10.3 Costs and Payments

Participants will not receive study incentives (compensation) nor will they incur costs for participating in this study.

10.4 Safety Monitoring:

In the unlikely event that side effects occur, patients will be requested to immediately inform the study coordinator/research nurse, PI or consultant gastroenterologist. A direct phone number will be given to patients so that they are able to call the study coordinator or assigned nurse in case of any adverse events. Depending on the severity of the event, the participant will be instructed whether to report to the Gastroenterology unit at the study site or to head to the emergency department of one of the hospitals covered by the trial's liability insurance, for further investigation and possible treatment.

Simultaneously, the PI, assisted by the clinical gastroenterologists on the team, and taking into account any other medical information available, will perform a causality assessment to ascertain whether the adverse event was related to participation in the trial. If the adverse event is deemed to be related to participation in the trial, the participant will be advised to immediately stop taking the study medication (whether Frondanol or placebo) and will be provided with the necessary medical care until the issue is resolved.

In the unlikely event that the adverse event is deemed to be caused by Frondanol and if the assessment by the study team results in a doubt that further adverse events may occur with other participants, the trial will be immediately suspended, and all participants will be informed and required to stop taking their assigned study medication and await further instructions from the PI or trial coordinator.

Any serious or unexpected adverse event will be reported by the PI to all ethical committees that have approved this study, no later than 24 hours for

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life threatening events and no later than 10 working days for non-life-threatening events. Reports to the involved ethical committees will include advice from PI as to whether in her opinion (supported by the opinions of the study team's gastroenterologists):

- The adverse event was related to the protocol or the study drug
- The adverse event necessitates an amendment to the study protocol and/or patient information/consent form

The PI will be obliged to conform with the course of action that the relevant ethical committees have deemed necessary to occur. In the unlikely event that the ethical committees recommend termination of the trial, participants will be contacted immediately and asked to stop taking their assigned medication and return it to the study centre at their earliest convenience. The entire process will be documented and safely stored electronically on the devices of the PI and trial coordinator.

10.5 Conflicts of Interest:
The Investigators and the research do not have a financial or commitment conflict of interest in relation to this study. Completed disclosure forms will be held at MCH.

10.6 Clinical Study Insurance and Compensation to Subjects
In the unlikely event that side effects occur, patients will be requested to immediately inform the study coordinator/research nurse, PI or consultant gastroenterologist. A direct phone number will be given to patients so that they are able to call the study coordinator or assigned nurse in case of any adverse events. Depending on the severity of the event, the participant will be instructed whether to report to the Gastroenterology unit at the study site or to head to the emergency department of one of the hospitals covered by the trial's liability insurance, for further investigation and possible treatment.

Subjects will not be offered compensation for their participation in the trial.

10.7 Independent Research Ethics Committee
Prior to commencement of the study the study protocol will be submitted together with its associated documents to the responsible REC for its favourable opinion/approval. The written favourable opinion/approval of the REC will be filed in the Investigator Site File.
The study must not start before the obtaining written confirmation of favourable opinion/approval from the concerned REC.
Amendments to the study will also be submitted to the concerned REC before implementation in case of substantial changes. Relevant safety information will be submitted to the REC during the course of the study in accordance with national regulations and requirements.

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11. CLINICAL STUDY REPORT AND PUBLICATIONS

11.1 Study Report

After completion of the study, a study report according to good clinical practice will be written by the Investigator.

11.2 Publication and Dissemination of results

The principle of research is to obtain data and results to benefit society and, in this context, research may be published. Due to the anonymizing nature of data collection, participants will not be personally identified in any publication. Research data may be kept indefinitely as use of such data is sometimes required by other researchers and occasionally by editors of publications.

Principal Investigator Signature: _____ _28/06/2021 ___ Date: DD/MM/YY

Sponsor

Signature: _____ _28/06/2021____ Date: DD/MM/YY

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