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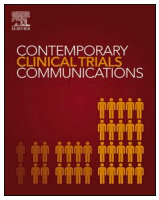

Study protocol for a pilot randomized, double-blind, placebo-controlled trial to investigate the anti-inflammatory effects of Frondanol in adults with inflammatory bowel disease

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD), consisting of Crohn's disease and ulcerative colitis, is a debilitating condition with a rising incidence globally over recent years. Frondanol, a widely available nutraceutical extract of the edible sea cucumber *Cucumaria frondosa* has been reported to possess potent anti-inflammatory effects, likely mediated by the inhibition of 5-lipoxygenase and 12-lipoxygenase pathways, whilst showing no signs of toxicity. The potent anti-inflammatory effects of Frondanol in a mouse model of IBD provide encouragement for investigating its effects in human IBD patients. Here we describe the study protocol of a pilot randomized, double-blinded, placebo-controlled trial of Frondanol in patients with mild to moderate IBD who are on standard therapy.

Material and methods: 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 stool samples will be obtained during routine visits at baseline, and after three months and six months of treatment, and tissue samples from colon biopsies will be obtained during clinically indicated colonoscopies at baseline and after six months of treatment. The levels of inflammatory markers will be compared in serum and tissue samples between patients treated with Frondanol and those treated with placebo, and findings will be correlated with clinical and histological parameters.

Discussion: If proven beneficial, treatment with Frondanol may increase the likelihood of patients remaining in remission and potentially provide an effective, natural and safe addition/alternative for treatment-naive patients in the future.

(Clinical trial registration number: NCT05194007).

1. Introduction

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 1800 people for Crohn's disease and one in 1000 people 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 mechanisms underlying the chronic gut inflammation are not fully understood, several cytokine networks are thought to be involved [4–6]. The conceptual framework for the pathogenesis of IBD is depicted in Fig. 1 [7]. Currently, treatment of IBD relies on minimizing symptoms and improving quality of life through the control of disease progression and complications; however,

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the drugs used for the treatment of IBD have significant systemic side effects that reduce their tolerability [8]. Moreover, up to 40% of patients are non-responsive to therapy, and these treatment-refractory patients require alternative therapeutic approaches [9,10]. Sea cucumbers and their extracts have been reported to have high nutritional value and several potential health benefits including anti-inflammatory effects and have been used for centuries as folk medicine or traditional foods in countries such as Japan, Indonesia, Korea and China [11–13]. Frondanol, a widely available nutraceutical extract of the edible sea cucumber, *Cucumaria frondosa*, has been reported to possess potent chemopreventive and anti-inflammatory effects in both animals and humans, whilst showing no signs of toxicity [14–17]. The potent anti-inflammatory effects of Frondanol in a mouse model of IBD provide encouragement for investigating its effects in human IBD patients [15]. 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.

2. Methods

2.1. Study site

Patients will be recruited from the gastroenterology units of two sites within the Mediclinic Middle East Hospitals network: Mediclinic City Hospital and Mediclinic Parkview Hospital in Dubai, United Arab Emirates (UAE). Data processing and analysis will be conducted at the research laboratories of the Mohammed Bin Rashid University of Medicine and Health Sciences – Biomedical Research Center (MBRU-BRC), Dubai, UAE.

2.2. Study aims

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

2.3. Study objectives

- To compare serum levels of anti-and pro-inflammatory markers between IBD patients treated with Frondanol and those treated with placebo at baseline, and at three and six months of treatment.

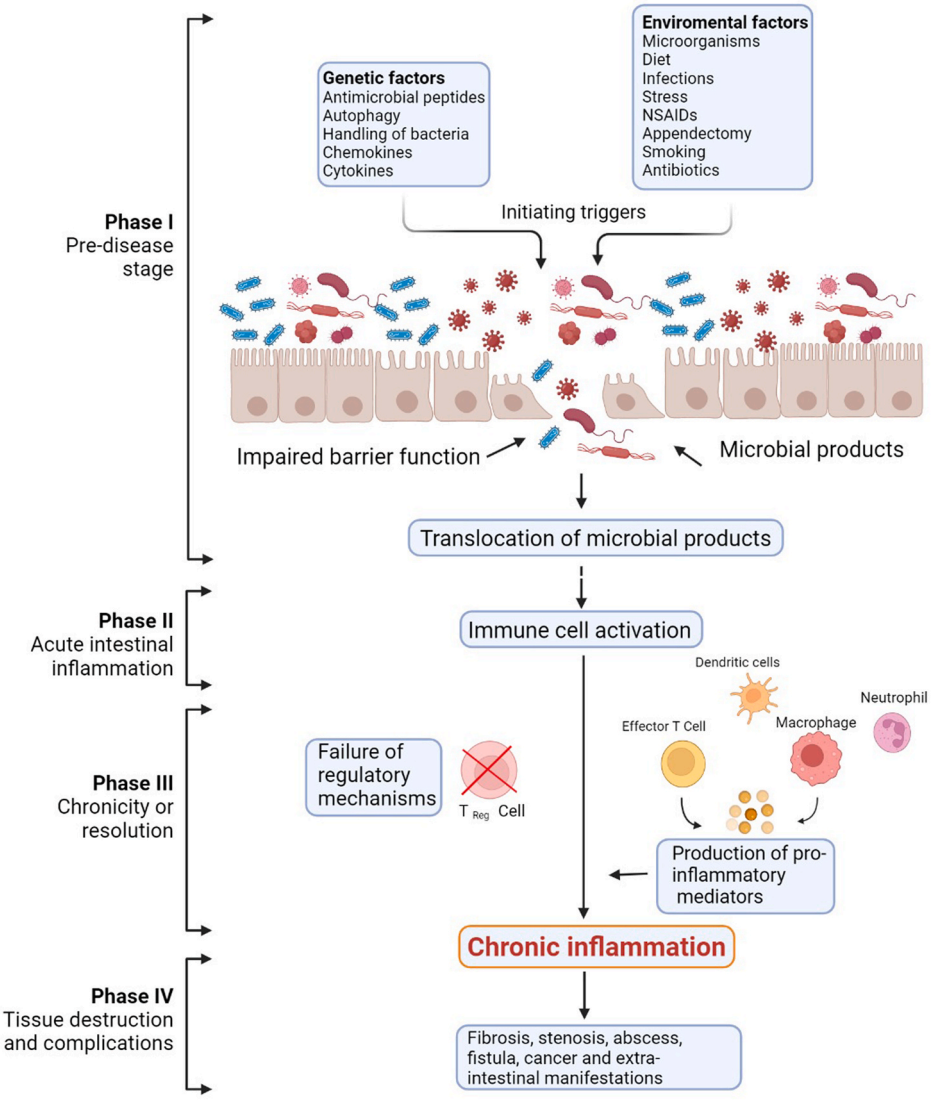

Fig. 1. Schematic diagram for the pathogenesis of IBD.
Adapted *from* Ref. [7].

- To compare colonic biopsy mRNA and protein expression levels of anti-and pro-inflammatory markers between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment.
- To compare routine clinical parameters between IBD patients treated with Frondanol and those treated with placebo at baseline, three and six months of treatment: patient symptoms, Disease Activity Index (also known as the Mayo Score), Inflammatory Bowel Disease Questionnaire (IBDQ), complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and albumin, fecal calprotectin, and faecal microbiota profile.

2.4. Study design

This is a pilot, prospective, double-blinded, two-arm, randomized controlled clinical trial to assess the efficacy of Frondanol in comparison to placebo in decreasing bowel inflammation in patients with stable mild to moderate IBD who are on standard of care treatment. Eligible patients with IBD who agree to participate in the trial will be randomized in a double-blinded fashion to one of two parallel arms to receive either one Frondanol 1000 mg capsule [14] or one placebo capsule twice daily for six months. Participants will be required to attend three visits with their consultant gastroenterologist, T0: baseline screening and randomization visit, T1: follow-up visit at three months, and T2: follow-up visit at six months. Routine blood and stool tests as well as extra blood and stool samples for research purposes will be obtained at these three time points (Fig. 2). Colonoscopies will be performed as part of routine clinical practice where appropriate at T0 and T2 only, and these include acquisition of routine biopsies as well as 2-4 additional biopsies for research purposes. This clinical trial protocol was designed following

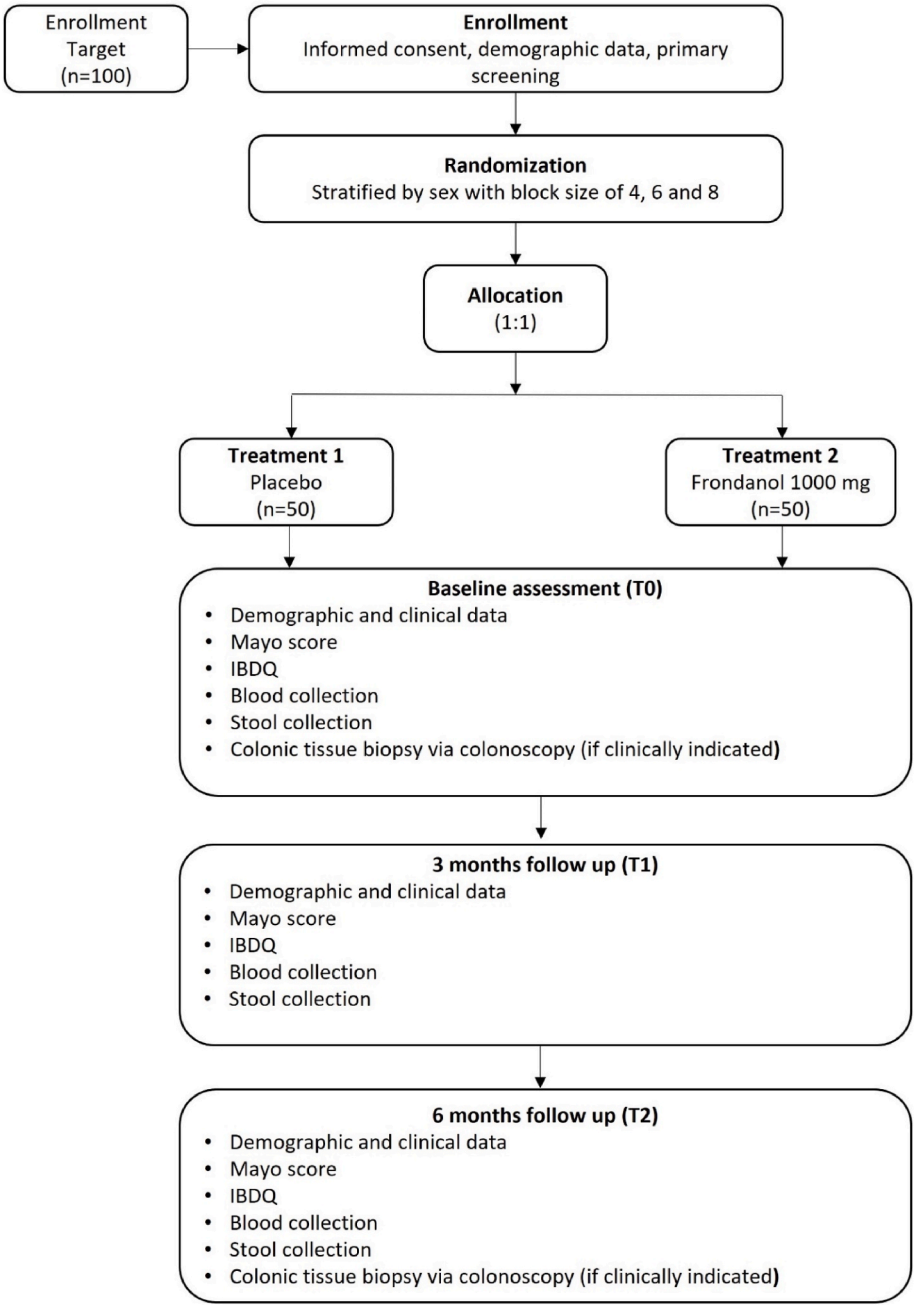

Fig. 2. Clinical trial study design
IBDQ = Inflammatory Bowel Disease Questionnaire.

International Council for Harmonisation (ICH)-Good Clinical Practices (GCP) guideline and has been prospectively registered at ClinicalTrials.Gov with registration no: NCT05194007.

2.5. Eligibility criteria

2.5.1. 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 diarrhea for more than four weeks, and evidence of active inflammation on endoscopy and chronic changes on biopsy. Patients with stable mild to moderate IBD (i.e., those who are not at risk of worsening disease to severe status and not requiring hospitalization) 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 [18]. 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). Crohn's colitis endoscopic severity will follow the standardized endoscopic Mayo scoring system used in Ulcerative colitis for consistency purposes.

2.5.2. Exclusion criteria

Pregnancy, breastfeeding, allergy to seafood or marine products or corn starch, severe medical illness such as uncontrolled diabetes (HbA1C $>$ 10), significant or unstable cardiovascular or pulmonary disease, impaired renal function (serum creatinine $>$2.0 mg/dL), current or recent ($<$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 [18], with active symptoms will not be eligible to participate in the study.

2.6. Informed 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 initiation of treatment. Participants will be requested to inform the principal investigator (PI)/study coordinator or the consultant gastroenterologist of any adverse events following the start of treatment. A direct phone number will be given to participants so that they are able to call the PI/study coordinator, or the post-doctoral fellow assigned to the trial in case of any adverse events. The PI/study coordinator, post-doctoral fellow, and coinvestigator gastroenterologists will have thorough understanding of the protocol and patient population. Retention of participants 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 Institutional Review Board (IRB)-approved protocol and with the guidelines of the Good Clinical Practices (GCP). Participants 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.

2.7. Trial drug and placebo

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. The strength (1000 mg) and dosage (one capsule twice daily) were chosen based on the manufacturer recommendation for the marketed Frondanol™ product, which were based on data from similar compounds originating from the same sea cucumber, and on consumer feedback of symptom control [14]. Frondanol capsules are supplied by Coastside Bio Resources, Stonington, ME, USA. The placebo hard gelatin capsules of matching size are supplied by ACG Associated Capsules Pvt. Ltd, Mumbai, India and contain food-grade corn starch, an inactive ingredient that is not expected to interact with the immune system. Patients will be instructed to take one capsule of their assigned medication twice daily. To promote adherence, participants will be sent periodic (monthly) reminder text messages or phone calls from the clinic to monitor compliance. Moreover, patients will be contacted one week before each follow up visit by the physician's nurse to remind them of their upcoming appointment. Patients will be informed of their clinical parameters at each visit and the final results after the trial data has been analyzed and unblinded.

2.8. Assignment of intervention

2.8.1. Randomization

Randomization to Frondanol/placebo will be performed to ensure that confounding variables will be equally distributed across both groups. Patients will be randomized (1:1) to receive either Frondanol or placebo using a randomization number sequence generator. Block randomization in blocks of 4, 6 and 8 patients will be used to ensure an equal number of patients in each treatment group (Sealed Envelope Ltd 2021. Simple randomization service) [19]. Stratified block randomization will be used to ensure comparability between genders in terms of Frondanol/placebo assignment. For this, two separate randomization sheets will be submitted for females and males by the study biostatistician and will only be shared with the PI/study coordinator.

2.8.2. Blinding

All patients and investigators, excluding the PI/study coordinator and study biostatistician, will be blinded to treatment allocation. Frondanol and placebo capsules will be placed in identical-looking bottles which will be labelled with the study name and number, and no patient-identifying information. The labelling of the bottle will be done with the open randomization sheet submitted by the PI/study coordinator. Patients will be instructed to take one capsule of their assigned medication twice daily. Hence, patients will receive their capsules in a bottle in a double-blind manner where patient and physician are not aware of treatment allocation as the treatment and placebo bottles will only indicate the pilot study name and the unique bottle number. Investigators involved in the laboratory analysis of samples will also be blinded to treatment allocation.

2.8.3. Recruitment

All eligible IBD patients visiting Gastroenterology Department of any of the study centers that will participate in patient recruitment (Mediclinic City Hospital and Mediclinic Parkview Hospital) under the care of any of the coinvestigator consultant gastroenterologists during the period of recruitment (24 months from the start date of the study) will be eligible to take part in the study. 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 PI/study coordinator) with the corresponding labelled medication bottle.

2.9. Outcomes

2.9.1. Primary outcome measures

- To assess the change in serum levels of pro-inflammatory (MPO, TNF-α, IFNγ, vascular endothelial growth factor [VEGF]-A, macrophage inflammatory protein [MIP]-2α, monocyte chemoattractant protein [MCP]-1, IFNα) and anti-inflammatory (IL-6, IL-17A, IL-22) cytokines between IBD patients treated with Frondanol and those

treated with placebo at baseline, after three months and after six months of treatment.

- To assess the change in serum levels of markers of inflammation leukotriene B4 [LTB4], 5-hydroxyeicosatetraenoic acid [5-HETE], and prostaglandin E2 [PGE2] between IBD patients treated with Frondanol and those treated with placebo at baseline, after three months and after six months of treatment.
- To assess the change in biopsy mRNA levels (using low density expression arrays) of the above-mentioned cytokines, inflammatory markers and transcription factors between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment.
- To assess the change in biopsy protein (using Luminex) expression levels of the above-mentioned cytokines, inflammatory markers and transcription factors between IBD patients treated with Frondanol and those treated with placebo at baseline and after six months of treatment.

2.9.2. Secondary outcome measures

- To assess the change in routine clinical parameters such as Mayo score, IBDQ, CBC, Frondanol and those treated with placebo at baseline, after three months and after six months of treatment.
- To evaluate potential adverse effects at each visit to evaluate for safety in this clinical cohort of IBD patients.
- To compare faecal microbiota profile between IBD patients treated with Frondanol and those treated with placebo at baseline, three and six months of treatment.

2.10. Study schedule

The participant schedule and timeline of the treatment are outlined in Table 1.

2.11. Sample size

The main objective of any pilot trial is to generate the primary data to design a definitive trial. The decision to recruit a total of 100 participants in the proposed trial was a pragmatic one based on statistical argument for sample size in pilot trials and the need to balance maximizing precision while minimizing the impacts of size on resources, time and cost [20,21]. Since the effect size of Frondanol compared to placebo in patients with IBD has not yet been reported in the literature, a time framed sampling has been implemented from February 2022 till the end date of the trial. It is estimated that in this pilot study 100 potentially eligible patients will attend the gastroenterology clinic at the two employed study sites, and that all patients will be approached to participate in this study. The literature indicates that for the pilot trial between 25 to 35 participants per group will generally provide sufficient power to conduct statistical analysis without being extravagant of resource [20–22].

2.12. Data collection, management and analysis

2.12.1. Data collection methods

Participants will give blood and stool samples at three time points, baseline (T0), 3 months (T1) and 6 months (T2). In addition to that, colonic tissue biopsy will be collected at baseline (T0) and 6 months (T2), if clinically indicated. All clinical and demographic data, Mayo score and IBDQ will be recorded at each visit. All data collection forms (DCFs) will be scanned, and copies saved electronically as well as in physical format in clinical research patient folders in study centers.

2.12.2. Blood sample collection

Minimal amount of blood (10 mL) will be collected from participants to minimize the burden of bleeding during each visit. Venepuncture will be done by a trained phlebotomist to ensure that distress during blood collection will be minimal and that correct procedures for collection,

Table 1
Study timeline from enrolment to end of the study.

	Study Timeline		
	Baseline/Allocation	Follow up	
Timepoint	T0 (Baseline)	T1 (3 months)	T2 (6 months)
Enrolment			
Eligibility criteria	X		
Informed consent	X		
Allocation	X		
Follow up visit		X	X
Treatment			
Frondanol group			
Placebo group			
Assessment			
Patient symptoms	X	X	X
Mayo score	X	X	X
IBDQ	X	X	X
Blood collection	X	X	X
Serum cytokines	X	X	X
Stool collection	X	X	X
Faecal microbiota	X	X	X
Tissue biopsy collection	X		X
Tissue mRNA (low density expression array)	X		X
Tissue protein (Luminex)	X		X
Routine clinical parameters			
CBC	X	X	X
ESR	X	X	X
CRP	X	X	X
Serum albumin	X	X	X
Faecal calprotectin	X	X	X

T0 = baseline visit, T1 = 3 months visit, T2 = 6 months visit, CBC = complete blood count, ESR = erythrocyte sedimentation rate, CRP=C-reactive protein, IBDQ=Inflammatory Bowel Disease Questionnaire.

processing and storage are followed. The blood will be centrifuged for the separation of serum and stored at −20 °C until further analysis.

2.12.3. Stool sample collection

At each visit, participants will be asked to provide a stool sample in a sterile stool pot (with small spoon inside) supplied by Pharm Land Medical Equipment, Dubai, UAE. The stool sample will be aliquoted and stored at −20 °C until further analysis.

2.12.4. Biopsy sample collection

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 placed in RNA*later* solution. The total mRNA and protein will be extracted from biopsy samples using PARIS™ kit as per standard protocol provided by the kit manufacturer and stored at −80 °C until further analysis [23].

2.13. Data management, confidentiality and access

2.13.1. Participant identification and privacy

A unique number will be assigned to each participant at inclusion, immediately after informed consent has been obtained. This number will serve as the participant's identifier in the study. The participant's data collected in the study will be stored under this number only. Only the PI at each site (and the PI/study coordinator in the event of an adverse event) will be able to link the participant's study data to the participant via an identification list kept with the PI at each site. Data protection and privacy regulations will be observed in capturing, forwarding, processing and storing participant data. Participants will be informed accordingly and will be requested to give their consent on data handling procedures in accordance with IRBs policies and guidelines.

2.13.2. Data handling and record-keeping

A data collection forms (DCFs) will be completed for each participant enrolled into the study. The investigator will review, approve and sign/date each completed 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 according to ICH-GCP standard for a period of 10 years, or until all regulatory and funder requirements are met.

2.13.3. 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, research ethics committee (REC) review, and regulatory inspections, providing direct access to source data.

2.13.4. Monitoring

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 Mediclinic Hospitals. Representatives of the relevant ethics committees will be permitted to inspect all study related documents and other materials at the site, including the ISF, completed DCFs and the participant's medical records/files.

2.14. Research governance

2.14.1. 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.

2.14.2. Benefits to participants

Currently, treatment of IBD aims to minimize symptoms, improve quality of life, prevent complications and modify the progression of the disease. 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 nutraceutical 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. Frondanol's anti-inflammatory properties may be attributed to its fatty acid constituents. For example, myristoleic acid, which is a potent 5-lipooxiginase (LOX) inhibitor [24], and astaxanthin, a carotenoid which has been shown to suppress gene expression of pro-inflammatory cytokines in dextran sulfate sodium-induced colitis mouse model [25]. Therefore, we decided to investigate the anti-inflammatory effects of Frondanol in human IBD patients. 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.

2.14.3. Costs and payments

Participants will not receive study incentives (compensation), nor will they incur costs for participating in this study. Patients existing insurance are expected to cover costs of routine clinical visits and testing.

2.14.4. Clinical study insurance and compensation to subjects

In the unlikely event that side effects occur, patients will be requested to immediately inform the PI/study coordinator or consultant gastroenterologist. A direct phone number will be given to patients so that they are able to call the PI/study coordinator or 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.

2.15. Safety considerations

The recommended dose of the marketed nutraceutical product of Frondanol is 1000 mg twice daily. Over the past 25 years more than three million Frondanol capsules have been consumed in the human market, with no reported side effects. An even larger amount has been consumed on the veterinary market without a single reported incident. 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 possible 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 [26]. 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. Simultaneously, the PI, assisted by the clinical gastroenterologists on the team, and considering 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 terminated, 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 h for 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 center at their earliest convenience. The entire process will be documented and safely stored electronically on the devices of the PI and trial coordinator.

2.16. Research ethics committee approval

The study protocol was submitted to together with its associated documents to the responsible REC for its favourable opinion/approval. The full ethical approval was granted by MBRU's Institutional Review Board (MBRU-IRB) on 09/12/21 (Approval ref: MBRU-IRB-2020-033), by Mediclinic Research Ethics Committee (MCME-REC) on 13/01/22 (Approval ref: MCME.CR.160.MCIT.2020) and by Dubai Scientific Research Ethics Committee (DSREC) on 23/12/21 (Approval ref: DSREC-12/2021_13). Approval was also received from the Ministry of Health & Prevention, UAE Government for the use of Frondanol in a human clinical trial in the UAE (Approval Date: 24/11/21, Approval ref: RCMOHP/CT1/0114/2021). The written favourable opinion/approval of the REC are filed in the ISF. 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.

2.17. 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 $\pm$ SEM and p values <0.05 will be considered statistically significant.

3. Discussion

Frondanol is a US-patented nutraceutical lipid extract of the intestine of the edible Atlantic Sea cucumber, *Cucumaria frondosa*, with potent anti-inflammatory effects. Despite its anecdotally reported benefits in humans, there have not been published research evaluating its efficacy and safety in humans. This is the first pilot randomized, placebo-controlled clinical trial to investigate the efficacy of Frondanol in reducing inflammatory markers in human patients with IBD. Frondanol has shown potent anti-inflammatory activity in the adjuvant arthritis rat model and ear edema mouse model when it was administered either orally or applied topically [14]. Moreover, Frondanol displayed potent inhibitory activity on both 5- LOX and 12-LOX pathways, suppressing the production of 12-HETE, 5-HETE, and LTB4 in human polymorphonuclear cells [14]. More recently, Frondanol was shown to markedly reduce inflammation in an ulcerative colitis rat model by reducing neutrophil and macrophage mRNA expression (F4/80 and MIP-2), and pro-inflammatory cytokine content (IL-1β, IL-6 and TNF-α) both at the protein and mRNA levels [15]. Although the anti-inflammatory activities of Frondanol in arthritis and IBD is well documented preclinically, to date the role of Frondanol in IBD human patient has not been explored. Therefore, in this study, we will explore the role of Frondanol supplementation in the patients with IBD as an adjuvant therapy together with their regular treatment. Results from this pilot clinical trial will provide novel, preliminary data on whether Frondanol will be a useful supplement in treating the underlying chronic gut inflammation in IBD patients and whether a large clinical trial will be warranted to investigate these effects further. If proven beneficial, treatment with Frondanol may increase the likelihood of patients remaining in remission and potentially provide an effective, natural and safe addition/alternative for treatment-naive patients in the future.

Author contributions

Hardik Ghelani: Methodology, Formal Analysis, Data Curation, Writing - Original Draft, Project administration. Thomas Adrian: Conceptualization, Resources, Methodology, Writing - Review & Editing. Aida Azar: Methodology, Data Curation, Writing - Review & Editing. Samuel Ho: Conceptualization, Resources, Methodology, Writing - Review & Editing. Jamil Akhras: Conceptualization, Resources, Methodology, Writing - Review & Editing. Reem Jan: Conceptualization, Resources, Data Curation, Methodology, Writing – Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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