**Clinical Trial Protocol**

**Version 1.0**

## **Title:** Efficacy of Fucoidan for eosinophilic oesophagitis: a phase 2 pilot study

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1. **BACKGROUND**

Eosinophilic oesophagitis (EoE) is a chronic, food triggered, inflammatory disease characterised by oesophageal dysfunction. The prevalence of EoE has increased in Australia consistent with other immune mediated allergy diseases. EoE is associated with elevated total and specific immunoglobulin E (IgE), largely to food and to aero-allergens, and is characterised with eosinophilic infiltration (when usually there is none in healthy individuals) to the oesophagus. Elevated IgE led to EoE initially being considered an atopic disease [1]. However, differential responses to therapeutic interventions have led to this classification of EoE as an atopic disease to be questioned [1]. More recently the oesophageal microbiome has been implicated in the inflammatory dysregulation of EoE [2]. The potential for the microbiome to influence local immune and inflammatory pathways in underlying tissues suggests that a range of complex immune mechanisms may underpin onset and development of EoE. Collectively this evidence suggests that mechanisms beyond traditional allergic pathways may underpin EoE and that additional treatment and management strategies are required.

Seaweeds comprise a diverse range of marine organisms containing biologically active metabolites that are being explored for their therapeutic effect(s) on a range of health conditions. Fucoidans are a group of high molecular weight, fucose-based, polysaccharides recognized as a key component of particular brown macroalgae species [1] that continue to receive attention for their potential bioactive properties. The bioactive properties of fucoidan preparations have been assessed in a range of *in vitro* and animal models [3] and include demonstrated anti-microbial [4], anti-viral [5] and anti-cancer [6] effects. Evidence from animal models also support the potential for fucoidans to possess immune-modulating effects [7, 8] and to modulate the composition of resident commensal microbial populations [9]. The ability of fucoidans to act as potential modulators of mucosal health generally, and mucosal immune function, are of particular interest considering the fucose-based structure of fucoidans, the role of fucose as a terminal sugar in human mucin glycoproteins [10], and evidence from *ex vivo* tissue preparations indicating fucose may regulate gut motility and secretory activity [11]. However, human clinical trials examining the impacts of fucoidans on immune markers and mucosal immune markers specifically are scarce and it is unclear how delivery of fucose via fucoidan supplementation may influence production and properties of mucins at mucosal surfaces.

The aim of this study is to examine the clinical effectiveness of fucoidan supplementation in alleviating symptoms of EoE. To better understand the local immune pathways and responsiveness to supplementation oesophageal biopsy samples will be used to characterise immune and inflammatory signalling and to assess microbiome composition at the mucosal oesophageal surface.

1. **RESEARCH PLAN**

## **2.1 Study Design**

This study is randomised, blinded placebo-controlled trial based on a modified phase two Simon Two-Stage design as previously utilised by the research team for atopy investigations [12]. Participants will be recruited in conjunction with specialist gastroenterologists at Gastro Care Gold Coast; only individuals with an endoscopy-confirmed diagnosis of EoE will be eligible for inclusion.

Following recruitment, participants will be asked to complete a series of symptom questionnaires and to attend an appointment with the Griffith University Research team to provide a blood and faecal sample. Patients will then undergo 12 weeks of fucoidan supplementation in conjunction with their routine treatment and complete a daily symptom and medication questionnaire. At the completion of supplementation patients will again be asked to complete a series of questionnaires and attend a follow-up appointment with the Griffith University Research team to provide a follow-up blood and faecal sample. Additional oesophageal biopsy specimens will be collected as part of routine clinical follow-up by clinicians at Gastro Care Gold Coast.

**Figure 1**



# **2.2 Population**

A total of 36 patients (male and female) aged between 18-60 years of age who have endoscopy-diagnosed EoE, will be recruited to the study. Patients will be treated with steroids and proton pump inhibitor (PPI) therapy for a minimum of 6 weeks as part of the routine clinical management and continue taking the study medication for a further 6 weeks. 24 participants will be in the active group and 12 participants will be in the placebo group. A further 12 participants without EoE will be recruited to participate as a healthy volunteer.

To be eligible for inclusion in the study participants will need to:

* Be aged 18-60 years;
* Have EoE diagnosed from oesophageal biopsy, based on eosinophil count greater than15 cells per high powered field;
* Be prescribed treatment with steroids and proton pump inhibitor (PPI) therapy for a minimum of 6 weeks as part of the routine clinical management and continue taking the study medication for a further 6 weeks.

Individuals will be excluded from participating if they:

* Report antibiotic, probiotic or symbiotic use in the previous 14 days;
* Report gastrointestinal or respiratory disease, autoimmune disease or other diseases, in particular Crohn’s Disease or Ulcerative Colitis;
* Return a finding of malignancy on biopsy results post gastroscopy procedure.
* Allergic, sensitive or intolerant to (one of) the ingredients of the study product

To be eligible to participate in the study as a healthy volunteer participants will need to:

* Be aged 18-60 years:
* Have no EoE/no abnormality detected from oesophageal biopsy, based on eosinophil count less than15 cells per high powered field.
1. **METHODS AND PROCEDURES**

**3.1 Patient recruitment**

Recruitment will be undertaken at the Gold Coast Gastro Care Clinic by Dr Ben Allen, Dr Aiden Lyon, Dr Alex Huelsen and Dr Alicia Braund. Patients presenting to the clinic with symptoms of EoE will be invited to participate in the study during an initial consultation prior to any gastroscopy procedure. Patients will be provided with the Patient Information and Consent Form and allowed time to consider their participation. Patients will be informed of the inclusion and exclusion criteria by their treating clinician and advised that their inclusion is based on pending pathology results after the biopsy sample is collected. Patients will be given the option of contacting the Griffith University Research team directly, or have the research team contact them to provide further information about what the study will involve and to answer any questions. If patients request the GU research team to contact them, they will then be provided with a ‘consent to share information’ form to allow Gold Coast Gastro Care clinicians to share patient contact details with the Griffith University Research team.

After signing the ‘consent to share information’ form, or directly making contact with the GU research team, patients with suspected EoE who have been identified by the clinicians at Gold Coast Gastro Care will be contacted by the Griffith University Research Team to discuss the requirements of the study further to ensure that the patient does not feel any pressure from their treating specialist to consent to participate.

After the gastroscopy procedure, patients that return a normal or ‘no abnormality detected’ finding from oesophageal biopsy may be invited to participate in the study as a healthy volunteer. Healthy Volunteers will not take any interventional medication, but will be asked to provide a blood and faecal sample at the start of the study only.

**3.2 Consent**

Consent will be documented by the participant’s dated signature on the Informed Consent Form. The trial will not commence until approval has been granted by the Griffith University and Ramsey Health Care Human Research Ethics Committee. This study will be conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines of good clinical practice, whichever represents the greater protection of the individual. The trial will be registered with the Australia New Zealand Clinical Trial Registry.

3.3 Intervention

Participants will be randomised to one of two treatment groups.

1. A formulation containing 100mg daily of 85% Maritech® (Marinova, Tasmania, Australia).
2. Placebo: same formulation as the fucoidan supplement but without the active ingredient

**3.4 Questionnaires**

Individuals will be asked to complete a series of questionnaires at various time points including:

* Dysphagia Symptom Questionnaire (DSQ) (14 days at screening and a further 14 days at weeks 11/12)
* Quality of Life questionnaires (Week 0, 6 and 12)
* 3-day food diary (baseline, Week 12)
* Symptom and medication diary (once per day during study from week 0 – week 12)

**3.5 Standardised medication regime**

Participants will be prescribed PPI/Steroid Puffer for a minimum of 6 weeks in accordance with standard clinical management and will be required to use the Fucoidan (or placebo) supplement concurrently for a period of 12 weeks (6 weeks of initial treatment plus an additional 6 weeks).

Study protocol:

|  |  |  |  |
| --- | --- | --- | --- |
| **Week** | **0** | **1-12 weeks** | **End** |
| Supplementation  | ✓ | ✓ | ✓ |
| Faecal, blood and biopsy sample | ✓ |  | ✓ |
| 3-day food diary | ✓ |  | ✓ |
| DSQ | ✓ |  |  |
| Symptom & Medication Diary | ✓ | ✓ | ✓ |

3.6 Sample Collection and Handling

**Blood:** Patients will provide a blood sample during an appointment with the Griffith University Research Team after receiving confirmation of EoE diagnosis. Blood will be collected via standard venepuncture into heparinised, serum and PAXgene tubes from an antecubital vein. Heparin and PAXgene tubes will be collected by the GU university team and processed in the MHIQ laboratories. Serum IgE and the clinical chemistry measures will be collected at the same time and sent to pathology for analysis. Peripheral blood mononuclear cells and whole blood will be stored at minus 80 for analysis should further funding become available.

**Faeces:** Participants will collect a faecal sample within their home. Participants will be provided with a collection kit that includes a collection container with a scoop in the lid. Participants will collect ~5g using the lid during a routine bowel motion. The diversity of bacterial species inhabiting the intestines will be determined by 16s rRNA sequencing. DNA will be extracted using a commercially available Stool Kit (Qiagen). The V4 region of the microbial 16S rRNA marker gene will be PCR amplified and sequenced using an Illumina MiSeq Personal Sequencer platform (Illumina). Sequence data will be processed using the Quantitative Insights into Microbial Ecology (QIIME) package. Analysis of microbial composition will include assessment of operational taxonomic units and beta-diversity distance metrics (using QIIME) with comparisons between time points.

**Oesophageal microbiome:** Participants who have consented to participate in the study prior to their endoscopy procedure, will also be asked to consent to having a microbiome sample collected by passing an endoscopy cytology sponge (Cook Medical, Bloomington, USA) 10 times back and forth in the following areas, 3cm proximal to the squamo-columnar junction, gastric cardia and mid-BE. Brush tips will be stored in sterile Eppendorf tubes at -80. DNA will be extracted using the Mo Bio DNeasy kit (Qiagen, USA) and bacterial DNA assessed by 16s rRNA sequencing as previously described above.

**Oesophageal biopsy:** During endoscopy, a total of eight biopsies will be collected. Four biopsies each will be collected from the mid and distal oesophagus. Biopsies will be sent to pathology for formalin fixation and paraffin embedding. Intra-epithelial eosinophils will be found in areas of great eosinophil density and reported as eos/hpf. Assessment for allergy inflammatory indices will be undertaken via digital spatial profiling with the GeoMx™ Digital Spatial Profiling Analyser using the Allergy Inflammation Panel (NanoString Technologies, Seattle, USA).

**DSQ:** The DSQ is a 3-question diary validated for the measurement of dysphagia frequency and severity in patients with EoE. DSQ scores are calculated on the basis of the responses to questions over a 7-14 day period.

**3.7 Laboratory Analysis and Sample Storage**

**Microbiome profiling:** Composition of the intestinal microbiota will be determined by 16S rRNA sequencing which is a well-established method for the phylogenetic identification of bacterial species. Faecal samples will be homogenised by repeated bead beating and DNA extracted using a commercially available Stool Mini Kit (Qiagen). The V3-V4 region of the microbial 16S rRNA marker gene will be PCR amplified and sequenced using an Illumina MiSeq Personal Sequencer platform (Illumina). Sequence data will be processed using the Quantitative Insights into Microbial Ecology (QIIME) package. Analysis of microbial composition will include assessment of operational taxonomic units and beta-diversity distance metrics (using QIIME) with comparisons between groups.

**Sample Storage:** All biological material collected for this study will be handled and processed in secure Menzies Health Institute Queensland Laboratories. Access to the research facility is for listed and inducted personnel only, access can be monitored through security systems. Biological specimens will be stored and monitored in lockable freezers in locations known to the research team members only.

1. **OUTCOMES**

**4.1 Primary Outcome**

Evidence supports the use of histologic scoring system and the DSQ as valid pediatric symptom measures for determining EoE disease activity [13]. Responders will be determined as patients who display:

* A reduction in oesophageal eosinophil count < 6 per high powered frame;
* Reduction in the DSQ >30%

#### 4.2 Secondary Outcomes

1. Oesophageal and faecal microbiome: metrics of faecal microbiome diversity and the abundance of key bacterial species;
2. Oesophageal biopsy immune gene expression profiling: the ratio of Th /Th2 inflammatory pathways and cells

**5. DATA RETENTION and ANALYSIS**

* 1. **Data Retention**

Respecting the valuable contribution of participants via the data which will be generated during the study and in accordance with the NHMRC Australian Code for the Responsible Conduct of Research (given the interventional nature of the study), data will be stored for a minimum of 15 years following completion of the study. All generated data (surveys and laboratory measures) will be stored in electronic files against coded IDs and accessible to key research team members only. The information from online surveys will be stored on a secure database hosted by the Griffith University Research Survey Tool Team. Participants clinical information will be collected and stored on the RedCap secure database and will be accessible by the Griffith University Research Team and the Gold Coast Gastro Care team only. Laboratory generated data will be stored as electronic files during and subsequent to the intervention period.

Future use of any generated data that is outside the scope of the proposed project will be subject to further human research ethics committee review.

**5.2 Data analysis**

Using the Simon two-stage design a sample size of 24 patients would provide 80% power and 95% confidence to exclude a non-interesting clinical rate of 30% to account for natural variability in symptoms for a meaningful clinical rate of 60%. Under these assumptions the treatment would be considered beneficial if >10 patients experienced a clinical benefit. A further 12 patients will be recruited to improve efficiency and reduce selection bias and allocated to a placebo arm in a 2:1 randomisation schedule. A total of 36 patients will be recruited (n=24 in the treatment arm; n=12 in the placebo arm).

This study is a non-comparative trial with repeated measures across time and with continuous and categorical outcome variables. For demographic and baseline characteristics, the categorical data will be tabulated by frequency and percentage. We will use an intention to treat analysis for DSQ score. For the primary outcome the proportion of patients in the treatment group meeting the criteria for a response in the DSQ and eos/hpf will be tabulated and compared to the criteria for determining treatment success. Faecal and oesophageal microbiome data, clinical chemistry, gene expression and pathology data changes will be analysed using a moderated within group t-statistic or Chi-Squared test (VAS). Adjustment for baseline values and clinical factors will be undertaken to improve the precision of estimation. Correlation analysis will be used to examine relationships between clinical and symptom scores with microbiome and inflammatory data. As a pilot study both adjusted and non-adjusted statistical tests will be considered with significance set at p<0.05.

1. **ETHICAL and REGULATORY CONSIDERATIONS**

Ethical approval for the study will be sought from the Ramsay Health Care Human Research Ethics Committee in the first instance. Subsequent approval will be sought from the Griffith University Human Research Ethics Committee. This study will be conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines of good clinical practice, whichever represents the greater protection of the individual.

Participants expressing an interest in the study will be provided with a Participant Information Sheet detailing what is involved for participants, including the purpose of the project, risks and benefits. Individuals wishing to participants will be required to provided informed consent prior to their participation. Consent will be documented by the participant’s dated signature on the Informed Consent Form.

The study will be registered with the Australia and New Zealand Clinical Trial registry (ANZCTR) prior to commencement. Approval from the Therapeutic Goods administration under the CTN scheme for use of the Fucoidan supplement in this trial is pending. The research team have previously obtained CTN approval (approval # CT-2019-CTN-00115-1) for prior trials utilising fucoidan supplements.

**7. RISKS and BENEFITS**

**7.1 Risks**

## For individuals attending the clinic for a blood collection, there is the risk of discomfort and bruising. A slight pinch may be experienced by participants as blood is being taken. The blood to be collected (approximately 45mL at each collection) is less than 1.5% total blood volume and therefore represents no hemodynamic risk to patients. Only suitably trained individuals will undertake collection of the blood samples.

## The faecal sample collection is undertaken at the participant’s house and can either be brought to the clinic during the appointment or couriered back to the Clinic.

Sensitivity to one or more of the product ingredients. During screening, participants will be asked if they have any allergies and will then be provided with a product information sheet and advised of the presence of artificial colours in the placebo intervention. As this is a blinded study, all participants will be notified as there is a chance of being randomised to the placebo group.

## Other rare but significant complications: 1. Perforation during upper endoscopy 2. Reaction to anaesthetic sedation given for upper endoscopy 3. Infection introduced through peripheral blood withdrawal site

## The research team will regularly discuss (at minimum weekly) progress of the study and any potential safety concerns should they arise.

**7.2 Benefits**

There is no specific benefit to individuals enrolled in the study. Fucoidan supplementation is not recognised as being a therapeutic intervention for EoE. The study will provide new knowledge on whether Fucoidan can modify oesophageal or faecal microbiome, oesophageal eosinophil count or clinical symptoms of EoE.

1. **SAFETY and MONITORING**

**8.1 Safety**

Investigators are responsible for monitoring the safety of participants in this study, and the Principal Researcher will be alerted to any event that appears unusual, even if the event seems to be of unanticipated benefit to the participant.

**8.2 Possible Side Effects**

Being a marine derived product there is some possibility of a mild “marine” after taste and belching, similar to that which is experienced by some individual who consume fish oil products, however this does not feature in published studies. It is also noted that fucoidan concentrate use as a food additive has been classified as “generally regarded as safe” (up to a daily dose of 250 mg/day) by the US Food and Drug Administration.

**8.3 Adverse Reactions (AR) / Adverse Events (AE)**

In accordance with the NHMRC statement on Safety Monitoring and Reporting for Clinical Trials involving Therapeutic Goods, an adverse reaction will be considered as any untoward or unintended response related to the administered supplement. The risk of adverse reactions in this trial in response to the supplement is considered very low. An adverse event will be considered as any untoward medical occurrence in a participant that does not necessarily have a causal relationship with the administered supplement.

Any serious AR/AE (life-threatening or requiring medication intervention) will be reported to the TGA and governing HRECs in accordance with reporting requirements and the participant will be immediately withdrawn from the trial. Any AR that are self-limiting in nature and do not require medical intervention, or other AE will be managed by the Chief Investigators or delegated research team member, and documented for periodic reporting to the HREC and TGA.

1. **PRIVACY and CONFIDENTIALITY**

All data collected during this study will be managed in accordance with Australian Code for the Responsible Conduct of Research. The information from online surveys will be stored on a secure database hosted by the Griffith University Research Survey Tool Team. Only the research team will have access to this information during the trial period. Upon closure of the trial surveys, the data will be exported to an electronic file for analysis. Survey responses will be maintained on a secure storage database hosted by the Griffith University Research Survey Tool team. Participants clinical information will be collected and stored on the RedCap secure database, and will be accessible by the Griffith University Research Team and the Gold Coast Gastro Care team only. Laboratory generated data will be stored as electronic files during and subsequent to the intervention period. This data will be accessible by key researchers only and information will only be stored against participant ID codes. All sample material will be identified only by coded ID and stored in a secure facility accessible to members of the research team.

We favour the generation and storage of electronic records and data files for this study. All generated data (surveys and laboratory measures) will be stored in electronic files against coded IDs and accessible to key research team members only. The file containing both participant details and coded IDs will be stored as a password protected file and will only be accessible by the Principal Researcher or nominated proxy. Access to survey response data via the Research Survey Tool requires a username and password while the trial is active. Access to participant’s clinical information via the RedCap online database requires a username and password while the trial is active. Upon cessation of the trail this data becomes inaccessible, except for access by specific Research Survey Tool/RedCap personnel for the purpose of statistical meta data. At the conclusion of the study, all data will be archived at Griffith University for minimum 15 years in accordance with national guidelines for clinical research studies.

Confidentiality of participants will be maintained. Individual participants will be provided with their own results only. Results from any specific individual will not be discussed with other participants in the study. Any group level and summary data relating to trial outcomes provided to participants will not contain participant-identifying information. It is anticipated that outcomes will be shared more broadly with scientific publication and presentation at appropriate scientific meetings. Any group level and summary data relating to trial outcomes disseminated in this way will not contain participant-identifying information.

1. **TIMEFRAME**

**Study approval:** June - July 2021

**Ethics submission:** July - August 2021

**Experimental protocol:** September 2021 - April 2022

**Sample and Statistical analysis:** May 2022 - September 2022

**Manuscript:** October 2022 – November 2022

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