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Efficacy and tolerability of a novel galactomannan-based formulation for symptomatic treatment of gastroesophageal reflux disease: a randomized, double-blind, placebo-controlled study

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Abstract. – OBJECTIVE: Proton Pump Inhibitors (PPIs) and traditional antacids are the common standard set of therapy for the management of gastroesophageal reflux disease (GERD) symptoms. The aim of the current study was to evaluate efficacy and safety of a novel galactomannan-based liquid formulation in reducing typical GERD symptoms in patients not taking PPIs.

PATIENTS AND METHODS: This was a single-center, randomized, double-blind, placebo-controlled study. Sixty patients met the eligibility criteria and were treated either with the investigational product (RefluG™) or placebo, one sachet three times per day for 14 consecutive days. Symptom intensity/frequency and quality of life were assessed over the course of the study by Reflux Disease Questionnaire (RDQ) and GERD-Health related Quality of life (HRQL) Questionnaire, respectively. The primary endpoint was to determine the number of subjects with at least 30% symptoms reduction from baseline to day 14 compared to placebo.

RESULTS: RefluG™ was statistically superior to placebo (p <0.001) as 100% of subjects experienced at least 30% symptoms reduction at the end of the study while none achieved a 30% reduction in the placebo group. For all domains both after 7 and 14 days of treatment, significant improvement in HRQL was seen in the active group in comparison to placebo. Tolerability and safety were good and comparable between groups.

CONCLUSIONS: The investigational product was safe and effective as mono-therapy in providing early resolution of troublesome GERD symptoms as well as for improving quality of life.

Key Words:
Galactomannan, GERD, Hyaluronic acid, Malva sylvestris, PPI, Calcium carbonate.

Introduction

Gastroesophageal reflux disease (GERD) is defined as the presence of recurrent troublesome symptoms induced by the reflux of the stomach contents into the esophagus. The Montreal definition and classification of GERD, on the basis of population-based studies, states that mild symptoms occurring 2 or more days a week or moderate/severe symptoms occurring more than 1 day a week are considered "troublesome" by patients[1]. Both typical esophageal symptoms such as heartburn and regurgitation and extra-esophageal symptoms (e.g., chronic cough, laryngitis, asthma and dental erosion) may impair the patient's quality of life independently of the presence of esophageal lesions[2]. At present, although a symptomatic response to acid suppression therapy with proton pump inhibitor (PPI) drugs can be achieved in 70-80% of patients with erosive GERD, this benefit is reduced by 20–30% in case of non-erosive reflux disease (NERD) probably because of a different pathophysiological pathway[3]. Furthermore, placebo-controlled trials including GERD patients treated with PPI, revealed that the therapeutic gain over placebo was around 17% for regurgitation and >20% less than that observed for heartburn[4].

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The relevance of GERD is witnessed by the fact that, with its estimated worldwide prevalence of 14.8%[5], it is one of the most common gastrointestinal (GI) abnormalities as well as one of the leading reasons for consultations encountered in both general practice and gastroenterological setting. Similar epidemiological findings have been reported in both Western and Asian Countries. As pointed out by the position statement of the Indian Society of Gastroenterology, the prevalence of GERD is almost 10% in the Indian rural and urban population, showing an incidence comparable to that reported by the Western countries while higher than that of other Asian countries[6].

Over the past two decades, the clinical relevance of low-grade esophagitis, i.e., Los Angeles (LA) classification grade A and grade B[7], has been de-emphasized because of the low likelihood of progression and the chance for spontaneous healing. This may be attributable to a different pathophysiology compared to LA grade C and grade D esophagitis[8]. In a recently published study conducted at Mayo Clinic Rochester, United States (US), none of the patients with LA grade A or grade B esophagitis progressed to more severe grades during a follow-up of 44 months. Furthermore, long-term healing of esophagitis occurred in the majority of patients despite discontinuation of PPI therapy. Hence, the authors concluded that a more conservative long-term PPI strategy should be considered in patients with low-grade esophagitis[9]. In accordance with these findings, the Lyon Consensus did not include LA grade A esophagitis as conclusive criteria for GERD[10].

GERD manifestations are the result of an imbalance between aggressive and protective factors of the esophageal mucosa. The disruption of the physiological mucosal barrier consisting of basal, intermediate and superficial cells layers, of the mucus that covers the luminal side of the mucosa, of the thickness of the internal lamina and the mucins contained in the intercellular spaces, is at the basis of GERD pathogenesis. When this mechanism of defense against refluxed materials is disrupted, structural and functional damages to the epithelium may occur depending on the frequency of the reflux episodes, the quantity and composition of the refluxed contents and the contact time with the esophageal mucosa[11]. Several studies[11,12] have shown that not only acid reflux but also non-acidic components such as pepsin, trypsin and bile acids, have a crucial role in contributing to histopathological alterations and triggering symptoms in patients with GERD. Among the mechanisms involved in the pathogenesis of GERD, transient lower esophageal sphincter (LES) relaxation which allows gastric contents to flow back up into the esophagus plays a well-established role[2]. All these factors may explain why routine treatments with antacids or PPI can fail to control endoscopic or symptomatic GERD. Considering the postulated pathways of GERD pathogenesis, hindering the passage of gastric contents into the esophagus and strengthening the esophageal mucosal resistance should be considered as the two main targets of treatment. However, the knowledge in this field, especially in patients with low-grade esophagitis, is limited and the optimal treatment remains debatable.

The aim of the current trial was to assess the efficacy and safety of a novel liquid formulation as mono-therapy in relieving symptoms in GERD patients. The investigated product contains a standardized complex of galactomannans that rapidly forms a physical barrier hindering reflux of gastric contents into the esophagus, calcium carbonate and sodium bicarbonate with antacid action, *Malva sylvestris* and hyaluronic acid to soothe and protect the mucosa, respectively.

Patients and Methods

This randomized, double-blind, placebo-controlled study was performed at Rajalakshmi Hospital in Bangalore (India). Sixty adult subjects with GERD symptoms, in accordance with the Montreal definition[1], were enrolled after signing the informed consent form. Patients with documented LA grade B, C or D esophagitis on endoscopy were excluded. Inclusion and exclusion criteria are shown in Tables I and II.

Placebo and investigational product evaluated in this study were supplied by Giellepi S.p.A. Health Science (Lissone, MB, Italy). The active formulation (RefluG™, medical device class IIa) and placebo were packaged in identical ready-to-take sachets containing a single dose of liquid solution (10 ml) for oral administration.

After the initial screening, subjects were randomly assigned to each arm equally using a blocked randomization method. The randomization list was computer-generated using the SAS 9.4 software. The study investigator, who was responsible for the enrolment of the subjects, was blinded to the treatment and assigned the test medication to the participants. All subjects were instructed to take one sachet of the investigation-

Table I. Inclusion criteria.

Diagnosis of GERD according to Montreal Consensus.
Greater than 18 years of age.
Able to give written informed consent.
Ability to follow a controlled diet (coffee and tea limited to not more than 2 cups per day; chocolate, alcoholic beverages and spices reduced as much as possible).

al product or placebo three times per day (after breakfast, lunch and dinner or at bedtime) for 14 days from the day of randomization.

The primary outcome was to determine the efficacy of the investigational product compared to placebo in inducing 30% symptoms remission from baseline to day 14. Efficacy data were obtained from the frequency and intensity/severity of patient's symptoms based on the Reflux Disease Questionnaire (RDQ, a 12-items questionnaire), Visual Analogue Scale (VAS, a 0-10 point scale), Health-Related Quality of Life (HRQL) according to the Short Form 36 (SF36) questionnaire, questionnaire for individual symptom, frequency scale for the symptoms of GERD (FSSG) questionnaire, and heartburn severity index (HSI, sum of all individual episodes of daytime and night-time heartburn per severity score per episode). Data collected at the screening (visit 1) and baseline (visit 2) were compared between groups after 7 days (visit 3) and 14 days of treatment (visit 4). GERD-related symptoms (heartburn, acid regurgitation, chest pain, dysphagia and acid taste in the mouth) were rated by patients on a 5-point Likert scale as follows: 0 = no symptoms, 1 = slight symptoms, 2 = moderate symptoms, 3 = severe symptoms, and 4 = very severe symptoms.

Secondary outcomes were the following:

- safety of the investigational product, assessed by adverse events (AEs) reporting;
- number of days required to achieve the first 24 hours without heartburn;
- change in symptom frequency and intensity using RDQ from baseline to day 7 and day 14, and from day 7 to day 14, as well as in between the groups;
- change in the HSI from baseline to day 7 and day 14, and from day 7 to day 14 and also in between the groups;
- change in FSSG from baseline to day 7 and day 14, and from day 7 to day 14, and also in between the groups;

Table II. Exclusion criteria.

Inability or unwillingness to return for study visits.
Patients with Los Angeles grade B-D esophagitis.
Diagnosis of Helicobacter pylori infection.
Presence of other significant gastrointestinal morbidities (including Barrett esophagus, esophageal stricture, pyloric stenosis, gastric and duodenal ulcers, infections or inflammatory conditions of the small or large intestine, irritable bowel syndrome, obstructions).
History of gastrointestinal surgery or malabsorption.
History of GERD refractory to 2 months of therapy with either an H2RA or a PPI drug.
Presence of extra-gastrointestinal comorbidities, such as diabetes, metabolic disease, atopy, scleroderma, thyroidal disease, severe cardiovascular, renal, hepatic, pulmonary, or mental disorders, malignancy, HIV infection or any other immuno-compromised condition.
Pregnant, lactating women.
History of alcohol or drug abuse within the past 5 years.
Presence of any condition which, in the opinion of the Investigators, may interfere with nutrient absorption, distribution, metabolism and excretion.
Deteriorating health status at the time of enrolment, rapid weight loss, terminal disease.
Currently participating in or has participated in another clinical trial in the preceding 3 months prior to the beginning of this study.
AST or ALT values $\geq$ 2.5 X ULN.
Serum creatinine $\geq$ 1.5 mg/dL.
Subject unwilling or unable to comply with the study procedures.
History of allergy to any component of the study product.

GERD: gastroesophageal reflux disease; H2RA: Histamine 2-receptor antagonist; PPI: proton-pump inhibitor; HIV: human immunodeficiency virus; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ULN: Upper Limit of Normal.

- change in GERD symptoms assessed by VAS from baseline to day 7 and day 14, and from day 7 to day 14, and also in between the groups;
- improvement in HRQL according to HRQL-SF36 at the end of treatment.

At baseline, a case report form (CRF) containing data such as date of birth, gender, ethnicity, weight, height, smoking and drinking habits was filled by investigator. Patients were told to refrain from using rescue medications (antacids) unless symptoms were severe and intolerable, and the number of tablets consumed daily was recorded. Eligible patients maintained a daily diary of symptoms during the 2-week study period and received the above-reported questionnaires. The number of administered study products, daily presence and severity of symptoms, time elapsed before onset of action, and any consumption of rescue medication were recorded. At each visit, filled questionnaires were collected and new copies of the same were dispensed to patients. Any AE that occurred during the study was recorded in the subject's CRF. The safety population included all randomized patients who had taken the study product at least once. Finally, patients' compliance was recorded at each visit by counting the remaining sachets in the provided container.

After the study period, each patient was clinically re-evaluated and PPI drugs were added, if necessary, based on investigator's assessment.

Ethical Information

The study was conducted according to the Good Clinical Practice guidelines, as issued by the International Conference on Harmonization (ICH/135/95, July 2002) guidelines, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and International Organization for Standardization (ISO) 14155 regarding the clinical investigation of medical devices carried out in human subjects.

The study protocol and informed consent were approved, prior to study initiation, by the Rajalakshmi Hospital Institutional Ethics Committee, Bangalore, India (approval code RH/IEC/AP-047/2020).

The trial was registered in the Clinical Trials Registry of India with the following number: CTRI/2020/03/023910.

Investigators responsible for study administration, data collection, intervention allocation and data analysis were blinded to treatment conditions until the final collection of all participant data.

Statistical Analysis

The primary outcome was calculated by collecting and computing the intensity/severity data of each patient's symptoms at the final visit and by comparing them with the baseline values. Continuous variables were reported as mean ± standard deviation (SD), median and interquartile [IQR] range depending on data distribution. Comparison of continuous variables between independent groups was performed using the Analyses of Variance (ANOVA) or the Mann-Whitney test. Comparison of paired measurements was carried out using the Student's t-test for paired measurements or the Wilcoxon test, depending on the shape of the distribution. Fisher's exact test was used for dichotomous variables. For all analyses, statistical significance was set at $p<0.05$. All statistical analyses were performed using R Statistical Software version 4.3.0.

To determine the sample size, type I error and power were considered 0.05 (two-tailed z-test of proportion) and 80%, respectively. Sample size calculation was based on the primary outcome. The latter was calculated by collecting and computing the proportion of subjects in each treatment group (subjects treated with the investigational product *vs.* placebo) with a symptom reduction of more than 30% at the final visit compared with the baseline values. For each arm, final sample size consisted of 27 patients while 3 additional subjects (10%) were considered to cover possible drop outs.

Results

A total of 71 subjects were screened, of whom 60 were enrolled and randomized into two groups: 30 patients were treated with the investigational product and 30 patients with placebo. Clinical and demographic features of patients included in the two trial arms were comparable (Table III). Among the enrolled patients, 57 completed the study while 3 were lost at follow-up, one from the placebo group and two from the active group. Treatment compliance ≥ 80% (range 92.8-100) was reported by all participants.

Primary outcome

Subjects on active treatment showed a statistically significant benefit in symptoms remission compared to baseline as well as to placebo (Figure 1). On the contrary, although 10 out of 29 patients on placebo experienced symptoms relief, none achieved a 30% remission at the end of the study

Table III. Baseline characteristics of the 60 enrolled patients.

	Test product	Placebo
Number of patients	30	30
Age (years), mean (SD)	51 (9.18)	46.4 (11.63)
Gender, M/F	21/9	26/4
BMI (kg/m^2), mean (SD)	26 (3.6)	27.7 (4.7)
RDQ at baseline (median IQR)	36 (34-36)	34 (32-36)
VAS at baseline (median IQR)	10 (8-10)	5 (5-6)
Heartburn severity index at baseline (median IQR)	53 (52-54)	9 (9-10)
FSSG at baseline (median IQR)	5 (5-6)	53 (52-56)

SD: standard deviation; M: male; F: female; BMI: Body mass index; RDQ: Reflux disease questionnaire; IQR: interquartile; VAS: Visual analogue scale; FSSG: frequency scale for the symptoms of GERD questionnaire.

(Figure 2). In detail, considering the RDQ based on presence of symptoms, all subjects on active group *versus* none in the placebo arm achieved 30% remission from baseline to visit 3 (p <0.001). This significant difference was maintained over the 2-weeks period of treatment, with 28 patients in the active group *versus* none in the placebo treatment achieving 30% symptoms remission (p <0.001) at visit 4 (Figure 3).

Secondary outcomes

Symptom frequency and intensity assessed by RDQ were progressively improved in patients treated with the investigational product from a median RDQ scores of 36 (IQR: 34-36), 4 (IQR: 3-6) and 0 (IQR: 0-0), at day 0 (baseline), day 7 and day 14, respectively (visit 2 *vs.* visit 3: p<0.001; visit 2 *vs.* visit 4: p<0.001; visit 3 *vs.* visit 4: p<0.001). Symptom frequency and intensity remained unchanged, 34 (IQR: 32-36) from the beginning to the end of treatment in the placebo arm.

In respect to pain assessment by VAS scale, 100% of 28 subjects in the intervention group showed a significant improvement in pain both after 7 days and at the end of treatment. On the contrary, out of 29 subjects in the placebo group, a reduction in pain was observed in 5 patients only at the final visit. Median VAS score for patients treated with the investigational product decreased over time, from 10 (IQR: 8-10) at day 0, to 1 (IQR: 1-1) at day 7 (p<0.001) and 0 (IQR: 0-0) at day 14 (visit 2 *vs.* visit 4: p<0.001; visit 3 *vs.* visit 4: p<0.001). On the other hand, the median VAS score remained unchanged (value: 9) for patients in the placebo arm (IQR: 9-10).

HSI significantly decreased in patients treated with the investigational product from 5 (IQR: 5-6) at day 0 (baseline) to 1 (IQR: 0-2) at day 7 and 0 (IQR: 0-0) at day 14 (p<0.001). On the contrary, in subjects in the placebo arm, HSI tended to increase from 5 (IQR: 5-6) at day 0 to 6 (IQR: 5-6) both at day 7 and at the end of the study (p>0.05).

As far as changes in FSSG were concerned, the median FSSG scores for patients treated with the investigational product at day 0 (baseline), day 7 and day 14 were 53 (IQR: 52-54), 24 (IQR: 24-28) and 14 (IQR: 13-15), respectively (visit 2 *vs.* visit 3: p<0.001; visit 2 *vs.* visit 4: p<0.001; visit 3 *vs.* visit 4: p=0.001). Among patients in the placebo arm, the median FSSG scores at day 0, day 7 and day 14 were 53 (IQR: 52-56), 54 (IQR: 53-57) and 54 (IQR:53-57), respectively (p was non-significant for all comparisons).

GERD-HRQL-SF36 for the assessment of quality of life (Table IV), showed that all items were ameliorated significantly over time in patients under active treatment while those in the placebo arm experienced only a significant general health perceptions improvement. When patients on active treatment *vs.* those on placebo were compared, the former achieved significant ameliorations in all parameters considered (Figure 4).

Overall, the treatment compliance to the investigational product administration ranged from 97.6% to 100% (mean ± SD: 99.31 ± 1.283) while in the placebo arm it ranged from 92.8% to 100% (SD: 95.37 ± 4.053).

There were no serious AEs reported throughout the study period. Seven participants, all treated with the investigational product, reported mild AEs such as headache and nausea on visit 4.

Discussion

A large American population-based survey found that despite PPI use, over 55% of subjects with GERD reported persistent symptoms and

Table IV. Assessment of health-related quality of life according to SF36 questionnaire (median score) at baseline and at end of treatment.

	Test Product		*p*-value	Placebo		*p*-value
	Day 0	Day 14		Day 0	Day 14	
Physical functioning	0 95% CI = 0-0	100 95% CI = 100-100	<0.001	50 95% CI = 30-54.2	50 95% CI = 30-50	0.63
Role limitations due to health	0 95% CI = 0-0	100 95% CI = 100-100	<0.001	0 95% CI = 0-95.8	0 95% CI = 0-100	ç
Reduction of 50% in physical pain	22 95% CI = 12-31	74 95% CI = 74-74	<0.001	30 95% CI = 30-30	30 95% CI = 30-30	ç
General health perception	15 95% CI = 10-25	100 95% CI = 90-100	<0.001	30 95% CI = 30-35	35 95% CI = 30-41.7	0.06
Vitality	20 95% CI = 15-25	85 95% CI = 85-90	<0.001	40 95% CI = 35-44.2	40 95% CI = 30.8- 5	0.85
Social role functioning	0 95% CI = 0-12	88 95% CI = 88-100	<0.001	50 95% CI = 37-50	50 95% CI = 37-50	0.84
Emotional role functioning	0 95% CI = 0-0	100 95% CI = 100-100	<0.001	0 95% CI = 0-94.2	0 95% CI = 0-100	ç
Mental health	40	60	0.005	48	40	0.03

SF: Short Form; CI: confidence interval; ç: inestimable *p* due to similar data.

consequently detrimental effects on their quality of life[13]. In 2006, Shaheen et al[14] revealed that in the US, cost of GERD management accounted for US$ 9-10 billion/year, in part linked to the overutilization of PPI drugs and the prescription of diagnostic procedures. Interestingly, as a consequence of GERD symptoms on quality of life, it has been reported that US patients are willing

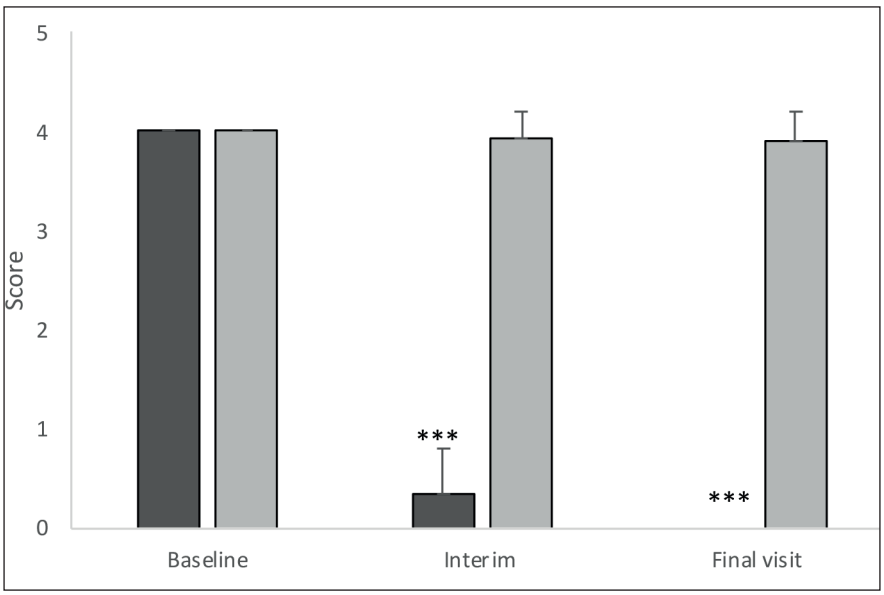

Figure 1. Reflux disease questionnaire (RDQ) score. Bar chart for comparison of intensity of heartburn at baseline (day 0), interim (day 7) and final (day 14) visit (***p <0.001 *vs.* baseline e p <0.001 *vs.* placebo). Dark grey: active; Light grey: placebo.

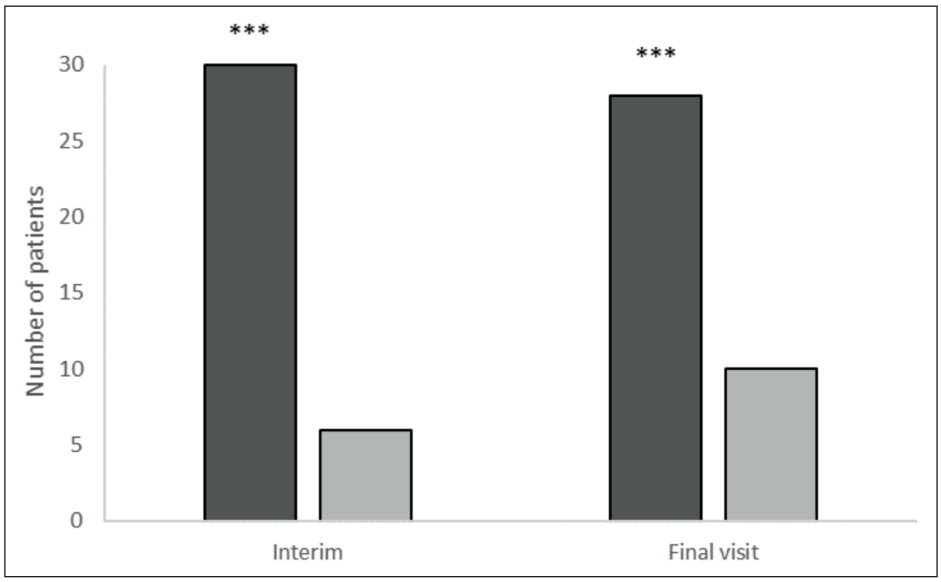

Figure 2. Number of patients who experienced symptoms reduction after one week and at the end of treatment (day 14). ***p <0.001 active *vs* placebo. Dark grey: active; Light grey: placebo.

to pay above their insurance prescription co-payments, for a medication that provides more complete and faster relief from common symptoms[15]. Furthermore, it has been demonstrated that up to 81% of patients with symptomatic GERD have unrecognized sleep disturbances with a severe impairment of quality of life[16].

An ideal therapy for symptomatic GERD patients, should have a multifaceted action, including forming a physical barrier hindering reflux of gastric content into the esophagus, mechanical protection against the residual aggressive components of the refluxate (i.e., weakly acidic and non-acidic gastric juice contents) as well as neutralizing action against hyperacidity. Although a number of products are currently available for improving symptom control and potentially combinable with PPIs, none is able to act simultaneously at all these levels. In fact, certain compounds act exclusively on mucosal protection[17], others offer an antacid effect[18], while hyaluronic acid and chondroitin sulphate-based formulations are able to protect from non-acidic reflux contents[19, 20] but are not designed to produce a raft for reducing reflux episodes. The novel oral formulation under investigation in this study has been registered in Europe as a class IIa medical device, since its mode of action is physical and does not depend on absorption into the systemic circulation. Its peculiarity is the presence of a standardized complex of galactomannan (fenugreek fiber) from *Trigonella foenum-graecum,* a

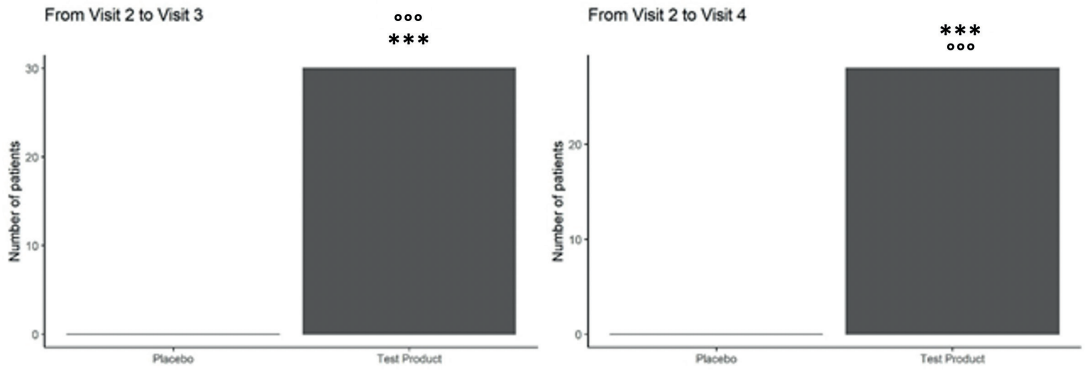

Figure 3. Number of patients with 30% symptoms reduction (***p <0.001 *vs.* baseline; °°°p <0.001 *vs.* placebo).

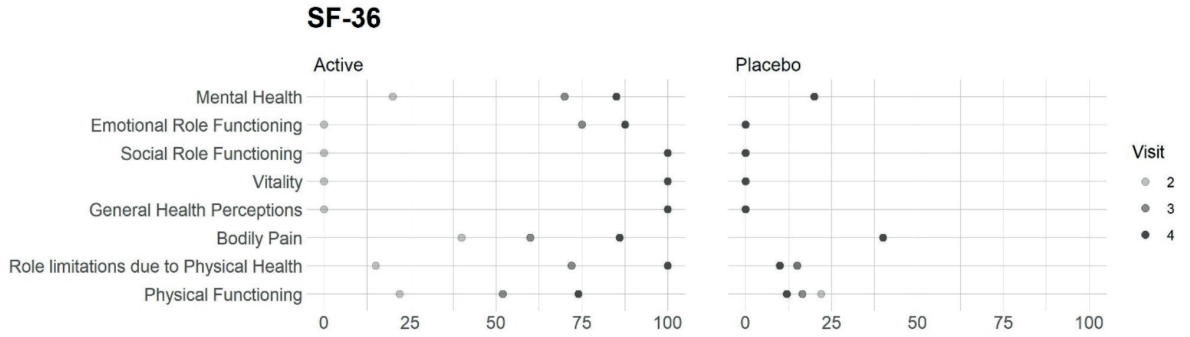

Figure 4. Dot plot comparing the median SF-36 scores at visit 2, at visit 3 and at visit 4 in the active and the placebo group. On the x-axis SF-36 scores ranging from 1 to 100 are reported. SF: Short Form.

high molecular weight polysaccharide composed of a long-chain mannose backbone with galactose molecules attached. Because of its unique molecular structure, fenugreek galactomannan resists enzymatic degradation in the stomach and binds the gastric content forming quickly a thick gel in the form of raft, which acts as a physical barrier against reflux into the esophagus. A two-week placebo-controlled, randomized clinical trial showed that fenugreek fiber was effective as the over-the-counter antacid ranitidine 75 mg/twice a day and significantly more effective than placebo in diminishing heartburn severity[21]. Other functional ingredients incorporated in the active formulation include calcium carbonate and sodium bicarbonate to allow the formation of carbon dioxide bubbles required to elevate the raft as well as to neutralize gastric acids. Hyaluronic acid, an anionic glycosaminoglycan that consists of a linear chain of D-glucuronic acid and N-acetyl-glucosamine fragments linked via glycosidic bonds is widely distributed throughout the extracellular matrix of connective, epithelial and neural tissues, where it is involved in several key processes such as control of epithelial cell turnover, acceleration of re-epithelialization and mucosal hydration in ulcer healing[19]. Hyaluronic acid's hygroscopic nature enables it to form a scaffold with binding sites for sulphur proteoglycans. Such structures can attain a large size and trap large quantities of water and ions, providing hydration and tissue distension[22]. Due to these effects, hyaluronic acid-based hydrogels are used as scaffolds to support tissue integrity and ensure proper barrier function. Finally, the addition of *Malva sylvestris* has a soothing effect on the irritated mucosal tissue[23]. Similarly, alginates and the investigational product act primarily as a raft-forming oral suspension providing a physical barrier[24]. However, the product under investigation is a galactomannan-based medical device and does not contain alginates from brown seaweed, thus avoiding the contraindication due to the higher sodium content of alginate-based products, in particular in those patients following a low-sodium diet[25].

In GERD, rapid onset of action is important for providing immediate relief from typical symptoms, especially after meals.

Laboratory tests (unpublished data) performed according to Hampson et al[24] showed that the investigational product acts very quickly, forming a complete raft within 2 minutes after dispensing the product in an acidic solution (100 ml HCl 0.1N) simulating the gastric content. In our clinical study, patients reported to feel better after taking the investigational product within 15 minutes at day 1 and within 5 minutes at day 14, thus confirming its ability to form a raft barrier quickly. Considering the detrimental effect of GERD symptoms on sleep disorders[26] the fast action of the combination taken before bedtime may be of particular relevance for improving patients' quality of life. Moreover, the investigational product provided complete relief from heartburn symptoms in all subjects at the end of the study with a median time of 6.4 days for experiencing a 24-hour heartburn-free.

In this single center, randomized, double-blind, placebo-controlled clinical study, 60 patients with symptomatic GERD were treated either with the investigational product or placebo. The results showed that all patients in the active group *vs.* none in the placebo arm achieved a 30% symptoms reduction from baseline to the end of treatment. Thus, the overall efficacy of the investigational product was statistically significant compared to the placebo, which did not induce any

measurable benefits. GERD may be associated with many symptoms with the most common ones being heartburn and regurgitation. In particular, in our study the heartburn frequency and severity scores evaluated by HSI were significantly decreased after treatment with the investigational product compared to placebo both at day 7 and day 14 supporting the effectiveness of the mechanism of action, which halts the reflux of acid contents, enhances the esophageal barrier and protects the epithelium from gastric contents.

In the past, the evaluation of the impact of GERD on patient's daily life has relied heavily on scales assessing symptom severity, such as heartburn, regurgitation, or pain, together with the endoscopic appearance of the esophageal mucosa. Nowadays, the assessment of HRQL has become a key focus in clinical research as a relevant tool for supporting health professionals' decisions. As confirmed by baseline measurements, in our study subjects suffering from GERD experienced a reduction in their HRQL driven by symptoms severity. The functional effect of the investigated therapy in this study was clearly seen already after one-week treatment as all the domains and items that measure HRQL were significantly improved compared to the placebo treatment.

Our study has several limitations. Firstly, the RDQ questionnaire was not validated in Indian language but applied in English language, with the study Investigators who translated the contents to the patients and filled the details based on patient's dairy and responses. This might have caused a potential assessment bias. Secondly, there was not a group of patients on PPI treatment, justified by the fact that the main endpoints of this study were to search for an improvement on symptoms and not on endoscopic features. Nevertheless, although this was a relatively small trial, its design was based on an adequate statistical power to show a significant effect.

RefluG™, a galactomannan-based formulation, acts primarily by a unique non-systemic mechanism of action, different from antacids, PPIs or histamine H2-receptor antagonists. Upon coming into contact with gastric acid, galactomannans rapidly form a gel in the form of raft that creates a physical barrier above the acidic gastric contents protecting the esophageal mucosa by limiting gastric reflux into the esophagus. Results from our investigation suggest that this combination provides quickly effective relief of GERD associated symptoms and may be considered as monotherapy in those subjects with a mild to moderate symptomatology that do not require the use of PPIs which are considered the gold standard treatment for patients with moderate to severe GERD. This strategy could both reduce the risk of long-term side effects of PPI drugs, which have raised several concerns in recent years[27], as well as avoid costly and not always appropriate pharmacological strategies, especially in patients with low-grade esophagitis[9].

Given that the investigational product offers a supplemental mechanism of action to acid suppression (i.e., raft-forming and mucoprotection), future studies will need to evaluate the usefulness of RefluG™ on demand as add-on therapy in patients with high-grade esophagitis, who remain symptomatic despite treatment with PPI standard dose.

Conclusions

This randomized study demonstrated that RefluG™ is safe and effective for short treatment of GERD symptoms as mono-therapy. These findings suggest that RefluG™ may be a reasonable alternative or add-on treatment option for patients with mild-to-moderate symptomatic GERD.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

Author's contribution

Ludovico Abenavoli contributed to manuscript preparation and revised the manuscript critically. Carmelo Luigiano contributed to manuscript preparation and revised the manuscript critically. Rajesh Pendlimari was investigator and acquired data. Sharmila Fagoonee contributed to data analysis and manuscript preparation, revised this article critically for important intellectual content. Rinaldo Pellicano* (corresponding author) analyzed, interpreted data and wrote the manuscript.

The study design, data analysis and interpretation were done by the academic authors without any industry involvement. The content of the manuscript was at the sole discretion of the academic authors.

All authors have read and approved the final version of this manuscript, including the authorship list.

References

1) Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R, Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol 2006; 101: 1900-1920.
2) Saracco M, Savarino V, Bodini G, Saracco GM, Pellicano R. Gastro-esophageal reflux disease: Key messages for clinicians. Minerva Gastroenterol Dietol 2020 Oct 26; doi 10.23736/S1121-421X.20.02783-X. Epub ahead of print.
3) Savarino E, Zentilin P, Marabotto E, Bodini G, Della Coletta M, Frazzoni M, de Bortoli N, Martinucci I, Tolone S, Pellegatta G, Savarino V. A review of pharmacotherapy for treating gastroesophageal reflux disease (GERD). Expert Opin Pharmacother 2017; 18: 1333-1343.
4) Kahrilas PJ, Howden CW, Hughes N. Response of regurgitation to proton pump inhibitor therapy in clinical trials of gastroesophageal reflux disease. Am J Gastroenterol 2011; 106: 1419-1425.
5) Nirwan JS, Hasan SS, Babar ZU, Conway BR, Ghori MU. Global prevalence and risk factors of gastro-oesophageal reflux disease (GORD): systematic review with meta-analysis. Sci Rep 2020; 10: 5814.
6) Bhatia SJ, Makharia GK, Abraham P, Bhat N, Kumar A, Reddy DN, Ghoshal UC, Ahuja V, Rao GV, Devadas K, Dutta AK, Jain A, Kedia S, Dama R, Kalapala R, Alvares JF, Dadhich S, Dixit VK, Goenka MK, Goswami BD, Issar SK, Leelakrishnan, Mallath MK, Mathew P, Mathew P, Nandwani S, Pai CG, Peter L, Prasad AVS, Singh D, Singh Sodi J, Sud R, Venkataraman J, MidhaV, Bapaye A, Dutta U, Jain AK, Kochhar R, Puri AS, Singh SP, Shimpi L, Sood A, Wadhwa RT. Indian consensus on gastroesophageal reflux disease in adults: a position statement of the Indian Society of Gastroenterology. Indian J Gastroenterol 2019; 38: 411-440.
7) Lundell LR, Dent J, Bennett JR, Blum AL, Armstrong D, Galmiche JP, Johnson F, Hongo M, Richter JE, Spechler SJ, Tytgat GN, Wallin L. Endoscopic assessment of oesophagitis: clinical and functional correlates and further validation of the Los Angeles classification. Gut 1999; 45: 172-180.
8) Katzka DA, Pandolfino JE, Kahrilas PJ. Phenotypes of gastroesophageal reflux disease: where Rome, Lyon, and Montreal meet. Clin Gastroenterol Hepatol 2020; 18: 767-776.
9) Bi D, Katzka DA, Lavey CJ, Geno DM, Ravi K. Erosive esophagitis portends a benign clinical course in the majority of patients. Dig Dis Sci 2020; 65: 3244-3252.
10) Gyawali CP, Kahrilas PJ, Savarino E, Zerbib F, Mion F, Smout AJPM, Vaezi M, Sifrim D, Fox MR, Vela MF, Tutuian R, Tack J, Bredenoord AJ, Pandolfino J, Roman S. Modern diagnosis of GERD: the Lyon Consensus. Gut 2018; 67: 1351-1362.
11) De Hertogh G, Ectors N, Van Eyken P, Geboes K. Review article: the nature of oesophageal injury in gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2006; 24 (Suppl. 2): 17-26.
12) Sharma P, Yadlapati R. Pathophysiology and treatment options for gastroesophageal reflux disease: looking beyond acid. Ann N Y Acad Sci 2021; 1486: 3-14.
13) Gupta N, Inadomi J, Sharma P. Perception about gastroesophageal reflux disease (GERD) and its impact on daily life in the general population: results from a large population based AGA survey. Gastroenterology 2012; 142(Suppl 1): S441-S441.
14) Shaheen NJ, Hansen RA, Morgan DR, Gangarosa LM, Ringel Y, Thiny MT, Russo MW, Sandler RS. The burden of gastrointestinal and liver diseases, 2006. Am J Gastroenterol 2006; 101: 2128-2138.
15) Kleinman L, McIntosh E, Ryan M, Schmier J, Crawley J, Locke R, De Lissovoy G. Willingness to pay for complete symptom relief of gastroesophageal reflux disease. Arch Intern Med 2002; 162: 1361-1366.
16) Chand N, Johnson DA, Tabangin M, Ware JC. Sleep dysfunction in patients with gastro-oesophageal reflux disease: prevalence and response to GERD therapy, a pilot study. Aliment Pharmacol Ther 2004; 20: 969-974.
17) Suzuki T, Matsushima M, Masui A, Tsuda S, Imai J, Nakamura J, Tsukune Y, Uchida T, Yuhara H, Igarashi M, Koike J, Mine T. Irsogladine maleate and rabeprazole in non-erosive reflux disease: a double-blind, placebo-controlled study. World J Gastroenterol 2015; 21: 5023-5031.
18) Reimer C, Lødrup AB, Smith G, Wilkinson J, Bytzer P. Randomised clinical trial: alginate (Gaviscon Advance) vs. placebo as add-on therapy in reflux patients with inadequate response to a once daily proton pump inhibitor. Aliment Pharmacol Ther 2016; 43: 899-909.
19) Pellegatta G, Spadaccini M, Lamonaca L, Craviotto V, D'Amico F, Ceriotti L, Meloni M, Repici A. Evaluation of human esophageal epithelium permeability in presence of different formulations containing hyaluronic acid and chondroitin sulphate. Med Devices 2020; 13: 57-66.
20) Savarino V, Pace F, Scarpignato C, Esoxx Study Group. Randomised clinical trial: mucosal protection combined with acid suppression in the treatment of non-erosive reflux disease--efficacy of Esoxx, a hyaluronic acid-chondroitin sulphate based bioadhesive formulation. Aliment Pharmacol Ther 2017; 45: 631-642.
21) DiSilvestro RA, Verbruggen MA, Offutt EJ. Anti-heartburn effects of a Fenugreek fiber product. Phytotherapy Res 2011; 25: 88-91.
22) Casale M, Moffa A, Sabatino L, Pace A, Oliveto G, Vitali M, Baptista P, Salvinelli F. Hyaluronic acid: perspectives in upper aero-digestive tract. A systematic review. PLoS One 2015; 10: e0130637.
23) Gasparetto JC, Ferreira Martins CA, Hayashi SS, Otuky MF, Pontarolo R. Ethnobotanical and scientific aspects of Malva sylvestris L.: a millennial

herbal medicine. J Pharm Pharmacol 2012; 64: 172-189.

24) Hampson FC, Farndale A, Strugala V, Sykes J, Jolliffe IG, Dettmar PW. Alginate rafts and their characterisation. Int J Pharm 2005; 294: 137-147.

25) Kaesler N, Baid-Agrawal S, Grams S, Nadal J, Schmid M, Schneider MP, Eckardt KU, Floege J, Bergmann MM, Schlieper G, Saritas T. Low adherence to CKD-specific dietary recommendations associates with impaired kidney function, dyslipidemia, and inflammation. Eur J Clin Nutr 2021 Feb 2. doi: 10.1038/s41430-020-00849-3. Epub ahead of print.

26) Jung H-K, Choung RS, Talley N. Gastroesophageal reflux disease and sleep disorders: evidence for a causal link and therapeutic implications. J Neurogastroenterol Motil 2010; 16: 22-29.

27) Lazarus B, Chen Y, Wilson FP, Sang Y, Chang AR, Coresh J, Grams ME. Proton pump inhibitor use and the risk of chronic kidney disease. JAMA Intern Med 2016; 176: 238-246..