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Translational evaluation of Gelsectan® effects on gut barrier dysfunction and visceral pain in animal models and irritable bowel syndrome with diarrhoea

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Abstract

Background: Gelsectan® is a formulation of xyloglucan (XG), pea protein, grape seed extract (PPGS) and xylo-oligosaccharides (XOS). Our aim was to examine the effect of Gelsectan® on rectal sensitivity in an animal model, abdominal pain in irritable bowel syndrome with diarrhoea (IBS-D) subjects and intestinal permeability in both conditions.

Methods: Animals: Wistar rats received gavage with XOS, XG + PPGS or XG + PPGS + XOS, as a single dose or for 7 days before a partial restraint stress (PRS). Visceromotor response to rectal distension and total gut paracellular permeability to ^{51}Cr-EDTA were assessed. Humans: IBS-D and control patients were involved. After initial colonoscopy with biopsy sampling Gelsectan® was administered to IBS-D patients for 12 weeks. Stool count and pain scores were documented. After treatment, colonoscopy was repeated. The permeability of biopsy samples was measured in Ussing-chambers. Adherent mucus layer, Muc-2 expression as well as TNFα, Interferon IFNγ were evaluated by histology/immunohistochemistry and ELISA assays, respectively.

Results: Animal studies: In control rats, PRS significantly increased visceromotor response as well as gut paracellular permeability. Single dose administration of XG + PPGS + XOS failed to reverse PRS, but 7 days of oral treatment reversed PRS-induced rectal hypersensitivity and gut hyperpermeability. Human studies: Gelsectan® treatment significantly reduced and abdominal pain. Intestinal permeability in IBS-D patients was elevated compared with controls, Gelsectan® restored permeability in the ascendent colon. Periodic acid–Schiff-stained mucus layer was significantly thinner in IBS-D patients compared with controls, In both segments, mucus thickness and the proportion of Muc-2 positive cells were not affected by

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Gelsectan® treatment. IFNγ tissue level in the sigmoid colon shows modest mucosal inflammation in IBS-D.

Conclusions: Gelsectan® prevented rectal hypersensitivity in rats, abdominal pain in human and intestinal hyperpermeability in rat and human studies respectively. These effects involve restoration of gut permeability. Based on this translational study, Gelsectan® can be considered as an effective therapy for IBS-D symptoms.

KEYWORDS
Gelsectan, gut hyperpermeability, irritable bowel syndrome, rectal hypersensitivity

BACKGROUND

According to Rome IV criteria, irritable bowel syndrome (IBS) is one of the most commonly diagnosed gastrointestinal disorders with a prevalence of 6.2%–44.2%.[1] IBS is a chronic condition which may severely impair the quality of life and work productivity.[2,3] Symptoms of IBS include chronic abdominal pain with altered bowel habits. Three main subtypes have been described: IBS with constipation, diarrhoea, or mixed episodes of constipation and diarrhoea.[2,4] The pathophysiology of IBS is not fully understood; disturbances of the microbiome-gut-brain axis,[3,5] increase of gut permeability and a low-grade mucosal inflammation, among others, play a role in the development of IBS symptoms.[6]

Around 30%–40% of IBS patients exhibit visceral hypersensitivity with increased sensitivity to colonic distension. This manifests through a reduced pain threshold, increased intensity of sensations and/or an amplified viscerosomatic reaction to colonic distension.[7] Animal models mimicking IBS pathophysiology have been developed, including partial restraint stress (PRS)-related models in rats. In the PRS model, first described by Williams and colleagues, small intestinal transit was inhibited, while large intestinal transit and faecal excretion were increased in stressed rats.[8] Furthermore, in rats, a PRS session promotes gut hyperpermeability and visceral hypersensitivity in response to rectal distension (CRD).[9]

Gelsectan®, a formulation containing xyloglucan (XG), pea protein, grape seed extract (PPGS) and xylo-oligosaccharides (XOS), has been developed to treat diarrhoeal IBS patients. It is designated as a Class III CE marked medical device. Clinical trials verified the efficacy of Gelsectan® treatment in symptom reduction in IBS-D patients.[10–12] Previous animal studies show that XG exerts protective intestinal effects such as prevention of gut hyperpermeability and reduction of mucosal inflammation[13] and exhibits efficacy for the treatment of adult and paediatric acute gastroenteritis.[14] XOS, another functional ingredient of Gelsectan®, exerts an antioxidant action by reducing reactive oxygen species[15] and has a prebiotic effect reflected by the increase of Bifidobacteria in human gut microbiota.[16]

The aims of this study were to evaluate the effect of an acute versus a chronic treatment of Gelsectan® versus its single components on rectal hypersensitivity and gut hyperpermeability induced by an acute stress in rat. We also aimed to examine the clinical effects of Gelsectan® on symptoms, intestinal permeability, mucus thickness and intestinal microinflammation in IBS-D patients.

Key summary

Summarize the established knowledge on this subject

- Irritable bowel syndrome (IBS) is one of the most commonly diagnosed gastrointestinal disorders.
- Symptoms of IBS include chronic abdominal pain with altered bowel habits.
- Gelsectan®, a formulation containing xyloglucan (XG), pea protein, grape seed extract (PPGS), xylo-oligosaccharides (XOS) and excipients, has been developed to treat diarrhoeal IBS patients.
- The mechanism of the beneficial therapeutic effect of Gelsectan® in irritable bowel syndrome with diarrhoea (IBS-D) is not understood.

What are the significant and/or new findings of this study?

- 7-day oral administration of Gelsectan® prevented the stress-induced rectal hypersensitivity and permeability increase in rats.
- Our human study showed that long-term treatment in humans with Gelsectan® had a significant effect on both abdominal pain and diarrhoea in IBS-D patients.
- Intestinal permeability in IBS-D patients is elevated compared with controls, Gelsectan® restores permeability in the ascendent colon.
- Periodic acid–Schiff-stained mucus layer is significantly thinner in IBS-D compared to controls. In both segments, mucus thickness and the proportion of Muc-2 positive cells were not affected by Gelsectan® treatment.

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MATERIALS AND METHODS

Animal studies

Ethical approval

The local ethical committee, according to the EU directive 2010/63/EU APAFIS#5143–2016042210305097 v3, approved the animal care and experimental protocols.

Animals

Two series of young adult female Wistar rats (Janvier SA, Le Genest St Isle, France) aged 8–10 weeks and housed individually in propylene cages were used. One set was designed to measure rectal sensitivity and the other to measure total intestinal gut permeability. Animals were allowed free access to water and standard food (Teklad Global Diet, Harlan laboratories).

Stress procedure

PRS, a relatively mild non-ulcerogenic model of stress, was performed as previously described[8] at the same time during the diurnal cycle from 10.00 AM to 12.00 PM. Briefly, animals were sedated with diethyl ether, and their upper forelimbs and thoracic trunk were wrapped in a confining harness of paper tape to restrict but not prevent their body movements. Rats were then placed in their home cages for 2 hours. Control sham-stressed animals were anaesthetized but not wrapped.

Surgery

Animals were surgically prepared for electromyography recordings (EMG). Briefly, they were generally anaesthetized by administration of acepromazine 0.4 mg/kg (Calmivet, Vetoquinol, Lure, France) and ketamine 75 mg/kg (Imalgene 1000, Rhône-Merieux, Lyon, France) intraperitoneally. After incision, three groups of electrodes of three Ni-Cr wires (80 μm diameter) were implanted in the oblique striated muscle of the abdomen. The ends of electrodes were exteriorised on the back of the neck and protected by a glass tube attached to the skin.

Rectal hypersensitivity

Prior to CRD, rats became accustomed to staying in polypropylene tunnels (7 cm diameter and 20 cm length) for 2 days to minimise recording artefacts due to movement. An arterial embolectomy catheter (Fogarty, Edwards Laboratories Inc.) was inserted into the colorectum, positioned at 1 cm from the anus and fixed at the base of the tail. CRD was performed by progressively inflating the balloon from 0 to 1.2 mL using steps of 0.4 mL, with each inflation lasting 5 min.

EMG recordings were started 5 days after surgery. Abdominal muscle electrical activity was recorded and analysed with the Powerlab Chart 5 program (AD Instruments, Oxford, United Kingdom). Rectal sensitivity was quantified as the number of spike bursts, reflecting abdominal contractions, for each balloon inflation applied for CRD.

Total gut permeability

Assessment of total gut permeability to ^{51}Cr-ethylenediamine-tetraacetic acid (^{51}Cr-EDTA; Perkin Elmer Life Science, Paris, France) was used as a marker of paracellular permeability. ^{51}Cr-EDTA (0.7 μCi) was diluted in 500 μL of saline and administered by gavage. Rats were then placed in metabolic cages and urine was collected for 24 h. Total radioactivity in urine was measured using a gamma counter (Cobra II, Packard). Total gut permeability was expressed as a percentage of the total radioactivity administered.

Experimental design

Groups of female Wistar rats (n = 8) were pre-treated with compounds administered by gavage before PRS. Compounds were administered either as a single dose administration 1 h before PRS or by oral repeated administration given once daily for 7 days before PRS. Compounds were vehicle (0.9% saline); XOS (76.8 mg/rat); XG (36.4 mg/rat) + PPGS (91.2 mg/rat); and XG (36.4 mg/rat) + PPGS (91.2 mg/rat) + XOS (76.8 mg/rat). Negative controls were administered with vehicle only, that is, no PRS (Supplementary 1).

Human studies

Ethical approval

The human study protocol was approved by the Ethical Committee of the University of Szeged (No.3645/2015).

Patients

Fourteen subjects were involved in the study (male: female = 9:5, age: 33.5 ± 2.8 years). Written and informed consent was obtained from all subjects. Six patients were suffering from IBS-D. All the patients went through detailed examinations before the enrolment: medical history and physical examination, screening routine laboratory tests, thyroid stimulating hormone, Tissue Transglutaminase antibody, stool Giardia antibody and abdominal ultrasound were performed to exclude other diseases. One patient could not complete the closing colonoscopy due

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to COVID pandemic quarantine regulations. Eight age matched control patients were involved. Every control patient was referred for colonoscopy with stool blood test positivity without abdominal pain or bowel habit changes and negative screening laboratory tests. Colonoscopy was normal in all control patients.

Study design

After enrolment, IBS-D patients received a stool diary and underwent initial colonoscopy. Patients received 2 caps. bid. Gelsectan® for 4 weeks followed by 8 weeks of 1 caps. bid. Closing colonoscopy was performed at the end of the therapy. Abdominal pain was assessed before and after the treatment. Control subjects did not undergo therapeutic interventions, they had only a colonoscopy for biopsy sample collection. During colonoscopy, the ascendent and sigmoid colons were biopsied.

Stool diary

IBS-D patients were asked to note their daily stool count. Data were collected and the average daily stool count was calculated for every week of the therapy.

Abdominal pain assessment

Abdominal pain was quantified using visual analogue scale (0–10), where 0 was 'no pain' and 10 was 'the worst pain', as previously described.[17]

Intestinal permeability measurement

Colonic biopsy strips were mounted with a flux area of Area = 0.031 cm^2 in Easymount Ussing-type chambers (Physiologic Instruments), bathed in Krebs solution and oxygenated at a maintained temperature of 37°C. After allowing 15 min for equilibrium, the buffer solution of the apical compartment (mucosal side) was enriched with fluorescein isothiocyanate (FITC)-labelled dextran (4 kDa, 0.022 g/mL, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). One hour later, fluorescent intensity was measured on the serosal side of the chamber (CLARIOstar® Plus plate reader [BMG Labtech, Germany]) as previously described.[18]

Periodic acid–Schiff staining

A detailed methodological description can be found in the Supplementary 2.

Muc-2 immunostaining

A detailed methodological description can be found in the Supplementary 3.[19]

TNFα and IFN γ cytokine measurements

A detailed methodological description can be found in the Supplementary 4.[20]

Statistical analysis

GraphPad Prism 4 software (GraphPad) was used for statistical analyses. Multiple comparisons were made using one-way ANOVA followed by Tukey's multiple comparison test. Abdominal pain was compared with a paired *t*-test. Data are reported as the means ± standard deviation and *p*-values lower than 0.05 were considered as significant.

RESULTS

Animal studies

Rectal hypersensitivity

In control rats, increasing the rectal distending volume produced an increase in the number of abdominal contractions (Figure 1). PRS significantly ($p < 0.0001$ and $p < 0.001$ respectively compared to vehicle) increased this visceromotor response in the experiments with acute and repeated administration of the compounds. These results illustrate both an allodynic response corresponding to a lowering threshold of gut sensitivity for an unpainful volume of colorectal distension, that is, 0.4 mL and a hypersensitivity response in pathophysiological conditions (after a PRS session) illustrated by a significant increase of abdominal contractions versus physiological conditions for a painful volume of colorectal distension, that is, 0.8 and 1.2 mL. Acutely administered compounds either alone or in combination failed to prevent PRS-induced hypersensitivity irrespective of the distending volume applied. In contrast, repeated oral administration of the combination XG + PPGS + XOS prevented stress-induced rectal hypersensitivity for the distending volumes of 0.8 and 1.2 mL ($p < 0.0001$ vs. vehicle for each). Neither XOS alone nor the combination of XG + PPGS chronically administered prevented stress-induced rectal hypersensitivity. Comparison of the effects of acute and repeated administration of XOS, XG + PPGS, or XG + PPGS + XOS on PRS-induced rectal hypersensitivity at a distending volume of 0.8 mL are shown in Figure 2.

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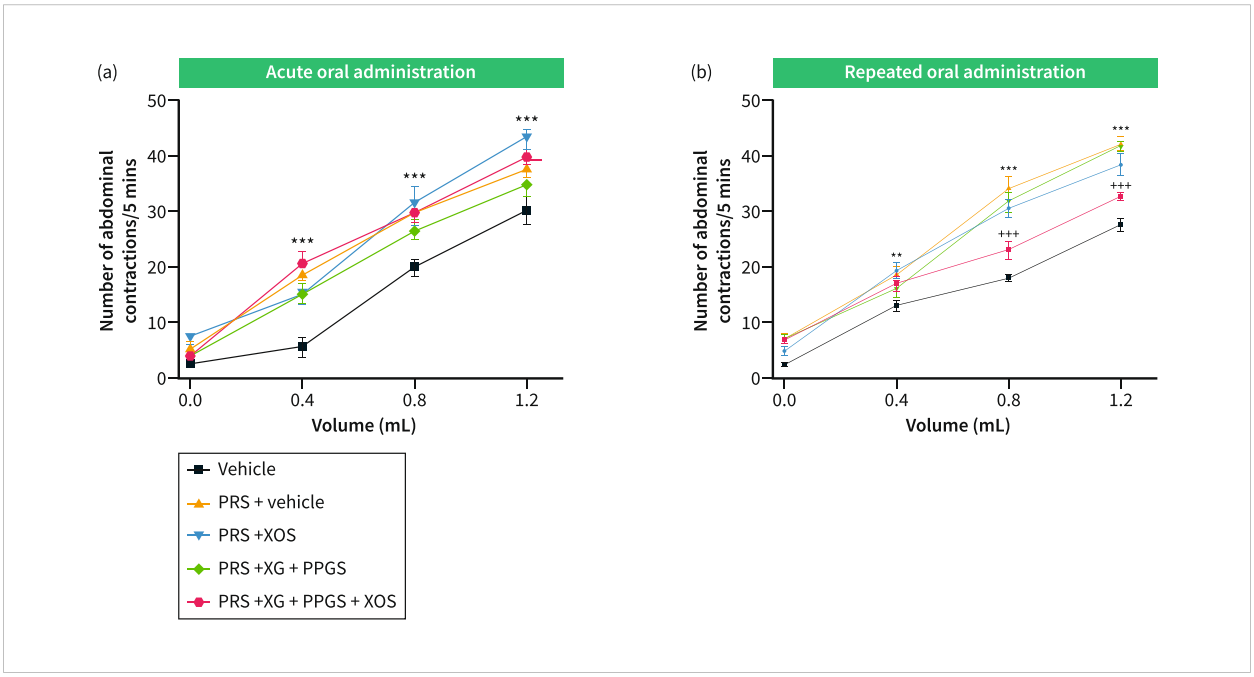

FIGURE 1 Effects of the tested compounds on PRS-induced allodynic and hypersensitivity response to colorectal distension. (a) Effect of an acute treatment (one single dose before PRS session) by XOS, XG + PPGS, or XG + PPGS + XOS ($n = 8$) on PRS-induced visceromotor response. None of these treatments have succeeded in reversing the PRS response to colorectal distension. (b) Effect of repeated oral treatment (once daily for 7 days) by XOS, XG + PPGS, or XG + PPGS + XOS ($n = 8$) on PRS-induced visceral hypersensitivity. Only the XG + PPGS + XOS combination prevented the PRS-induced hypersensitivity response to colorectal distension at 0.8 and 1.2 mL volumes. Error bars show standard deviation (SD). PRS, partial restraint stress; XOS, xylo-oligosaccharides; XG, xyloglucan; PPGS, pea protein and grape seed extract. $^{**}p < 0.001$ and $^{***}p < 0.0001$, vehicle versus negative control; $^{+++}p < 0.0001$ XG + PPGS + XOS versus vehicle. PPGS, pea protein, grape seed extract; PRS, partial restraint stress; XG, xyloglucan; XOS, xylo-oligosaccharides.

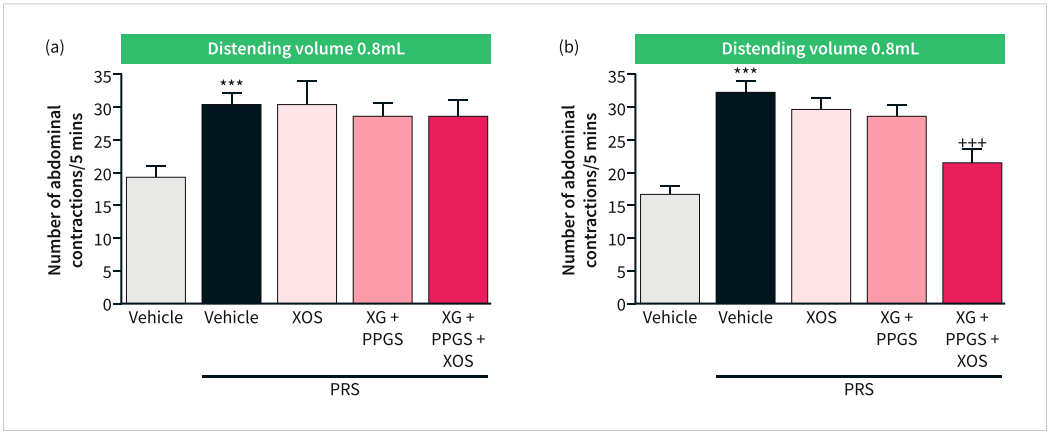

FIGURE 2 Comparison of the XOS, XG + PPGS or XG + PPGS + XOS treatments ($n = 8$) on the PRS-induced hypersensitivity response to colorectal distension at the distending volume 0.8 mL. (a) Acute administration (one single dose before PRS session) of the different compounds. All treatments tested failed to reverse the PRS-induced visceral hypersensitivity. (b) Repeated oral treatments (once daily for 7 days) by the different compounds. Only the XG + PPGS + XOS combination prevented the PRS-induced visceromotor response. Error bars show standard deviation (SD). PRS, partial restraint stress; XOS, xylo-oligosaccharides; XG, xyloglucan; PPGS, pea protein and grape seed extract. $^{***}p < 0.0001$, vehicle versus negative control; $^{+++}p < 0.0001$, XG + PPGS + XOS versus vehicle. PPGS, pea protein, grape seed extract; PRS, partial restraint stress; XG, xyloglucan; XOS, xylo-oligosaccharides.

Total gut permeability

PRS induced a significant increase in total gut paracellular permeability. Comparisons between control rats and rats receiving vehicle acutely or chronically before the PRS session produced p values of <0.0001 and < 0.001, respectively (Figure 3). Pre-treating rats with a single dose of XOS, XG + PPGS, or XG + PPGS + XOS failed to prevent the stress-induced increase in gut paracellular permeability.

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However, pre-treatment with repeated oral administration of XG + PPGS + XOS for 7 days significantly reduced this stress-induced hyperpermeability ($p < 0.05$). In contrast, repeated oral administration of XOS alone or XG + PPGS failed to protect against the stress-induced increase in gut paracellular permeability.

Human studies

Gelsectan® reduces diarrhoea in IBS-D patients

Our results show that stool count was significantly reduced during the therapy, significancy level was reached in the 3rd week of treatment and the beneficial effect was maintained till the end of the therapy (Figure 4a).

Gelsectan® reduces abdominal pain

Gelsectan® treatment significantly reduced the abdominal pain compared with the pretreatment values (Figure 4b).

Intestinal permeability

Measurements showed significantly elevated permeability in the IBS-D patients compared with the healthy controls in the ascendent colon. This permeability elevation was significantly reversed after the 12 weeks of Gelsectan® therapy. Similar observations were made in the sigmoid colon, although the results did not reach the significancy level (Figures 4c,d). No significant differences were observed between the ascendent and sigmoid colon samples in control subjects.

Mucus thickness

Our analysis showed a significantly more prominent adherent mucus layer in the sigmoid colon compared with the ascendent colon in control subjects. In both the ascendent and sigmoid colon segments, we found a significantly thinner mucus layer in IBS-D patients compared to the identic segment of the control subjects. After Gelsectan® therapy, no alterations were observed in mucus thickness in the examined colonic segments (Figure 5).

Muc-2 immunostaining

A significantly higher proportion of Muc-2 positive cells was observed in the sigmoid colon of the healthy controls compared to the ascendent colon, which is in agreement with the thicker adherent mucus layer measured in this segment. In the ascendent colon, we did not observe alteration of the Muc-2 positive cells in IBS-D compared with the controls. In contrast, in the sigmoid colon, the IBS-D group (treated or untreated) had a significantly lower proportion of Muc-2 positive cells compared to the controls. Gelsectan® treatment had no effect on the mucus secreting cell proportion in any of the segments examined (Figure 6).

IFNγ and TNFα ELISA assay

No significant differences were observed in TNFα and IFNγ levels in the ascendent colon compared with controls and IBS-D patients before Gelsectan® treatment. In the sigmoid colon, we observed a significantly elevated IFNγ level in IBS-D compared with healthy controls. No significant differences were documented in the TNFα

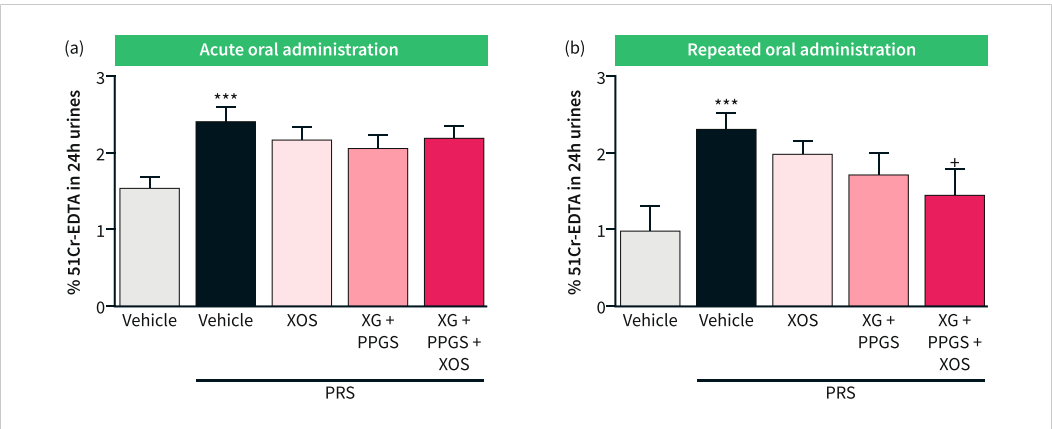

FIGURE 3 Effects of the tested compounds on PRS-induced intestinal paracellular hyperpermeability. (a) Effect of an acute treatment (one single dose before PRS session) by XOS, XG + PPGS, or XG + PPGS + XOS ($n = 8$) on PRS-induced hyperpermeability. None of the tested compounds had an effect upon PRS-induced intestinal epithelial barrier impairment. (b) Effect of repeated oral treatment (once daily for 7 days) by XOS, XG + PPGS, or XG + PPGS + XOS ($n = 8$) on PRS-induced total gut hyperpermeability. Note the efficacy of the XG + PPGS + XOS combination repeated treatment to prevent the PRS-induced intestinal epithelial barrier impairment. Error bars show standard deviation (SD). PRS, partial restraint stress; XOS, xylo-oligosaccharides; XG, xyloglucan; PPGS, pea protein and grape seed extract ***$p < 0.0001$, vehicle versus negative control; $^{+}p < 0.05$, XG + PPGS + XOS versus vehicle.

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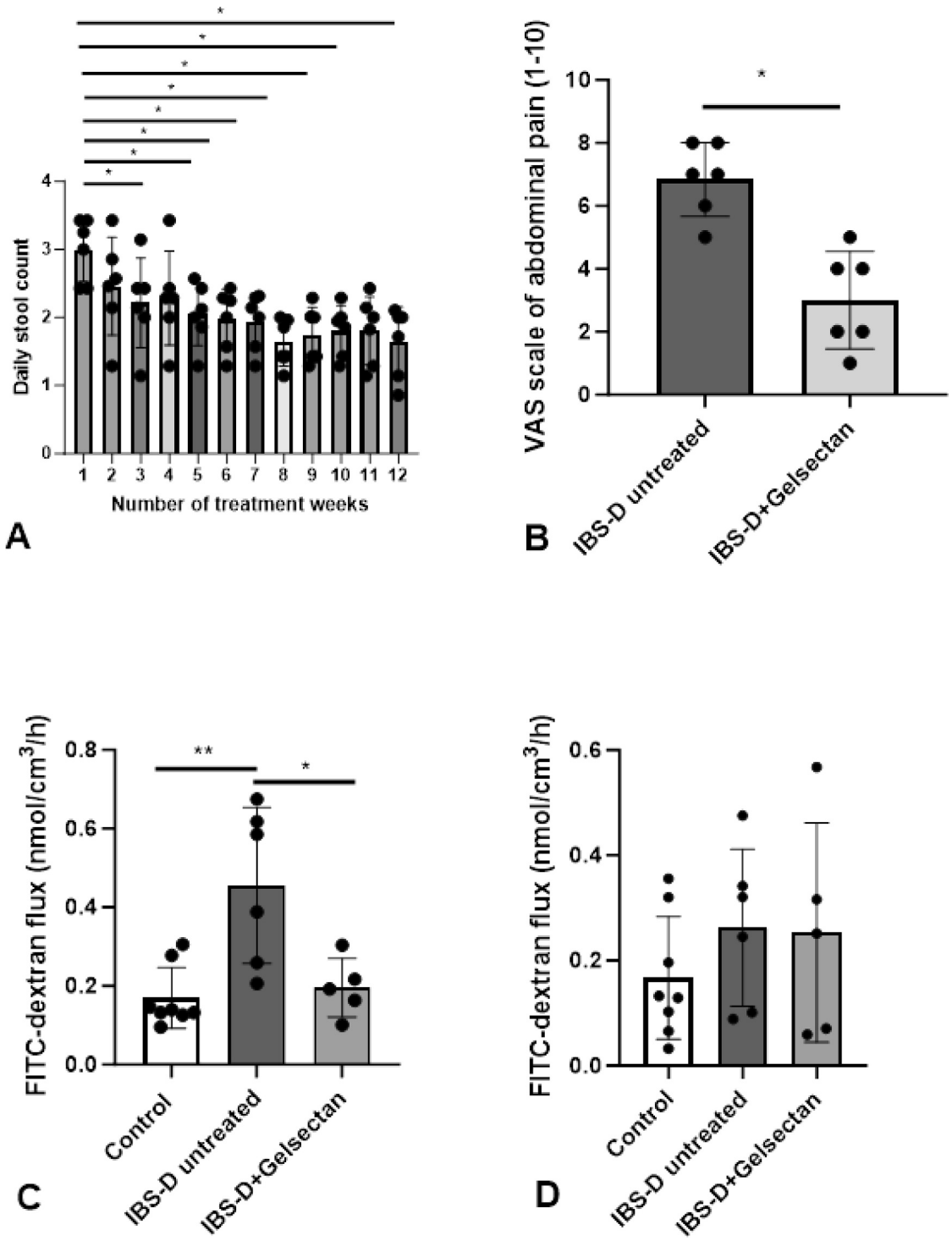

FIGURE 4 (a) Daily stool count during the study period. Stool count was significantly reduced during Gelsectan® therapy. Significant stool count reduction was observed for the 3rd therapeutic week (*$p < 0.05$) and maintained till the end of the study. (b) VAS scores before and after 12 weeks of Gelsectan® treatment: Abdominal pain reflected by the patients using VAS scale. Gelsectan® treatment significantly reduced the abdominal pain compared with the pretreatment values. (*$p < 0.05$) (c) Intestinal permeability of 4 kDa FITC-dextran on Ussing chambers system of the ascendent colon in control subjects and irritable bowel syndrome with diarrhoea (IBS-D) patients before and after Gelsectan® treatment: Ascendent colon biopsy samples of IBS-D patients showed significantly elevated intestinal permeability compared to the healthy individuals. After therapy, the permeability elevation was significantly reduced. (*$p < 0.05$, **$p \leq 0.01$). (d) Intestinal permeability of 4 kDa FITC-dextran on Ussing chamber system of the sigmoid colon in control subjects and IBS-D patients before and after Gelsectan® treatment: Sigmoid colon samples' results did not reach a significant level.

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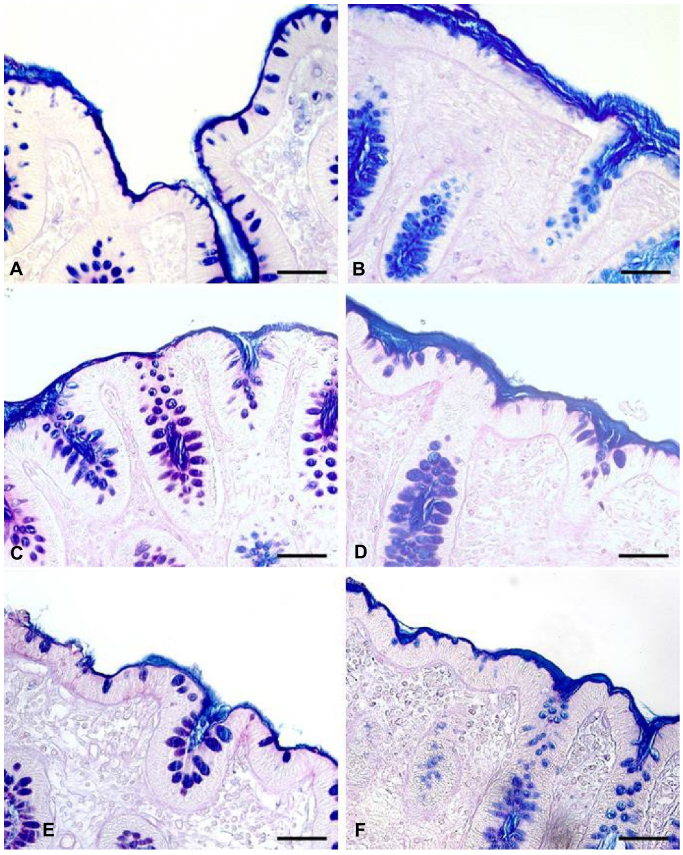

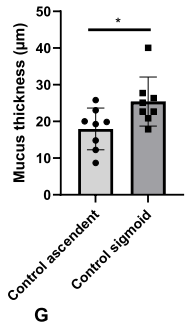

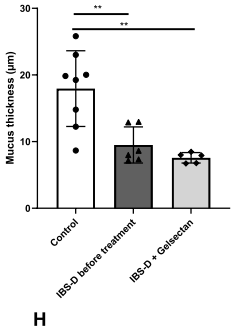

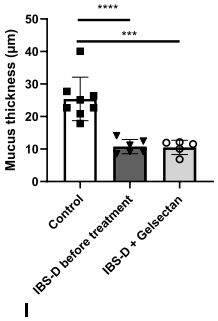

FIGURE 5 (a–f): Representative microscopic pictures of PAS staining, (a) control subjects' ascendent colon, (b) control subjects' sigmoid colon, (c) ascendent colon of irritable bowel syndrome with diarrhoea (IBS-D) patient before treatment, (d) sigmoid colon of IBS-D patient before treatment, (e) ascendent colon of IBS-D patient after Gelsectan® treatment, (f) sigmoid colon of IBS-D patient with Gelsectan® treatment. (g–i) Thickness of the adherent mucus (PAS staining): (g) mucus thickness of the control subjects' colon segments: Sigmoid colon segments had significantly thicker mucus layer compared to the ascendent colon. (*$p < 0.05$) (h) comparison of the ascendent colon segments. (i) Comparison of the sigmoid colon segments. IBS-D patients' mucus layer before Gelsectan® treatment was significantly thinner in both segments compared to the control patients' colon. Gelsectan® treatment did not influence the adherent mucus layer thickness in both segments. (**$p \leq 0.01$ ***$p \leq 0.001$, ****$p \leq 0.0001$). PAS, Periodic acid–Schiff.

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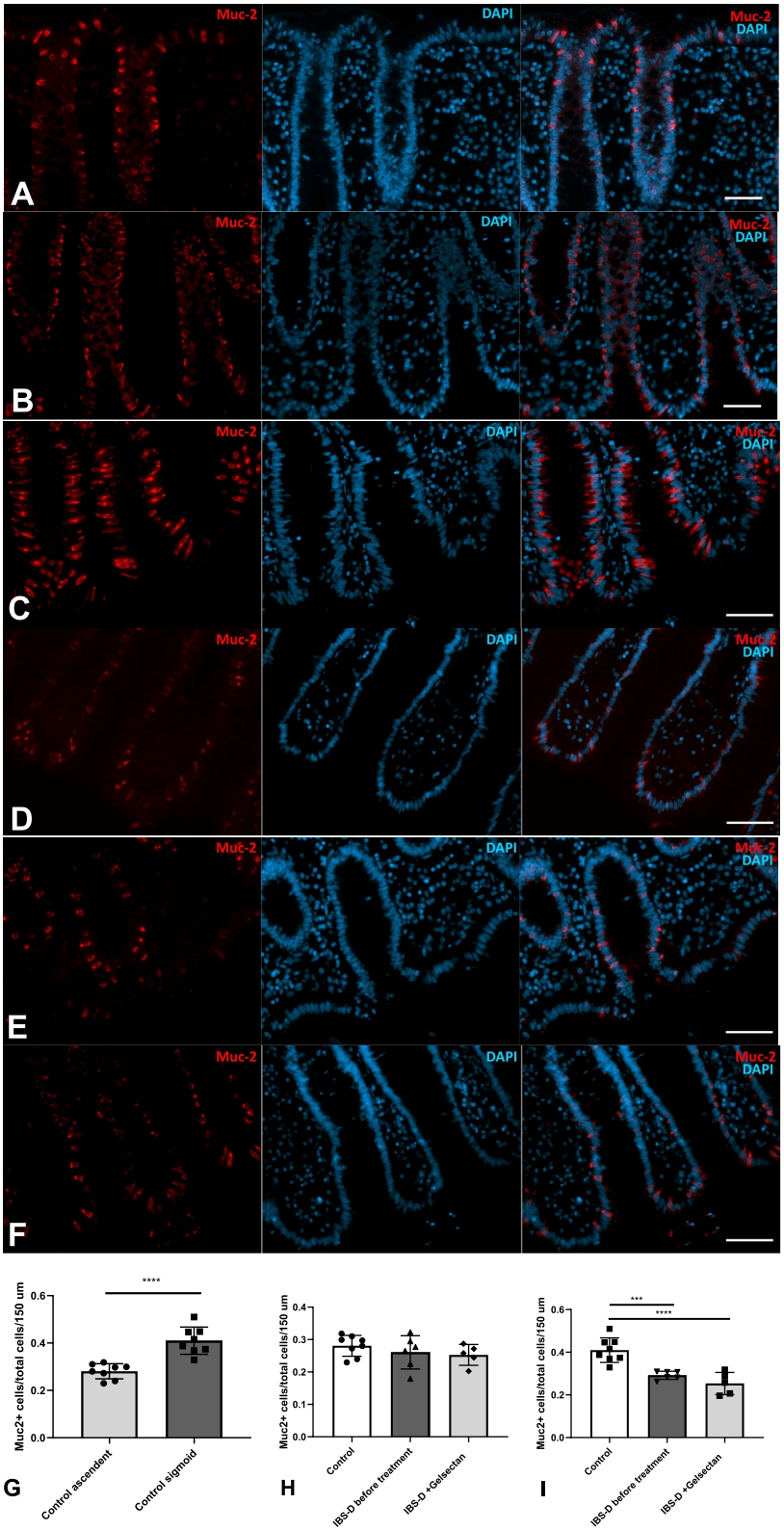

FIGURE 6 (a–f) Representative microscopic pictures of Muc-2 immunostaining, (a) control subjects' ascendent colon, (b) control subjects' sigmoid colon, (c) Ascendent colon of irritable bowel syndrome with diarrhoea (IBS-D) patient before Gelsectan® treatment. (d) Sigmoid colon of IBS-D patient before Gelsectan® treatment. (e) Ascendent colon of IBS-D patient after Gelsectan® treatment, (f) sigmoid colon of IBS-D patient after Gelsectan® treatment. (g) Proportion of Muc-2-immunoreactive (IR) cells to the total cell count in the healthy ascendent and sigmoid colon: Muc-2-IR cells are significantly more abundant in the sigmoid colon compared to the ascendent colon in healthy individuals. (h) Muc-2-IR cells in the ascendent colon: No significant difference was observed comparing control IBS-D before treatment and IBS-D + Gelsectan® group. (i) Muc-2-IR cells in the sigmoid colon: In the IBS-D group before Gelsectan® treatment a reduction in the Muc-2-IR cells in the sigmoid colon was detectable compared to the control. Therapeutic intervention with Gelsectan® did not have an effect on the Muc-2-IR cells proportion. (***$p \leq 0.001$, ****$p \leq 0.0001$).

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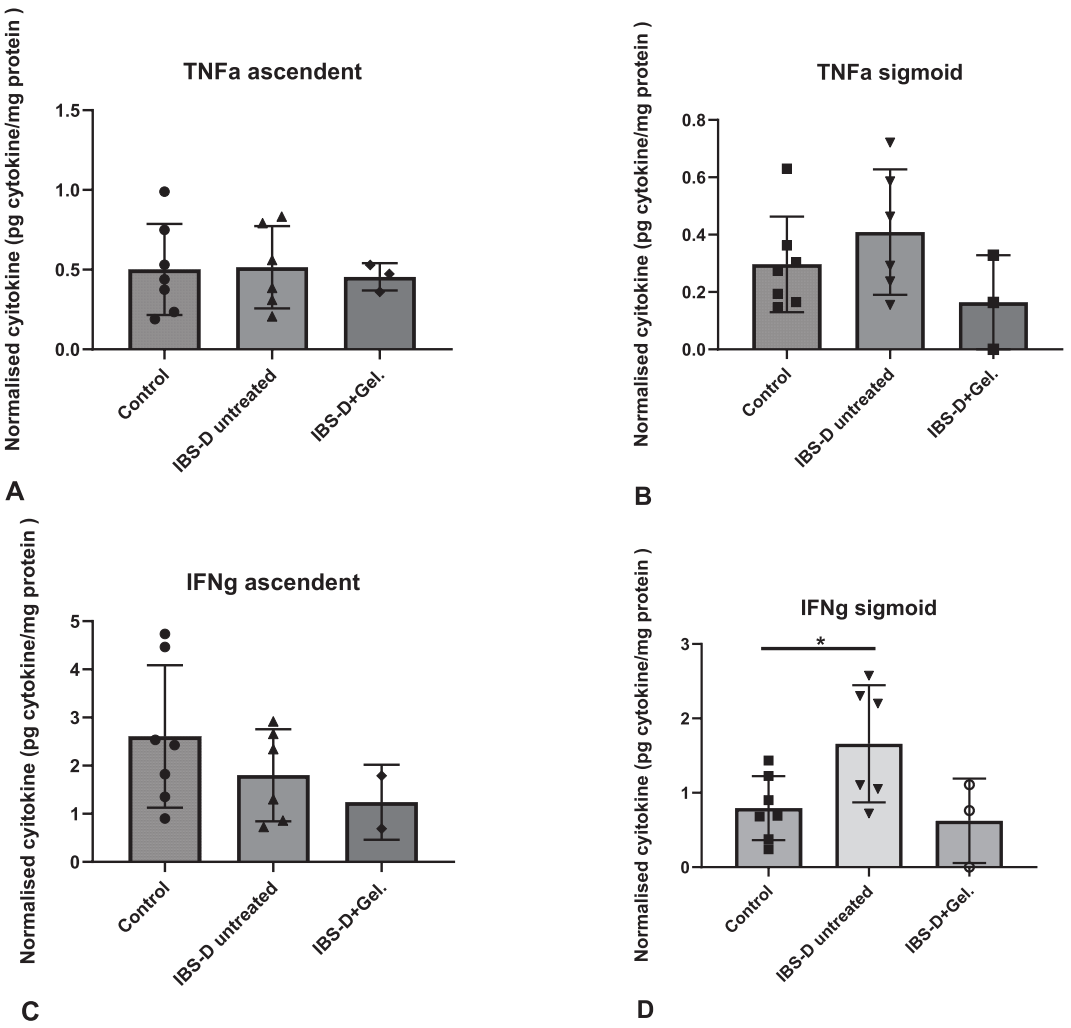

FIGURE 7 (a) Tissue TNFα levels in the ascendent colon (b) Tissue TNFα levels in the sigmoid colon (c) Tissue IFNγ levels in the ascendent colon (d) Tissue IFNγ levels in the sigmoid colon No significant change was observed in the tissue TNFα levels neither in the ascendent nor in the sigmoid colon. Tissue IFNγ profile alteration was observed in irritable bowel syndrome with diarrhoea patients in the sigmoid colon but not in the ascendent colon ($^*p < 0.05$).

levels. The limited number of data in the Gelsectan® treated group did not allow us to perform statistical analysis; data are shown in Figure 7.

DISCUSSION

This study examined the effect of Gelsectan® on stress-induced rectal hypersensitivity and gut hyperpermeability in rats and the clinical efficacy of Gelsectan® in the treatment of diarrhoea and abdominal pain in IBS-D patients. We also aimed to investigate the possible mechanism of action of the product.

Our animal model confirmed that a progressive increase in CRD produced a proportionate increase in the visceromotor response. PRS significantly increased this visceromotor response of the rats for each distending rectal volume, in agreement with previously published results.[21] 7-day oral administration of Gelsectan® prevented this stress-induced rectal hypersensitivity. In addition, Gelsectan® significantly reduced stress-induced increases in gut permeability. An individual compound (XOS) or a combination of compounds (XG + PPGS) failed to protect against these adverse effects of acute stress. These results emphasise that the use of a combination of XG + PPGS + XOS is required for efficacy and provide a preclinical rationale for the use of Gelsectan® in the management of IBS. Further, the protective effects of Gelsectan® observed in the animal study were linked to the repeated administration of this formulation, providing also rationale for long-term treatment by Gelsectan® in the clinical use for IBS management.

Rome IV criteria define[22] IBS-D as a combination of abdominal pain and diarrhoea. Our human study showed that long-term treatment in humans with Gelsectan® had a significant effect on both symptoms. We demonstrated that similar to animal experiments on rectal hypersensitivity, abdominal pain reported by human participants was ameliorated by Gelsectan® treatment. A significant

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reduction in pain was also observed after 3 month of treatment with Gelsectan® in IBS-D subjects.

Intestinal permeability was elevated in IBS-D patients in most of the studies[23] similar to stressed animals.[24–26] Our data confirmed this permeability elevation, and showed that Gelsectan® treatment was able to decrease permeability in both animals and humans.

Clinical data regarding the intestinal microinflammation in IBS are very controversial.[27–29] Our study showed a significant increase of INFγ tissue levels in the sigmoid colon in IBS-D. Given the limitations of the available data, it is not possible to draw any conclusions regarding the impact of Gelsectan treatment on the inflammatory process.

Intestinal mucosa is covered by a self-produced viscoelastic gel referred to as mucus. The mucus layer is highly dynamic, allowing nutrient access to the epithelium and acting as a niche for commensal bacteria while confining pathogens into the lumen. Mucus actively participates in the intestinal barrier function. From a structural point of view, mucus is mainly composed of mucins either secreted by epithelial goblet cells or anchored at the apical surface. The mucus layer differs along the gastro-intestinal tract, reaching 800 µm thickness in the colon where microbiota is the most abundant. Interestingly, in that part of the gut, two mucus layers can be distinguished: an inner layer, firmly attached to epithelial cells and considered as sterile, and an outer loose mucus layer that may harbour microorganisms.[30,31] It has been shown in rats submitted to chronic water avoidance stress, a widely used model of IBS in rodents, that mucus composition and structure were strongly modified.[32] In our knowledge, our study shows for the first-time alterations in the mucus layer in IBS-D patients. Indeed, we found that the adherent mucus layer in the ascendent and sigmoid colon is thinner in IBS-D patients versus controls, suggesting consequent vulnerability of the colonic mucosa and supporting the rationale of mucoprotectant use in the IBS-D management. Indeed, our study suggests that the symptom ameliorating effect of Gelsectan® in IBS-D patients is not associated with alteration of the adherent mucus thickness and the mucus secreting Muc-2 positive goblet cells. We hypothesise the reinforcing effect on the outer mucus layer through a non-adherent mucus-like structure[33] of XG in the Gelsectan® formulation. The restoration of the mucus layer could improve the function of the intestinal barrier, which may reduce mucosal microinflammation and subsequent abdominal pain, leading to an improvement in IBS-D symptoms.

The limitation of our work is the small number of patients enrolled in the human study. Beyond the current translational study, further clinical trials with a larger number of patients are needed to confirm the associations found.

CONCLUSIONS

Our translational data show the efficacy of the Gelsectan® treatment on the stool frequency and abdominal pain in IBS-D patients, rectal hypersensitivity and intestinal permeability in an animal model. Our preliminary data suggest that Gelsectan® may act as a mucoprotectant with a reinforcing effect on the outer mucus layer of the colon, which may lead to improvement in intestinal permeability and IBS-D symptoms.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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