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A randomised trial of pleuran in paediatric acute gastroenteritis

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Pleuran, as a potent immunomodulator targeting intestinal immunity with a strong safety profile, could be a potential treatment for acute gastroenteritis. This study evaluates the effect of pleuran on the duration and severity of acute infectious diarrhoea in children. This is a multi-centre, randomised, double-blind, placebo-controlled, CONSORT statement superiority trial. Children aged 2–10 years presenting to hospital with acute gastroenteritis were included. The primary outcome measure was the duration of diarrhoea. Twenty-seven children were enrolled. There were no significant differences between the experimental and control groups regarding duration of diarrhoea, hospitalisation, intravenous rehydration and symptom severity. The administration of Pleuran was well tolerated. In this study, Pleuran was ineffective in the treatment of acute gastroenteritis in children. Further studies are needed to investigate its potential as a nutraceutical in children.

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Keywords Infectious diarrhoea, Beta-glucan, Management of acute gastroenteritis, Mucosal immunity, Innate immunity, Interventional trial.

Acute gastroenteritis (AGE) is a major cause of childhood morbidity and mortality worldwide. Oral or intravenous rehydration has been shown to be effective in reducing mortality associated with AGE. However, it does not affect disease progression. New treatment that can effectively relieve the symptoms are therefore needed. Pleuran (β-(1,3/1,6)-D-glucan) is of particular interest in the treatment of acute gastrointestinal infections. It has potential immunomodulatory properties targeting the innate intestine immunity and a strong safety profile. However, the efficacy of pleuran in AGE treatment has not been investigated. In this manuscript, we present the results of an original randomised, double-blind, placebo-controlled trial to assess the efficacy of pleuran in the treatment of AGE in children, conducted according to the CONSORT Statement (See Supplementary information).

Background

The prevalence of AGE in children aged < 3 years in Europe is 0.5- 2 episodes per child per year[1]. AGE is the third leading cause of child mortality worldwide. It kills more than 400,000 children under the age of five each year[2]. Most deaths occur in developing countries because of limited access to safe water and health care. In developed countries, the death rate from AGE is much lower. However, infectious diarrhoea remains a common cause of paediatric emergency department visits and hospital admissions[1]. Age under 6 months, early weaning, immunodeficiency and malnutrition are risk factors for severe or persistent diarrhoea[3]. Prolonged hospitalisation is associated with exposure to infectious agents, stress, catheter-related bloodstream infections and high costs. In addition, prolonged illness contributes to lost parental working days[4]. Therefore, finding an effective way to shorten and alleviate AGE symptoms would not only benefit children's health, but also reduce social costs.

Few interventions to reduce the duration and severity of diarrhoea have been shown to be effective in AGE. Adequate rehydration is recommended as a first-line treatment, preferably with oral rehydration solutions (ORS)[3,5]. Oral rehydration reduces mortality, the need for hospitalisation and intravenous rehydration, but has no effect on the duration or intensity of AGE. Probiotics may reduce the length of hospital stay and relieve symptoms of AGE in children[3,5–7]. However, recommendations for their use are inconsistent[8,9]. European Society for Paediatric Gastroenterology Hepatology and Nutrition/ European Society for Paediatric Infectious Diseases (ESPGHAN/ESPID) and the Infectious Diseases Society of America (IDSA) currently recommend 4 probiotic

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strains for AGE: *Lacticaseibacillus rhamnosus GG* and *Saccharomyces boulardii* (strong recommendation), *Limosilactobacillus reuteri* DSM 17,938 and heat-like *Lacidophilus LB* (weak recommendation). The authors of these guidelines rated the quality of the evidence as low or very low[1,3,5]. In contrast, the National Institute for Health and Care Excellence (NICE) found the evidence insufficient to recommend routine use of probiotics in this indication[10]. According to ESPGHAN/ESPID, both diosmectide and racecadotril can be considered for treating AGE in hospitalised and outpatients[3,5,10]. A lactose-free diet shortens the duration of diarrhoea, but only in inpatients[11]. Most guidelines do not support routine zinc supplementation in high-income countries, although it may be beneficial for children in developing countries[3,8,9]. Evidence for the effectiveness of other therapies (symbiotics, prebiotics, gelatine tannate, activated charcoal, kaolin-pectin) in treating and reducing the symptoms of diarrhoea is insufficient to recommend their routine use[3].

Further studies are needed to evaluate new therapeutic interventions that are effective in reducing the duration and severity of diarrhoea and the length of hospital stay.

Beta-Glucans

Beta-glucans are high molecular weight polysaccharides found in the cell walls of bacteria, fungi, marine algae and cereal grains. They exert multi-directional beneficial effects on the human body. The immunomodulatory, anti-infective, anti-tumour, hypocholesterolemic, hypoglycaemic and antihypertensive properties are the most studied[12,13]. The natural origin of β -glucans from common dietary components reduces concerns about their safety. They are well tolerated. No serious adverse events have been reported following β-glucan administration[14–18].

The anti-infective properties of β-glucans may be related to their immunomodulatory effect. However, direct antiviral and antimicrobial activity of β-glucans has also been described in several studies[12,19]. In clinical trials, oral β-glucans have been associated with reduced incidence of respiratory infections in paediatric populations[14,16,17,20], healthy adults[21], the elderly[22], athletes[23] and chronically stressed adults[24]. It has been suggested that they may help to reduce the severity of SARS-CoV-2 infection and play an important role in regulating cytokine production in patients with the cytokine storm and hyperinflammation observed during COVID-19[25,26]. Recently, it has been reported that β-glucans selectively stimulate the growth of a small number of beneficial bacteria, therefore they have prebiotic properties[27,28].

The structure and physicochemical characteristics of β-glucans strongly influence their properties. β-1,3-glucans with β-1,6 side chains are the most active immunomodulators[29]. Medium or large molecular weight, triple helix conformation in aqueous environment and a low degree of branching (between 20 and 33%) correlate with the highest immunostimulatory and antitumour activity[12,13,18,30]. Water-insoluble β-glucans have stronger immunostimulating properties than water-soluble ones[31]. Although intestinal absorption of insoluble polysaccharides is very low (systemic blood levels less than 0.5%) or undetectable, significant systemic immunostimulation is observed following oral ingestion of insoluble β-glucans. This phenomenon can be explained by the role of M cells and dendritic cell pseudopods, which transport insoluble β-glucan or its fragments from the intestinal lumen to the lymph fluid and gut-associated lymphoid tissue (Peyer's patches)[32–34].

β-glucans never occur naturally in the human body. Therefore, they are recognised by pattern recognition receptors (PRRs) as pathogen-associated molecular patterns (PAMPs). They trigger signalling pathways to stimulate the innate immune response, as well as mucosal immunity via induction of various inflammatory cytokines, chemokines and interferons[31,35]. PRRs activation initiates pathogen-specific humoral responses by activating B and T lymphocytes. It has also been suggested that β-glucans are inducers of an innate immune memory called Trained Immunity (TRIM)[26,36]. After exposure to a specific stimulus, innate immune cells undergo metabolic, mitochondrial and epigenetic changes, followed by phenotypic memory of enhanced immune responses to secondary heterologous stimulus. It remains controversial whether β-glucans can induce an adaptive immune response. However, studies have shown increased T-cell activity and interferon-ϒ production following interaction with the β-glucan-dendritic cell complex[37].

Pleuran

Pleuran is an insoluble β-(1,3/1,6)-glucan isolated from the popular dietary oyster mushroom (*Pleurotus ostreatus*). It was identified and chemically characterised by Karacsonyi and Kuniak[38]). It has a β-1,3-linked main chain with D-glucosyl side groups attached to every third backbone unit with β-1,6 links and a degree of branching of 0.25. The molecular weight of pleuran is between 600 and 700 kDa. Many studies have reported pleiotropic properties of pleuran, in particular: immunostimulatory, antitumour, hypocholesterolemic, hypoglycaemic and antidiabetic[39–42]. The immunomodulatory properties of pleuran have been demonstrated in numerous studies. Much of this research has focused on the treatment and prevention of infections in children and adults, including reducing the frequency of respiratory tract infections (RTI) in children with recurrent RTI, relieving symptoms associated with upper RTI, and treating HSV infections[14–17,20]. In all of these studies, pleuran was administered on a chronic basis, with treatment durations ranging from 3[14–16] to 6 months[17]. Pleuran is derived from a common dietary ingredient and is therefore considered to be a highly safe nutraceutical. No serious adverse events were observed in any of the above studies.

Pleuran is a promising candidate for the treatment of AGE in children due to its immunomodulatory and antiviral properties, the intestinal mucosa as the site of action, and its strong safety profile.

Objectives

This study aims to evaluate pleuran's efficacy in reducing the duration and severity of AGE. To ensure the accuracy of the results, we decided to use a placebo as a comparator.

Research hypothesis
Pleuran is more effective than a placebo in reducing the duration and severity of diarrhoea in children.

Methods
Trial design
This study is a multicentre randomized, double-blind, placebo-controlled superiority trial with two parallel groups and 1:1 allocation ratio. We used the CONSORT Statement to prepare this manuscript[43].

Protocol
The protocol of this trial was registered in the Clinical Trials platform (NCT03988257) before the study commenced and published in the British Medical Journal[44].

Research ethics approval
The Bioethics Committee of The Medical University of Warsaw approved the study protocol with all the following amendments and other required documents (an information sheet for patient's caregivers, informed consent forms in the local language version, and information on personal data management). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Study settings
We have recruited the study group from patients requiring hospitalization in the pediatric department or counselling in the emergency department (ED) due to AGE. The recruitment took place in three hospitals in Poland: The Paediatric Teaching Clinical Hospital of the Medical University of Warsaw, the Department of Pediatrics, St Hedwig of Silesia Hospital in Trzebnica, and the Department of Pediatrics of Warsaw Medical Center "KOPERNIK".

Eligibility criteria
Eligibility Criteria are presented in Table 1.

Interventions
Pre-intervention assessment
We assessed the eligibility criteria for the study by conducting physical examination and interviewing caregivers within 24 h of the patient's admission to the hospital or presentation into the ED. We provided the patient's caregiver with comprehensive information about the purpose, methods, safety, and duration of the study intervention. We have obtained written consent for the participation from the legal guardians of all patients. The patient's legal guardian had the right to withdraw the patient from the study at any time without negative consequences. We calculated body mass index (BMI) and converted it into percentiles using WHO percentile charts. Before starting the intervention, we collected stool samples to determine the aetiology of AGE (rotavirus, adenovirus and norovirus antigens from stool sample). When indicated, we also performed microbiological stool cultures.

Used scales
We used appropriate scoring systems to ensure comparability of the parameters and outcomes analysed. The severity of diarrhoea was assessed with the Modified Vesikari Score[45]. The degree of dehydration was evaluated by the WHO Scale[46] and the Clinical Dehydration Scale (CDS)[47]. The Bristol Stool Form Scale was applied to objectively assess stool consistency[48].

Inclusion criteria
• Children aged 13–120 months, hospitalized or requiring counselling in ED due to AGE, defined as a decrease in the stool consistency (grade 6 or 7 in Bristol Stool Form Scale[46]) or an increase in the frequency of stool evacuations > 3 in 24 h.
• The duration of symptoms: 24–72 h at the time of inclusion.
• A caregiver's written, informed consent.
Exclusion criteria
• The duration of symptoms > 72 h at the time of inclusion.
• Co-occurring other systemic infectious diseases (e.g., pneumonia, sepsis).
• Diagnosed immune deficiency.
• Malnutrition (defined as the body mass index (BMI) <-2 SDS (Standard Deviation Scores) or < 3rd percentile based on the World Health Organization (WHO) percentile charts.
• Chronic diarrhoeal gastrointestinal disease (inflammatory bowel disease, short bowel syndrome, cystic fibrosis, celiac disease, food allergy).
• The use of antibiotics, probiotics, prebiotics, or anti-diarrhoeal medicines (diosmectite, racecadotril) within seven days before enrolment into the study.
• The use of probiotics, diosmectite, and racecadotril during the current AGE episode.
• The use of pleuran in the 14 days before enrolment into the study.

Table 1. Eligibility criteria.

Intervention
Children in the experimental group received Imunoglukan PH4 oral suspension (10 mg of pleuran and 10 mg of vitamin C in 1 ml of syrup) at a dose of 1 ml per 5 kg of body weight. Patients in the control group received a placebo (10 mg of vitamin C in 1 ml of syrup) at a dose of 1 ml per 5 kg of body weight. Both suspensions were administered daily in the morning before the first meal. Treatment continued until symptoms of AGE resolved, as indicated by fewer than 3 stools in 24 h and normalisation of stool consistency (Bristol Stool Form Scale grade 2–5), or until day 14.

Concomitant treatment
Patients in both groups received an ORS or intravenous fluids as needed, based on their daily fluid requirements, to maintain adequate hydration. Regular diet was recommended. Each patient received antipyretic and analgesic (ibuprofen, acetaminophen) and anti-emetics (ondansetron) as needed. No other medications that could potentially interfere with the course of the disease, such as probiotics, diosmectide, or racecadotril, were administered. When indicated, antibiotics were prescribed according to the ESPGHAN/ESPID guidelines[3].

Intervention modification
If vomiting occurred within 30 min after administration of the study drug, the dose was repeated. However, if vomiting occurred more than 30 min after administration, we considered the dose to be absorbed. In the event of severe, persistent or troublesome adverse events (AEs), we were prepared to discontinue the intervention. Mild allergic reactions were treated with antihistamine medication. All adverse events were documented in the study records and reported to the manufacturer according to the established procedure. If a child had difficulty tolerating the preparation, it could be mixed with milk, tea or fruit juice.

Outcomes
All outcome measures were evaluated daily until the 14th day of intervention, using the Symptoms Diary completed by the physician and the caregiver. Each day in Symptoms Diary was divided into 8-hour intervals. The number and consistency of stools were assessed at each time interval. Outcomes are presented in Table 2. An example of a Symptoms Diary, accompanied by a comprehensive demonstration illustrating the calculation of endpoints, can be found in the Supplementary Information.

Participant timeline and Follow-up
During the hospitalisation or the emergency department visit, the physician examined the patients to assess their dehydration status, and the need for intravenous rehydration, or concomitant treatment. For hospitalised children, this assessment was performed daily. The evaluation results were documented in the Case Report Form (CRF). The patient's caregiver recorded AGE symptoms, such as the number of stools per day, their consistency, and any accompanying symptoms (fever, vomiting, abdominal pain), in a Symptom Diary during and after the hospitalisation. Following discharge, the physician contacted the caregiver via phone, daily during their treatment and every second day after the treatment ended (after the diarrhoea subsided), until the 14th day of observation. They recorded informations on the intensity of symptoms in CFR. After discharge, caregivers could contact the physicians if they needed advice.

Sample size calculation
We used the Altman nomogram to determine the sample size[47]. To demonstrate a clinically significant difference of 24 h in the duration of AGE symptoms in experimental and control groups, with a 5% significance level and a power of 90%, assuming the standard deviation of the variable in each group of 40 h, a sample of 110 patients was established as needed. To account for 10% of potential dropouts or attrition during the study, we increased the sample size to 120 patients (60 patients in each group).

Recruitment
Study participants were recruited from patients admitted to hospital or consulted in the ED due to AGE in three centres: The Paediatric Teaching Clinical Hospital of the Medical University of Warsaw, the Department

Primary outcome
The duration of diarrhoea is defined as the time (hours) until the stool consistency normalization (grade 2, 3, 4, or 5 according to the Stool Form Scale[46]) and until normalization of the number of stools per day.
Secondary outcomes
1. The number of days with the need for intravenous rehydration. A physician used CDS[49] and WHO[48] dehydration scales and physical examination to assess the indication for intravenous rehydration and the possibility of oral rehydration.
2. The number of hours until normalization of stool consistency (defined as the beginning of the first 8-hour time interval when Bristol Stool Form Scale was 2–5 [46]).
3. The number of hours until the normalization of a number of stools per day (defined as the beginning of the first 24 h period (3 consecutive time intervals) when the total number of stools did not exceed 3).
4. The duration of hospitalization due to AGE (number of days) in hospitalized children.
5. The percentage of children with moderate and severe diarrhoea was evaluated with the Modified Vesikari Scale[47] after the 14th day of observation.
6. The number of hours until the child's general condition improved according to the caregiver's assessment.

Table 2. Outcome measures.

of Pediatrics of Warsaw Medical Center "KOPERNIK", and the Department of Pediatrics, St Hedwig of Silesia Hospital in Trzebnica. The inclusion and exclusion criteria were assessed in every patient admitted to paediatric departments or ED due to AGE.

Randomization

We implemented a stratified randomization, using two subgroups based on the duration of diarrhoea at enrolment: 24–48 h (referred to as short-lasting diarrhoea - SLD) and 48–72 h (referred to as long-lasting diarrhoea - LLD). The participants of each subgroup were randomly allocated to either group A (experimental) or B (control) in blocks of four.

Allocation and blinding

The manufacturer prepared and packaged the active product and the placebo in identical bottles. These bottles were then labelled with random numbers, the meaning of which was kept blind. The randomisation list prepared by the manufacturer was placed in a sealed envelope in the administrative part of the department and kept by an independent person not involved in the study. The study product was delivered to the principal investigator in boxes of four, ensuring that each box contained two active and two placebo samples. Subsequent boxes were used to allocate consecutive patients within the subgroups. On opening each subsequent box, the researcher randomly assigned the numbers on the bottles to the next four patients in a subgroup. The contents of all bottles looked and tasted the same to maintain blinding. The researchers, carers, patients and the person responsible for statistical analysis were blind to the intervention until the end of the trial.

Data collection, data management and confidentiality

A coded paper CRF was completed to record each patient's scales and Symptom Diaries. Two independent researchers transferred the data from the CRFs into two electronic databases at the end of the study to ensure data accuracy. In case of drop-out, data and endpoints were collected up to the date of dropout, with the reason and date of dropout documented. Confidentiality of data, including personal data of study participants, was maintained.

Statistical methods

The analyses were performed using R V. 4.1.3 (2022-03-10) and Rstudio V.2022.12.0 + 353 (2022-12-0) with the help of "rstatix"[49], "tidyverse"[50], and "flextable"[51] packages. The Shapiro-Wilk W test was employed to ascertain whether the sample exhibited non-normality evidence. The Student's t-test was used to compare the means of continuous variables that conformed to a normal distribution. In contrast, the Mann-Whitney U test was used for non-normally distributed variables. The mean difference (M_{diff}) with a 95% CI was calculated for normally distributed variables, while the Hodges-Lehmann estimator was used to estimate the median difference (Me_{diff}) and its confidence interval for non-normally distributed variables. The χ^2 test, together with Phi or Cramer's V effect size, as appropriate, was used to compare proportions for the categorical variables. The difference between study groups was deemed significant when the 95% CI for mean/median difference did not include 0 (equivalent to $p < 0.05$) and the p-value for χ^2 was smaller than 0.05. All statistical analyses were conducted using a two-tailed test with a significance level of 5%. The IQR in Tables 3 and 4 is defined as the difference between the 25th percentile (Q1) and the 75th percentile (Q3). These percentiles were calculated using R's default Type 7 quantile method, which follows the interpolation approach recommended by Hyndman and Fan (1996). We have decided to use interquartile range as one of several established methods for computing sample quantiles.

Harms

We carefully recorded all AEs on the CRF after enrolment and throughout the observation period. These AEs were promptly reported to the manufacturer according to a predefined procedure. An AE was considered serious if it met one of the following criteria: death, life-threatening condition, serious or permanent disability, prolonged hospitalisation, or significant risk.

Results

Recruitment started in June 2019 and ended in December 2022. Twenty-seven children were enrolled and randomly assigned to one of the two groups – 13 patients in group A (experimental) and 14 in group B (control). Due to a lost contact with the caregivers and therefore inability to collect measurements, it was necessary to exclude one patient from the analysis of the duration of diarrhoea, the need for antibiotics, the severity of diarrhoea according to the Vesikari scale, the time to normalisation of stool consistency, the time to normalisation of the number of stools, and the time to improvement of the child's general condition according to the caregiver's assessment. For these outcomes, 26 patients were included in the analysis (12 and 14 in groups A and B, respectively). All 27 children were included in the assessment of intravenous rehydration duration (13 and 14 in groups A and B respectively). We excluded children who only had an emergency department visit from the analysis of length of hospitalisation ($n = 9$), because the intervention did not affect the length of hospital stay. Therefore, we evaluated 18 patients (8 and 10 from groups A and B, respectively) for this outcome. All analyses were performed in the groups as originally assigned.

After the study was completed, we were able to contact the caregiver of the lost patient from group A - the reason for not answering the phone was a difficult family situation; they did not observe any adverse events and the patient stopped taking the study medication after leaving the hospital.

Baseline demographic and clinical characteristics are shown in Table 3. Group A had a higher proportion of female participants, more prolonged diarrhoea, and more episodes of vomiting and abdominal pain. In group B, the aetiology of diarrhoea was more often related to rotavirus. However, all observed differences were deemed

Characteristics	Group A $N=13$	Group B $N=14$	P values
Duration of diarrhoea at the time of the intervention (hours), mean (SD)	42.46 (13.62)	41.71 (12.62)	0.804
Duration of diarrhoea at the time of the intervention (hours), median (IQR)	48.0 (24)	40.0 (14)	
Age (months), mean (SD)	32.85 (28.05)	38.29 (31.45)	0.481
Age (months), median (IQR)	30.0 (20)	26.5 (23)	
Sex n (%) (W)	8 (61.5)	4 (28.6)	0.182
Duration of diarrhoea at the time of enrolment (strata SLD/ LLD n (%) (LLD)	5 (38.5)	3 (21.4)	0.585
Vomiting n (%)	9 (69.2)	5 (35.7)	0.175
Stomach Pain n (%)	12 (92.3)	8 (57.1)	0.100
Fever n (%)	8 (61.5)	7 (50)	0.830
Need for antibiotic use n (%)	2 (16.7)	2 (14.3)	1.00
Etiology of acute gastroenteritis n (%)			0.566
Norovirus	1 (7.7)	0 (0)	
Rotavirus	3 (23.1)	6 (42.9)	
Salmonella	1 (7.7)	1 (7.1)	
Unknown	8 (61.5)	7 (50)	
Dehydration level before enrolment according to the Clinical Dehydration Scale (no/some/moderate-severe) n (%)			1.000
Mild	9 (69.2)	9 (64.3)	
Not present	4 (30.8)	5 (35.7)	
Dehydration level before enrolment according to the WHO scale (no/some/severe) n (%)			0.560
Mild	3 (23.1)	4 (28.6)	
Not present	9 (69.2)	10 (71.4)	
Severe	1 (7.7)	0 (0)	

Table 3. Baseline demographic and clinical characteristics. W- woman, SLD- short-lasting diarrhea, LLD- long-lasting diarrhea, WHO- World Health Organisation, SD- standard deviation, IQR- interquartile range.

Outcomes	Group A	Group B	*P* values	MD/V	95% CI
Duration of diarrhoea (hours), median (IQR)	96.0 (70.0)	116.0 (32.0)	0.836	-8.00	-48 to 40
Duration of hospitalization (days), mean (SD)	3.5 (1.8)	3.4 (1.1)	0.891	0.10	-1.47 to 1.67
Duration of intravenous rehydration (days), median (IQR)	1.0 (1.0)	2.0 (1.8)	0.545	0.00	-1 to 1
Time until normalization of stool consistency (hours), median (IQR)	96.0 (70.0)	92.0 (64.0)	0.485	8.00	-24 to 64
Time until normalization of the number of stools (hours), median (IQR)	56.0 (52.0)	84.0 (52.0)	0.226	-24.00	-64 to 24
Time until the improvement of the child's general condition according to the caregiver's assessment (days), median (IQR)	2.5 (2.5)	1.5 (3.0)	0.598	0.00	-1 to 2
Severity of diarrhoea according to the Vesikari Scale, mean, mean (SD)	10.6 (1.6)	9.6 (2.9)	0.311	0.94	-0.94 to 2.82
Duration of diarrhoea since intervention (hours), median (IQR)	56.0 (76.0)	84.0 (44.0)	0.605	-8.00	-48 to 40
Time until normalization of stool consistency since intervention (hours), median (IQR)	56.0 (76.0)	56.0 (66.0)	0.757	8.00	-32 to 56
Time until normalization of the numbers of stools since intervention (hours), median (IQR)	12.0 (54.0)	32.0 (70.0)	0.268	-24.00	-72 to 24
The severity of diarrhoea according to the Vesikari Scale (mild/moderate/severe) (n), %			0.653	0.18	
Mild	1 (8.3%)	3 (21.4%)			
Moderate	8 (66.7%)	8 (57.1%)			
Severe	3 (25.0%)	3 (21.4%)			
Adverse events (n), %			0.970	0.01	
No	12(92.3%)	14(100.0%)			
Yes	1 (7.7%)	0 (0.0%)			

Table 4. Clinical endpoints measures summary. MD- mean or median difference, as appropriate; NA- not applicable; V- Cramer's V or Phi, as appropriate; SD- standard deviation, IQR- interquartile range.

statistically insignificant which means that the two groups were similar in terms of baseline demographics and clinical characteristics.

Clinical endpoints

A summary of the endpoint measures is shown in Table 4. The duration of diarrhoea since diagnosis (Me_{diff} = -8 h, 95% CI -48 to 40) as well as from intervention (Me_{diff} = -8 h, 95% CI -48 to 40), the duration of hospitalisation (M_{diff} = 0,1 days, 95% CI -1,47 to 1,67) and the duration of intravenous rehydration (Me_{diff} = 0 days, 95% CI -1 to 1) were comparable in both groups. Similarly, there were no significant differences in time until normalisation of stool consistency both since diagnosis (Me_{diff} = 8 h, 95% CI -24 to 64) as well as intervention (Me_{diff} = 8 h, 95% CI -32 to 56). No differences in time until normalisation of the numbers of stools, since diagnosis (Me_{diff} = -24 h, 95% CI -64 to 24) and intervention (Me_{diff} = -24 h, 95% CI -72 to 24) was found. Groups also did not differ in time until the improvement of the child's general condition according to the caregiver's assessment (Me_{diff} = 0 days, 95% CI -1 to 2) and severity of diarrhoea according to Vesikari Scale (M_{diff} = 0.94, 95% CI -0.94 to 2.82). 26 children did not experience adverse events ($V = 0.01$, $p = 0.970$). One patient from experimental group experienced a rash. The AE was classified as mild and managed with antihistaminic medication. The causal relationship between the study medication and the rash was assessed as possible. Chronology was compatible as the adverse event appeared 2 days after the treatment initiation. However, a viral infection could be an alternative explanation.

Discussion

Principal findings

The aim of this study was to evaluate the efficacy of pleuran in reducing the duration and severity of AGE. We demonstrated that pleuran was ineffective in shortening the duration of AGE and alleviating its symptoms in a paediatric population. A small advantage towards the experimental group in terms of duration of diarrhoea, duration of intravenous rehydration and time to normalisation of stool count was not statistically significant.

Apart from small differences, the baseline characteristics of the experimental and control groups were comparable. We excluded children who only attended an emergency department from the analysis of the endpoint "The duration of hospitalisation", because the intervention did not affect the length of hospital stay. Therefore, there was no cause-and-effect relationship. Given that the average duration of acute infectious diarrhoea is 5–7 days, we decided to exclude patients with diarrhoea lasting longer than 72 h from the study. We assumed that these children's symptoms may have resolved spontaneously regardless of treatment.

No serious adverse events were observed during the study. This observation is consistent with other studies using pleuran and other β-glucans. β-Glucans are derived from popular dietary sources and show no signs of toxicity. They are therefore considered to be safe.

To the best of our knowledge, this is the first and only RCT to evaluate the efficacy of pleuran or other β-glucans in the treatment of acute gastroenteritis in children. However, several reports have evaluated the efficacy and safety of pleuran in the prevention of recurrent respiratory tract infections (RRTIs) in children. A significant reduction in the incidence of both upper and lower respiratory tract infections was reported in RCT by Jesenak et al. ($n = 175$)[17]. The authors of multicentre open-label trial demonstrated a statistically significant reduction in the incidence of RTIs in children after minimum 3 months of pleuran supplementation, compared to the same group in the year before the study ($n = 1030$)[14]. Urbancikova et al. reported a shorter duration of HSV infection after short-term administration of pleuran, as well as shorter upper respiratory tract symptoms after long-term therapy in both children (above 6 years of age) and adults[15].

Strengths of the study

The strength of this trial is its randomised, fully blinded, placebo-controlled design. Standard Protocol Items: The Recommendations for Interventional Trials (SPIRIT) Checklist was used to prepare the study protocol, to ensure the implementation of appropriate methods[52]. In addition, the CONSORT statement was used to prepare this manuscript[43] (See Supplementary Information). Stratified randomisation with two subgroups ensured an even distribution of children with shorter and longer duration of diarrhoea at inclusion in the experimental and control groups. We decided to present the duration of diarrhoea, the time to normalisation of stool count and the time to normalisation of stool consistency as two values - from the onset of symptoms and from the start of the intervention. Although the sample size is small, this study can be used as a pilot study for further research. Our experience has allowed us to point in the direction of future studies in this area.

Limitations

The main limitation of this study is the small sample size. Before the study started, we calculated that 120 patients would be an adequate sample size. However, we have managed to recruit 27 patients, which is 23% of the expected number. During the first year of the trial, the main factor contributing to delayed recruitment was a significant number of patients meeting at least one exclusion criterion. The most common exclusion criteria were symptoms lasting more than 72 h at the time of presentation to hospital, parents administering probiotics before seeking medical advice, and patients younger than 13 months. We saw a positive trend: parents now have easy access to knowledge enabling them to confidently provide basic treatment for gastroenteritis, such as oral rehydration and probiotics, at home. As a result, they only report to the hospital after a few days if the home treatment fails. Two protocol amendments were introduced during the trial to improve the pace of recruitment (see Supplementary Information for a detailed description of these amendments). In the following months, the profile of hospitalised patients changed significantly due to the SARS-CoV-2 pandemic. Most children with acute gastroenteritis were treated as outpatients. This made it even more difficult to recruit the planned number of patients. Since the global SARS-CoV-2 pandemic subsided (January 2022), the pace of recruitment has increased significantly, but

not enough to make up for the previous two years. However, the probability of a significant effect with a larger group appears to remain low, given the considerable width of the confidence interval for the observed difference between the two groups in the endpoints analysed (at the 95% confidence level).

In this study, we focused exclusively on the clinical effect of pleuran. It would have been beneficial to test the effect of β-glucan supplementation on laboratory parameters demonstrating immune responses. In particular, mucosal immunity markers in pleuran users, e.g. salivary lysozyme or IgA levels, would provide a more comprehensive understanding of the mechanisms underlying the enhancement of the immune response. However, there is a wealth of data available on the effects of β-glucans on various laboratory markers of immune stimulation in children[17,53–56]. Another limitation of the study is that the follow-up was based on a conversation with the patient's caregivers, without a personal examination of the patient, or controlling the amount of used product. We decided to use telephone follow-up because we expected poor compliance if parents had to return to hospital with a healthy child or return the bottle with the study medication at the end of the treatment. The assessment of the patient's symptoms and condition after discharge from hospital was based on the parents' subjective assessment, which could be a source of bias.

Vitamin C as comparator

In our study, we chose to use a commercially available pleuran preparation that also contains vitamin C. In order to minimise the effect of this vitamin on the results of the study, a matching placebo was manufactured, containing the same concentration of vitamin C. The used dose of vitamin C (2 mg/kg of body weight, maximum 50 mg/day) meets the EFSA recommended daily allowance for children[57]. The longest ingestion of the preparation was 13 days. To our knowledge, there are no data indicating a possible effect of this dose on the duration or severity of diarrhoea, either in children or adults. We found no studies concerning the role of vitamin C in acute gastroenteritis treatment. Although an effect of vitamin C on immune mechanisms in humans has been suggested for years, studies to date have not confirmed those hypotheses. According to Cochrane Systemic Review, chronic use of much higher doses of vitamin C led to a small and clinically insignificant reduction in the duration of the common cold of 14% and 18% with the use of 200 mg /day and 1000 mg/ day respectively[58].

Probiotics as exclusion criteria

In order to increase the reliability of the study results, we decided to exclude patients who had been given probiotics during the episode of AGE. Probiotics have been shown to reduce the length of hospital stay and to relieve the symptoms of AGE in children[3,5–7]. However, there is not enough evidence that they work to recommend them routinely. Guidelines from major medical societies are inconsistent, ranging from "should be considered"[3,5] to "are not recommended"[10]. The administration of probiotics prior to recruitment was one of the main factors contributing to the under-enrolment of the sample. Given that a significant number of patients now receive a probiotic prior to medical advice, the authors of this study believe that further research should not exclude the use of probiotics, but instead administer pleuran as an adjunctive treatment. This approach is also supported by the potential prebiotic properties of β-glucans, which could enhance the effectiveness of probiotics. It is important to note that all study participants should receive the same strain of probiotic bacteria. Moreover, a β-glucan with proven prebiotic properties for particular probiotic strain should be used. For example, studies have shown the stimulatory effect of pleuran on the growth of *Lacticaseibacillus* strains, so this β-glucan-probiotic pair could be one of potential choices for further trials[59].

Conclusions

Despite promising results in children with respiratory infections, our experimental study shows that the use of pleuran does not reduce the duration and symptoms of acute gastroenteritis in children. Therefore, there is no evidence to support its use in infectious diarrhoea. However, given pleuran's ability to stimulate intestinal innate immunity, prebiotic properties and good tolerability, larger studies are needed to further investigate its potential as a nutraceutical in the paediatric population. The authors of this article hypothesise that the combination of pleuran with probiotic *Lacticaseibacillus* strains, particularly those demonstrated to alleviate the symptoms of gastroenteritis in children (e.g. *Lacticaseibacillus rhamnosus* GG or *Limosilactobacillus reuteri* DSM 17938), may represent a promising avenue for further investigation. This study can be used as a pilot study for further research.

Data availability

The study protocol is publicly available via the ClinicalTrials platform (NCT03988257) and published in BMJ. The data that support the findings of this study are available upon reasonable request from corresponding author. Data requestors would need to sign a data access agreement to gain access.

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Author contributions

All authors meet The ICMJE Guidelines for Authorship. Anna Piwowarczyk (AP) initially conceptualized the study. Katarzyna Wzorek-Łyczko (KWŁ), Ernest Kuchar (EK), AP, and Henryk Szymański (HSz) initiated the study design and helped with its implementation. KWŁ and WW (Weronika Woźniak) conducted the study. KWŁ and AP provided statistical expertise and analysed the data with blinded statistician supervision. EK was a grant holder. KWŁ and AP, wrote the first draft of the manuscript. All authors have contributed to and approved the final version.

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Declarations

Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. An agreement between the Medical University of Warsaw (WUM) and the PLEURAN s.r.o. was described above.

Additional information

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