

Original Article

A new approach to the treatment of acute infection diseases with antibiotic-pectin formulae

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Abstract

Introduction: Intestinal infections are a significant health issue; antibiotics are essential in treating acute intestinal infections. However, evidence in the literature shows that the excessive use of antibiotics has created many threats to human health. This work aimed to study the impact of apple pectin in combination with antibiotics on treating patients with amebiasis and dysentery.

Methodology: Patients suffering from acute intestinal diseases (amebiasis and dysentery) were treated with traditional antibiotic therapy and a new formula containing antibiotics with low and high methoxylated apple pectin in a randomized block design. Four clinical trials were performed at the Infection Disease Hospital from 1998 until 2013.

Results: The study demonstrated that the antibiotic-pectin formulae (APF) significantly reduced the severity of acute intestinal infection diseases and allowed patients to recover faster than conventional treatment. APF reduced the patient's stay in the hospital by 3.0 ± 1.0 days. The clinical trial findings demonstrated that applying APF in intestinal infection diseases helped maintain a constant concentration of the antibiotic in the blood and accelerated the clinical recovery of the patients.

Conclusions: It was concluded that using pectin with antibiotics could improve clinical outcomes in patients with acute infectious diseases. Research on elucidating the mechanisms of pectin digestion in the colon, polyphenol content, and its role in dysbiosis recovery, etc., is also considered.

Keywords: amebiasis; dysentery; pectin; metronidazole; ciprofloxacin; tetracycline.

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Introduction

Gastrointestinal (GI) infections are an essential reason for disease and death worldwide, particularly in developing countries [1]. Diarrheal illness ranks among the top ten causes of mortality globally and poses a significant threat to children under five years in areas with limited resources. Conversely, in affluent regions, diarrhea is typically viewed as a minor inconvenience for healthy adults [2,3].

Bacterial-induced diarrhea can lead to severe cases of acute gastrointestinal distress. Dysentery, characterized by bloody stools with or without mucus, indicates a more invasive bacterial infection. The primary culprits behind bacterial diarrhea include *Escherichia coli* (prevalent globally), *Shigella*, *Salmonella*, *Campylobacter* (especially common in children), *Yersinia*, and various *Clostridium* species. Traveler's diarrhea commonly stems from shiga-toxin producing *E. coli* (STEC), along with *Shigella*, *Salmonella*, *Entamoeba histolytica*, *Giardia*, *Cryptosporidium*, *Cyclospora*, and enteric viruses.

Predictions suggest that the ongoing climate change will escalate diarrhea cases in Asia between 2030 and 2050 [5].

Treating intestinal infections is a significant health issue worldwide, and antibiotics are essential in treating patients suffering from severe diarrhea or acute intestinal infections. However, antibiotic resistance is a significant danger to global health, food security, and development. Although new antibiotics are in the developmental pipeline, none are anticipated to be efficacious against the most harmful strains of antibiotic-resistant bacteria [6]. At the same time, virtually any antibacterial agent can have life-threatening side effects [7] because normal intestinal flora can prevent the formation of a colony of microbes in the intestine [8,9]. Hence, protecting this unique system from various antigens is the first and most crucial step in treating GI diseases.

Pectin, found in fruits and vegetables, is increasingly recognized as a precursor for intestinal flora and may facilitate intestinal recovery after

enterocolitis [10-17]. Our reports on the effect of pectin in the complex treatment of acute intestinal diseases and typhoid fever and in the treatment of patients with cholelithiasis [12] were based on the beneficial effect of the fruit pectins on the microbial ecosystem and the elimination of toxic compounds from the body, including the secondary bile acids.

Pectins, commonly present in our daily diet and utilized as food additives, resist digestion by human enzymes. However, they can be broken down by gut bacteria in the colon into short-chain fatty acids (SCFAs). Our understanding of the impact of pectin on gut homeostasis and gut microbial communities remains limited. Research has demonstrated that orally administered pectin can suppress the development of intestinal wall inflammation in experimental animals, and the anti-inflammatory effects of pectins are focused explicitly on commercial pectins derived mainly from citrus and apple sources [13]. Due to its confirmed immunomodulating, anti-inflammatory, antioxidant, hypolipidemic, antidiabetic, anticarcinogenic, antitussive, gastroprotective, and wound-healing properties, pectin has recently been termed “universal medicine” [14]. Recently, there's been widespread concern about the global risks posed by particulate matter (PM). Research suggests that adding fermentable fiber like pectin to diets can change the composition of the gut microbiota, boosting the presence of Bacteroidetes and reducing the ratio of Firmicutes to Bacteroidetes. Pectin can relieve PM-induced pulmonary inflammation by altering intestinal microbiota composition and SCFA production [15].

A group of Italian scientists led by professors R Ciriminna and M Pagliaro [16] has provided an updated overview of the antimicrobial behavior of pectins, including pioneer works of the 20th century from early 1937 and 1970-1990 [16]. Furthermore, Ciriminna *et al.* proposed that polyphenolic compounds enhance pectin's antibacterial properties. They discovered that lemon pectin obtained by mild hydrolysis via hydrodynamic cavitation of the citrus industry's waste (IntegroPectin) displays significant antibacterial activity against *Staphylococcus aureus*. This Gram-positive pathogen easily contaminates food. Their findings demonstrated that the new “IntegroPectin”'s antibacterial properties exceed those of traditional citrus pectin, paving the path for innovative uses. It can be produced cheaply from waste generated by the citrus juice industry.

Various studies have indicated a significant decrease in the risk of *Helicobacter pylori* infection with higher intake of fruits and/or vegetables and

increased consumption of vitamin C, beta-carotene, and pectin [17]. The findings from this study indicate that pectin exhibits antibacterial effects against all 16 clinical isolates and two reference strains of *H. pylori*. Notably, the most substantial antibacterial effect occurs at a low pH (5.0) compared to higher pH levels, and the lowest minimum inhibitory concentration was 16 mg/L. Kothandaraman *et al.* [18] conjugated a water-insoluble polyene antibiotic to oxidize pectin through an imine linkage and then assessed its effectiveness against fungal strains such as *C. albicans* and *A. fumigatus*. The study highlighted that oxidized pectin conjugates have promising anti-fungal and anti-leishmanial properties.

The rise in antimicrobial resistance has resulted in a surge of infectious diseases worldwide, leading to accelerated rates of illness and mortality. In response, there has been an increased demand to develop novel and enhanced antimicrobial agents and explore innovative and effective methods for administering and targeting antibiotics [19]. Because of the high cost, lengthy process, and limited success rate of discovering new classes of compounds, one approach to address this challenge could involve enhancing existing antimicrobial compounds to reduce their impact on the intestinal microbiota and minimize their side effects. Therefore, developing a new approach to increase the effectiveness and decrease the toxicity of existing antimicrobial drugs is essential.

In recent years, different sources and types of pectin have been evaluated in humans. Some clinical studies have provided evidence that pectin can help with the problems associated with the side effects of antibacterial agents [20–24]. The study's results demonstrated that the source of pectin and type (degree of esterification, DE; and molecular weight, MW) affected the cholesterol level [20]. Clinical evidence suggests the ingestion of pectin-containing fruits (e.g., apple) mitigates niacin-induced flushing [21]. A randomized trial of fecal microbiota transplantation pectin treatment in patients with active ulcerative colitis (UC), there was a reduction in the Mayo score, potentially attributed to the preservation of gut flora diversity after controlling UC [22]. The findings from four clinical trials evaluating the effectiveness and safety of a thickening complex containing pectin in managing regurgitation in infants revealed a significant reduction in the daily number of regurgitation episodes on days 3 and 14 compared to the baseline in all studies ($p < 0.001$). Thus, the most significant decrease was observed in the formula with the highest pectin content (study 1; $p < 0.001$) [23,24].

Table 1. Study design.

STUDY 1			
Patient Group	First trial	Patient Group	Second trial
A (n = 16)	Metronidazole	C (n = 20)	Tetracycline
B (n = 16)	Metronidazole + LMAP	D (n = 20)	Tetracycline + LMAP
STUDY 2			
Patient Group	Third trial	Patient Group	Fourth trial
E (n = 15)	Metronidazole	G (n = 18)	Ciprofloxacin
F (n = 18)	Metronidazole + HMAP	H (n = 16)	Ciprofloxacin + HMAP

LMAP: low methoxylated apple pectin; HMAP: high methoxylated apple pectin.

Ried *et al.* tested the Nutrition Care Gut Relief Formula containing curcumin, aloe vera, slippery elm, guar gum, pectin, peppermint oil, and glutamine over three months. They found it effective in improving GI symptoms and gut health in adults with digestive disorders [25]. Bian *et al.* found evidence that an extract containing low molecular weight citrus pectin beneficially improved gut microbiota dysbiosis induced by antibiotics, improved gut mucosa integrity, and increased the production of short-chain fatty acids [26].

Thus, pectin as soluble dietary fibers (DF) can be a potential therapeutic agent for both diarrhea and constipation events during GI disorders. However, a well-controlled clinical study still needs to assess these effects. We hypothesized that when compared to antibiotics alone, a combination of apple pectin with antibiotics protects the intestinal mucosa from the adverse effects of toxic metabolites of antibacterial agents and reduces the severity of acute intestinal infections, which allows the patients to recover faster.

We aimed to study the impact of two types of apple pectin in combination with the antibiotics on the treatment progress of patients with amebiasis and dysentery in a series of clinical trials.

Methodology

Study design

The study was designed as a randomized block experiment where patients were selected only after identification of amebiasis and dysentery bacteria in their feces. Patients suffering from diabetes mellitus and liver diseases were excluded from all studies. The study design is presented in Table 1. The following sections describe the details of the clinical trial. The clinical trial registration number was TPhA MHSP RT230021-14, valid till 2019. The original TPhA 42-

0395 was not registered with the National Institutes of Health US Library of Medicine.

Pectin treatment formulae

Two apple pectin formulae were used in this study. The first formula was a mixture of 2.0 g low molar mass and low methoxylated apple pectin (LMAP) with 10 g sugar dissolved in 200 mL of deionized water. The second formula was a 200 mL freshly extracted high molar mass and high methoxylated pectin (HMAP) solution purified by dialultrafiltration and 10 g sugar. The control formula was 10 g sugar dissolved in boiled water and designated as sugar water solution (SWS). These formulae were assigned to the experimental and control groups of patients who suffered from acute gastrointestinal tract diseases. The patients were required to drink an antibiotic dose after pectin or SWS solution. The content of the pectin formulae was described in Pectin Pharmacopeia [27] which was approved by the Pharmacological Committee of the Ministry of Health of the Republic of Tajikistan in 1995 for five years, and extended twice until 2025.

It is worth noting that the amount of pectin administered to the patients of any adult group did not exceed the recommendation of the European Food Safety Authority Panel on Contaminants in the Food Chain regarding pectin exposure [28].

Pectin characteristics

Pectin was extracted from apple pomace by steam-assisted flash extraction and purified by dialultrafiltration methods [29]. The main characteristics of the pectin samples, such as anhydrogalacturonic acid (AGA) content, DE, neutral sugar (NS) composition, polyphenol content (mg gallic acid equivalent (GAE) on gram pectin powder), and MW are presented in Table 2.

Table 2. Pectin’s sample characterization.

Pectin formula	AGA %	DE %	Neutral sugar composition, %					Polyphenol content, mg GAE/g	M _w , KD
			Rha	Ara	Xyl	Gal	Clc		
LMAP	65.5	38.0	2.02	7.50	0.80	4.32	12.68	0.652	113
HMAP	59.4	68.6	5.62	9.58	3.43	4.94	0.70	0.834	165

LMAP: low methoxylated apple pectin; HMAP: high methoxylated apple pectin; AGA: anhydrogalacturonic acid; DE: degree of esterification; Rha: rhamnose; Ara: arabinose; Xyl: xylose; Gal: galactose; Clc: glucose; GAE: gallic acid equivalent; M_w: molar mass; KD: Kilo Dalton.

The specifications for apple LMAP and HMAP pectins that were purified by diaultrafiltration and centrifugation with regards to the content (mg/kg) of the toxic components were as provided in the Pectin Pharmacopeia [26], and assessed by the Atomic absorption spectroscopy method. The values were as follows (mean of 3-year analysis): total arsenic: 1.24 ± 0.3 ; lead: 1.32 ± 0.4 ; mercury: 0.16 ± 0.2 ; cadmium: 0.17 ± 0.3 ; and aluminum: 1.13 ± 0.6 . The lower amounts of toxic metals in the pectin sample were due to the removal of the microgels that these metals formed with the pectin by centrifugation. Microbiological data were provided for five batches of LMAP and six extracts of HMAP (documentation provided to Pectin Pharmacopeia). In all the samples, the total viable count was < 10 CFU/g, yeasts < 10 CFU/g, molds < 10 CFU/g, *E. coli* negative in 10 g, and *Salmonella* spp. negative in 15 g. The analyses were performed with the Research Institute of Prophylactic Medicine (Health Ministry of Tajikistan Republic) in accordance with International Organization for Standardization (ISO – 6579-1) methods.

Determination of metronidazole in the blood and feces

Blood samples were collected by venipuncture to collect 2 mL of blood, or 5 g of feces sample was taken from each patient. Serum was obtained by low-speed centrifugation for 30 min. Metronidazole concentration on the day of treatment in blood plasma or feces was determined by the simple, accurate, and cost-efficient UV spectrophotometric method [30]. The proposed method involves dissolving metronidazole in a 0.1 HCl and acetonitrile mixture. The maximum absorption was at 278 nm using a UV-Vis Spectrometer (Thermo Scientific UV 1, London, United Kingdom). Standard metronidazole (Sigma Aldrich, Berlin, Germany) was dissolved in a 0.1 M HCl solution and used for calibration. The concentration range was 1-30 $\mu\text{g/mL}$ ($y = 0.035x - 0.0073$, $R^2 = 0.99$). The sample solution was stable for up to 36 hours. Metronidazole was extracted after plasma protein precipitation by methanol. 2 mL of acetonitrile and 0.1 N hydrochloric acid solution (1:1) were added to 1 mL of deproteinated plasma. The tubes were vigorously shaken for 10 minutes and centrifuged (Hermle Labnet, Z 323, Kaiserslautern, Germany) at 10 000 revolutions per minute (rpm). The mother liquor was filtered using a 0.45 microns membrane filter. The plasma concentration of metronidazole was determined using a calibration curve.

Study 1 design and patients

The Institutional Review Board at the Department of Infectious Diseases of Avicenna Tajik State Medical University in Dushanbe reviewed the research protocols approved by the Infectious Diseases Hospital (Protocol No. 08 dated 1998). The study was a randomized (using MS Excel), parallel-group comparative study implemented in the Dushanbe City Infection Disease Hospital over a long period, mostly in the summer season, from 1998 until 1999. The patients, both men ($n = 42$) and women ($n = 30$), who suffered from acute infectious diseases (amebiasis and dysentery), were selected from the outpatient department and admitted into a special study ward. Patients suffering from diabetes mellitus and liver diseases were excluded from all studies. The duration of the trials was 8 days. All variable symptoms, such as body temperature, stool frequency, mucin and blood in the stool, number of bowel movements, clinical findings, and medications, including pectin and antibiotics intervention, were recorded in the personal health records. Each patient or his/her legally authorized representative provided written informed consent before the randomization trials.

First trials

Thirty-two patients, aged 18 to 48 years, with intestinal amebiasis were divided into two groups. Patients of Group A ($n = 16$) were assigned as the control group who received the conventional treatment of metronidazole 500 mg with 200 mL SWS. Patients of Group B ($n = 16$) received metronidazole 500 mg and 200 mL of pectin solution (LMAP), administered three times a day before meals.

Second trials

Forty patients diagnosed with dysentery, aged 18-50, were divided into two groups. Patients in Group C ($n = 20$) received tetracycline 200 mg thrice a day and 200 mL of SWS. Patients in Group D ($n = 20$) received tetracycline 200 mg and 200 mL of LMAP in the same manner as the control group.

Study 2 design and patients

The second study was conducted in the City Clinical Infectious Diseases Hospital in Dushanbe (Protocol No. 01 dated 2012) as a randomized block (using MS Excel); a parallel-group comparative study implemented over long periods, mostly summer season, from 2012 until 2014. The Institutional Review Board reviewed the research protocol at the Department of Infectious Diseases of Avicenna Tajik State Medical

University in Dushanbe and the Infectious Diseases Hospital approved it. The randomized, open-label, parallel-group study included 33 patients with intestinal amebiasis, aged 21-50 years and 34 patients in almost the same age group with dysentery. The use of the antibiotics-pectin formula in the therapy of both patient groups was carried out with their voluntary consent. The duration of the trials was 8 days. The treatment data and results were recorded as study 2.

Third trials

Thirty-three patients aged 18-48 with intestinal amebiasis were divided into two groups. Patients of Group E (n = 15) were assigned as the control group, who received the conventional treatment of metronidazole 500 mg with 200 mL SWS. Group F (n = 18) patients received metronidazole 500 mg and 200 mL of HMAP thrice daily before meals.

Fourth trials

Thirty-four patients aged 21-50 years and diagnosed with dysentery were divided into two groups. Patients in Group G (n = 18) received ciprofloxacin 200 mg 3 times a day and 200 mL of SWS. Patients of Group H (n = 16) received tetracycline 200 mg and 200 mL of HMAP in the same manner as the control group.

Clinical management

The diagnosis of patients was established by bacteriological and bacterioscopic detection of pathogenic microorganisms (*Shigella*, *Salmonella*, *Escherichia coli*, *Entamoeba histolytica*) in feces using the standard technique [31,32]. For the *Shigella* study, native feces were diluted with saline (dilution 1:10) and filtered to remove larger sediments and fats. The filtered fecal preparation was stored at 4 °C for retests. 0.1 mL of filtrate was inoculated with selective medium - SS (*Shigella*, *Salmonella*) agar in an incubator for 37 hours at 37 °C. An absolute number of colonies grown on plates and their content in 1 g of feces was calculated with dilutions and the amount of seeded material. To identify *Entamoeba histolytica*, at least 5-6 swabs were prepared from freshly isolated warm stool. Detection of erythrophages in feces indicates activity of the process or the presence of a widespread lesion of the colon. The smears were stained with Lugol's solution, and the vegetative forms of amoeba were observed under a microscope.

Body temperature, stool frequency, mucin presence, and blood in the feces were monitored every 4 hours. Recovery for individual patients was defined based on normal body temperature, stool frequency,

absence of blood and mucin in stool, and constitutional symptoms (such as tension during bowel movements, fever, etc.).

Statistical analysis

The data were processed statistically using OriginPro 2023 10.0.0.154 software. The normality of the distribution was determined using the Shapiro-Wilk test. The results showed that the distribution follows a Gaussian curve. The results were presented as mean (M) ± standard deviation (SD). Data were compared between groups using Student's t-test for independent samples. The difference was considered statistically significant if $p < 0.05$.

The recovery rate was determined on the complete analysis set for each study using the percentage of recovered patients, which was calculated according to the equation [33]:

$$E (\%) = \left(\frac{Pat_1}{S} \times Pat_0 \right) \times 100$$

Where: Pat_1 was the number of recovered patients, Pat_0 was the total number of patients, and S was the number of symptoms.

Results

Study 1. Treatments of acute infection diseases with LMAP formula

First trials

There was no significant difference in age ($p > 0.05$) between the patients in the first and second groups and no difference in the length of hospital stay during the two experiments. Although 25% of intestinal amebiasis patients in the first group and 75% in the second group had characteristic symptoms of recovery (within 3-4 days), they were left for follow-up. The patients of the study groups were discharged at the end of day 8.

The patients with intestinal amebiasis were diagnosed by bacterioscopic determination of the enteric pathogen *Entamoeba histolytica*. Among the 40 diagnosed patients with dysentery, 18 were determined by *Shigella flexneri* observation from feces, and the others were clinically determined. Although patients who had clinically defervesced, suffered from increasing lower abdomen pain and tenderness on the first intervention day, fecal urgency, tenesmus, and the passage of blood mucoid feces. In both cases, the intensity of diarrhea was observed from the 2nd to the 7th day. The stool frequency was 10-15 times daily in 6 patients, 15-20 times in 10 patients, and 20-25 times in other patients.

The results of treating patients with intestinal amebiasis control metronidazole + SWS formula and

Table 3. Normalization of symptoms in patients with intestinal amebiasis treated with control metronidazole + SWS formula (A) and metronidazole + LMAP formula (B).

Symptoms	Days													
	1		2		3		4		5		6		7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Normalization of stool frequency	0	0	2	3	6	8	8	14	10	16	14	-	16	-
Absence of mucus in stool	0	0	0	4	0	8	3	14	8	16	15	-	16	-
Absence of blood in stool	0	0	0	4	2	8	6	12	12	16	16	-	-	-
Vomit cessation	1	6	5	12	10	16	14	-	16	-	-	-	-	-
Body temperature	0	0	0	8	6	12	10	16	14	-	16	-	-	-

The patients did not receive the prescribed formula or have been discharged. SWS: sugar water solution; LMAP: low methoxylated apple pectin.

metronidazole + LMAP formula [33] are presented in Table 3. Normalization of stool frequency was observed in 2 patients on the 3rd day of therapy after control treatment of metronidazole with SWS formula administration. Full stool normalization of all examined patients occurred only on day 7.

Unlike the control group, the intervention of pectin in the second group resulted in stool normalization in 3 patients on the 2nd day, 8 patients on the 3rd day, and the rest on day 5 of treatment. Moreover, the other symptoms' recovery was observed 2 days earlier than the normalization of the stool frequency. Six patients stopped vomiting on the first day after pectin administration, and the rest of the patients did not complain of vomiting on the 3rd day.

Second trials

A similar picture was observed in the second trial on the patients (Group C and D) with acute dysentery induced by *Shigella flexner*. In this case, the patients had significantly higher body temperatures and more cases and duration of visible blood in feces than those with *Entamoeba histolytica* infection. It should be particularly emphasized that the temperature of patients increased up to 40 °C due to total intoxication of the body. As shown in Table 4, treatment of acute diarrhea with pectin-antibiotics formula causes normalization of all symptoms faster than administering the conventional procedure.

Study 2. Treatments of acute infection diseases with HMAP formula

Given the clinical outcome of the efficiency of pectin in two human trials, and as prebiotics maintain intestinal microbiota and reduce the severity of antibiotic toxicity, it is timely and essential to understand how pectin's structure delivered those effects. Two more human clinical trials were carried out to evaluate this finding. The same pectin was tested under controlled conditions but with different DE and MW. Subsequent studies discuss treating acute infectious diseases and the consumption of HM pectin (freshly prepared) differently by MW.

Third trials

In contrast to the previous experiments, we investigated the complementary application of fresh extracted HMAP, the correlations of antibiotics concentration in the patient's plasma and feces, and the restoration of amebiasis and dysentery symptoms. In this study, patients with intestinal amebiasis were treated in hospital wards using metronidazole + SWS and metronidazole + HMAP as described in the methodology section. The antibiotic-HMAP formula was well-tolerated by patients without apparent side effects. It is well known that the bioavailability of metronidazole oral formula is around 80% via the gut, and C_{max} occurs after 1-2 hours [34,35]. Metronidazole and its metabolites are primarily eliminated through the kidneys (approximately 77%), with a smaller portion excreted via the feces (around 14%) [35].

Table 4. Normalization of symptoms in patients with acute dysentery after treatment with (C) control antibiotic formula (tetracycline + SWS) and (D) antibiotic-pectin formula (tetracycline + LMAP).

Symptoms	Days													
	1		2		3		4		5		6		7	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D
Stool frequency	0	0	1	4	2	8	6	16	10	20	16	-	20	-
Absence of mucus in the stool	0	0	0	4	2	10	4	16	8	20	14	-	20	-
No blood in feces	0	0	0	4	2	8	4	16	10	20	16	-	20	-
Vomit cessation	1	6	6	16	10	20	16	20	20	-	-	-	-	-
Body temperature	0	6	2	10	6	14	8	20	14	-	20	-	-	-

The patients did not receive the prescribed formula or have been discharged. SWS: sugar water solution; LMAP: low methoxylated apple pectin.

The metronidazole concentration in the plasma (Group E) reached 17.93 mg/L in 2 hours and decreased to 8.26 mg/L in 8 hours after oral administration of metronidazole as M + SWS formula. In contrast, drug concentration in the plasma of patients after oral administration of metronidazole + HMAP formula in the 2nd group (Group F) was 18.67 mg/L after 2 hours and 13.38 mg/L after 8 hours. The determination of metronidazole in feces failed due to the lower drug excretion level and matrix interferences.

Fourth trials

To investigate the effect of antibiotics and antibiotics in combination with pectin as a new approach for treating acute dysentery, we examined 18 patients, including 10 men and 8 women. In the case of acute dysentery, regardless of the severity, it proceeded mainly with a colitis form. At the same time, 50% of patients had loose stools. Stool frequency is one indicator of the severity of acute dysentery. In our observations, stool frequency was up to 5 times a day in 45% of patients, 6 to 10 times in 40%, 11 to 20 times in 10%, and more than 20 times in 5% of patients. Another characteristic manifestation of dysentery is the appearance of tenesmus - excruciating pulling pains in the rectum after the act of defecation, which we noted in 24% of patients. Vomiting was observed in 48% of cases, and nausea was reported in 62% of patients.

The concentration of ciprofloxacin in the blood and feces was determined by the capillary electrophoresis (CE) method [36]. The fourth clinical study showed that ciprofloxacin concentration in the blood serum of Group H patients was 69.048 ± 3.921 mg/L on the first days and slowly dropped to a steady state. However, in Group G patients, plasma concentrations were: 39.109 ± 3.230 mg/L on day 3 and 35.606 ± 3.456 mg/L on day 6. At the same time, drug concentration in the plasma of Group G patients was significantly low on the first day (14.557 ± 3.923 mg/L), followed by a reduction up to 2.5 ± 4.0 mg/L. The data showed that the dynamics of the antibiotic concentration in feces changed in the same manner as in plasma. The mean value of the drug in Group H patients was 50.607 ± 4.201 mg/L on the first day. The value slowly decreased to 47.995 ± 3.257 on day 3 and 46.721 ± 3.161 on day 6. In the case of patients who received only ciprofloxacin, its concentration was low from the 1st day (16.134 ± 4.289 mg/L, reduced to 7.336 ± 4.673 mg/L). There was also clinical improvement in the patients who received the HMAP pectin formula. Stool normalization was observed after 4.0 ± 1.0 days; the disappearance of pain syndrome after 2.0 ± 1.0 days; and leveling tenesmus

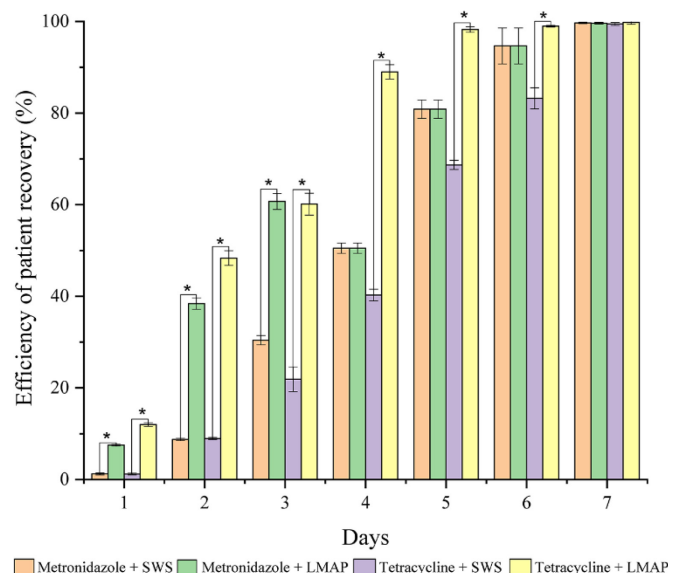
after 3.0 ± 1.0 days of treatment; while in the group of patients who received ciprofloxacin with SWS formula the above-listed parameters were 6.0 ± 1.0 days, 4.0 ± 1.0 days and 5.0 ± 1.0 days, respectively.

Discussion

Tetracyclines and ciprofloxacin are broad-spectrum antibiotics used to treat many common infections [37,38]. Tetracyclines have a broad spectrum and low cost. However, their use is limited because of side effects like permanent dental discoloration, enamel hypoplasia, and reversibly impaired bone growth [37,38]. It is because of these important side effects that tetracyclines were replaced by drugs with lower toxicity and similar effectiveness, such as the fluoroquinolone antibiotic ciprofloxacin. In this study, treatment of patients with pectin in combination with antibiotics (ciprofloxacin 200 mg tablet form instead of tetracycline as was used in the second trial) was carried out in a hospital setting. Ciprofloxacin has a serum half-life of approximately 4–6 hours, with around 50–70% of the administered dose excreted unchanged through the urine [38].

Metronidazole, ciprofloxacin, and their metabolites are primarily excreted through kidneys and a small part via feces (14%) [34,35]. Nevertheless, in this work, the application of pectin increased the bioavailability of antibiotics, which turned it into advantageous drug pharmacodynamics. From this point of view, the new formula of pectin-polyphenol-antibiotic can act as a

Figure 1. Comparative effectiveness of antibiotics + sugar water solution and antibiotics + low methoxylated apple pectin on recovery of patients with intestinal amebiasis and acute dysentery (Mean ± SD, *p < 0.05).



foundation for enhancing the effectiveness of current antibiotics. Therefore, the use of natural antimicrobials could play a positive role in reducing the rates of infection.

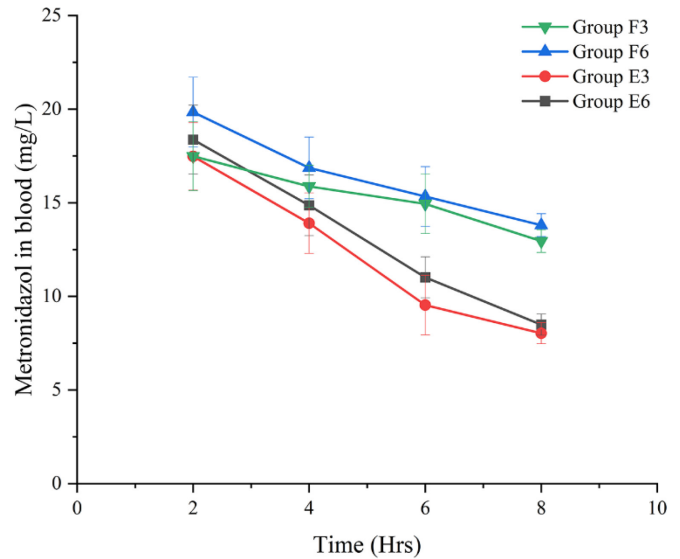
Figure 1 expresses the efficiency of the treatment in the first study (Groups A, B, and C, D) as a percentage of recovered patients. The findings indicated that the combination of pectin and antibiotics notably reduced the severity of acute intestinal infections, leading to faster patient recovery compared to traditional treatment methods.

Study 2 (trial 3) showed that the use of the metronidazole-HMAP pectin formula in the treatment of intestinal amoebiasis contributes to maintaining a high constant concentration of the leading drug in the blood and feces during the entire course of treatment. This leads to an acceleration of the clinical recovery of patients with the extinction of the main symptoms of the infectious process. The result was a reduction in the patient’s stay in the hospital by 3.0 ± 1.0 days.

Analysis of the mean drug concentration value distribution (Figure 2) showed that the metronidazole plasma concentration in patients who received the HMAP formula was higher than in the control group. The concentration level of metronidazole in the control group patients also reached its maximum value after 2 hours of administration. Still, during the monitored days (day 3 and day 6), it significantly decreased, and reached a minimum level of 8.26 mg/L within 8 hours.

As demonstrated in Figure 2, the use of the metronidazole-HMAP pectin formula in the treatment

Figure 2. Dynamics of metronidazole distribution in the blood plasma of patients with intestinal amebiasis on the 4th and 6th day of treatment.



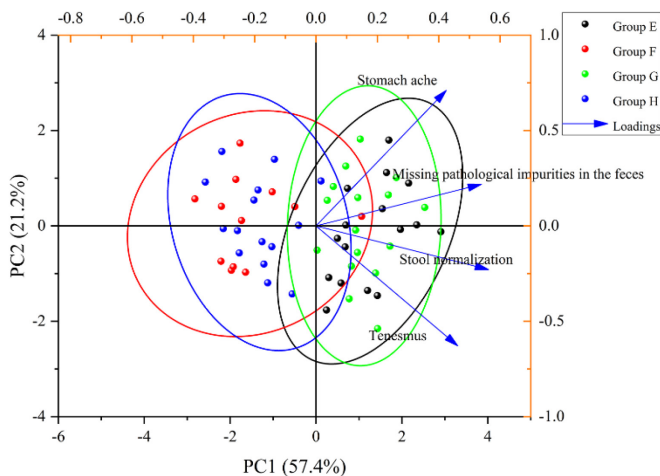
(▼) - the mean plasma metronidazole value in the blood of 6 patients of Group E on the 4th day of treatment; (▲) - the mean plasma metronidazole value of Group E on the 6th day of treatment, respectively. (●) - the mean plasma value of the metronidazole concentration of Group F on the 4th day, (□) - on day 6, respectively. * the differences are significant ($p < 0.05$).

of intestinal amoebiasis contributes to maintaining a concentration of the leading drug in the blood during the entire course of treatment, which leads to an acceleration of the clinical recovery of patients with the extinction of the main symptoms of the infectious process. The result is a reduction in the patient’s stay in the hospital by 3 days.

In the case of Group H patients receiving the HMAP-ciprofloxacin formula, all the patients tolerated the new procedure well, and no side effects were observed. The concentration of ciprofloxacin in the blood and feces was determined using the CE method. CE is particularly suitable for analyzing diverse, active pharmaceutical ingredients, including cationic, anionic, and other analytes, and offers a wide range of separation modes combined with various detection techniques. The advantages of high-performance CE over the existing methods are simple sample preparation (centrifugation, filtration) and the ability to determine small amounts of a substance within a short period [39].

The results obtained from the fourth trial also showed that the use of ciprofloxacin + HMAP in acute dysentery helps maintain a constant concentration of the antibiotic in the blood and feces and accelerates the clinical recovery of patients.

Figure 3. Principal component analysis score scatter plots of the disappearance of the main clinical symptoms in Groups E and F (metronidazole + sugar water solution and metronidazole + high methoxylated apple pectin) versus G and H (ciprofloxacin + sugar water solution and ciprofloxacin + high methoxylated apple pectin) patients with intestinal amebiasis. Ellipses indicate 95% confidence for corresponding groups.



We performed principal component analysis (PCA) to compare two groups (E-F and G-H) based on the treatment and control groups' main clinical symptoms to evaluate the treatment components' severity and reduction sufficiency. This method is very effective in dimensionality-reduction of the extensive data set by transforming a large set of variables into a smaller one to visualize them. Here, we used the statistical analysis method of PCA to find differences and cluster groups of patients treated with antibiotic + SWS and antibiotic + HMAP formulae based on the disappearance of the main clinical symptoms in treated and control groups. The results are shown in Figure 3.

As demonstrated in Figure 3, the principal components could account for 78.6% of the total variance. PC1 accounted for 57.4% of the conflict, and PC2 accounted for 21.2%. Except a few abnormal discrete samples, they can be grouped into two regions. The first region is grouped in blue and red, corresponding to patients treated with the antibiotic+HMAP formula. The second region is grouped with green and black corresponding to patients of the control group treated with antibiotic + SWS. This indicates that the variables of the experimental groups in the negative PC1 have higher score values of missing pathological impurities in the feces, stomachache, tenesmus, and stool normalization compared to the control groups. Principal component analysis based on the disappearance of the main clinical symptoms in Groups E, F, G, and H patients clearly showed a strong effect of the antibiotic + HMAP formula.

Overall, this research also opens new horizons for further study of the properties of pectin to bind and deliver antibiotics to the intestine. A review of the latest literature sources on the application of pectin polysaccharides shows that pectin's biological and physiological properties are paramount to further research and developments other than their use in the food industry. Further, it is essential to perform research on more precise elucidation of the mechanisms of pectin metabolism in the colon, the role of dysbiosis recovery, etc. Here we discuss available literature data on the possible action mechanisms of pectin and pectin conjugated from nature.

The effectiveness of pectin as both an antibacterial agent and an immune-modulating substance has been thoroughly investigated using *in vitro*, animal models [11,13,15,18,26,29,31], and subsequently in clinical trials [22–26]. Its action as dietary fiber (DF) in the human gastrointestinal tract was examined with a focus on four key aspects: (1) how plant cell walls influence its bioavailability, (2) impact on the rheological and

colloidal properties of digested materials, (3) ability to bind with phenolic compounds, bile salts, metal ions, and enzymes, (4) fermentation in the colon and the consequent effects on gut microbiota [16,32,39]. The interaction of DF and colon cells occurs directly via what are known as pattern recognition receptors. Citrus pectins, for instance, have shown health-promoting effects by directly engaging with Toll-like receptor 2 (TLR2) and the distribution patterns of methyl esters across homogalacturonan contribute to this immunomodulatory activity, prompting further investigation into molecular interactions with TLR2 [40].

Research has demonstrated that pectin-supplemented nutrition is effective, safe and improved clinical outcomes of patients in the intensive care unit [41]. A pilot study assessed the effect of pectin based on an infant formula on regurgitation and stool patterns in children less than 5 months old [23, 24]. Maruyama *et al.*, in a randomized clinical trial investigated the clinical effects of a pectin-containing oligomeric formula (POF) in tube-feeding patients compared to a standard polymeric formula (SPF). Results showed that patients receiving POF experienced significantly fewer composite events, including diarrhea, defecation treatments, and other enteral nutrition management-related events, than those receiving SPF. Specifically, diarrhea occurred less frequently in the POF group. The findings suggest that POF may be associated with a lower incidence of adverse events related to enteral nutrition than SPF. [42].

Recent research has demonstrated that non-digestible carbohydrates (NDCs) can benefit infants through at least two mechanisms. Firstly, they can interact directly with the epithelium or immune cells in the intestine. Secondly, they can support the development and colonization of gut microbiota in the colon. NDCs can act as substrates for microbiota fermentation, leading to alterations in microbiota composition, which in turn stimulate the immune system [43,44]. Pectin enhances mucus layer health and the integrity of the intestinal epithelium, thereby enhancing gut barrier function in a manner dependent on its structure. Low methoxylated (LM-) and high methoxylated (HM-) pectins influence mucin secretion through distinct mechanisms. LM pectin, characterized by non-esterified galacturonic acid residues, cannot interact with mucins due to its negative charge, allowing it to penetrate the mucus layer and directly stimulate goblet cells to produce mucus. Conversely, HM pectins can form hydrogen bonds with mucin,

resulting in a gel formation that reinforces the barrier function of the mucus layer [45].

Study 2 (trial 3) showed that the use of the metronidazole-HMAP pectin formula in the treatment of intestinal amoebiasis contributes to maintaining a high constant concentration of the main drug in blood and feces during the entire course of treatment, which leads to an acceleration of the clinical recovery of patients with the extinction of the main symptoms of the infectious process. The result is a reduction in the patient's stay in the hospital by 3.0 ± 1.0 days. The finding obtained from the 4th trial also showed that the use of ciprofloxacin + HMAP in acute dysentery helps maintain a constant concentration of the antibiotic in the blood and feces, and accelerates the clinical recovery of patients.

Other mechanisms involved in the apple pectin-antibiotics formula's effect concern the presence of polyphenol compounds in the pectin (Table 2). The synergetic effects of the pectin-polyphenol complex with antibiotics act as antimicrobial polymers that can inhibit bacteria [16]. The antibacterial effect of the new IntegroPectin was largely superior to that of commercial citrus pectin due to the polyphenols it contained, which were removed from the last sample by alcohol.

Taking advantage of the results of the two last trials, we suppose that the mean antibiotic concentration levels maintained in the blood of the patient's group with the pectin formula indicate a complexation of apple pectin containing a polyphenol moiety with both antibiotic molecules. The data of FTIR spectra of the pectin-antibiotic complex are not yet fully discussed and are hereby not presented in this article.

Previous researchers have explored the presence of phenols in pectins sourced from different origins. For instance, sugar beet pectin contains up to 0.7% (ferulic acid equivalents per gram of pectin) phenols [46]. In orange peel pectin, the phenolic content ranges from approximately 0.160 mg/g for acid extraction and 0.740 mg/g for water extraction. In comparison, both LM- and HM- apple pectins used in this study exhibited high levels of polyphenols (calculated as gallic acid equivalents), with 6.52 and 8.34 mg/g, respectively. However, it is worth noting that commercial pectin preparations, including apple pectin, do not demonstrate any specific anti-cancer actions, while pectins extracted from various sources and subsequently modified to low molecular weight, highly branched fractions rich in RG-I, have been shown to reduce proliferation rate and migration, as well as induce apoptosis in numerous cancer cell lines [46]. A

recent study [47] revealed that the structural properties of LM- and HM- pectins determine fermentability, affect microbial composition and metabolite production, and modulate immune responses. The authors stated that consumption of HM-pectin preferentially altered the gut microbiota and suppressed pro-inflammatory immune responses.

Numerous studies have investigated the antibacterial properties of flavonoid-rich products, revealing that novel flavonoid compounds exhibit bactericidal rather than bacteriostatic effects. These compounds have been shown to enhance the activity of antibiotics through synergistic interactions [48-51]. Aprikain *et al.* [49] demonstrated that consuming phenolic-rich apple extract alongside apple pectin significantly impacted gut microbiota metabolism in the colon and lipid metabolism compared to consuming the phenolic-rich apple extract alone. This indicates a beneficial interaction between fiber and phenolic compounds.

Thus, future research should include the application of complex therapies with antibacterial or anti-inflammatory drugs, prebiotics, or probiotics to improve mucosal healing after colonic surgery. We particularly noticed the rapid cessation of vomiting and diarrhea, normalization of body temperature, appearance of appetite, and feeling of satiation among the examined patients with acute intestinal infections after administration of pectin, which indicates a favorable effect on the GI tract.

Conclusions

A comparative observational study of the effect of antibiotics in combination with apple pectins (antibiotic-pectin formula) was carried out. Compared to conventional therapy (control group), the benefits of the new treatment method included reduction of the recovery time of patients with an acute intestinal infection. Symptomatic observation demonstrated that the inclusion of pectin in the treatment of patients suffering from *Entamoeba histolytica* and *Shigella flexneri* infections contributes to the rapid cessation of vomiting, diarrhea, normalization of body temperature, and appearance of appetite, which indicates a favorable effect of the pectin against the traditional progression of the disease.

Additionally, pectin can mitigate the side effects associated with antibiotics. Further understanding these mechanisms could open the way for more effective therapeutic modulation of the human gut microbiota. It is safe to say that pectin-polyphenol conjugate with antibiotics was another finding of our two clinical trials.

Nevertheless, in this work, the application of pectin increased antibiotics' bioavailability, which turned it into advantageous drug pharmacodynamics. Prescribing pectin with an antibiotic can significantly change the extent of intestinal damage and help establish the bowel's physical barrier function by reducing intestinal permeability in the gut, as stated in several studies elsewhere. From this point of view, the new formula of pectin-polyphenol-antibiotic can serve as a base to improve the potency of existing antibiotics. Additional studies must include a larger ethnic group and a dual-mode combined pectin-antibiotics and polyphenol-antibiotics to be tested in the early stage. Further research that considers the influence of pectin metabolism in the colon and the knowledge of methanol-aldehyde formation on overall functionality will potentially improve the effect of clinically meaningful symptoms.

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Authors' contributions

Conceptualization: ZM, ER, LL; methodology: RK, JB, ZSh, GK; software: JB; validation: JB, ZM; formal analysis: ZSh.; investigation: RK; data curation: ZM, ER; writing - original draft preparation, ZM, ER; writing - review and editing: JB and LL; visualization: JB; supervision: LL, ZM, ER. All authors have read and agreed to the published version of the manuscript.

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