ELSEVIER

Enterex: Anti-diarrheic drug based on purified natural clinoptilolite

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A new anti-diarrheic drug for humans has been developed based on the physical and chemical properties of the purified natural clinoptilolite NZ. A series of physical, chemical, technological, pharmacological, microbiological, and clinical studies were successfully conducted to meet the requirements of the Cuban Drug Quality Agency. The most important results concerning the properties and biological mechanism of NZ are decribed in this paper. © Elsevier Science Inc. 1997

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INTRODUCTION

Since 1974, when Torii[1] reported a low incidence of death and sickness produced by scours and other intestinal diseases in swine fed on a diet containing 6% natural clinoptilolite from a Japanese deposit, the list of studies with similar results has grown by the year. The most remarkable works were done by England,[2] Mumpton and Fishman,[3] Vrzgula and Bartko,[4] Castro and Pastrana,[5] Vrzgula et al.,[6] and some other authors who reproduced and observed the prophylactic effect of 5–6% clinoptilolite added to the animals' diet on scours. Wells and Kilduff[7] studied the use of rat cake supplemented with clinoptilolite at the rate of 50 g/kg in male rats infected with *Nippistiongylus brasiliens*; these authors have reported that the dietary zeolite facilitated the restoration of the enzymes α-D-glucosidase and aminopeptidase activity in the small intestine of those rats recovering from infection.

Sardiñas et al.[8] conducted exciting research on the therapy of the diarrheic syndrome in three test groups of growing pigs: One group was administered doses of 20% natural clinoptilolite in the diet, whereas a second group of pigs was given Diarrex® an antibiotic drug, in doses of 200 mg/kg of body weight and a control group was kept without medical treatment. The recovery rates were 71.0% for Diarrex®, 58.5% for natural zeolite, and 17.1% for the control group. The mortality rate in each group was 13.0% for Diarrex®, 16.0% for natural zeolite, and 34.3% for the control group. The results were considered adequate taking into account the presence of bacteria as the etiology of diarrhea, *Treponema spp* (48.7%), *Escherichia coli* (12.8%), *Salmonella spp* (10.3%), and *Salmonella spp* + *Treponema spp* (10.3%), and the fact that the natural zeolite is not an antibiotic.

Bartko et al.[9] presented their results at the Natural Zeolites '93 Conference on the use of clinoptilolite-enriched tuff in doses of 2 g/kg of body weight for therapy and 1 g/kg of body weight for the prevention of diarrhea in calves. The effectiveness of clinoptilolite in preventing diarrhea was of 68.7% as compared to 18.0% in the control group. The authors did not report their results on the treatment of diarrhea. The doses of clinoptilolite for the treatment of diarrhea are large as reported in the work of Sardiñas et al.,[8] "who were based on the criteria that the suggested and non confirmed mechanisms of effect are: (1) alteration of metabolic acidosis through effects on osmotic pressure in the lumen of intestines; (2) slowing of the passage of the ingest; and (3) increasing the retention of enteropathogenic *E. Coli*."[9,10]

Based on the fact that the gastrointestinal system of pigs is most similar to that of humans and on the

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results of the above-mentioned researchers, we seriously evaluated the possibility of developing an anti-diarrheic drug based on natural clinoptilolite as an active material in the therapy of acute diarrheic diseases (ADD) in humans. Some important aspects were considered to meet the requirements of the pharmaceutical industry and drug administration agencies for a practically unknown raw material.[11] The subjects under analysis were:

1. The lack of homogeneity in the physical and chemical properties of natural zeolitic materials.
2. The technological properties of zeolitic materials.
3. The quality requirements.
4. The analytical methods of zeolites for the pharmaceutical industry.
5. The toxicological tests.
6. The pharmacological tests.
7. The stability of the zeolite raw material and its pharmaceutical form.
8. The clinical studies.

A comprehensive study has been conducted since 1988 by the experts of the University of Havana's Project Zeolitic Active Principles. This paper describes the most interesting results obtained by the group for the first generation of the anti-diarrheic drug Enterex, approved by the Cuban Drug Quality Control Agency in September 1995.[12] The first presentation of the ENTEREX as a drug under study to the medical community was made during the XXIII Pan-American Congress of Digestive Diseases in Buenos Aires in December 1993.

EXPERIMENTAL

There are in Cuba 21 occurrences of natural clinoptilolite of which 11 have been studied geologically and have been certified as deposits with an industrial reserve. In four of these deposits—Tasajeras, Castilla, Chorrillo, and San Andrés—there are working factories that crush, dry, mill, and classify by size the zeolitic rock[13]; the one used in developing drugs for humans was chosen from one of these four natural zeolite deposits with about 55–70% clinoptilolite content.

The well-characterized natural zeolite from the Tasajeras deposit, located in the Villa Clara province in the central region of Cuba,[14] was the raw material of choice for this study. This zeolitic rock was widely used in animal research by Castro and Pastrana,[5] Zaldivar et al.[15] Galindo et al.,[16] and other Cuban scientists. This zeolitic rock, as do other natural zeolites, presents enough variation in its phase and chemical composition to classify the rock as a nonhomogeneous pharmaceutical raw material, however, it meets the Quality Requirement NRIB 1152.[17] Nevertheless, this zeolitic raw material must be treated to improve its properties.

Beneficiation of zeolitic raw material

Few methods are described in literature that allow clinoptilolite recovery at 95% purity. We have developed a method for the beneficiation of the zeolitic raw material from the Tasajeras deposit considering the nonzeolitic mineral phases contained in the rock and their physical properties. The process can be used by the pharmaceutical industry without a significant modification of its technological basis. The purified clinoptilolite obtained by this method is a nontoxic pharmaceutical raw material.

This process was designed using the possibilities of the fluidized bed technique, which allows for the separation of particles suspended in a viscous fluid depending on size and mass density. In a cylindrical reactor with a bottom water inlet, a calculated mass of zeolitic material with narrow particle size range was fluidized. The water flux was altered to produce the desired separation of nonzeolitic mineral phases, quartz, feldspar, montmorillonite, iron oxide, noncrystalline alumisilicates, and detrital material, from zeolitic phases. The following operations were also part of the process:

1. Final cleaning and drying steps of the zeolitic material in a centrifugal machine.
2. Hot drying in a furnace at 150°C.
3. Separation of particle conglomerates.
4. Sterilization of NZ.

Physical, chemical, and technological properties

The characterization of NZ was made in compliance with the requirements of NRIB 1152[17]:

Mineral phase composition by X-ray diffraction analysis.
Chemical composition of major elements.
Toxic elements content (Pb, Cd, As, Hg, F).

The technological properties of NZ were tested according to United States Pharmacoepia (USP) XXIII requirements for solid pharmaceutical products:

Particle size.
Real and apparent density.
Humidity.
Rest angle.

The physical, chemical, and technological properties of the Enterex tablets were tested according to the USP XXIII requirements. The determination of NZ content and its stability as an active ingredient in the Enterex tablets were evaluated by 3 methods: (1) quantitative X-rays diffraction analysis[18]; (2) infrared spectroscopy; and (3) hydrogen ion exchange capacity.

The first one is a well-known subject to material science experts, but it is not so for pharmacists. In the second technique we used the assignment of vibration modes for the clinoptilolite from Tasajeras deposit proposed by Alonso.[19] The third technique was designed based on the ion exchange capacity of clinoptilolite with H^+ ions with the natural cations of the zeolitic phases.[20] The amount of H^+ exchanged in NZ is a linear function of NZ mass, from which a straight calibration line was obtained with a good linear regression coefficient; this method was presented to the Cuban Drug Quality Control Agency, which gave its approval.

The quality of the sterilization was evaluated by a

Table 1 Microorganisms used to determine the microbicide activity of NZ

Microorganism	NZ sensibility	ZZ sensibility
1. *Bacillus cereus* BSG 001	–	+
2. *Klebsiella pneumoniae* ISA	–	+
3. *Streptococcus cloacae* ATCC 23355	–	–
4. *Escherichia coli* ATCC 25922	–	+
5. *Shigella sonnei* ATCC 75931	–	+
6. *Salmonella anatum* BSG 012	–	+
7. Arizona 67341	–	+
8. *Staphylococcus epidermidis* BSG 021	–	+
9. *Escherichia coli* 44	–	+
10. *Basilus subtilis* ATCC 6633	–	–
11. *Pseudomona aeruginosa* ATCC 27853	–	–
12. *Shigella flexneri* BSG 009	–	–
13. Arizona 173-6734	–	–
14. *Proteus vulgaris* BSG 015	–	+
15. *Pseudomona aeruginosa*	–	+
16. *Proteus vulgaris* ATCC 13315	–	+
17. *Citrobacter freundii* BSG 032	–	+
18. *Escherichia coli* ATCC 25922	–	+
19. *Klebsiella pneumoniae* BSG 032	–	+
20. *Staphylococcus aureus* BSG 031	–	+
21. *Salmonella typhimurium* ATCC 14628	–	+
22. *Serratia marcencens* BSG 033	–	+
23. *Citrobacter freundii* ATCC 2090	–	+
24. *Klesiella pneumoniae* BSG 034	–	+
25. *Streptococcus freundii* BSG 036	–	+
26. *Enterobacter aerogenes* BSG 023	–	+
27. *Proteus mirabalis* BSG 013	–	+
28. *Salmonella typhimurium* BSG 030	–	+
29. *Providencia* ISA 21	–	–
30. *Shigella flexneri* ATCC 12027	–	–
31. *Proteus rettgeri* ATCC 1407	–	–
32. *Serratia marcencens* ATCC 8100	–	+
33. *Candida albicans* BSG 002	–	+
34. *Candida albicans* BSG 003	–	+
35. *Candida albicans* BSG 007	–	+

microbiological test following the procedure established for these products.[21]

Toxicological studies

A well-documented toxicological study for the gastrointestinal use of natural clinoptilolite from the Tasajeras deposit—Quality NRIB 1152—was conducted by Tillán et al.[22] The authors did not observe any biological damage in the rats to which different doses of this zeolite were administered for 90 days. Delgado et al.,[23] conducted simultaneous toxicological studies with this zeolite and proved that the gastric's proteolitic capacity and intestinal disacaridase potential were not modified. The low content of mordenite (<10%) present in this natural clinoptilolite did not produce biological damage to the gastrointestinal system and other organs of the animals.[22,23] To know the capability of mordenite as a hazardous material a toxicological study[24] was conducted in rats fed for 90 days with a diet supplemented with 5 and 10% of the natural mordenite rock from the Palmarito deposit, in the eastern region of Cuba (70% of mordenite). The results have indicated that the gastric's proteolitic capacity and intestinal disacaridase potential were not modified, as was the case for clinoptilolite. Nonbiological damage was detected in the histological studies of various organs of the animals submitted to the test.

The Cuban Drug Quality Control Agency has accepted these results and has permitted the use of clinoptilolite—Quality NRIB 1152—as a raw material by the pharmaceutical industry.

Microbiological test

Test no. 1

The possible microbicide activity of NZ was evaluated using 35 enteric strain bacteria Gram +, Gram −, and yeast (*Table 1*). Different doses of NZ, 0.5 to 15 g, were added to 100 ml of agar nutrient media in Erlenmeyer flasks. The Erlenmeyer flasks were sterilized in an autoclave at 121°C and 1 atm of pressure for 15 min. After this operation the sterilized nutrient media with NZ was placed on Petri plates at room temperature. The bacteria were disseminated in each plate using a Drigalski spatula. The incubation was done at 37°C, and the observation of the bacteria growth was observed at 24, 48, and 72 h.[25] The zeolite bactericide product ZZ developed from the same clinoptilolite[25] was used as a positive control of microbicide activity.

Test no. 2

The interaction between NZ and antibiotics—Tetracycline and Cloranfenicol—was studied using *Vibrio cholerae* serogroup 01 (Lima biotype El Tor, serotype Inaba)[26] cultivated in Soy Triton broth (TSB) at 37°C for 18 h. Tetracycline and Cloranfenicol were used at their minimum inhibitory doses, 12.9 and 3.125 μg/ml, respectively, whereas NZ was added at 10%. The sodium- and potassium-exchanged forms of NZ were used as controls to find a possible antibiotic capability against this micro-organism.

Table 2 Efficiency of the beneficiation process of zeolitic raw material

Lot	Zeolitic raw material (kg)	Zeolitic phase content (%)	By-product I (montmorillonite + detritus) (kg)	By-product II (quartz+feldspar+calcite) (kg)	NZ[a] (kg)	Zeolitic phase (%)
1	20	55.0	1.32	2.60	13.55	77.0
2	20	56.0	1.25	1.72	15.03	77.0
3	20	54.2	1.48	2.68	14.62	76.0

[a] Mineral phase analysis of NZ determined by X-ray diffraction.

Table 3 Chemical composition of NZ

	NZ (%)	Requirement (%)
Elements		
SiO_2	66.00	64.00–66.00
Al_2O_3	10.96	10.00–12.00
Fe_2O_3	1.80	1.50–2.20
FeO	0.5	0.20–0.5
MgO	0.90	0.30–0.90
CaO	4.51	2.50–6.00
Na_2O	0.97	1.00–2.00
K_2O	1.00	1.00–2.00
P_2O_5	0.05	0.05–0.07
H_2O	4.83	4.00–7.00
Toxic elements		
F	1 ppm	<10 ppm
Pb	2 ppm	<10 ppm
As	0.1 ppm	<3 ppm
Cd	0.5 ppm	<2 ppm
Hg	0.2 ppm	<5 ppm

Pharmacological studies

The effectiveness of Enterex and its mechanisms of action were proved in pharmacological tests conducted in animals.

Test no. 1

The DE50 test was performed using the intestinal transit test in Wistar rats. Two known anti-diarrheic drugs—Loperamide (2 mg/kg of body weight) and Kaoenterin (Kaolin + pepsin) (112 mg/kg of body weight), an absorber drug—were used as controls. The dosages of Enterex were 30 and 90 mg/kg of body weight. The drugs were administered to the animals using intragastric cannulas, and 5 min after administration activated charcoal was introduced as marker. Fifteen minutes later the animal was dissected to measure the intestinal dimensions and the transit length of the drugs.

Test no. 2

Two-day-old piglets affected by dysentery were separated into three groups: (1) for Enterex therapy (30 mg/kg of weight); (2) for Tetracan, a pool of tetracycline therapy, (200 mg/kg); and (3) for no therapy. The test was conducted for 72 h, and the therapy was applied every 24 h.

Different studies were conducted to establish the possible action mechanisms of NZ, basically adsorption and ion exchange reactions in gastric and intestinal juices.

Table 4 Physical and mechanical properties of NZ

Property	Sample 1	Sample 2	Sample 3
Particle size (mm)	<0.2	<0.2	<0.2
Apparent density (g/cm^3)	0.67 ± 0.02	0.69 ± 0.03	0.68 ± 0.05
Real density (g/cm^3)	1.50 ± 0.03	1.49 ± 0.05	1.50 ± 0.02
Porosity (%)	55.40	54.37	55.26
Rest angle (degree)	None	None	None
Flux velocity ($g/cm^2.sec$)	None	None	None
Humidity (%)	2.70	2.72	2.6

Table 5 Mineralogical analysis of Enterex tablets by X-ray diffraction

Sample	Mineral phases[a]	Zeolitic phases (%)
NZ	Heul-Clinop, Mord; Quartz; Montm(−); Calc(−)	72.8
Lot 1	Heul-Clinop, Mord; Quartz; Montm(−); Calc(−)	65.4
Lot 2	Heul-Clinop, Mord; Quartz; Montm(−); Calc(−)	69.4
Lot 3	Heul-Clinop, Mord; Quartz; Montm(−); Calc(−)	63.6

[a] Heul, Heulandite; Clinop, Clinoptilolite; Mord, Mordenite; Montm, Montmorillonite; and Calc, Calcite.

Clinical studies

Four different clinical studies were conducted following the recommendations of the Cuban Drug Quality Control Agency. Experimental procedures of each test are described in detail in the Enterex drug file No. 0823.

1. Effectiveness study of Enterex in 30 volunteer patients with nonspecific diarrhea.
2. Use of Enterex in the therapy of acute diarrheic diseases. Etiological study.
3. Enterex in the therapy of diarrheic disease in diabetic patients with vascular impairment (neuropathic diarrhea).
4. Enterex in the therapy of acute diarrheic diseases resulting from food intoxication.

RESULTS AND DISCUSSION

Beneficiation of zeolitic raw material

The beneficiation process was evaluated for different zeolitic raw materials obtained from the Tasajeras deposit with a wide range of nonzeolitic mineral phase compositions (30–50%). The results were considered good because the zeolitic phases were enriched by at least 15%, and a raw material with 50% of zeolitic phases meets the NRIB 1152 requirements of 60% zeolitic phases. In *Table 2* the results of the benefit process for three samples of 20 kg are presented. It may be observed that the enrichment of zeolitic phases is about 15%. A higher recovery of clinoptilolite could be achieved by changing the density of the fluid, but that would imply the use of hazardous substances, and the quality of the nontoxic material of NZ would be thus modified.

The NZ that we need for the anti-diarrheic drug

Table 6 Antibiotic effect of NZ and its sodium and potassium forms against *Vibrio cholerae* serogroup 01 (Lima biotype El Tor, serotype Inaba)

Sample	Number of colonies	%
Control	105	100.0
Na-NZ	104	99.0
K-NZ	97	92.3
NZ	81	77.0

Table 7 Effectiveness comparison of ENTEREX and two anti-diarrheic drugs for length intestinal transit test

Drugs (dosage)	Length of intestinal transit (%)
Loperamide (2 mg/kg)	23.12
Kaoenterin (112 mg/kg)	42.00
Enterex (478 mg/kg)	45.00

should be the one enriched in clinoptilolite-heulandite zeolite, but the process developed cannot improve the zeolitic phase content by over 20%. Therefore, as producers of the new drug we have established a new requirement for the reproduction of our results; NZ must have a zeolitic phase content in the range of 75–80%.

Physical, chemical, and technological properties

The chemical composition of NZ is presented in *Table 3* and is compared with the NRIB 1152 quality requirements. The analysis showed that NZ meets the quality controls.

The physical and technological properties of NZ as a raw material for pharmaceutical tablets are shown in *Table 4*. The results of flux velocity and rest angle tests are not adequate for a technological process where the powder should be dosed to a pressing machine. On the other hand, the pressing test of the NZ was negative, as expected. These results led us to design a NZ granule. The granule should run fluidly and be adequate for pressing. The pressing quality of the NZ granule was tested, and the results allow for the production of three lots of Enterex tablets. The values of the different properties determined for the tablets are in the range of acceptance for pharmaceutical solid products according to USP XXIII, as reported earlier.[27] The stability of the tablet's properties was also tested after 1, 2, and 3 years. The nonsignificant variations of the properties due to the passage of time was confirmed and accepted by the Cuban Drug Quality Control Agency.[12]

As expected, the zeolitic phase content in Enterex tablets did not change. *Table 5* shows the XRD analysis results for the three lots of Enterex tablets compared with the NZ analysis. The difference in the number of zeolitic phases results from the addition of inactive ingredients to shape the tablets. The i.r. spectroscopy also confirmed the stability of NZ in time, whereas neither frequency shifts nor new band appearance were observed in i.r. spectra during the 3 years of study.

The sterilization quality of NZ and Enterex tablets was tested after production at time zero and every 6 months thereafter. The results confirmed that the quality requirements were met. Three years later no microbiological contamination was detected in Enterex tablets stored in the cellophane film container.

Microbiological test

Test no. 1

In *Table 1* we present the results of the microbicide activity of NZ and ZZ, a proven zeolitic antiseptic, against enteropathogenic bacteria. The increasing retention of enteropathogenic *E. coli* observed by Bartko et al.[28] in a natural clinoptilolite was not confirmed by us. The study of pH variation, optic density, protein variation by electrophoresis, ionic penetration capacity, and osmotic activity permitted us to establish that the natural purified clinoptilolite NZ used in the production of the Enterex anti-diarrheic drug has no microbicide activity.

Test no. 2

In *Table 6* the results of the antibiotic effects test of NZ and its sodium and potassium forms against *V. cholerae* 01 are presented in comparison with the control group. It has been observed that the number of colonies of the microorganism is not significantly different from the control. In all cases the viability was over 50%, the standard figure for bactericide test. Therefore, we may conclude that none of the clinoptilolite forms tested has bactericide activity against *V. cholerae* 01 because the survival level of the vibrium was higher and comparable with the control.[26]

The test for the interference of NZ and its sodium and potassium forms with the action of the antibiotic drugs Tetracycline and Cloranfenicol, like the test with the nonmixed antibiotics, showed a total inhibition of bacteria growth, whereas in the control plate 180 colonies were detected. Therefore, it could be established that NZ and its Na and K forms do not interfere with the action of these two antibiotics in the *V. cholerae* 01 therapy.[26] This result is most significant inasmuch as the use of adsorbent anti-diarrheic drugs is not recommended for cholera therapy due to interference with the antibiotics.

Pharmacological studies

In *Table 7* the results of Test no. 1 are shown. As expected, Loperamide reduced the intestinal transit of

Table 8 Clinic recovery of growing swine with scours produced by Rotavirus

Therapy time (days)	Enterex[a] (n = 84)		Tetracan (n = 88)		Control (n = 64)	
	Recover	%	Recover	%	Recover	%
1	28	33.3	46	52.3	13	20.3
2	31	70.2	17	71.5	16	45.3
3	13	85.7	12	85.2	12	64.2
Nonrecover	6	8.1	5	5.6	18	28.1
Deaths	2	2.4	3	3.4	4	6.3

[a] Dosage: Enterex, 800 mg; Tetracan, 400 mg.

Table 9 Effectiveness of ENTEREX in ADD of nonspecific etiology in the urgency room of Hospital Dr. Carlos J. Finlay

	Men		Women		Total	
Therapy time (h)	*n*	%	*n*	%	*n*	%
<12	9	56.25	14	100.0	23	76.67
12–24	7	43.75			7	23.33
>24						
Total	16	100.0	14	100.0	30	100.0
Number of tablets[a]						
2	8	50.0	7	50.0	14	50.0
2–4	6	37.5	5	35.7	11	36.67
4–6	2	12.5	2	14.3	4	13.33

[a] Dose: 2 tablets every 4 h.

ingest, whereas Kaoenterin and Enterex did not have a significant influence. Enterex is not an antimotility drug such as Loperamide. This fact does not coincide with the proposal by other authors[3,5,9] who explained the action of natural clinoptilolite in animal nutrition based on a reduction of the intestinal transit. Zaldivar et al.[29] have confirmed this result in a test conducted in broiler chickens where they observed that the intestinal transit of ingest was the same in the control group with no additive in the diet as in the group fed with a diet using NZ as an additive, whereas the animals that received a diet with raw natural clinoptilolite had a faster intestinal transit. The authors demonstrated that the presence of Ca-carbonate and Mg-silicate in the zeolitic rock produces a Ca and Mg disorder in the animal's metabolism that prompts a faster intestinal transit.

The results of Test no. 1 also confirmed the hypothesis that NZ is not an absorber such as Kaoenterin. If water adsorption were the action mechanism of NZ (like kaolin), the dosage of Enterex would have to be 478 mg/kg of body weight, which is an excessive dosage. However, the effective dosage of Enterex obtained in Test no. 2 was 30 mg/kg of body weight. The results presented in *Table 8* are significant when compared with those by Sardiñas et al.,[8] who reported only a 58.5% recovery rate in piglets using a dosage of 20% of raw natural clinoptilolite in the diet, whereas the percentage of animals that recovered with Enterex was higher (85.7%) using a lower dosage. Our results also showed that Enterex and Tetracan are similarly effective in the therapy of diarrheas produced by rotavirus.

Action mechanisms

The series of studies conducted allows us to establish that NZ is an adsorber of:

Bile acids, one of the endogenic causes of diarrhea. This result led us to improve this property of NZ and to develop CZ, the active product of the hypocholesteremic drug Colestina.[30]

Aflatoxine B_1, a mycotoxin that produces severe toxicity in animals and humans.[31]

Glucose, whose high content in intestinal fluid acts as an irritant factor and whose transport through the intestinal cells is reversed during diarrhea. FZ, a hydrothermal modification of NZ, acts as a selective adsorber of glucose.[32]

Clinical studies

1. *Effectiveness study.*[33] *Table 9* shows the first documented results of a clinical study conducted in humans who were treated with an anti-diarrheic drug based on a purified natural zeolite and approved by the Cuban Drug Quality Control Agency. The study allowed us to establish the effective dose of Enterex—4 to 6 tablets—and the evolution time of diarrhea during the therapy—24 h. It should be noted that the patients under treatment were affected by acute diarrhea, whose etiology was not studied.
2. *Etiological study.*[34] In this second clinical study 73 volunteer patients affected by acute diarrheic disease were treated with Enterex using the doses established in the first study. The patients were also subjected to bacteriological and parasitological studies to establish the

Table 10 Results of the clinic study no. 2 (Doctor's office no. 26 Polyclinic Cristobal Labra)

Number of patients according the bacteriologic test and diarrhea evolution time						
	Bacteriologic test positive		Bacteriologic test negative		Total	
Therapy time ADD[a] (h)	*n*	%	*n*	%	*n*	%
<24	0	0	72	98.6	72	98.6
>24	1	1.4	0	0	1	1.4
Total	1	1.4	72	98.6	73	100.0
Number of patients according to parasitologic test and diarrhea evolution time						
	Parasitologic test positive		Parasitologic test negative		Total	
Therapy time ADD[a] (h)	*n*	%	*n*	%	*n*	%
<24	8	10.9	64	87.7	72	98.6
>24	0	0	1	1.4	1	1.4
Total	8	10.9	65	89.1	73	100.0

[a] Dose: 2 tablets every 4 h.

cause of the diarrhea. In *Table 10* the results of these studies are presented. It is observed that, even when the bacteriological and parasitological tests were positive, the diarrhea and its symptoms could be removed, whereas the pathogenic agents (*Shigella, Entoamoeba hystollitic,* and *Giardia lamblia*) were eliminated using the proper antibiotic after the treatment with Enterex. The microorganisms were not hidden by the presence of NZ at the intestinal lumen, contrary to kaolin or clay mineral antidiarrheic drugs. Also note that the effectiveness time (24 h) was the same as in study no. 1.

3. *Clinical study in diabetic patients with vascular impairments (neuropathic diarrhea).*[35] This was a comparative study with diphenoxilate of atropine, an antimotility drug; and it was the clinical study no. 3. The results (*Table 11*) did not show a significant difference between the two drugs, which is remarkable because neuropathic diarrhea is a syndrome that affects patients with vascular impairments produced by diabetes mellitus. Thus the recovery of the patients must be achieved in the first 24 h, and this was made possible by the application of Enterex therapy. It was demonstrated that a second dosage of our drug had no adverse side effects, contrary to diphenoxilate of atropine, which does not allow for a second dosage without adverse side effects.
4. *Clinical study in patients with acute diarrhea resulting from food intoxication.*[36] The main cause of acute diarrhea in adults is food intoxication, therefore, the fourth clinical study was conducted in a large population of volunteers (434) affected by this diarrhea. The results of the study are presented in *Table 12* where it is shown that 75.6% of the patients recovered from diarrhea in the first 24 h and 24.4% in 36 h. These figures confirm the results obtained in previous studies such as a significant reduction in time for the physiological evolution of an acute diarrhea—72 h—without adverse side effects.

Table 11 Comparative clinical test of Enterex and diphenoxilate of atropine in diabetic patients suffering of neuropathic diarrhea conducted at the Institute of Angiology and Vascular Surgery

Evolution of the patients	Enterex[a]		Diphenoxilate of atropine[b]	
	n[c]	%	*n*	%
Healed	29	80.5	9	75.0
Not healed	4	11.1	3	25.0
Healed with double dosage	3	8.3	no double dosages allowed	
Total healed	32	88.8	9	75.0

[a] Dosage: 10 tablets every 1 h (9 g).
[b] Dosage: 2 tablets every 4 h.
[c] Actually the number of patients healed by Enterex therapy is higher than 200.

Table 12 Enterex effectiveness in the therapy of patients with diarrhea produced by food intoxication (Hospital Dr. Carlos J. Finlay)

Therapy time ADD (h)	*n*	%
<24	328	75.6
24–36	106	24.4
Total	434	100.0

Side effects

Most patients have shown good tolerance to treatment with Enterex, though, like with all the prescription anti-diarrheic drugs, Enterex could have side effects. However, no patient dropped out of the four clinical studies with Enterex because of side effects.

Drug interactions

Enterex does not interact with Tetracycline and Cloranfenicol, the drugs normally used in the treatment of patients affected by enterobacteria such as *V. cholerae* 01. However, during the clinical studies the physicians suspended the use of parasymptomatic drugs usually involved in the therapy for diarrhea's symptoms. Some low physical adsorption of aspirin, thyophiline, propanolol, and phenobarbital on NZ have been determined by *in vitro* tests, therefore, further studies are still called for.

CONCLUSIONS

Enterex is the registered trade mark for the anti-diarrheic drug based on the physical and chemical properties of purified natural clinoptilolite-heulandite. In September 1995, the Cuban Drug Quality Control Agency approved Enterex as a new drug.

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