

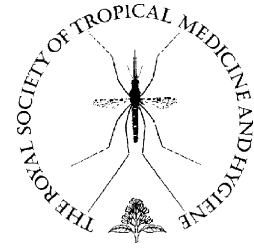


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Nitazoxanide in the treatment of amoebiasis

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KEYWORDS

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Treatment;
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Summary Amoebiasis is a significant cause of morbidity worldwide and is the third leading cause of death from parasitic diseases. This study evaluated nitazoxanide, a thiazolide anti-infective, in the treatment of intestinal and hepatic amoebiasis. Prospective, randomised, double-blind, placebo-controlled studies were conducted in outpatients with intestinal amoebiasis from the Nile Delta of Egypt. Nitazoxanide was administered twice daily for 3 days at doses of 500 mg (age ≥ 12 years), 200 mg (age 4–11 years) or 100 mg (age 1–3 years). Seventeen adults hospitalised with hepatic amoebiasis were treated with 500 mg nitazoxanide twice daily for 10 days. Four days after completion of therapy, 32 (94%) of 34 nitazoxanide-treated patients with intestinal amoebiasis resolved symptoms compared with 15 (50%) of 30 patients who received placebo ($P < 0.001$). Thirty-two (94%) of 34 nitazoxanide-treated patients were free of *Entamoeba histolytica* in two post-treatment stool specimens compared with only 13 (43%) of 30 patients receiving placebo ($P < 0.0001$). All patients with hepatic amoebiasis responded to nitazoxanide therapy. Nitazoxanide is effective in treating invasive intestinal amoebiasis and in eliminating *E. histolytica* colonisation of the intestinal tract. Further studies are warranted in patients with hepatic amoebiasis.

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1. Introduction

Amoebiasis is a widespread parasitic disease caused by *Entamoeba histolytica* and is the third leading cause of death

from parasitic diseases, surpassed only by malaria and schistosomiasis. It is a common disease in developing countries and an important health risk to travellers (Haque et al., 2003; Petri et al., 1999).

Entamoeba histolytica infection presents in a variety of forms; 90% of infections present as asymptomatic colonisation of the intestinal tract. Symptomatic disease ranges from transient colitis to fulminant colitis with an array of manifestations that may include dysentery, toxic megacolon and peritonitis to extraintestinal disease. Liver abscess or

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hepatic amoebiasis is the most common manifestation of extraintestinal disease. Unfortunately, the factors that control the invasiveness of *E. histolytica* are incompletely understood (Haque et al., 2003; Petri et al., 1999).

Treatment of *E. histolytica* infection depends on diagnosis, which differentiates *E. histolytica* from its more prevalent non-invasive, morphologically identical counterpart *E. dispar*. Standard ova and parasite examinations are useful for identifying *E. histolytica* or *E. dispar*, but they are unable to differentiate between the two. Serological tests to detect *E. histolytica* antibodies can be performed by indirect haemagglutination assay (IHA) or countercurrent immunoelectrophoresis. Antigen-based ELISAs are sensitive and specific for diagnosis of *E. histolytica* in stool but suffer from the need to examine fresh or frozen (not preserved) specimens. PCR techniques may also be useful but remain impractical in most parts of the world (Tanyuksel and Petri, 2003).

Symptomatic intestinal or extraintestinal amoebiasis is treated successfully with metronidazole or another nitroimidazole drug followed by a luminal amoebicide such as iodoquinol, paromomycin or diloxanide furoate. Given the risk of transmitting *E. histolytica* infection and the serious nature of invasive disease, treatment of asymptomatic infection with a luminal agent is recommended (Anonymous, 2004; Haque et al., 2003).

Nitazoxanide is a thiazolide anti-infective with activity against anaerobic bacteria, parasites and viruses (Fox and Saravolatz, 2005; Pankuch and Appelbaum, 2006; Rossignol et al., 2006a, 2006b, 2006c). It is approved in the USA (Alinia®; Romark Laboratories, Tampa, FL, USA) for treating diarrhoea and enteritis caused by *Cryptosporidium* spp. and *Giardia lamblia* and is undergoing clinical development for treating *Clostridium difficile*-associated disease, rotavirus and norovirus gastroenteritis, and chronic hepatitis C (Amadi et al., 2002; Musher et al., 2006; Ortiz et al., 2001; Rossignol and El-Gohary, 2006; Rossignol et al., 2006d).

In vitro, nitazoxanide and its active circulating metabolite tizoxanide are at least as potent as metronidazole against metronidazole-sensitive isolates of *E. histolytica* and maintain activity against isolates that are resistant or poorly susceptible to metronidazole (Adagu et al., 2002). Nitazoxanide was previously reported to be effective in treating diarrhoea associated with *E. histolytica/dispar*-positive stools, but that study did not differentiate between *E. histolytica* and *E. dispar* (Rossignol et al., 2001).

We conducted two double-blind, placebo-controlled studies of nitazoxanide in treating diarrhoea and enteritis associated with *E. histolytica* as the sole identified enteric pathogen, one study in adults and another in children. We also report results from 17 patients with hepatic amoebiasis treated with nitazoxanide.

2. Materials and methods

2.1. Study design

Two prospective, randomised, double-blind, placebo-controlled studies were performed, one in patients aged ≥ 12 years using nitazoxanide tablets and another in children aged 1–11 years using nitazoxanide oral suspension,

in order to evaluate the effectiveness of nitazoxanide in treating diarrhoea and enteritis caused by *E. histolytica* (*E. histolytica/dispar* cysts or trophozoites observed in stool by microscopic examination and antigen-based ELISA test-positive for *E. histolytica*). The studies were designed in compliance with published guidelines for evaluation of new anti-infective drugs for treating diarrhoea caused by *E. histolytica* (Cooperstock et al., 1992). In view of the variability of the disease in patients excreting *E. histolytica* cysts or trophozoites and the lack of randomised placebo-controlled trials of other drugs in patients with this disease, a placebo control was used for these studies. Any patient with worsening symptoms, such as blood in the stool, was removed from the study to receive rescue therapy and was accounted for as a treatment failure. The primary endpoint of the studies was clinical response recorded at the Day 7 follow-up visit. Clinical response was defined as either 'well' (no symptoms, no watery stools and no more than two soft stools, and no haematochezia within the past 24 h or no symptoms and no unformed stools within the past 48 h) or 'continuing illness'. Microbiological response, defined as either 'eradicated' (no *E. histolytica* cysts or trophozoites observed in either of two stool samples collected between study Days 7 and 10) or 'persistence', was evaluated as a secondary endpoint. Each study was designed to enrol 50 patients. Previous studies of nitazoxanide in treating diarrhoea caused by enteric protozoan pathogens suggested that response rates to nitazoxanide therapy using this study design should be at least 80%, whereas placebo response rates should be no more than 35%. Using these assumed response rates, a sample size of 50 patients (25 patients per treatment group) was deemed sufficiently powerful (88%) to demonstrate that treatment with nitazoxanide is superior to treatment with placebo using a two-sided Fisher's exact test and a 5% significance level.

2.2. Patients

Patients presenting with diarrhoea at the outpatient clinics of the Benha University Hospital in Benha, Egypt, or the University of Alexandria Hospital in Alexandria, Egypt, were screened for enrolment in the study. The screening was part of a broader programme to identify patients for placebo-controlled studies of nitazoxanide in treating diarrhoea and enteritis associated with enteric protozoa including *Cryptosporidium parvum*, *Giardia intestinalis* and *Blastocystis hominis*. Before screening, written informed consent was obtained from each of the adult patients, and in the case of children from their parents or guardians. Where possible, written informed consent was also obtained from the children. Patients with diarrhoea (≥ 3 unformed stools per day), one or more enteric symptoms (e.g. abdominal pain, nausea, vomiting, flatulence), *E. histolytica/dispar* trophozoites identified in stool by microscopic examination and stool-positive for *E. histolytica* by antigen-based ELISA were eligible for enrolment. Patients with other identified enteric pathogens, pregnant and lactating females, patients using any drug with antiprotozoal activity within 2 weeks of enrolment, and patients known to have or suspected of having AIDS or other immune deficiencies were excluded from the studies.

2.3. Assessment of the cause of diarrhoea and enteritis

All stool samples were subjected to a direct examination, an examination after concentration, a Ziehl-Neelsen stain and immunofluorescence assay (MeriFluor®; Meridian Diagnostics, Cincinnati, OH, USA) for parasitic causes of diarrhoea and enteritis. Stool samples were also subjected to an antigen-based ELISA test (*E. histolytica* II; Techlab Inc., Blacksburg, VA, USA) to differentiate between *E. histolytica* and *E. dispar*. The *E. histolytica* II ELISA test is approved by the United States Food and Drug Administration, with sensitivity ranging from 96.9% to 100% and specificity ranging from 94.7% to 100%. A stool culture was carried out on the baseline stool sample to identify bacterial causes of diarrhoea, including adherent or toxigenic *Escherichia coli*.

2.4. Study procedures and follow-up

Patients enrolled in the studies underwent a complete physical examination, including recording of systolic and diastolic blood pressure, pulse rate, body weight and temperature and an assessment of stool characteristics (frequency, consistency, presence of mucus or blood). Patients ≥ 12 years of age received one nitazoxanide 500 mg tablet or a matching placebo tablet twice daily for 3 consecutive days. Patients aged 4–11 years received 10 ml of nitazoxanide 100 mg/5 ml suspension or a matching placebo twice daily for 3 consecutive days. Patients aged 1–3 years received 5 ml of nitazoxanide 100 mg/5 ml suspension or a matching placebo twice daily for 3 days. Patients were instructed to take their medication with food and were given a diary with instructions to record administration of the medication, stool frequency and consistency, and other symptoms. In addition to the blinded study medication, all patients received routine care, including fluid replacement therapy and nutritional and metabolic management of diarrhoea. The patients returned to the clinic on Day 7 following initiation of treatment for a physical examination and evaluation of clinical response. Two stool samples collected at least 24 h apart between Days 7 and 10 and a third stool sample collected on Day 14 were subjected to microscopic examination as described above (see Section 2.3), including ELISA testing for *E. histolytica*. The Day 14 stool examination was conducted for its scientific value, but the results were not considered as part of the definition of microbiological response (prospectively defined) owing to the potential for re-infection. Adverse events were recorded on the appropriate case report forms, and the severity of each adverse event was graded on a four-point scale: mild, moderate, severe or life threatening. Where applicable, adverse events were classified as serious or unexpected, and the relationship to the study drug was recorded.

2.5. Randomisation

Upon enrolment, each patient was sequentially assigned a number corresponding to the number on his/her package of study medication. The computer-generated randomisation list and the packaging of study medications were

prepared by the study sponsor, Romark Laboratories. The patients, principal investigators and their staffs, laboratory personnel and study monitors were blinded so that critical data for each of the endpoints (clinical response, results of post-treatment stool examinations and adverse events) were generated without knowledge of treatment assignment.

2.6. Statistical analysis

Statistical analyses were conducted using JMP® software version 5.1.1 (SAS Institute Inc., Cary, NC, USA). The population used for efficacy analyses was a modified intention-to-treat population defined prospectively as all patients randomised to the study, excluding: (1) patients with no *E. histolytica* cysts or trophozoites in their baseline stool sample; and (2) patients with other identified enteric pathogens in the baseline stool sample. For the purpose of defining this population, *B. hominis* was treated as an enteric pathogen. Whilst the pathogenicity of *B. hominis* has been subject to some debate, a recent study showed that nitazoxanide was effective in treating diarrhoea and enteritis in patients with *B. hominis* as the sole identified potential enteric pathogen (Rossignol et al., 2005). Patients who failed to complete the study were treated as failures. Proportional clinical and microbiological response rates and the frequency of adverse events were compared by treatment group using two-sided Fisher's exact tests with an α of 0.05. Time from first dose to last unformed stool was analysed by survival analysis using a Prentice-modified Wilcoxon test. A secondary analysis was performed for all patients randomised to the study.

2.7. Hepatic amoebiasis cases

Patients presenting at the University of Alexandria Hospital were screened for enrolment in an uncontrolled study of nitazoxanide in treating hepatic amoebiasis. Inclusion criteria included fever, abdominal pain, hepatomegaly, positive serological titre for *E. histolytica* infection by IHA and ultrasound showing a single hypoechoic mass on the liver compatible with abscess. Pregnant or lactating females, patients with multiple abscesses (indicative of more serious disease) and patients in danger of imminent rupture were excluded. Pre-treatment study procedures included a physical examination, evaluation of symptoms, collection of a stool sample for ova and parasite examination, and collection of serum for serological studies (IHA), erythrocyte sedimentation rate (ESR), haematology and blood chemistry. Patients enrolled in the study received one nitazoxanide 500 mg tablet with food every 12 h for 10 days and remained hospitalised for the duration of treatment. Serum was collected on study Days 5, 10, 15 and 30 to monitor ESR, haematology and clinical chemistry parameters (Day 10 only). A stool sample was collected for ova and parasite examination at the end of treatment and at study Day 30. Clinical response was defined as resolution of clinical symptoms of invasive amoebiasis (fever, abdominal pain and hepatomegaly) and normalisation of leukocytosis at the end of treatment without any recurrence of hepatic amoebiasis through study Day 30.

3. Results

3.1. Study population

Stool samples from 4587 symptomatic patients were screened between 17 February 2004 and 2 October 2005. *E. histolytica/dispar* cysts or trophozoites were observed in the stools of 254 patients (5.5%), and 158 (3.4%) of these were confirmed to be *E. histolytica* by the antigen-based ELISA test. One hundred patients fulfilling the inclusion criteria were enrolled in the two placebo-controlled studies. The distribution of the patients by study and treatment group is shown in Figure 1. Two patients in the tablet study (both from the placebo group) failed to return for follow-up and were considered treatment failures according to the protocol. Patients were distributed well among the active and placebo treatment groups, with no significant differences in age, gender, weight, duration of diarrhoea or symptoms. Demographic and disease-related characteristics of the patients included in the primary efficacy analysis are summarised by treatment group in Table 1.

3.2. Efficacy

Clinical and microbiological response rates are presented by treatment group in Table 2. Times from first dose to passage of last unformed stool are presented in Figure 2.

The correlation of clinical response with microbiological response was near perfect in the nitazoxanide groups, with 30 of the 32 clinical responders being free of *E. histolytica* cysts or trophozoites in the Day 7–10 stool samples. Twelve of the 15 clinical failures in the placebo groups had *E. histolytica* cysts or trophozoites in their follow-up stool samples. Ten of the 15 clinical responders in the placebo groups were negative for the parasite in both Days 7–10 follow-up stool samples.

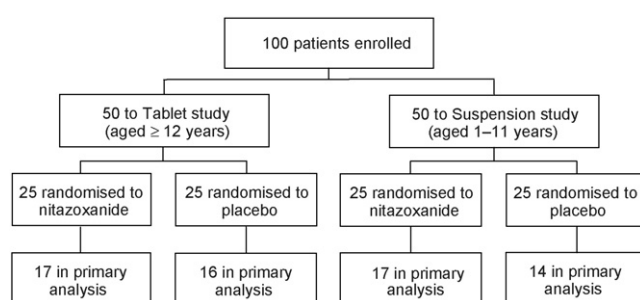


Figure 1 Patient distribution flow chart. Patients excluded from the efficacy analysis in accordance with the protocol were: (a) for the tablet study nitazoxanide group, four *Entamoeba histolytica*-negative at baseline, four *Blastocystis hominis*-positive at baseline; (b) for the tablet study placebo group, six *E. histolytica*-negative at baseline, two *B. hominis*-positive at baseline, one *Salmonella*-positive at baseline; (c) for the suspension study nitazoxanide group, five *E. histolytica*-negative at baseline, three *B. hominis*-positive at baseline; (d) for the suspension study placebo group, six *E. histolytica*-negative at baseline, four *B. hominis*-positive at baseline, one *Giardia lamblia*-positive at baseline.

One (2%) of 47 microbiological responders in the nitazoxanide treatment group (all patients randomised analysis) had *E. histolytica* identified in the Day 14 stool sample, indicating either re-infection or an incomplete response to treatment. The other 46 microbiological responders remained *E. histolytica*-negative at study Day 14. Four (17%) of 23 microbiological responders in the placebo treatment group had *E. histolytica* identified in the Day 14 stool sample.

3.3. Safety and tolerability

Upon questioning at follow-up, 14 patients reported one or more adverse events irrespective of causality. The adverse events consisted of drowsiness (four nitazoxanide), abdominal pain (two nitazoxanide, one placebo), headache (two nitazoxanide, one placebo), fatigue (one nitazoxanide, one placebo), yellowish urine (one nitazoxanide, one placebo), nausea (one placebo), vomiting (one placebo) and dyspepsia (one nitazoxanide). Each of the adverse events was mild and transient in nature with none requiring discontinuation of treatment.

3.4. Hepatic amoebiasis cases

Seventeen adults (14 males and 3 females) with a mean age of 29 ± 5.4 years (range 19–41 years) and mean body weight of 63.8 ± 5.9 kg (range 54–76 kg) were enrolled in the hepatic amoebiasis study. Each of the patients presented with fever, abdominal pain and hepatomegaly, and on ultrasound each patient showed a single hypoechoic mass on the liver compatible with abscess. Serology for *E. histolytica* (IHA) showed an elevated titre in all 17 patients. Leukocytosis was noted for 12 patients (71%). *Entamoeba histolytica* was identified in the stool of only one patient before treatment.

Each of the 17 patients responded to treatment, resolving all symptoms before the end of treatment. Defervescence ($<37.5^\circ\text{C}$) occurred before the fifth day of treatment for 14 patients (82%) and all patients had normal body temperature by the end of treatment. Leukocytosis also resolved in each of the patients by study Day 10. Each of the 17 patients remained well, with no complications 20 days after the end of treatment. None of the patients discontinued treatment or required aspiration of pus from the liver. Stool samples collected from the 17 patients at study Days 10 and 30 were all negative for *E. histolytica*. No adverse events were reported.

4. Discussion

Our studies describe the effectiveness of nitazoxanide in treating invasive disease caused by *E. histolytica* infection in the intestinal tract and the liver. They also demonstrate the effectiveness of nitazoxanide in clearing *E. histolytica* from the intestinal tract.

The studies in intestinal amoebiasis were very rigorously designed and conducted. Strengths of these studies include the double-blind design, use of a placebo control, differential diagnosis of *E. histolytica* versus *E. dispar*, exclusion of other potential enteric pathogens, demonstration of

Table 1 Demographic and disease-related characteristics at baseline

Characteristic	Tablet study ^a		Suspension study ^b	
	Active (n = 17)	Placebo (n = 16)	Active (n = 17)	Placebo (n = 14)
Gender (n)				
Male	8	10	11	5
Female	9	6	6	9
Age (years)				
Mean ± SD	34 ± 15	33 ± 10	7 ± 3	7 ± 3
Range	13–54	14–48	2–11	3–11
Weight (kg)				
Mean ± SD	72 ± 19	78 ± 15	22 ± 7	26 ± 9
Range	39–120	40–110	10–40	14–48
Duration of diarrhoea (days)				
Mean ± SD	8 ± 2	8 ± 2	8 ± 2	8 ± 2
Range	4–11	5–11	5–12	4–10
Stool frequency (n)				
3–4 per day	8	6	10	11
≥5 per day	9	10	7	3
Stool consistency (n)				
Liquid	13	9	13	11
Soft	4	7	4	3
Other symptoms (n)				
Abdominal pain/cramps	16	12	12	14
Mucus in stool	2	1	1	3
Nausea	2	4	4	2
Vomiting	1	1	1	1
Anorexia	—	—	—	2
Haematochezia	—	—	—	1
Urgency	1	1	1	—
Flatulence	—	2	2	—
Abdominal distention	1	1	1	—
Malabsorption	—	1	1	—
Fever	—	—	—	—

^a The tablet study included patients aged ≥12 years.

^b The suspension study included patients aged 1–11 years.

Table 2 Response rates by treatment group

	Primary analysis ^a			All patients randomised		
	Active	Placebo	P-value ^b	Active	Placebo	P-value ^b
Clinical response ^c	32/34(94%)	15/30 (50%)	<0.001	47/50(94%)	22/50 (44%)	<0.001
Microbiological response ^d	32/34(94%)	13/30 (43%)	<0.001	47/50(94%)	23/50 (46%)	<0.001

^a Modified intention-to-treat population excluding patients with no *Entamoeba histolytica* cysts or trophozoites detected in baseline stool sample as well as patients with other enteric pathogens identified in baseline stool sample.

^b Fisher's exact test, two-sided.

^c Proportion of patients resolving symptoms by study Day 7. Clinical response rates for each study were: tablet study primary analysis, 16/17 (94%) vs. 9/16 (56%) ($P=0.017$); tablet study all randomised, 24/25 (96%) vs. 11/25 (44%) ($P=0.001$); suspension study primary analysis, 16/17 (94%) vs. 6/14 (43%) ($P=0.004$); suspension study all randomised, 23/25 (92%) vs. 11/25 (44%) ($P<0.001$).

^d Proportion of patients with no *E. histolytica* organisms detected in post-treatment stool samples. Microbiological response rates for each study were: tablet study primary analysis, 16/17 (94%) vs. 7/16 (44%) ($P=0.002$); tablet study all randomised, 23/25 (92%) vs. 11/25 (44%) ($P<0.001$); suspension study primary analysis, 16/17 (94%) vs. 6/14 (43%) ($P=0.004$); suspension study all randomised, 24/25 (96%) vs. 12/25 (48%) ($P<0.001$).

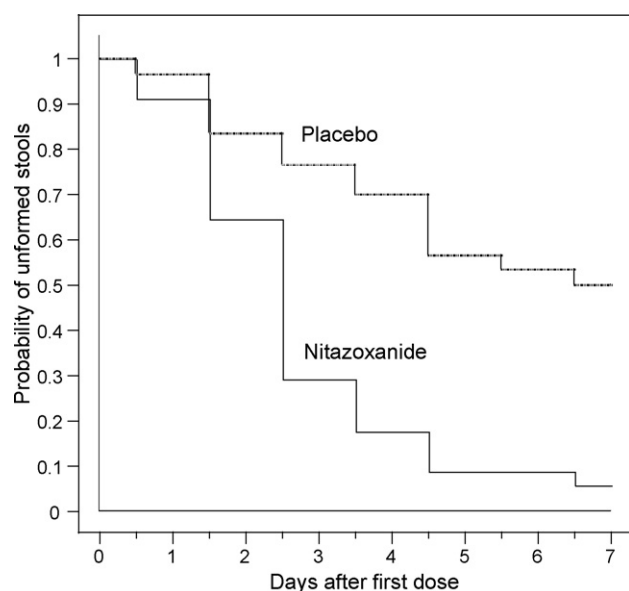


Figure 2 Survival analysis of time from first dose to passage of last unformed stool. Analysis shows combined results for the tablet study and suspension study ($P < 0.001$) (Prentice-modified Wilcoxon test). Results were also significant for each of the two studies: tablet study ($P = 0.001$); suspension study ($P = 0.025$).

activity both in adult and paediatric populations and the use of multiple clinical and microbiological endpoints. Our screening for causes of diarrhoea did not include testing for *C. difficile* toxins, but patients were not hospitalised and the likelihood of *C. difficile*-associated disease in this population was low. Likewise, we did not test for enteric viruses and whilst we cannot exclude the possibility that diarrhoea in some of these patients may have been caused by an enteric virus against which nitazoxanide is effective, the correlation of microbiological responses with clinical responses in these patients strongly suggests that diarrhoea and colitis in this population were caused by *E. histolytica*.

The study in patients with hepatic amoebiasis was the first clinical trial of nitazoxanide conducted in treating this disease and whilst the results are encouraging, the number of patients was small, the study was not controlled and it excluded patients with multiple abscesses indicative of more serious disease. Further studies in patients with hepatic amoebiasis will be required to establish the effectiveness of nitazoxanide in treating this disease.

Our studies indicate an important role for nitazoxanide in resolving symptoms associated with intestinal amoebiasis and in clearing organisms from the intestinal tract. A 3-day course of nitazoxanide could replace much longer regimens of metronidazole followed by a luminal amoebicide, providing an advantage with respect to convenience and potential advantages with respect to cost and availability (versus luminal agents). Unlike metronidazole, nitazoxanide is licensed in the USA for use in children as young as 12 months of age and is available as a paediatric suspension. In cases of more serious or severe disease, the duration of treatment with nitazoxanide may be extended beyond the 3-day regimen typically used for treating enteric protozoan infections.

The rationale for use of nitazoxanide becomes more robust when consideration is given to the spectrum of activity of nitazoxanide against enteric pathogens, the practical difficulties involved in diagnosing *E. histolytica* infection, the possibility of asymptomatic infection and the multiplicity of potential infectious causes of diarrhoea and enteritis. Diagnosis of *E. histolytica* infection is important, but in many cases the diagnosis is impractical or impossible owing to lack of adequate and reliable diagnostic procedures or because of the time or cost involved in making the diagnosis. In such cases, empirical treatment with nitazoxanide based on a clinical diagnosis may be an appropriate option.

In conclusion, we note that the management of amoebiasis has improved with recognition of *E. histolytica* and *E. dispar* and the development of modern diagnostic techniques. The results reported here suggest a new option for treating intestinal and hepatic amoebiasis that may lead to further improvements in the management of this disease.

Authors' contributions: J-FR designed the study protocol; SMK and YE-G carried out the clinical assessment; AMY carried out the comprehensive stool examinations and immunoassays; J-FR carried out the analysis and interpretation of the data and drafted the manuscript. All authors read and approved the final manuscript. J-FR is guarantor of the paper.

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Conflicts of interest: J-FR is an employee of, and owns an equity interest in, Romark Laboratories, L.C., Tampa, FL, USA. All other authors have no conflicts of interest.

Ethical approval: The study was conducted in compliance with the human experimentation guidelines of the US Department of Health and Human Services. The protocol and informed consent forms were approved by the ethical committees of the University of Alexandria Faculty of Medicine, Alexandria, Egypt, and the University of Benha Faculty of Medicine, Benha, Egypt.

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