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Effect of Combined Therapy with Albendazole and Sutrim in Experimental Cryptosporidiosis

[1]Eman Ali Mohamed, [1]Rabab S. Zalat, [2]Mona Magdi, [1]Nevine Guirguis and [1]Soheir Mahmoud

[1]Department of Parasitology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt
[2]Pathology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt

Abstract: *Cryptosporidium* is a protozoan parasite that infects the gastrointestinal tract of vertebrate animals. This work aimed to elaborate the effect of a combination of a broad spectrum antihelmintics; albendazole and Sutrim (Sulpamethazole+Trimethoprin) on experimental *C. parvum* infection. Forty male albino mice obtained from Theodor Bilharz Research Institute (TBRI) were infected orally with 10,000 *C. parvum* oocysts per mouse then divided into four groups: group I- infected control, groupII- infected animals treated with Albendazole orally (0.4 mg/ mouse) daily for five consequence days two weeks postinfection, groupIII- infected mice treated with Sutrim orally (Trimethoprim + Sulphamethoxazde) (1.2 mg orally/ mouse) daily for five consequence days two weeks postinfection and groupIV infected mice treated with half the doses of both drugs orally (0.2 mg Albendazole orally / mouse + 0.06 mg Sutrium orally/ mouse) daily for five consequence days two weeks postinfection. Oocysts counts in feces and intestinal contents and histopathological changes in intestines of mice were observed. Results: A highly significant reduction found in the mean number of *Cryptosporidium* oocysts/ gm stool five days posttreatment in groups II, III, IVwas 49.9, 74.3 and 75.7%, respectively. Reduction percentages in *Cryptosporidium* stages in intestinal villi were 54.7, 81.1and 80.4%, respectively. Conclusion: Sutrim is the most useful drug for treatment of cryptosporidiosis.

Key words: Albendazole · Trimethoprim · Sulpamethazole · Sutrim · Cryptosporidium · Parasitological · Histopathological

INTRODUCTION

Cryptosporidium, a unicellular coccidian protozoan, causes several outbreaks [1, 2]. Two species of *Cryptosporidium* are mainly found to cause disease in man, *C. hominis* which shows anthroponotic transmission and *C. parvum* with zoonotic transmission [3]. Oocysts are highly resistant to dessication, disinfectants and other environmental stresses [4, 5]. Infection with *Cryptosporidium spp.* was reported in immunocompromised, as well as immunocompetant children and adults [6]. Transmission of the parasite takes place through the fecal-oral route and with the use of recreational waterways in developed countries [7]. Clinical diseases are characterized by mucous to hemorrhagic diarrhea, lethargy and may be pyrexia [8]. Infection is diagnosed by conventional microscopic examination of *Cryptosporidium* oocysts [9]. Paromomycin is the most commonly used drug against cryptosporidiosis. Azithromycin shows partial results against the disease [10]. In vitro and in vivo repeated doses of Nitazoxanide and Albendazole are also effective against cryptosporidiosis [11]. This study aimed at evaluating the efficacy of Albendazole, alone and/or in combination with Sutrim on *C. paruim* infected mice.

MATERIALS AND METHODS

Animals: Male C57BL/6 strain, albino mice, 6 to 8 weeks old (18 ± 28 gm), clean from parasitic infection, were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI). Animals were maintained under standard laboratory care (controlled temperature and light environment); and were given filtered drinking water and balanced diet. Appropriate number of animals to produce statistically reproducible results was used.

Parasite: The stool samples of infected cows were collected in sterile clean stool cups; they were repeatedly

Corresponding Author: Eman Ali, Department of Parasitology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt.

washed and sieved by using 100-, 200- and 400-mesh sieves. Oocysts were concentrated by a modified Formalin-Ether sedimentation technique in which phosphate-buffered saline (PBS) (pH 7.2) was used instead of Formalin [12].The oocysts were suspended in a phosphate-buffered saline (PBS) with 0.01% Tween 20, containing 200 U/mL Penicillin, 0.2 mg/mL Streptomycin and 2.5 µg/mL amphotericin B at 4°C.

Drugs: Albendazole was obtained from Sigma and Sutrim (Trimethoprim + Sulphamethoxazole) from Memphis Co.

Studied Groups: All infected animals received 10,000 *C. parvum* oocysts orally. Animals were divided into four groups:

Group I: Infected control mice.
Group II: Infected animals treated with Albendazole orally (0.4 mg/ mouse) daily for five consequence days, two weeks post infection.
Group III: Infected mice treated with Sutrim orally 1.2 mg orally/ mouse daily for five consequence days, two weeks post infection.
Group IV: Infected mice treated with half the doses of both drugs orally (0.2 mg Albendazole orally / mouse + 0.06 mg Sutrim orally/mouse) daily for five consequence days, two weeks post infection. Animals were sacrificed four weeks post infection.

Parasitological Examination: Two weeks post infection, 3 and 5 days post treatment stool samples were collected daily in sterile clean stool cups. The samples were stained by modified Zeihl–Neelsen (MZN) [13].

Sacrifice of mice was done two weeks after administration of drugs by intraperitoneal anesthesia. The upper part of small intestine was removed and subjected to histopathological examination. The duodenal contents were subjected to parasitological examination.

Histopathological Examination: The small intestine of mice was fixed in 10% neutral buffered formalin. Sections stained by Hematoxylin and Eosin (H&E) and (ZN stain) then examined by light microscopy according to standard operation procedures [14].

Statistical Analysis: Comparison was done between each treated group and its respective untreated control. The percentage change between each two groups to be compared was assessed using the formula:

$$\frac{\text{(Mean value of the infected untreated group - mean value of infected treated group)}}{\text{Mean value of infected untreated group}} \times 100$$

Difference between the mean scores of any of the two groups to be compared, were tested for significance using an unpaired 2 tailed studentis t-test. The data were considered significant if P values were less 0.05.

Ethical and Regulatory Guidelines: The experimental animal studies were conducted in accordance with international valid guidelines and they were maintained under convenient conditions at the SBSP animal house of TBRI.

RESULTS

Parasitological Examination: The number of oocysts/gm stool was recorded between control and treated *Cryptosporidium* infected groups 3 days post infection. The percent reduction was 28.6%, 54.2% and 55.3% in infected treated groups with Albendazole, Sutrim and Sutrim + Albendazole, respectively. (Table 1). A highly significant reduction was found in the mean number of *Cryptosporidium* oocysts/ gm stool five days post-treatment. The reduction in oocysts excreted in the stools of groups II, III, IV were 49.9%, 74.3%, 75.7%, respectively compared to infected control group. Again, the reduction in *Cryptosporidium* stages in intestinal villi was 54.7%, 81.1% and 80.4%, respectively, compared to infected control group (Tables 2, 3).

Table 1: Effect of treatment with Sutrim and/or Albendazole on *C. parvum* oocysts/ gm stool three days post- treatment.

Groups	Oocysts/ gm stool (Mean±SE)	%Reduction
Group I: infected control	7943±1342	------
Group II: infected treated with Albendazole.	5670±940*	28.6%
Group III: infected treated with Sutrim.	3640±978**	54.2%
Group IV: infected treated with Sutrim + Albendazole.	3549±1036**	55.3%

Data are expressed as means ± SD. *P<0.05 and **P<0.01 are of significant difference in comparison to infected control group

Table 2: Effect of treatment with Sutrim and/or Albendazole on *C. parvum* oocysts/ gm stool five days post- treatment.

Groups	Oocysts/ gm stool (Mean±SE)	%Reduction
Group I: infected control	8179±1254	-
Infected treated with Albendazole.	4097±875**	49.9%
Infected treated with Sutrim.	2100±698***	74.3%
Infected treated with Sutrim + Albendazole.	1989±724***	75.7%

Data are expressed as means ± SD. ***P*<0.01 and ****P*<0.001 are of significant difference in comparison to infected control group

Table 3: Effect of treatment with Sutrim and/or Albendazole on mean count of *C. parvum* in intestinal contents five days post- treatment.

Groups	*Cryptosporidium* stages in intestinal contents (Mean±SE)	%Reduction
Infected control	14.8±4.1	-
Infected treated with Albendazole.	6.7±2.7**	54.7%
Infected treated with Sutrim.	2.8±0.78***	81.1%
Infected treated with Sutrim + Albendazole.	2.9±0.93***	80.4%

Data are expressed as means ± SD. ***P*<0.01 and ****P*<0.001 are of significant difference in comparison to infected control group

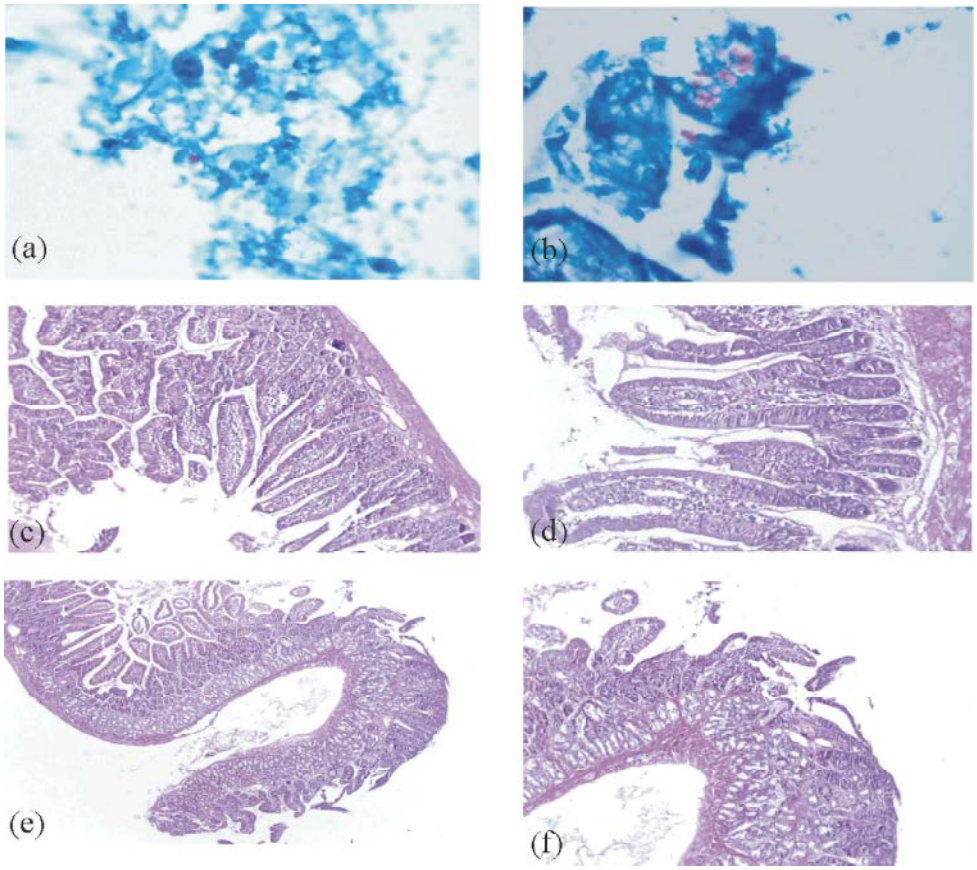

Fig 1a-f: a: Cryptosporidium oocytes stains purple (red arrow) in comparison to blue stained yeast (orange arrow). Modified ZN on tissue paraffin sections x 1000.
b: Cryptosporidium oocytes stains purple (red arrow) in the intestinal glandular epithelium. Infected control group (N). Modified ZN on tissue paraffin sections x 1000.
c: Normal intestinal villi. Normal crypt villous ratio. H&E x100.
d: Normal intestinal villi. Normal crypt villous ratio. H&E x200.
e: Infected control group showing moderate and severe villous atrophy (arrows) and moderate exudation of mononuclear inflammatory cells . H&E x100.
f: Infected control group showing moderate and severe villous atrophy (arrows) and moderate exudation of mononuclear inflammatory cells . H&E x200.

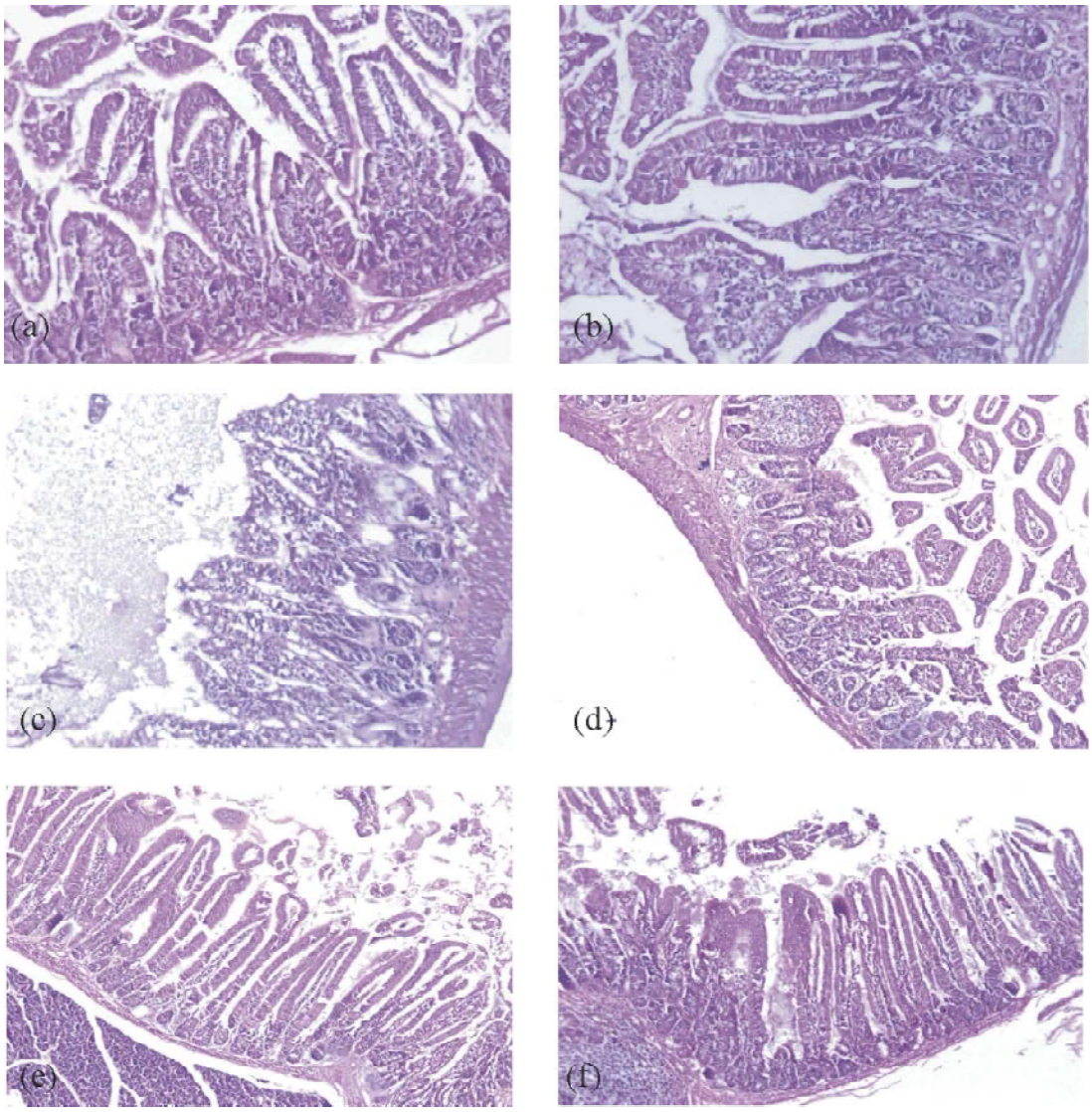

Fig. 2a-f: a: Group treated with Albendazole treatment only showing foci of mild to moderate villous atrophy (arrow) and exudation of mononuclear inflammatory cells. H&E x200.
b: Group with Sutrim only treatment showing relatively normal crypt villous ratio and moderate exudation of mononuclear inflammatory cells. H&E x200.
c: Group treated with Albendazole shows persistent foci of moderate villous atrophy, surface erosions and mild mononuclear inflammatory exudation. H&Ex100
d: Group with Sutrim only showing normal villi but persistent foci of mild mononuclear inflammatory exudation.H&E X100.
e: Group with Sutrim only treatment showing normal villi but still foci of mild mononuclear inflammatory exudation.H&E X100.
f: Group treated with Sutrim and Albendazole combination showing normal villi but still foci of mild mononuclear inflammatory exudation (similar effect to Sutrim only treated group). H&E X100.

Histopathological Examination: The infected control group showed complete, severe and moderate degrees of villous atrophy as well as presence of *Cryptosporidium* oocysts on the villi surface and in the glands (Fig 1a-f). Furthermore, the study showed persistent degrees of villous atrophy after albendazole

treatment in contrast to absent villous atrophy in Sutrim treated group. On the contrary, there were foci of mild inflammation in Sutrim treated group more than the quite normal inflammatory component in albendazole treated group. In the same context, the drug combination of sutrim + albendazole gave similar results to sutrim alone (Fig. 2a-f). Accordingly, sutrim considered the most useful drug on the parasitological and histopathological bases.

DISCUSSION

Cryptosporidium is a protozoan parasite that infects the gastrointestinal tract of vertebrate animals, including mammals, birds, reptiles, amphibians and fish [15]. Outbreaks of cryptosporidiosis are regularly occurring throughout the world. New drugs against this parasite became consequently urgently needed. Among the most commonly used treatments against cryptosporidiosis are Paromomycin and Azithromycin, which are partially effective [10]. Despite recognition of *Cryptosporidium* as an important human pathogen, there is a relatively limited number of agents available for chemotherapeutical purposes and more research is required in order to identify existing or new compounds that may be effective against cryptosporidiosis. The aim of this work was to compare the effect of treatment with albendazole, sutrim wether alone or in combination for the treatment of experimental cryptosporidiosis. This study showed a high significant difference between control and all treated infected groups, especially in groups treated five consequence days post infection. Albendazole resulted in a percentage reduction of 49.9% and 54.7% in both cystic and intestinal contents stages form respectively, compared to 74.3% and 81.1% when Sutrim was given. These finding are in agreement with those of Sabiqaa Masood *et al.,* [16] who found that oocyst per gram (OPG) count showed an increasing trend in control (untreated) animals. A single dose of 10mg/kg body weight of Albendazole caused a significant decrease in OPG count from 6th day post treatment and onward ($P<0.05$). Similar findings in relation to the efficacy of Albendazole at 20mg/kg body weight in calves against cryptosporidiosis were reported by Xiao *et al.*[8] and Johny *et al.* [17]. Metronidazole treatment caused a significant decrease in oocyst per gram (OPG) count from *6th* day post treatment and onward ($P<0.05$). Paromomycin when used against cryptosporidiosis under experimental conditions showed better results than Albendazole and Metronidazole. The successful experimental trial of Xiao *et al.* [8] then Johny *et al.* [17], reported that a single dose of Albendazole 10mg/kg body weigh caused a significant decrease in OPG, stool count from 6th day past treatment and onward ($P<0.05$) in cattle. In this study, the drug combination gave 75.7% and 80.4% percentages oocysts and intestinal contents reduction, respectively. These data were more or less similar to those detected in groups given Sutrim alone (74.3% and 81.1%, respectively). Although a large number of compounds had been tested against cryptosporidiosis yet only limited ones showed effective results, Kelly *et al.* [18] observed improvement in symptoms of cryptosporidiosis and eradication in four Zambian AIDS patients by Albendazole used at a dose of 80 mg twice daily.

However a control large scale study was recommended. Only Paromomycin had been shown to have anti-cryptosporidial activity [19]. Comparable results were documented in relation to efficacy of Paromomycin by Leitch & He [15].

In AIDS patients, combination therapy restoring immunity along with antimicrobial treatment of *Cryptosporidium* infection is necessary. Recent investigations focused on the potential of molecular-based immunotherapy against this parasite. Others tested the effects of probiotic bacteria, but were unable to demonstrate eradication of *C. parvum*. paromomycin was the most commonly used drug against cryptosporidiosis. azithromycin showed partial results against the disease [10]. Moreover, histopathological study supported our parasitological results, in which there were still degrees of villous atrophy after Albendazole treatment, but in case of treatment with Sutrim there was no villous atrophy, however, there were foci of mild inflammation (that might indicate some degree of immune response in comparison to Albendazole). The drug combination induced similar results to sutrim alone. So Sutrim is the most useful drug on parasitological and histopatholoical levels. It was reported that albendazole was clinically effective against related protozoan parasites causing microsporidiosis in humans [20]. In conclusion, albendazole and sutrim are efficient anti-cryptospoidial drugs. Sutrim gave the highest efficacy. The albendazole and sutrim drug combination would be suggested as a new therapeutic approach.

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