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IN VITRO STUDY ON THE ANTIPARASITIC EFFECT OF OMEPRAZOLE ON *CRYPTOSPORIDIUM PARVUM*

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Abstract

Cryptosporidiosis is an important zoonotic diarrheal disease of worldwide distribution in man animals as well water canals, causing about one million deaths annually. This study investigated the anti-parasitic and anti-inflammatory effects of omeprazole versus nitazoxanide and as a combined therapy to treat *Cryptosporidium parvum* infection in tissue culture. Histopathological and SEM examinations on infected tissue cultures evaluated treatment.

The results showed marked treated and inflammation improvement with omeprazole over nitazoxanide combined treatment as indicated by histopathological and SEM studies.

Key words: Cryptosporidiosis, Culture, Omeprazole, Nitazoxanide Histopathology, SEM

Introduction

Cryptosporidium parvum is the causative agent of an enteric disease with variable severity depending on the immune system and age; it affects mainly children and immunocompromised individuals (Liu *et al,* 2012). About one million individuals suffered from *C. parvum* gastroenteritis worldwide in the early 2020th (Mohamed, 2022). Infective oocysts are transmitted by fecal-oral route, or with contaminated raw vegetables, fruits, or water and/or contact with a patient or farm or domestic animals (El-Bahnasawy *et al*, 2018). Human-to-human was reported (Bouzid *et al*, 2013).

Nitazoxanide is the only drug against *C. parvum* with approval from the FDA up till now, but with limited efficacy depended on patient's immunity (Widmer *et al,* 2020). Also, it was not allowed for children below 12 months of age (Abubakar *et al,* 2007).

Omeprazole proved to have many medical values and anti-parasitic (Hernández-Ochoa *et al,* 2020) and anti-inflammatory effects (El-Nezhawy *et al,* 2013; Ittiyavirah and Shenika, 2014), and well tolerated with mild or side effects (Ataş *et al,* 2022).

The study aimed to evaluate anti-parasitic and anti-inflammatory effects of Omeprazole® versus Nitazoxanide® and combination of both on *Cryptospoidium parvum* oocysts in buffalo's ileum tissue cultures.

Materials and Methods

This study was done in Department of Medical Parasitology, Faculty of Medicine, Tana University from January to June 2023.

Ethical committee: The study protocol was approval by Faculty of Medicine with the Code No. 36229/12/22.

Buffalo ileum infected with *Cryptosporidium parvum* oocysts were obtained from the Biological Unit, Theodore-Bilharz Research Institute, Imbaba, Giza. They obtained infected tissues from slaughtered buffaloes in sterile cold saline solution with 1% (v/v) Gentamicin 50µg/ml (Garamycin; 80mg/2ml ampoules, Memphis, Pharmaceuticals, Egypt).

Study design: Tissues cultures were washed with ethanol 70% and then washed with sterile warm normal saline solution for five times. The ileum were cut into small pieces of 40-50mg each, and put with mucosa upwards into 6-well culture plates (a piece/well), supplemented with 1.5ml Roswell Park Memorial Institute 1640 medium with L-glutamine, with 50µg/ml of Gentamicin 1% & 10% fetal bovine serum. Pieces were incubated for 2hr at 37°C and 5% CO_2 in an incubator, moved into new culture plates and media were added (Aboelsoued *et al,* 2019).

Oocysts excystation to infect tissue cultures: Freeing of sporozoites was achieved by completing oocysts pre-treatment for 10min. in acidified (pH 2.0) and pre-warmed (37°C) 1x Hank's balanced salt solution calcium & magnesium free (HBSS), centrifuged and then incubation with 37°C warmed non-acidified 1x HBSS for 10min. Oocysts were washed, suspended in an excystation medium with warmed RPMI 1640 with L-glutamine & 10% of FBS for 30min. Put into 2ml tubes and centrifuged at 4000×g at 4°C, for 3min. Oocysts within the excystation media were immediately added to wells in a dose of 10^4/well (Varughese *et al,* 2014).

Experimental design; Culture plates were divided into 5 groups of 6 wells each as follows: G1: Normal tissue (negative control). G2: Ileal tissue with oocysts (positive control). G3: ileal tissue with oocysts and nitazoxanide treated. G4: ileal tissues with oocysts and omeprazole treated. G5: ileal tissues with oocysts and omeprazole and nitazoxanide combined treated.

All tissue culture plates (except GI) were oocysts infected in a dose of 10^4/well, and incubated at 37°C & 5% CO2 in an incubator. Drugs were added 6hrs post infection (PI) as follows: 50µl of omeprazole in 145 µM (Matsui *et al,* 2015) and 50µl of nitazoxanide as 32.5µM (Theodos *et al,* 1998). After 24 hours all mice were collected and examined by light and SEM microscopy.

Histopathological drugs evaluation: Tissues were removed and fixed in 10% neutral buffered formalin, dehydrated in an ascending ethanol series, cleared in xylol, for paraffin processing, sectioned (5µm), and hematoxylin & eosin stained (Abdel-Bary *et al,* 2012). Smears were microscopically examined and evaluated inflammatory scoring (Houghton, 2012).

SEM examination: The tissue specimens were fixed for 15 minutes in glutaraldehyde 3.7%, washed with PBS two times, dehydrated in ethanol, and left to dry at room temperature. They were mounted on stubs of aluminum microscopy and coated with 20 nanometer gold particles in a serial peripheral interface (SPI) Module ™ Vac/ Sputter and examined by JEOL, JSM-5200 LV Japan at EM Unit, Tanta Faculty of Medicine.

Results

GI tissues were completely normal picture. G2 showed high inflammatory changes with many basophilic oocysts at the damaged villi surf-ace, brush border disruption and congested blood vessels surrounded by numerous inflammatory cells. G2 showed severe inflammations with many follicles of inflammatory cells extended up to muscle layer. G3 showed less change and multiple basophilic oocysts on surface with partial brush border disruption and thick wall congested blood vessels surrounded with few inflammatory cells. G4 showed a few scattered basophilic oocysts on surface, partial focal brush border disruption and normal blood vessels with normal or mild wall thickness. G5 showed least inflammatory changes, intact brush border with few basophilic oocysts on outer surface compared to positive control, G5 showed minimal inflammation with significant scattered inflammatory cells in mucosa (P=0.003*). G4 showed insignificant mild inflammation cells in mucosa and submucosa (P=0.006*). G3 showed insignificant moderate inflammation cells in mucosa and submucosa lamina propria (P=0.080).

SEM showed that G1 was completely normal. G2 showed different sized parasitic stages scattered on irregular disturbed intestinal surface which showed microvillus border loss at the infected sites with multiple hole like lesions, mucous and cellular debris were on surface. G3 showed many parasitic stages having different size and shape, loss of the microvillus border and also, the hole like lesions were still present. G4 showed some parasitic stages and focal areas of disturbed intestinal villi and microvillus border, less hole-like lesions. G5 showed microvillus border of intestinal villi and crypts as in the normal control.

Details were in tables (1& 2) and figures (1, 2 & 3)

Table 1: Degree of inflammation in different groups as to *in vitro* inflammatory score.

Group	Degree	Inflammation score
Negative control.	0.0	No
Positive control.	++++	Many follicles of inflammatory cells up to muscle layer
Nitazoxanide treated.	+++	Nodules of inflammatory cells in lamina propria of mucosa & submucosa
Omeprazole treated.	++	Few aggregates of inflammatory cells in mucosa & submucosa
Combined therapies.	+	Scattered inflammatory cells in mucosa.

0= no, **+** = minimal, ++ = mild, +++ moderate, ++++ severe

Table 2: Comparison between four infected groups according to inflammatory score *in vitro*.

Variations	GII (n = 6)		GIII (n = 6)		GIV (n = 6)		GV (n = 6)		χ^2	^{MC}p
	No.	%	No.	%	No.	%	No.	%		
Minimal	0	0.0	0	0.0	1	16.7	4	66.7	22.235*	<0.001*
Mild	0	0.0	1	16.7	4	66.7	2	33.3		
Moderate	1	16.7	4	66.7	1	16.7	0	0.0		
Severe	5	83.3	1	16.7	0	0.0	0	0.0		
p_1			$^{MC}p=0.080$		$^{MC}p=0.006^*$		$^{MC}p=0.003^*$			
Significance			$^{MC}p_2=0.127$, $^{MC}p_3=0.014^*$, $^{MC}p_4=0.246$							

χ^2: Chi square test MC: Monte Carlo, $^*P \leq 0.05$, p_1: between GII and each Gs, p_2: between GIII & GIV, p_3: between GIII & GV &p_4: between GIV & GV.

Discussion

In the present study, the cryptosporidiosis oocysts *in vitro* cultures showed different results when treated with omeprazole and/or nitazoxanide only and with combination of both drugs. This agreed with Randall *et al.* (2011), who reported *in vitro* has many advantages over the traditional *in vivo* one by ensuring accurate dose(s) and minimized mode animal usage, and improved statistical data outcome. Also, the present histopathological and SEN examinations of buffalo ileal tissue cultured of *C. parvum* oocysts gave correct drug evaluation. This agreed with Vélez *et al.* (2021), who reported the parasite replication by qPCR, and Browning and Trier (1969), who found that tissue culture for 24hr maintained tissue viability.

In the present study, histopathological analysis showed that positive control showed severe inflammation score) with numerous oocysts on the surface of the damaged villi. This agreed with Aboelsoued *et al.* (2019), who detected numerous parasitophorous vacuoles attached to *C. parvum* infected ileal tissue surfaces. However, the inflammatory changes were mild in the omeprazole treated tissues accompanied with few scattered basophilic oocysts on ileal surface with multiple basophilic oocysts, but without significant difference (P=0.006*). Nitazoxanide treated culture showed moderate inflammation, along the ileal surface, but without significant difference (P=0.080).The least inflammatory changes with rare oocysts were in culture treated with combined nitazoxanide and omeprazole, with rare significant inflammations (P =0.003*).

In the present study, SEM showed that the most severe pathological changes were in the positive control scattered intestinal parasitic stages. This agreed with Vélez *et al.* (2021), who reported more or less similar changes in cryptosporidiosis bovine small intestine. They added that development of *C. parvum* in human ileocecal adenocarcinoma (HCT-8) cell culture and the parasitic stages with the hole-like defects on the surface of host cells induced by parasite release. Nitazoxanide (32.5µM) treated culture showed a sort of pathological improvement, but omeprazole treated one showed marked improvement with regression of parasitic stages. The combined treated culture showed a rare pathology with rare parasites. This agreed with Theodos *et al.* (1998), who declared that nitazoxanide (32.5µM) *in vitro* efficacy on *C. parvum* by inhibiting its growth. This also, agreed with Gargala *et al.* (2000), who reported that anti-parasitic affected *C. parvum* sexual & asexual stages. But, this disagreed with Shalaby *et al.* (2024), who detected insufficiency of nitazoxanide against *C. parvum* in tissue culture and Romidepsin was more effective than nitazoxanide in reducing oocysts development. Whitta *et al.* (2024)

reported a higher *in vitro* safety and efficacy of pyrazolopyridine analogue KDU731 than nitazoxanide in treating cryptosporidiosis.

It was worth to mention that none available report for omeprazole against tissue culture cryptosporidiosis. But, Ellakany *et al.* (2019) reported that omeprazole with PZQ significant reduction of *Schistosoma mansoni* adult worm counts, liver egg counts, diameter of hepatic granulomas, hepatic fibrosis, and GFAP efficacy of PZQ in treated.

Conclusion

The outcome data showed that omeprazole was better than nitazoxanide in cryptosporidiosis treatment and their combination gave best achievement.

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Authors' contribution: They all equally shared in the theoretical and practical studies as well as writing, revising and approving the manuscript publication.

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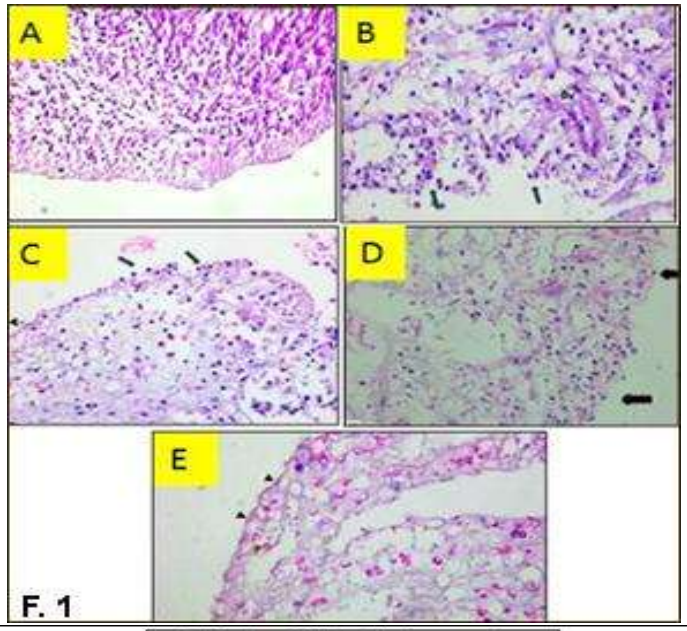
Explanation of figures

Fig. 1: Histological stained sections of bovine ileum (H & E, X400) showed A: Normal surface with intact brush border (arrow) without oocysts, (B) GII basophilic oocysts spreading on surface (arrows) with complete brush border loss, C: Basophilic oocysts spread on ileal surface (arrows) with partial brush border disruption, D: Few scattered basophilic oocysts on ileal surface (arrows) with partial disruption of brush border, (E) GV ileal surface with intact brush border (arrow heads).

Fig. 2: Comparison between four infected groups as to *in vitro* inflammatory score.

Fig. 3: SEM of bovine ileal tissues showed A: Normal surface without parasitic stages (X2000), B: Different sized and shaped parasitic stages (yellow arrows) scattered on irregular disturbed surface (star), injured underlying connective tissue (white arrow head) with multiple hole like lesions (yellow arrow heads, X2000, C: Many parasitic stages, few with regular surface (yellow arrow) mostly granular and irregular surface (white arrow), Also, surface underlyed disturbed connective tissue (arrow head, X2000), D: A parasitic stage with granular surface (yellow arrow) with projecting sporozoite (arrow head, X3500), E: Regular surface as in GI, with some parasitic stages (arrow, X3500}.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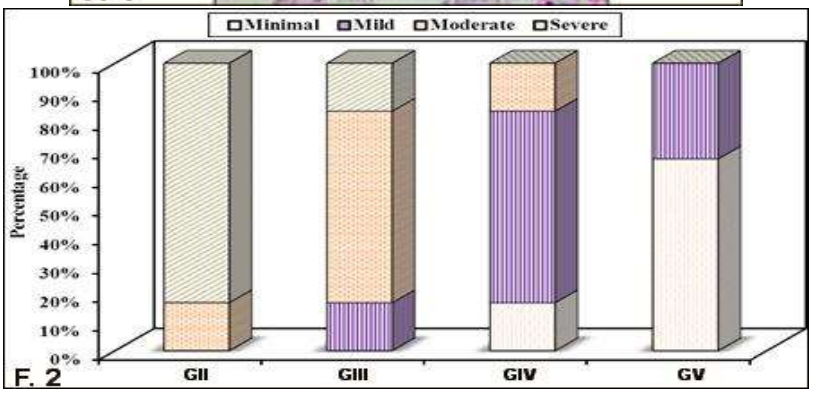
F. 1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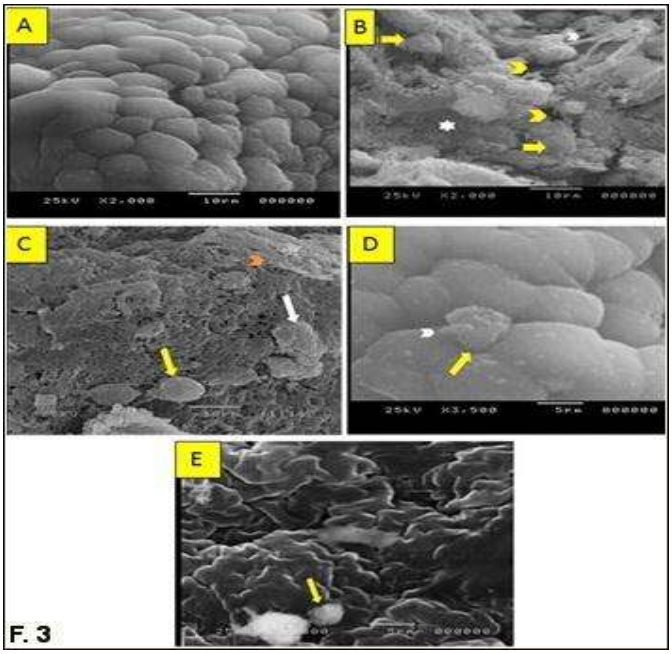
F. 2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F. 3