Journal of the Egyptian Society of Parasitology, Vol.52, No.2, August 2022
J. Egypt. Soc. Parasitol. (JESP), 52(2), 2022: 183 – 192
(Online: 2090-2549)

EVALUATION OF *LACTOBACILLUS ACIDOPHILUS*, CHITOSAN AND/OR AZITHROMYCIN IN TREATING CRYPTOSPORIDIOSIS IN NEONATAL MICE

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Abstract

There are various limitations on the efficacy of anticryptosporidal drugs with a critical need to have better alternatives. This study evaluated the effect of *Lactobacillus acidophilus* (*L. acidophilus*), a probiotic and chitosan, a natural polysaccharide as compared to azithromycin (AZT), as single therapy and in combination in treatment of cryptosporidiosis on infected neonatal mice.

The three therapeutics individually showed significant decrease in *Cryptosporidium* oocysts shedding compared to control (P <0.001). However, *L. acidophilus* caused a higher reduction (91.07%) compared to both AZT (83.19%) and chitosan (65.61%). Also, they reduced the oocysts numbers detected in the intestine, resolved totally the neutrophilic activity, without affecting the intestinal inflammation, but caused liver inflammation improvement. AZT/*L. acidophilus* combination caused highest reduction in oocysts shedding (93.44%), followed by chitosan/*L. acidophilus* combination (92.76%). These results represented a significant improvement compared to AZT single therapy (*P*-values 0.012 & 0.024 respectively) or chitosan single therapy (*P* <0.001 for both combinations). Besides, all therapeutic combinations removed any apparent parasite in the intestine and halted acute neutrophilic activity. Intestinal inflammation totally disappeared with chitosan/*L. acidophilus* combination. Liver inflammations improved with drug combinations, but did not resolve totally as mostly observed with single therapy.

Key words: *Cryptosporidium*, Neonatal mice, *Lactobacillus acidophilus*, Chitosan, Azithromycin.

Introduction

Cryptosporidium is an intracellular protozoon of phylum Apicomplexa, recognized as a cause of pediatric diarrhea worldwide (Ashigbie *et al,* 2021). *Cryptosporidium* outbreaks occurred in developing and developed countries due to its low infective dose, extended range of reservoir hosts and chlorination resistance leading to chronic and severe symptoms in immunosuppressed patients (Diptyanusa and Sari, 2021). The Egyptian endemicity of cryptosporidiosis was reported in polluted water (El Shazly *et al,* 2007), farm animals (Mahfouz *et al,* 2014), man (El-Bahnasawy *et al,* 2018), particularly school children (Shalaby and Shalaby, 2015), and even nosocomial transmission (el-Sibaei *et al*, 2003). In Egypt and many African countries, depend on chlorine in water disinfection to which *Cryptosporidium* was highly tolerant (Ahmed *et al,* 2019). Reports of pediatric cryptosporidiosis ranged from 10.9% (Sadek, 2014) to 49.1% (Helmy *et al,* 2013) in children with diarrhea and 19.5% - 22.4% in children with or without diarrhea (El-Badry *et al,* 2017; Fathy *et al,* 2014 respectively).

The infection burden was significantly underestimated with a very low case reporting (Sparks *et al*, 2015). Indian infants < 2 years were the most susceptible up to 75% (Checkley *et al,* 2015). As clinical symptoms cannot be an accurate method to assess tried therapeutics efficacy, assessment of oocyst shedding reduction per gram was useful and helped to estimate transmission risk (Dixit *et al,* 2022).

Cryptosporidium infects the microvillous layer of small intestine epithelial cells. Villous atrophy, white blood cells increase in lamina propria and histopathological damage occurred with persistent infection (Fahmy *et al,*

2021), which led to cell damage by cytokines and immune cells stimulation and recruitment to infection site (Zakir *et al,* 2021). In immunosuppressed patients infection extended to gastrointestinal tract, bile ducts and respiratory tract led to chronic liver inflammation or even cirrhosis (Wolska-Kusnierz *et al,* 2007). Its intracellular extracytoplasmic location limited the drug efficacy (Dixit *et al,* 2022).

Azithromycin is one of the most active macrolides against cryptosporidiosis (Rehg, 1994 b) that improved symptoms in immunocompromised children & chronic patients (Mead, 2002). Long-term AZT low dose caused stable remission of cryptosporidiosis in AIDS patients and eradicated infection, even with high immune suppression (Dionisio *et al,* 1998).

Chitosan (chitin) is a water-soluble unbranched polymer chains of N-acetyl-D-glucosamine (Shepherd *et al,* 1997), found in exo-skeletons of arthropods, aquatic invertebrates & fungi (Yong *et al,* 2015), as the most available natural polysaccharide after cellulose (Saber *et al,* 2017), used in drugs (Tozaki *et al,* 1997), against pathogens (Hayashi *et al,* 2007), and parasites (Silva *et al*, 2021) including cryptosporidiosis (Mammeri *et al,* 2018).

Lactobacillus is one of the widely used probiotics (Ajanya *et al,* 2018), as natural safe effective on intestinal microbial (Vemuri *et al,* 2018). *L. acidophilus* anticryptosporidial effect was due to releasing antimicrobial products affected *Cryptosporidium* (Foster *et al*, 2003), acting as immunomodulatory agents in intestinal and urogenital infections, cancer or liver diseases (Patel *et al,* 2014) with pro-inflammatory responses reduction (Charania *et al,* 2020). Their products hindered survival and physiology of many parasites (Berrilli *et al,* 2012). *Lactobacilli* inhibited the *Cryptosporidium* habitation and reproduction (Gupta and Garg, 2009). Also, they counteracted *Cryptosporidium* damage caused by the D-amino acids product-ion promoting micro-biome-host signaling by attaching to intestinal epithelium and related immune cells (Karpe *et al,* 2021).

This study aimed to assess the effects of *L. acidophilus,* a probiotic, and chitosan, a natural polysaccharide, as compared to AZT in the treatment of cryptosporidiosis, in experimentally infected neonatal mice, given as a single therapy and combined. This was evaluated by analyzing parasitic burden (oocysts shedding) and histo-pathological changes in duodenum and liver.

Materials and Methods

Experimental animals: The current study was carried out on 80 baby male Swiss albino mice, 2-3 weeks of age, weighing about 10-15gm, bred in Theodor Bilharz Research Institute (TBRI) in Giza. The mice were free from any parasitic infection as determined by the inspection of their stools for three successive days. All animal handling and experimental procedures were carried out according to national and institutional rules and ethics for laboratory animals' care and use. They were housed in a twelve-hour light/dark cycle and were given conventional pelleted feed with unlimited water.

Cryptosporidium oocysts*:* Oocysts were obtained from the laboratory of the Parasitology Department, TBRI, Giza. Using an oesophageal tube, 10^3 oocysts were given orally to each mouse on day one (Mostafa *et al,* 2018). After a week, fecal pellets were collected from the inoculated mice (Rehg, 1991) for parasitologically examined using Modified Zeihl-Neelssen stain (Garcia, 2007), to confirm infection.

Therapeutics: AZT (Epizithro) 500mg, manufactured by the Egyptian International Pharmaceutical Industries Company (E.I.P.I.C.O.) was given at a dose of 200mg/kg/day for five consecutive days (Rehg, 1991). The chitosan (500mg), manufactured by DBK Pharmaceuticals was given at a dose of 1mg/mouse/day for five consecutive days (Mammeri *et al,* 2018). *L. acidophilus* in capsule form, manufactured by Natrol LLC, United States, was given orally for 5 consecutive days at a dose of 10^8 CFU in 0.2 ml/day (Alak *et al,* 1997).

Experimental design: Mice were randomly

divided into eight groups of ten mice each. GI: negative control mice (neither infected, nor treated), GII: positive control mice (infected, non-treated); GIII: infected mice treated with AZT; GIV: infected mice treated with chitosan; GV: infected mice treated with *L. acidophilus*; GVI: infected mice treated with AZT combined with chitosan; GVII: infected mice treated with AZT combined with *L. acidophilus* and GVIII: infected mice treated with *L. acidophilus* combined with chitosan. All groups were compared to control group and one another. Treatment started simultaneously on day 8 post-infection (PI), continued for 5 days and all were sacrificed on the 20th day PI (Mostafa *et al,* 2018).

Parasitological examination: At scarification, fresh fecal pellets were collected from each mouse and labeled individually for assessment of oocysts count per gram. To detect *Cryptosporidium* oocysts, Modified Ziehl-Neelsen stain was employed followed by microscopical examination using the oil immersion lens (x100) according to Garcia (2007) with modifications. The reduction percentage of parasitic count was calculated as follows:

$$\frac{\text{mean number of parasite in control - mean number of parasite in treated group}}{\text{mean number of parasite in control group}} X100$$

Histopathological examination: A part of duodenum and liver were collected from each mouse, fixed with 10% neutral buffered-formalin, processed for paraffin block, sectioned at 5µm thickness, and stained in hematoxylin and eosin (Cardiff *et al,* 2014).

Duodenal samples were evaluated for the recognized criteria of duodenitis; neutrophil infiltrate (activity), mononuclear infiltrate in form of lymphocytes and plasma cells (inflammation), villous atrophy and gastric metaplasia (Dixon *et al*, 1996). Inflammatory cells were assessed in epithelial layer and lamina propria according to the Sydney classification, while gastric metaplasia and villous atrophy were evaluated (Wyatt *et al,* 1987; Blomquist *et al*, 1994) respectively. Each feature received a score ranged from zero to three: 0= normal, 1= mildly abnormal, 2= moderately abnormal and 3= severely abnormal.

Grading of inflammation of liver samples was done in accordance with ISHAK scoring system that assigns numbers to indicate the severity level of the necro-inflammatory features (interface, lobular & portal inflammations). Scores of the interface inflammation were assessed as follows: (0) if absent, (1) if mild (focal, few portal areas), (2) if mild/ moderate (focal, most portal areas), (3) if moderate (continuous around < 50% of tracts or septa) and (4) if severe (continuous around > 50% of tracts or septa); lobular inflammation: (0) if not present, (1) one focus (2) presence of two to four foci, (3) presence of five to ten foci and (4) if >10 foci were seen. Portal inflammation: (0) indicated absence, (1) mild in some or all portal tracts, (2) moderate in some or all portal tracts, (3) moderate/marked in all portal tracts and (4) marked inflammation in all portal tracts (Westin *et al,* 1999).

Statistical methods: Statistical package for the social sciences (SPSS) version 28 was used to code and data entry (IBM Corp., Armonk, NY, USA). The mean and standard deviation were used to summarize data. Analysis of variance (ANOVA) with multiple comparisons post hoc test was used to compare groups (Chan, 2003). If the *P*-values were less than 0.05 considered statistically significant.

Results

Oocysts count: A week post-treatment, the three single therapy groups (AZT, chitosan & *L. acidophilus*), showed a significant reduction in shedding compared to the control group ($P < 0.001$). But, *L. acidophilus* caused a higher reduction (91.07%) compared to the regular therapy AZT (83.19%) and chitosan; which showed the least reduction (65.61%).

All combination therapy groups resulted in significant decrease in *Cryptosporidium* oocysts in examined samples compared to the

control mice. AZT/*L. acidophilus* combined resulted in the highest reduction in oocysts shedding (93.44%), followed by chitosan/*L. acidophilus* combination (92.76%). This represented a significant improvement compared to AZT single therapy (*P*-values 0.012 & 0.024 respectively) or chitosan single therapy (*P*-value <0.001 for both combinations). This combination was comparable to *L. acidophilus* single therapy (91.07%). Combined chitosan and AZT was not significant (86.03%) compared to AZT single therapy (83.19%).

Histopathologically small intestine: In positive control, marked oocysts were detected. Mild intestinal inflammation in form of lymphocytic and plasma cells infiltration and acute immune cells activity in form of neutrophil infiltrates were seen.

Three therapeutics given individually reduced the oocysts numbers in intestine and totally resolved neutrophilic activity, but didn't affect intestinal inflammation. All therapeutic combinations wiped away any parasitic infection in intestine and halted any acute neutrophilic activity. Intestinal inflammation totally disappeared with chitosan/*L. acidophilus,* but increased on combined chitosan with AZT.

Liver samples of positive control showed moderate grades of widely spread liver inflammation, portal, lobular and interface, associated with mild degeneration. Three therapeutics caused in marked improvement in liver inflammation when given individually, without interface or portal inflammation (except a mild form of portal inflammation with chitosan). Also, lobular inflammation decreased to a mild form, but hepatocytes degenerative changes remained. Three therapies combined improved all forms of liver inflammation, as compared to positive control, but didn't resolve them totally as seen with single therapy; still, mostly mild portal, interface and lobular inflammations could be detected in liver tissues. Strikingly, Chitosan/*L. acidophilus* combined caused marked lobular inflammation, also, degeneration did not improve.

Details were given in table (1, 2 & 3) and figures (1, 2 & 3).

Table 1: Mean, standard deviation & reduction % of *Cryptosporidium* oocysts fecal shedding (per gram) in positive control and treated ones received single or combined regimens.

Oocysts fecal shedding 'count ($X10^3$) /1gm'	Positive control	AZT	Chitosan	*L. acidophilus*	AZT & Chitosan	AZT & *L. acidophilus*	Chitosan & *L. acidophilus*	*P*-value
Mean	253.40	42.60 *	87.14 *	22.63 *	35.39 *	16.63 *	18.35 *	<0.001
SD	42.60	4.21	4.55	1.29	2.30	2.02	2.14	
Percent reduction		83.19 %	65.61 %	91.07 %	86.03 %	93,44 %	92.76 %	

AZT= Azithromycin, *Significance at P < 0.05 compared to positive control.

Table 2: Intestinal tissue examination in positive control and treated ones received single or combined regimens.

Intestinal tissue (duodenitis score)	Positive control	AZT	Chitosan	*L. acidophilus*	AZT & Chitosan	AZT & *L. acidophilus*	Chitosan & *L. acidophilus*
Parasite	3	1	1	1	0	0	0
Neutrophilic activity	1	0	0	0	0	0	0
Inflammation	1	1	1	1	2	1	0
Villous atrophy	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD = No abnormality detected. Each feature received a score as follows: 0 if normal, 1 if mildly abnormal, 2 if moderately abnormal and 3 if severely abnormal.

Table 3: Liver inflammation in positive control and treated ones receiving single or combined regimens.

Liver inflammation score	Positive control	AZT	Chitosan	*L. acidophilus*	AZT & Chitosan	AZT & *L. acidophilus*	Chitosan & *L. acidophilus*
Portal	2	0	1	0	1	2	1
Interface	2	0	0	0	1	1	1
Lobular	2	1	1	1	1	1	3

Grading according to ISHAK scoring system; lobular inflammation absent=0 , one focus or less =1, 2-4 foci=2, 5-10 foci=3 and >10 foci=4; interface hepatitis absent = 0, mild = 1, mild/moderate = 2, moderate = 3 and severe = 4; and portal inflammation absent =0, mild some or all portal tracts=1, moderate in some or all portal tracts=2, moderate/marked in all portal tracts =3 and marked in all portal tracts =4.

Discussion

In the present study, AZT administration resulted in a significant decrease in *Cryptosporidium* oocysts shedding (83.19%). Similar studies using AZT, reported oocysts shedding reduction of 68% in experimental animals (Obiad *et al,* 2012) and parasitic clearance in 83% of the treated human immunodeficiency virus (HIV) patients (Diptyanusa and Sari, 2021). On the other hand, Bitsue *et al.* (2017) reported a 33.33% reduction in mice on using AZT. Also, Wolska-Kusnierz *et al.* (2007) reported that AZT was ineffectiveness in treating cryptosporidiosis in children with primary immunodeficiencies.

A single therapy, histopathologically, AZT caused decrease of the intense parasitic load and elimination of the acute neutrophilic activity but it was not able to resolve the inflammatory activity. In liver tissues, a decrease in inflammation was observed except for a mild grade of lobular inflammation. Also, significant decrease of cryptosporidial stages in intestinal epithelium was reported by Fayer and Ellis (1993). Besides, Al-Jarjary *et al.* (2010) reported milder degenerative and inflammatory effects in examined liver tissues with AZT. Dong and Yuanyuan (2013) reported a decrease in cellular infiltration, but without intestinal histopathological damage. Nevertheless, a higher dose of AZT (400mg/kg/day) in immunosuppressed rats was the best results in treating biliary duct infection (Regh, 1994a).

In the present study, *L. acidophilus* resulted in a significant decrease in *Cryptosporidium* oocysts shedding (91.07%). Similarly, Gaber *et al.* (2022) reported an oocyst reduction rate of 95.77% on using *L. acidophilus* in infected mice. Also, Rotkiewicz *et al.* (2001) reported its ability to hinder oocyst excretion by the 15th day of infection in piglets. In an *in vitro* study, *L. acidophilus* caused significant decrease (81%) in oocysts viability & (95%) infectivity (Foster *et al,* 2003; Glass *et al,* 2004 respectively). But, others didn't show beneficial effects when *Lactobacillus* was orally administered to children with cryptosporidiosis (Sindhu *et al,* 2014). Guitard *et al,* (2006) reported that *Lactobacillus* didn't eradicate the *Cryptosporidium parvum* in the neonatal rats.

In the current study, *L. acidophilus* decreased the intense parasitic infection, observed in the small intestine of the control group, to just a mild grade. This was in agreement with a study done on immunosuppressed mice which related the use of probiotics (including *L. acidophilus*) to a decrease in *Cryptosporidium* load in intestinal epithelium (Al-Khaliq *et al,* 2021). Similarly, *Lactobacillus casei* decreased *Cryptosporidium* in intestine starting the 3rd day PI (Khalifa, 2016).

In this study, *L. acidophilus* administration was accompanied with disappearance of the acute neutrophilic activity in intestinal tissues but not the inflammatory infiltration. Also, *Lactobacillus reuteri* (Fahmy *et al,* 2021) and *Lactobacillus brevis* (Ueno *et al,* 2011) helped cure intestinal inflammation. On the contrary, Al-Khaliq *et al.* (2021) didn't find change in intestinal cellular infiltration in *L. acidophilus* treated mice in comparison to positive control. Also, Rotkiewicz *et al.* (2001) detected numerous epithelial lymphocytes, lamina propria mononuclear cells and enlargement of mucosal lymph nodes (with large numbers of lymphocytes in their periphery) after *L. acidophilus* administration to *Cryptosporidium* infected piglets.

In the present study, chitosan resulted in a significant decrease in oocyst production; a 65.61% reduction compared to positive controls. This agreed with Mammeri *et al.* (2018) who reported a 95.7% to 98.9% reduction in oocysts *in vitro*, but disagreed with Moawad *et al.* (2021), who reported a 14.3% and 7.68% in immunocompetent and immunocompromised mice respectively.

In this study, chitosan single therapy was associated with a decrease in parasitic load in intestinal sections, from a marked to a mild state. Also, Kayser (2001) related the decrease in parasitic stages in intestinal tissues to add-

ing chitosan in treatment combinations. This anticryptosporidial potential of chitosan was related to its direct effect on oocysts viability and development (Mammeri *et al*, 2018), its ability to inhibit *Cryptosporidium* attached to enterocytes and creating a physical barrier (Blanco-Garcia *et al,* 2016).

In this study, chitosan alone caused disappearance of the acute neutrophilic activity, but not intestinal inflammation. This agreed with Ahmed *et al.* (2022) reported that using chitosan nanoparticles partially reversed colitis in rats and decreased but not resolved inflammatory infiltrate. But, Moawad *et al.* (2021) reported marked intestinal histopathological changes when treating *Cryptosporidium*-infected mice with chitosan nanoparticles.

In the current work, chitosan resulted in disappearance of interface form of liver inflammation and the decrease of lobular and portal inflammations to a mild grade. The liver enzymes; AST, ALT, ALP decreased and liver histology improved with chitosan treatment in hepatic fibrosis rat model, suggesting that chitosan has a hepato-protective effect (Wang *et al.,* 2018). But, Moawad *et al.* (2021) reported marked inflammation in liver tissue similar to that observed in the control group with chitosan nanoparticles.

Combining AZT/ *L. acidophilus* resulted in the highest percent reduction of oocysts shedding perceived in this work (93.44%) which varied significantly compared to control and AZT single therapy. *L. acidophilus* added to AZT, helped restoring gut flora acting as a natural barrier against *Cryptosporidium* proliferation, which was altered by using the AZT (Rehg, 1994a). Also*,* Gargala (2008) reported that the most often used medications, such as paromomycin, AZT, & NTZ were more successful when administered in conjunction with immune-boosting therapeutics (including probiotics).

In the current work, histopathological examination revealed that chitosan improved drastically the effectiveness of AZT, when given together. Complete disappearance of the parasites and neutrophilic activity from intestinal tissues were reported. In the same context, other protozoa; *Entamoeba histolytica*, benefited most from combined chitosan (nanoparticles) with paromomycin (Hamad, 2021).

In this study, using AZT combined with *L. acidophilus* or chitosan for 5 days caused the disappearance of infection from intestinal tissues which were maintained till scarification, a week after treatment. This agreed with Rehg (1991) who found disappearance of *Cryptosporidium* from intestinal tissues using AZT alone for 9 days.

In the present study, chitosan combined with *L. acidophilus* was the first trial to assess the synergistic effect of the natural therapies. This caused a significant decrease in oocysts shedding compared to control and AZT alone, with a reduction of 92.76%. This was a comparable to combined synthetic AZT with *L. acidophilus* (93.44%), which caused remarkable histopathological improvement as compared to *Lactobacillus* alone. Chitosan/*L. acidophilus* was the only group that gave total re-solution of intestinal inflammation, and complete disappearance of parasitic infection.

On examining liver specimens, all drug combinations were capable to decrease but not to totally resolve portal and interface inflammations (as mostly observed with single therapy). Chitosan/*L. acidophilus* combined caused increased lobular inflammation. This incomplete cure of inflammation with combined therapy might be related to the immaturity of the neonatal mice body systems and their inability to cope with combined therapies (Bugelski *et al,* 2010). This required more study to assess the hepatic effects caused by such combinations, especially in the young aged.

Conclusion

Lactobacillus acidophilus showed a highly promising anticryptosporidial activity, which exceeded the synthetic drug AZT, in addition to its ability to down-regulate intestinal and hepatic inflammations even when administer-

ed alone. Chitosan, also, represented a rather attractive anticryptosporidial alternative.

Further studies are needed to standardize *L. acidophilus* administration modalities to achieve total infection elimination (as a single or combined therapy), especially for younger ages.

References

Ahmed, O, Abdel-Halim, M, Farid, A, Elamir, A, 2022: Taurine loaded chitosan-pectin nanoparticle shows curative effect against acetic acid-induced colitis in rats. Chem. Biol. Interact. 351: 109715.

Ahmed, SA, El-Mahallawy, HS, Karanis, P, 2019: Inhibitory activity of chitosan nanoparticles against *Cryptosporidium parvum* oocysts. Parasitol. Res. 118, 7:2053-63.

Ajanya, BU, Attah, F, Mahmud, ME, Owolabi, BI, Adetoro, RO, *et al*, 2018: Therapeutic potency of probiotics in the treatment of gastrointestinal parasites. J. Publ. Hlth. Dent. 1, 2:22-30.

Alak, JI, Wolf, BW, Mdurvwa, EG, Pimentel-Smith, GE, Adeyemo, O, 1997: Effect of *Lactobacillus reuteri* on intestinal resistance to *Cryptosporidium parvum* infection in a murine model of acquired immunodeficiency syndrome. J. Infect. Dis. 175, 1:218-21.

Al-Jarjary, SA, Al-Kennany, ER, Abbu, OA, 2010: Histopathological study on liver of mice infected with *Cryptosporidium parvum* and treatment with azithromycin, spiramycin alone and mixed with vitamin E. AJVS. 3, 2:63-70.

AL-Khaliq, IMA, Nasser, AJ, Ghadban, MM, 2021: Role of *Lactobacillus plantarum* and *Lactobacillus acidophilus* as a treatment of Cryptosporidiosis in mice. Int. J. Drug Deliv. Technol. 11, 3: 812-16.

Ashigbie, PG, Shepherd, S, Steiner, KL, Amadi, B, Aziz, N, *et al*, 2021: Use-case scenarios for an anti-*Cryptosporidium* therapeutic. PLOS Negl. Trop. Dis. 15, 3: e0009 057.

Berrilli, F, Di Cave, D, Cavallero, S, D'Amelio, S, 2012: Interactions between parasites and microbial communities in the human gut. Front. Cell Infect. Microbiol. 2:141-6.

Bitsue, H K, Zeru, F, Gebreyesus, N, Hadush, B, Afera, B, *et al*, 2017: *In vivo* evaluation of the therapeutic efficacy of *Allium sativum* against cryptosporidiosis. Ethiopian J. Vet. Sci. Anim. Para. (EJVSAP). 1, 1:46-56.

Blanco-García, E, Guerrero-Callejas, F, Blanco-Méndez, J, Gómez-Couso, H, Luzardo-Álvarez, A, 2016: Development of particulate drug formulation against *C. parvum*: Formulation, characterization and *in vivo* efficacy. Eur. J. Pharm. Sci. 92:74-85.

Blomquist, L, hirata, I, Slezak, P, Ohshiba, S, 1994: Duodenitis-distinguishing features in a retrospective endoscopic and histological study. Hepatol. Gastroenterol. 41: 537-41.

Bugelski, PJ, Volk, A, Walker, MR, Krayer, J H, Martin, P, *et al*, 2010: Critical review of preclinical approaches to evaluate the potential of immunosuppressive drugs to influence human neoplasia. Int. J. Toxicol. 29, 5:435-66.

Cardiff, RD, Miller, CH, Munn, RJ, 2014: Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb. Protoct. 6: 073411.

Chan, YH, 2003: Biostatistics102: Quantitative Data: Parametric & Non-parametric Tests. Singapore Med. J. 44, 8:391-6.

Charania, R, Wade, BE, McNair, NN, Mead, J R, 2020: Changes in the micro-biome of *Cryptosporidium*-infected mice correlate to differences in susceptibility and infection levels. Microorganisms 8, 6:879-82.

Checkley, W, White Jr, AC, Jaganath, D, Arrowood, MJ, Chalmers, RM, *et al*, 2015: A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect. Dis. 15, 1:85-94.

Dionisio, D, Orsi, A, Sterrantino, G, Meli, M, Di Lollo, S, *et al*, 1998: Chronic cryptosporidiosis in patients with AIDS: Stable remission and possible eradication after long-term, low dose azithromycin. J. Clin. Pathol. 51, 2:138-42.

Diptyanusa, A, Sari, IP, 2021: Treatment of human intestinal cryptosporidiosis: A review of published clinical trials. Int. J. Parasitol.: Drugs Drug Resist. 17:128-38.

Dixit, P, Rao, MLV, Dixit, AK, Shukla, PC, 2022: Comparative efficacy of different therapeutic regimen for natural cryptosporidiosis in kids. Haryana Vet. 61, I:S108-10.

Dixon, MF, Genta, RM, Yardley, JH, Correa, P, 1996: Classification and grading of gastritis. Am. J. Surg. Pathol. 20:1161-81.

Dong, WA, Yuan-Yuan, ZH, 2013: Therapeutic efficacy of Allitridin and Azithromycin on *Crypt-*

osporidium infection in mice. Chin. J. Parasitol. Parasit. Dis. 31, 6:15-9.

El Shazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, TA, 2007: Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol. 37, 1: 55-64

El-Badry, AA, Abdel Aziz, IZ, Shoeib, EY, Ghallab, MM, 2017: *Cryptosporidium* genotypes and associated risk factors in a cohort of Egyptian children. Comp. Clin. Pathol. 26, 5:1017-21.

El-Bahnasawy, MMM, Morsy, ATA, Morsy, T A, 2018: A mini-overview on zoonotic cryptosporidiosis. J. Egypt. Soc. Parasitol. 48, 1:35-44.

el-Sibaei, MM, Rifaat, MM, Abdel Hameed, D M, 2003: Nosocomial sources of cryptosporidial infection in newly admitted patients in Ain Shams University Pediatric Hospital. J. Egypt. Soc. Parasitol. 33, 1:177-88

Fahmy, A, Abuelenain, G, Abdou, A, Elhakeem, M, 2021: Comparative study between wild and commercial Egyptian *Lactobacillus reuteri*-antagonizing effects in cryptosporidiosis models. Adv. Anim. Vet. Sci. 9, 6:802-10.

Fathy, MM, Abdelrazek, NM, Hassan, FA, El-Badry, AA, 2014: Molecular copro-prevalence of *Cryptosporidium* in Egyptian children and evaluation of three diagnostic methods. Indian Pediatr. 51, 9:727-9.

Fayer, R, Ellis, W, 1993: Glycoside antibiotics alone and combined with tetracyclines for prophylaxis of experimental cryptosporidiosis in neonatal BALB/c mice. J. Parasitol. 1:553-8.

Foster, JC, Glass, MD, Courtney, PD, Ward, L A, 2003: Effect of *Lactobacillus* and *Bifidobacterium* on *Cryptosporidium parvum* oocyst viability. Food Microbiol. 20, 3:351-7.

Gaber, M, Galal, LA, Farrag, HM, Badary, D M, Alkhalil, SS, *et al*, 2022: The effects of commercially available *Syzygium aromaticum*, *Anethum graveolens*, *Lactobacillus acidophilus* LB, and zinc as alter-natives therapy in experimental mice challenged with *Cryptosporidium parvum*. Infect. Drug Resist. 15:171-6.

Garcia, LS, 2007: Macroscopic and microscopic examination of fecal specimens. In: Diagnostic Medical Parasitology. Part II, 5th ed. Washington, DC: American Society for Microbiology (ASM) Press.

Gargala, G, 2008: Drug treatment and novel drug target against *Cryptosporidium*. Parasite.15, 3: 275-81.

Glass, MD, Courtney, PD, LeJeune, JT, Ward, LA, 2004: Effects of *Lactobacillus acidophilus* and *Lactobacillus reuteri* cell-free supernatants on *Cryptosporidium* viability and infectivity *in vitro*. Food Microbiol. 21, 4:423-9.

Guitard, J, Menotti, J, Desveaux, A, Alimardani, P, Porcher, R, *et al*, 2006: Experimental study of the effects of probiotics on *Cryptosporidium parvum* infection in neonatal rats. Parasitol. Res. 99, 5:522-7.

Gupta, V, Garg, R, 2009: Probiotics. Indian J. Med. Microbiol. 27, 3:202-9.

Hamad, HK, 2021: Evaluation of paromomycin loaded chitosan nanoparticles and oxidative stress activities against *Entamoeba histolytica*. J. Phys. Conf. Ser. 2114, 1:1-7.

Hayashi, Y, Ohara, N, Ganno, T, Ishizaki, H, Yanagiguchi, K, 2007: Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. J. Dent. 35, 11:871-4.

Helmy, YA, Krücken, J, Nöckler, K, von Samson-Himmelstjerna, G, Zessin, KH, 2013: Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt. Vet. Parasitol. 193, 1/3:15-24.

Karpe, AV, Hutton, ML, Mileto, SJ, James, M L, Evans, C, *et al*, 2021: Cryptosporidiosis modulates the gut microbiome and metabolism in a murine infection model. Metabolites 11, 6:380-6.

Kayser, O, 2001: A new approach for targeting to *Cryptosporidium parvum* using mucoadhesive nano-suspensions: research and applications. Int. J. Pharm. 214, 1/2:83-5.

Khalifa, EA, 2016: Probiotics as a promising treatment of experimental cryptosporidiosis in an immuno-suppressed mouse model. Int. J. Curr. Microbiol. 5, 3:97-106.

Mahfouz, ME, Mira, N, Amer S, 2014: Prevalence and genotyping of Cryptosporidium spp. in farm animals in Egypt. J. Vet. Med. Sci. 76, 12: 1569-75.

Mammeri, M, Chevillot, A, Thomas, M, Polack, B, Julien, C, *et al*, 2018: Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum in vitro* and *in vivo* in neonatal mice. Exp. Parasitol. 194:1-8.

Mead, JR, 2002: Cryptosporidiosis and the chall-

enges of chemotherapy. Drug Resist. Update 5, 1: 47-57.

Moawad, HS, Hegab, MH, Badawey, MS, Ashoush, SE, Ibrahim, SM, *et al*, 2021: Assessment of chitosan nanoparticles in improving the efficacy of nitazoxanide on cryptosporidiosis in immunosuppressed and immunocompetent murine models. J. Parasit. Dis. 45, 3:606-19.

Mostafa, NE, Abdel Hamed, EF, Fawzy, EM, Zalat, RS, Rashed, HE, *et al*, 2018: The new trend in the treatment of experimental cryptosporidiosis and the resulting intestinal dysplasia. Colorect. Cancer 7, 4:CRC06.

Obiad, HM, Al-Alousi, TI, Al-Jboori, AH, 2012: The in vivo effect of some medicinal plant extracts on *Cryptosporidium* parasite. J. Univ. Anbar Pure Sci. 6:3-6.

Patel, AR, Shah, NP, Prajapati, JB, 2014: Immunomodulatory effects of probiotics in the treatment of human immunodeficiency virus (HIV) infection. Biomed. Prev. Nutr. 4, 1:81-4.

Rehg, JE, 1991: Activity of azithromycin against *Cryptosporidia* in immunosuppressed rats. J. Infect. Dis. 163, 6:1293-6.

Rehg, JE, 1994a: A comparison of anticryptosporidial activity of paromomycin with that of other aminoglycosides and azithromycin in immunosuppressed rats. J. Infect. Dis. 170, 4:934-8.

Rehg, J, 1994b: New potential therapies for cryptosporidiosis: an analysis of variables affecting drug efficacy. Folia Parasitol. 41, 1:23-6.

Rotkiewicz, T, Rotkiewicz, Z, Depta, A, Kander, M, 2001: Effect of *Lactobacillus acidophilus* and *Bifidobacterium* sp. on the course of *Cryptosporidium parvum* invasion in new-born piglets. Bull. Vet. Inst. Pulawy 45, 2:187-95.

Saber, AE, Abdelwahab, AK, El Amir, AM, Nassar, MI, Zohdi, HF, 2017: Bee venom loaded chitosan nanoparticles as treatment for amoebiasis in mice. J. Egypt. Soc. Parasitol. 47, 2:443-58.

Sadek, GS, 2014: Use of nested PCR-RFLP for genotyping of *Cryptosporidium* parasites isolated from calves and children suffering from diarrhea. PUJ 7, 2:129.

Shalaby, NM, Shalaby, NM, 2015: *Cryptosporidium parvum* infection among Egyptian school children. J. Egypt. Soc. Parasitol. 45, 1:125-31.

Shepherd, R, Reader, S, Falshaw, A, 1997: Chitosan functional properties. Glycoconj. J. 14, 4: 535-42.

Silva, NS, Araújo, NK, Daniele-Silva, A, Oliveira, JW, Medeiros, JM, *et al*, 2021: Antimicrobial activity of chitosan oligosaccharides with special attention to antiparasitic potential. Mar. Drugs 19, 2:110.

Sindhu, KN, Sowmyanarayanan, TV, Paul, A, Babji, S, Ajjampur, SS, *et al*, 2014: Immune response and intestinal permeability in children with acute gastroenteritis treated with *Lactobacillus rhamnosus* GG: A randomized, double-blind, placebocontrolled trial. Clin. Infect. Dis. 58, 8:1107-15.

Sparks, H, Nair, G, Castellanos-Gonzalez, A, White, AC, 2015: Treatment of *Cryptosporidium*: What we know, gaps, and the way forward. Curr. Trop. Med. Rep. 2, 3:181-7.

Tozaki, H, Komoike, J, Tada, C, Maruyama, T, Terabe, A, 1997: Chitosan capsules for colon-specific drug delivery: Improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86, 9: 1016-21.

Ueno, N, Fujiya, M, Segawa, S, Nata, T, Moriichi, K, *et al*, 2011: Heat-killed body of *Lactobacillus brevis* SBC8803 ameliorates intestinal injury in a murine model of colitis by enhancing the intestinal barrier function. Inflamm. Bowel Dis. 17, 11:2235-50.

Vemuri, R, Shinde, T, Gundamaraju, R, Gondalia, SV, Karpe, AV, *et al*, 2018: *Lactobacillus acidophilus* DDS-1 modulates the gut microbiota and improves metabolic profiles in aging mice. Nutrients 10, 9:1255.

Wang, ZF, Wang, MY, Yu, DH, Zhao, Y, Xu, HM, *et al.* 2018: Therapeutic effect of chitosan on CCl4-induced hepatic fibrosis in rats. Mol. Med. Rep. 18, 3:3211.

Westin, J, Lagging, LM, Wejstål, R, Norkrans, G, Dhillon, AP, 1999: Interobserver study of liver histopathology using the ISHAK score in patients with chronic hepatitis C virus infection. Liver 19, 3:183-7.

Wolska-Kusnierz, B, Bajer, A, Caccio, S, Heropolitanska-Pliszka, E, Bernatowska, E, *et al*, 2007: *Cryptosporidium* infection in patients with primary immunodeficiencies. J. Pediatr. Gastroenterol. Nutr. 45, 4:458-64.

Wyatt, JI, Rathbone, BJ, Dixon, MF, Heatley, RV, 1987: *Campylobacter pyloridis* and acid induced gastric metaplasia in the pathogenesis of duodenitis. J. Clin. Pathol. 40:841-8.

Yong, SK, Shrivastava, M, Srivastava, P, Kun-

hikrishnan, A, Bolan, N, 2015: Environmental applications of chitosan and its derivatives. Rev. Environ. Contam. Toxicol. 233:1-43.

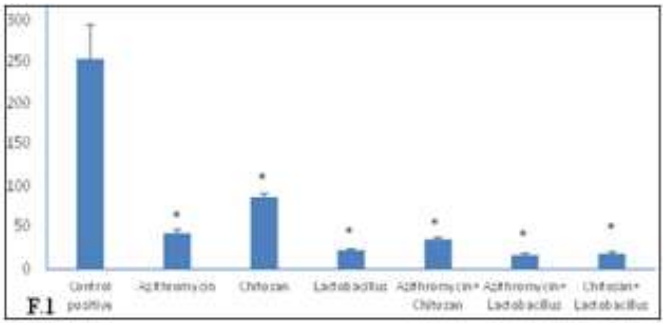
Zakir, S, Tafese, W, Mohamed, A, Desa, G, 2021: Review of cryptosporidiosis in calves, children and HIV/AIDS patients. Healthcare Rev. 2, 1:1-5.

Explanation of figures

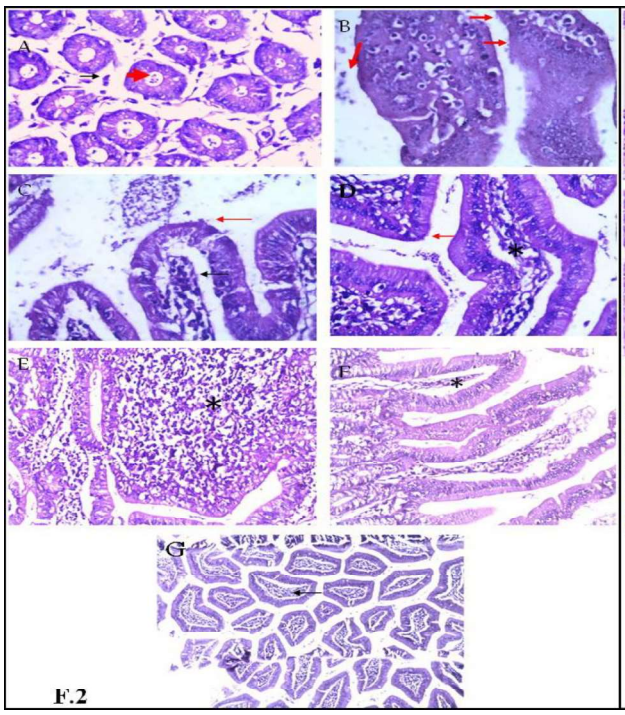
Fig.1: *Cryptosporidium* oocysts/gm., in positive control and treated with single & combined therapeutic regimens (*significant to control).

Fig.2: Small intestine H&E-stained sections: A- Positive control. Lamina propria showed mild lymphoplasmacytic cellular infiltrate (black arrow), scattered neutrophils & mild edema. Trophozoites related to brush borders (red arrow), B- AZT-treated one showed scattered trophozoites related to brush borders (red arrows), C- Chitosan-treated one with lamina propria showed mild lymphoplasmacytic cellular infiltrate (black arrow) and mild edema. Trophozoites related to brush borders (red arrow), D- *L. acidophilus*-treated one with lamina propria showed mild lymphoplasmacytic cellular infiltrate (asterisk) and mild edema. Trophozoites related to brush borders (red arrow), E- AZT/Chitosan-treated one showed lamina propria with moderate lymphoplasmacytic cellular infiltrate (asterisk), mild edema, no parasite, F- AZT/ *L. acidophilus* -treated one showed lamina propria with mild lymphoplasmacytic cellular infiltrate (asterisk), mild edema but no parasite, G- Chitosan/ *L. acidophilus*-treated one with lamina propria within normal lymphoplasmacytic cellular infiltrate (black arrow), but no parasite. Sections (A-F) with average goblet cell population but no gastric metaplasia, ulceration, atrophy, atypia or malignancy; sections A, C, D ,E, F (X 200), B (X 400) & G (X 100).

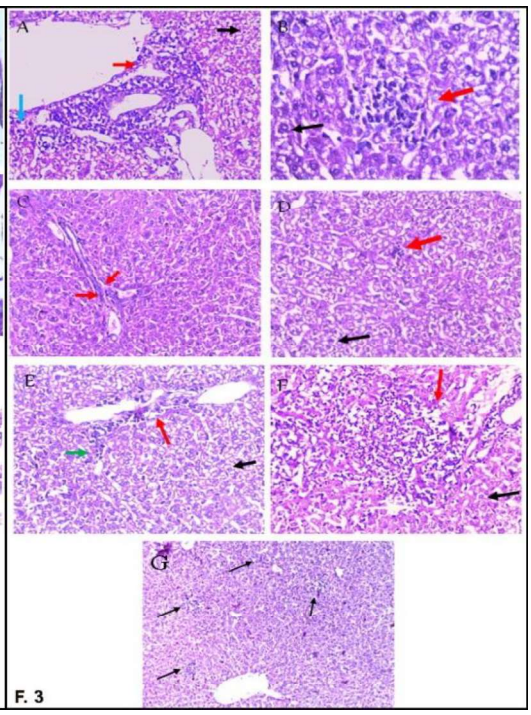
Fig.3: Liver H&E-stained sections: A- Positive control showed hepatocytes with mild hydropic degeneration (black arrow). Portal tract with moderate portal inflammation (red arrow) and moderate interface (periportal) hepatitis (green arrow), B- AZT-treated one showed liver tissue hepatocytes with mild hydropic degeneration (black arrow) and lobular inflammation focus (red arrow), C- Chitosan-treated one, hepatocytes showed mild hydropic degeneration. Portal tract showed mild portal inflammation (red arrows), D- *L. acidophilus*-treated one showed hepatocytes with mild hydropic degeneration (black arrow) and of lobular inflammation focus (red arrow), E- AZT/Chitosan -treated one showed hepatocytes with mild hydropic degeneration (black arrow). Portal tract with mild inflammation (red arrow) and focal periportal hepatitis foci (green arrow), F- AZT/*L. acidophilus*-treated one hepatocytes showed mild hydropic degeneration (black arrow). Portal tract with moderate portal inflammation (red arrow) and G- Chitosan/*L. acidophilus*-treated one showed hepatocytes with mild hydropic degeneration and lobular inflammation multiple foci (black arrow). Central veins patent. Sections (A-F) with lobular architecture without steatosis, cholestasis, dysplasia or malignancy seen. Sections A,C,D,E,F (X200), B (X400) and G (X100).



F.1



F.2



F. 3