MJMR, Vol. 34, No. 2, 2023, pages (132-142).

Access Open
ISSN:2682-4558

Research Article

The efficacy of natural ginger (Zingiber officinale) on experimentally infected mice with *Cryptosporidium parvum*

Noha H. Abdelgelil[1], Manar M. Sanadeki[1], Mohamed M. Abdel-Fatah[1], Reham A. M. Abd Rabou[1*]

[1]Department of Parasitology, Faculty of Medicine, Minia University, Minia 61519, Egypt.

DOI: 10.21608/MJMR.2023.218999.1427

Abstract

Cryptosporidium is a protozoan parasite that cause diarrhea with significant morbidity and mortality. Few antiparasitic drugs are effective against it. Therefore, finding an alternative is necessary. The current study aimed to evaluate the therapeutic efficacy of ginger (*Zingiber officinale*) in experimentally infected mice. Mice were divided into five groups: G1: healthy controls, G2: infected/untreated, G3: infected/ginger-treated, G4: infected/nitazoxanide-treated and G5: uninfected/ginger-treated. On day 7 post-inoculation, each group started its specific treatment. Diarrhea, weight, daily oocyst shedding, serum levels of IL12, IFNγ, TNFα, IL10, and IL4 levels were recorded. Furthermore, ileal histopathology was done. The mean weight gain of G3 and G4 mice was significantly higher than that in G2 ($P< 0.001$). Oocyst shedding was significantly reduced in G3 and G4 by day 10 pi ($P< 0.02$), till zero by day 20 pi. On day 10, IFNγ, TNFα and IL12 were significantly lower in G3 and G4 than that in G2. On day 21, G2 showed a significant upregulation of TNFα compared to that in G3 and G4. Both G3 and G4 showed upregulation of IL4 vs. G2 (p value $\leq$ 0.0001). The histological architecture of villi in G3 and G4 showed visible improvement compared to that in G2. These results suggest that ginger is a promising anti-*cryptosporidium* treatment without side effects.

Key words: *Cryptosporidium*, Mice, *Zingiber*, Nitazoxanide, Cytokines.

Introduction:

Human cryptosporidiosis is caused by infection with apicomplexan protozoa of the genus *Cryptosporidium*. Molecular studies have demonstrated that it is caused by at least 15 different species. Among the more common species are *Cryptosporidium hominis* (*C. hominis*), for which humans are the only natural host, and *Cryptosporidium parvum (C. parvum*), which infects bovines as well as humans[1]. *C. parvum* is highly resistant to the current drug treatments[2]. Though the pharmaceutical industry has made several attempts to develop an effective treatment for cryptosporidiosis, this disease still constitutes a major health problem[3].

Infection with *Cryptosporidium* is associated with an acute clinical illness characterized by diarrhea in humans and many domestic and wild animals. Its infection is most pathogenic in neonates and elderly people[4]. In immune-competent patients, the disease causes profuse watery diarrhea, abdominal cramps, nausea, vomiting and low grade fever, which may last 2–12 days but these symptoms are usually self-limiting [5]. On the other hand, the infection may persist for longer duration with mal-absorption and severe dehydration in immune-compromised or malnourished patients, which may require fluid replacement therapy [6, 7].

The United States Food and Drug Administration has approved nitazoxanide (NTZ), a broad-spectrum anti-parasitic drug that is effective against cestodes, trematodes, nematodes, and protozoa in humans[8]. However, NTZ does not show any curative

effect against *C. parvum* infection in calves[9]. Furthermore, other anti-parasitic treatments have received considerable attention due to the increased development of chemo-drug resistance[10]. Consequently, the available drugs are considered ineffective in the treatment of cryptosporidiosis. The development of drug-resistant strains of this organism evokes the importance of using alternative medicine.

Ginger (*Zingiber officinale*) is known worldwide as a spice and flavoring agent. It has an antioxidant effect that enhances the immune response, allowing the body to fight the infections naturally. Moreover, ginger has other activities that may help in parasitic clearance, such as its ability to increase digestive fluids, absorb and neutralize toxins. Studies were conducted on the antibacterial, antiviral, antifungal[11] and antiparasitic activity of ginger and its constituents [12, 13, 14].

Therefore, this study was carried out to evaluate the protective and therapeutic effects of ginger in experimentally infected mice with *C. parvum.*

Materials and methods

Ethical consideration:

The study protocol was accepted by the Ethics Committee of the Department of Medical Parasitology, Faculty of Medicine and by the Institutional Committee of Research Ethics (approval No. 3332022).

Type of the study:

This case–control study was conducted during the period from July 2022 to September 2022 in the Parasitology Department, Faculty of Medicine, Minia University, Minia, Egypt.

Preparation of *C. Parvum* oocysts (Source of *C. parvum* and genotyping)

C. parvum oocysts were collected from naturally infected humans. Oocysts were concentrated by the water ether technique and identified by modified Zeihl Neelson stain [15]. The purified oocysts were confirmed as *C. parvum* by Nested polymerase chain reaction (nPCR) amplification to amplify a 214-base pair fragment of the *Cryptosporidium* 18S ribosomal RNA gene encompassing the polymorphic region between nucleotides 179 and 271. Restriction fragment length polymorphism (RFLP) was done for the product of nPCR that initially digested with the restriction endonuclease *TaqI* (Thermo Scientific, EU, Lithuonia, #ER0671, 3000U, Lot: 00399406, concentration: 10U/ µl, supplied with; 1ml of 10X buffer TaqI), then the *Taq1* +ve product was digested with *AseI* restriction enzyme (Thermo Scientific, Thermo Fisher Scientific, EU, Lithuonia) to differentiate *C. hominis* from *C. parvum* as previously described by Gabr et al., [16]. Oocysts were kept in a BPS solution and counted using a hemocytometer [17].

Preparation of drugs:

Ginger solution: 50 gm of ginger powder was soaked in distilled water for about 2 days to prepare a stock solution of 100 mg/ml. The solution was filtered through grade 1 filter paper (Whatman UK). The solution was stored in a sterile dark bottle at -20^{o} c until use [13]. The concentration of ginger extract was adjusted to a final concentration of 50mg/kg body weight/day[18]. Nitazoxanide (NTZ) suspension: It was given at a dose of 100 mg/kg to mice [14].

Animals:

Fifty, eight-week-old male albino laboratory mice, each weighing 20±5 gm., were obtained from the experimental house, Faculty of Medicine, Minia University. The animals had free access to standard rodent food and water.

Experimental design:

Mice were divided into five groups (G1–G5), ten mice each. G1 represented healthy control, while G2 represents infected/untreated group.
G3 mice were infected/ginger-treated, G4 mice were infected/ NTZ-treated, and G5 mice were uninfected/ginger-treated. Each mouse in G2, 3 and 4 was infected by oral administration of 1 x 10^4 *C. parvum* oocysts[19] using gastric gavage. On day 7 post-inoculation (pi), G3 and G5 received an aqueous suspension of ginger and G4 received NTZ daily by gastric tubes 1 h before meals for 7 consecutive days. To determine the potency of the treatments, the animals were given a recovery period of 7 days at the end of the treatment period.

Follow up of mice:

Behavioral observations and mice survival:

The activity of animals in different groups and survival were recorded every other day.

Body weight measurements:

All animals were weighed on days 0, 7, 14 and 21(pi).

Fecal examination:

Fecal samples were collected and examined on day 0 to exclude mice infection (not shown data). Then fecal samples were collected every other day starting from the second day (pi). The number of oocysts was detected per mg of feces using a hemocytometer.

Cytokines measurements:

Blood samples were taken from the medial canthus on days 10 and 21. The blood was left and then centrifuged to obtain sera. The sera were kept at -20° C for cytokine detection. In different study groups, a two-site sandwich enzyme-like immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Wuhan Elabscience Biotechnology Co., Ltd.) measured IFNγ, IL-12, TNFα, IL-4, and IL-10 levels[20].

Histopathological analysis:

All the mice were sacrificed on day 21. Small intestine (ileum) sections were excised and fixed in 10% formalin. The obtained tissue sections were fixed on glass slides, deparaffinized and stained with hematoxylin and eosin stain. Then the sections were examined under a light microscope at 10, 40 and 100 X magnifications [21].

Statistical analysis:

Statistical significance was determined using Kaplan meier, Chi-square tests, and Kruskal Wallis test. Data are presented as means ± standard error (SE) using Statistical SPSS for Windows, issue 15.8 with P 6 .05 as significant and P 6 .01 as very significant.

Results

Behavioral observations and survival of mice

Pathological symptoms were observed from day 7, with maximum severity on day 14 pi in the infected untreated group (G2). Symptoms involved lack of energy, diarrhea and stool contamination of the back. Other groups (G1, 3, 4 and 5) did not display any pathological symptoms.

Mice in all studied animal groups were alive till day 13. From day 15, the survival rate of infected/untreated (G2) mice started to decrease until on day 21, it became 50%. It was statistically significantly lower than the other groups (p value≤ 0.02). There was no difference in the survival rate of mice between the infected/ginger-treated (G3) and the infected/ NTZ-treated (G4) groups, as shown in Fig. 1.

The effect of ginger extract treatment on body weight of mice:

On day 7, there was a significant difference in the body weight reduction of mice of the three infected groups (G2, G3 and G4) compared to the healthy control group (p value ≤0.03). While on days 14, and 21, the weight loss of mice in the infected/untreated group (G2) was statistically significant (P ≤0.0001 and 0.001, respectively). There was no significant difference in body weight of mice between the infected/ginger-treated group (G3) and the infected/NTZ-treated (G4), as shown in Table 1

The effect of ginger extract treatment on fecal oocyst levels:

Oocysts started to appear in stool from day 4 pi in all infected animal groups (G2, G3 & G4) until day 8 without any statistically significant difference. On day 10, (3 days after starting the treatment regimen), the number of shed oocysts significantly decreased in both the infected/ ginger treated (G3) and the infected/NTZ treated (G4) compared to the infected/untreated G2 (P≤ 0.02).

This significant difference showed more increases on successive days (12, 14, 16, 18 and 20) (P≤ 0.0001). The number of shed oocysts became nearly zero on day 20 in G3 and G4 (Table 2).

Cytokines:

On day 10, the three infected groups (G2, G3 & G4) showed significant upregulation of proinflammatory cytokines (IFNγ, TNFα, IL12) compared to that in healthy control group (G1) and the uninfected/ginger treated (G5) (p value ≤ 0.002). While there was no statistical difference in IL4 and IL10 between all of them.

The infected untreated G2 showed higher upregulation of proinflammatory cytokines (IFNγ, TNFα, IL12) compared to that in G3and G4 (p value ≤ 0.001, 0.001, 0.01 respectively).

On day 21 pi, G2 showed significant upregulation of only TNFα compared to that in G3 and G4 (p values ≤0.0001). Both G3 and G4

showed upregulation of IL4 vs. G2 (p value ≤0.0001). There was no significant change in the levels of both IFNγ and IL10 in all groups (Fig. 2).

Histopathological examination

The healthy control group displayed normal architecture of the villi of the ileum epithelium (Fig. 3f). On the other hand, small intestinal sections of infected untreated mice (G2) showed classical cryptosporidiosis-associated pathological changes, including villi blunting and ulceration, bleeding as a result of dilated blood vessels and capillaries, lymphocytic infiltration in the lamina propria and oedema . *C. parvum* parasites were present on the brush border (Fig. 3a, b and c). While both groups of mice that were infected and treated by ginger or NTZ (G3 and G4) showed improved villi symmetry, lengthened villi, improvement in the cellular structure of the absorptive cells, and reduced bleeding compared to G2 with complete absence of oocyts. (Fig. 3 d and e).

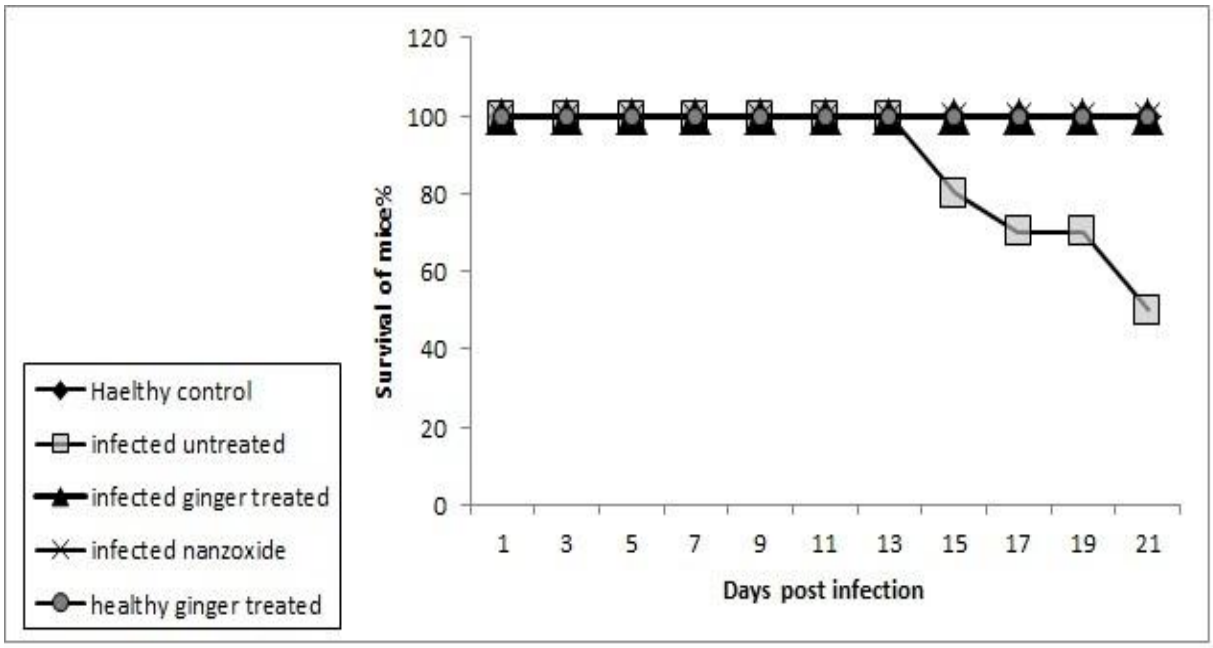

Fig.1: Survival rate of the different groups of mice.
(♦) Diamonds show the data from the healthy group G1.
(■) Squires show the data from the infected untreated G2.
(▲) Triangles show the data from the infected treated + Ginger G3.
(×) X-shapes show the data from the infected treated+ NTZ G4.
(*) Stars show the data from healthy taking ginger G5.
Data are presented as the mean ± SEM.

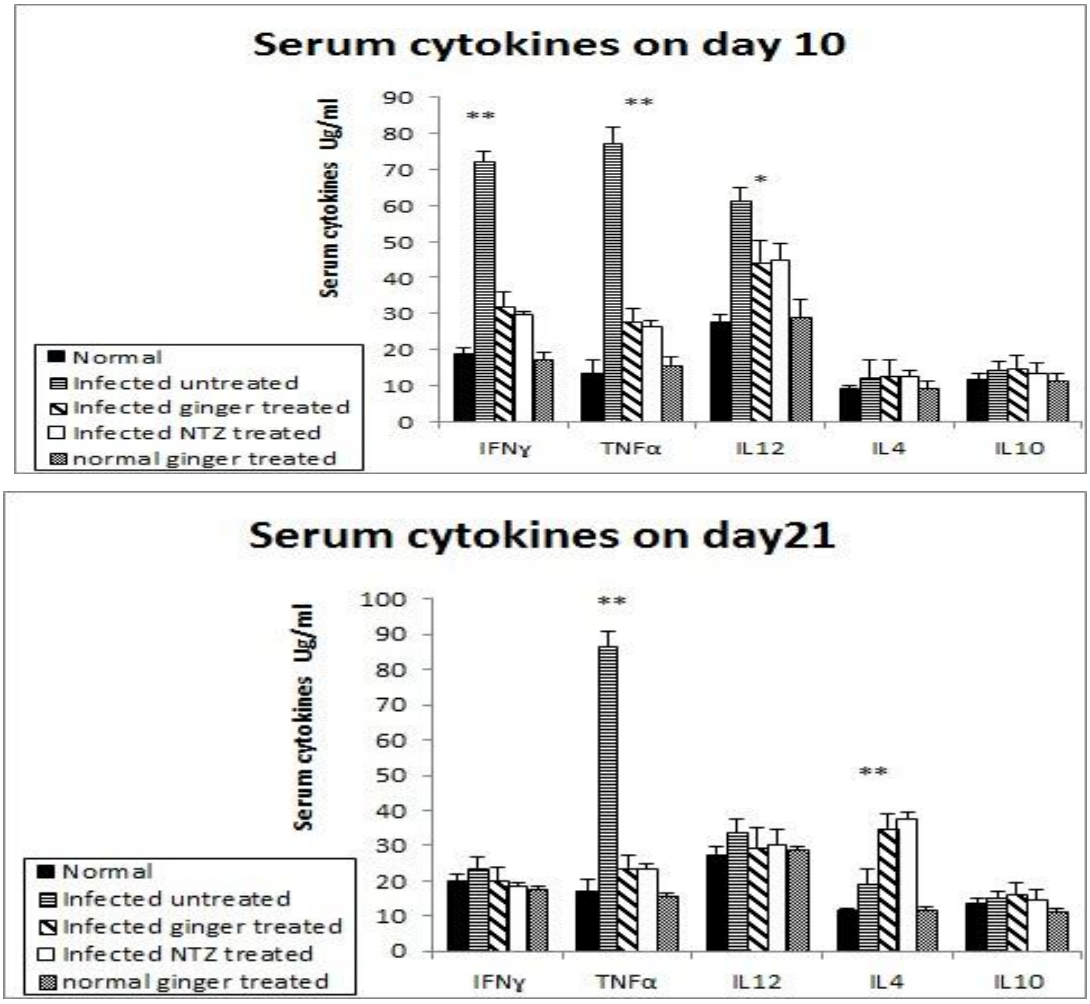

Fig. 2: Serum cytokines on day 10 and day 21.

*: P value: ≤ 0.05, **: P value: ≤ 0.001

shows the data from the healthy group G1.

shows the data from the infected untreated G2.

shows the data from the infected treated + Ginger G3

shows the data from the infected treated+ NTZ G4.

show the data from healthy taking ginger G5.

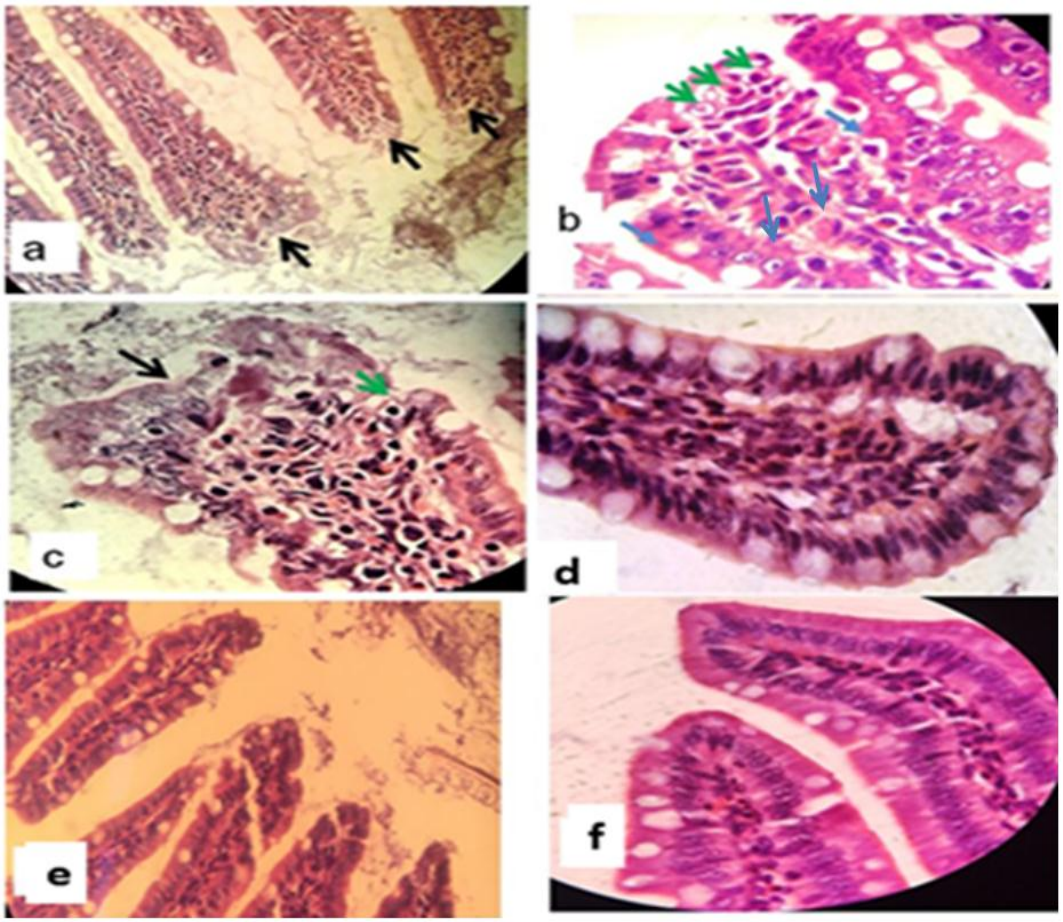

Fig. 3 Histological photos of cryptosporidiosis in intestinal sections of different study groups of mice stained with H&E.

(a-c): Photomicrograph of intestinal section of infected untreated mice shows (a) changes in the villi architecture, including a blunting, villi ulceration (black arrow) (X40). (b) Dilated blood vessels and capillaries with bleeding (blue arrow), lymphocytic infiltration in the lamina propria and several *Cryptosporidium* oocysts (green arrows) (X100). (c) The tips of the microvilli were denuded (black arrow) and *Cryptosporidium* oocysts (green arrows) (X100).
(d, e): Photomicrograph of intestinal section of infected treated mice either with ginger and/ or NTZ show improvement of villi symmetry, lengthened villi, improvement in the cellular structure of the absorptive cells, reduced bleeding **d** (X100) and **e** (X40).
(f) Photomicrograph of intestinal section of healthy control shows normal mucosal walls with average villi, crypts and submucosa.

Table 1: Changes in the body weight of the mice of the various studied groups:

Groups/N*	Day 0	Day 7	Day 14	Day 21
Healthy group	21.45±1	21.3±1.5	22.79±0.89	23.43±0.84
Infected untreated	22.18±0.6	20.54. ±0.6	18.44±1.4	18.15±1.4
Infected (+ ginger)	21.81±0.6	20.23±0.8	21.47±1.4	22.08±1.4
Infected (+ NTZ)	22.39±0.9	20.89±0.91	21.76±0.92	22.5±1.3
Healthy (+ginger)	21.55±0.8	21.85±0.85	22.18±0.9	22.55±0.92

N*= 10 mice in all groups except in infected un-treated , here N equals 7 mice on day 14 and 5 mice on days 21.
P value ≤ 0.05 (significant).

Table 2: *Cryptosporidium* oocyst's shedding intensity in the stool of mice of the various studied groups.

days post infection Groups	2	4	6	8	10	12	14	16	18	20
Healthy group	0	0	0	0	0	0	0	0	0	0
Infected untreated	0	1.9± 0.14	3.96± 0.13	8.12± 0.48	9.81± 0.45	12.77 ± 0.9	15.6± 0.6	19± 0.5	21.25 ±0.75	23.46 ±0.4
Infected ginger treated	0	1.87± 0.15	4.01± 0.14	7.97± 0.1	*8.86 ±0.68	**10.1 3±0.2	**6.84 ±0.85	**2.41 ±0.41	**1.3 ±0.3	**0.23 ±0.16
Infected NTZ	0	1.79± 0.12	4.02± 0.17	8.06± 0.168	8.98± 0.17	9.84± 0.28	6.49± 0.22	2.13± 0.33	1.3± 0.34	0.18± 0.17
Healthy ginger treated	0	0	0	0	0	0	0	0	0	0

*: P value: ≤ 0.05, **: P value: ≤ 0.001
N= 10 mice in all groups except in infected un-treated G2, here N equals 7 mice on day 14 and 5 mice on days 21

Discussion

Ginger (Zingiber officinale) is one of the most widely used spices due to its taste and pungency. The rhizome of this plant is one of the most commonly used medicinal herbs because it has many pharmacological effects such as antioxidant effect, potent antibacterial activity[22], strong antifungal activity[23] and anthelmintic activity[24].

In this study, by observing the pathological symptoms, the infected untreated group (G2) displayed lack of energy, diarrhea, and soiling of the back area with feces, while the other groups (G1, 3, 4 and 5) did not display any pathological symptoms. Also, the survival rate was significantly lower in the infected/untreated (G2) mice (50% on day 21) than in that of the infected/ginger-treated (G3) and the infected/ NTZ-treated (G4) ones. Our study is in accordance with a study done by Fayer [6] who reported that the most significant pathological manifestations of cryptosporidiosis observed in the infected animals were diminished activity and the presence of diarrhea, weight loss, and mortality. The absence of pathological symptoms and increased survival rate in the infected ginger treated group could be attributed to ginger's wide range of biological activities, which include anti-inflammatory, anti-microbial, antioxidant and analgesic actions, anti-parasitic effects, and gastro-protective effects[21].

Regarding weight reduction, the infected/ untreated G2 animals experienced significant weight loss on days 14 and 21 of the experiment compared to that of the healthy control mice (G1). Whereas there was no significant difference in body weight between the infected/ginger treated (G3) and infected/NTZ treated (G4) groups of mice. The weight loss in G2 could be attributed to parasite multiplication and immune response, which resulted in atrophy and

sloughing of the upper tips of the intestinal villi, resulting in malabsorption[25].

Infected mice treated with ginger (G3) & NTZ (G4) showed weight gain during the treatment period. This may be explained by ginger limited the parasite multiplication, minimized the parasite load and the pathology associated with *C. parvum* infection. These led to better absorption and increased appetite[26].

Our results agreed with a study done by Shahbazi [27] who stated a significant reduction in the mean body weight of infected mice compared to that of the healthy control.

As previously reported, ginger (*Zingiber officinale*) extract has anti-*cryptosporidium*[16, 20], anti-*Toxoplasma gondii* activity [28], anti-*Giardia lamblia* activity [29], and anti-protozoan activity against *Blastocytis* [13]. It also has an antioxidant effect that enhances the immune response, allowing the body to fight infections naturally [26]

C. parvum oocyst shedding started in all infected groups 4 days after inoculation and continued to increase in the infected/untreated group (G2) throughout the duration of the experiment. On the other hand, *C. parvum*-infected mice treated with ginger and NTZ showed a significantly decreased number of shed oocysts until they disappeared on day 21. The reduction and elimination of fecal oocyst shedding in response to ginger treatment described here may be attributable to a direct effect on parasite growth in the intestines, the production of the sexual stages, and/or the formation of oocysts. Ginger's anti-*Cryptosporidium* effect may be due to gingerols, shogaols and phenolic compounds are the major bioactive components present in *Zingiber officinale* (ginger) [30, 31].

Abouel-Nour and others [18] reported that oocyst shedding in ginger-treated mice decreased gradually till it disappeared. Moreover, another study reported a statistically significant reduction in oocyst shedding in ginger and NTZ treated groups till no oocysts were found at days 21 and 23 PI, respectively [14].

C. parvum infection and pathogenesis are controlled by interaction of both Th1 (IFN γ, TNF α, and IL12) and Th2 (IL4 and IL10) cytokine responses. In the infected untreated group, there was a significant increase in proinflammatory cytokines in an attempt to control infection and parasite multiplication, but also this led to severe pathogenesis with no significant change in the level of Th2 (anti-inflammatory cytokines) compared to that in the healthy control group.

Both ginger and NTZ treatments modulate immune responses. In the early stage of infection, G3 and G4 showed a significantly lower level of Th1 cytokines (IFN γ, TNF α and IL12) compared to that of the G2 to control infection. Later, more balanced responses through Th2 cytokines (IL4) occurred, which increased significantly to limit intestinal pathology and facilitate cure. This result matches that of Abouel-Nour and others [18] (except that they measured IL5 as a Th2 cytokine) and Fawzy and others [32].

Many medicinal plants and drugs have been tested on *C. parvum* infection, primarily through immune response modulation, as they reduce TH1 cytokines (pro-inflammatory cytokines) and increase anti-inflammatory cytokines (IL4, IL5, or IL10). This immune response matches the immune response in this experiment [33].

Ginger had a therapeutic effect in many disorders by modulating immune response by decreasing proinflammatory cytokine secretion (IFNγ, TNF α, IL6, IL12, IL17) and increasing anti-inflammatory (IL4, IL5 & IL10) cytokines [28, 34]. Also, 6-gingerol showed efficacy in the treatment of DSS-induced ulcerative colitis in mice [35] and against chemical induced cutaneous inflammation [36].

Many medicinal plants and drugs have been tested on *C. parvum* infection, primarily through immune response modulation, as they reduce TH1 cytokines (pro-inflammatory cytokines) and increase anti-inflammatory cytokines (IL4, IL5, or IL10). This immune response matches with the immune response in this experiment [18, 33].

Regarding histopathological examination, the infected/untreated group (G2), showed sub-epithelial cell edema, bleeding, atrophy and sloughing of the upper tips of the intestinal villi of the ileum with inflammatory cell infiltration. These pathological changes can be attributed to the multiplication of the parasite, secreted toxins, increase of the levels of proinflammatory

cytokines, causing an asymmetrical loss of epithelial cells resulting in shortening and fusing of the villi. Toxins secreted by *C. parvum* that directly damage epithelial cells [37].

Both the infected/ginger-treated group (G3) and infected /NTZ -treated group (G4), showed obvious improvement in the villi symmetry, length and the cellular structure of the absorptive cells with reduced bleeding. This may be explained by ginger has many compounds such gingerols and shogaols and phenolic compounds which might block or compete for receptor sites on the intestinal surface, which leads to a reduction in *C. parvum* colonization [38] or the inhibitory effect of immunomodulation on parasite growth and multiplication [39]. Similar findings were found in studies conducted by Abouelsoued and others [14] and Abouel-Nour and others [18].

Conclusions

The potency of aqueous suspensions of ginger in the treatment of *C. parvum* has been recognized in all measurement parameters used in this study. Infected mice that were treated with ginger showed continuous weight gain and improved intestinal histopathology throughout the course of the experiment. Furthermore, ginger-treated mice stopped shedding fecal oocysts by day 21. These data presented here indicate that ginger is a promising natural treatment for cryptosporidiosis.

References

1. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. *Cryptosporidium* pathogenicity and virulence. Clinic microbiol reviews. 2013; 26:115-134.
2. Armson A, Reynoldson JA, Thompson RC. A review of chemotherapeutic approaches to the treatment of *Cryptosporidium*. *Cryptosporidium*. 2003; 1:395-403.
3. Del Coco VF, Córdoba MA, Basualdo JA. Criptosporidiosis: una zoonosis emergente. Rev argent de microbiol. 2009;41:185-196.
4. Anderson BC, Donndelinger T, Wilkins RM, Smith J. Cryptosporidiosis in a veterinary student. J Am Vet Med Assoc. 1982; 180:408-409.
5. Klein P, Kleinová T, Volek Z, Šimůnek J. Effect of *Cryptosporidium parvum* infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves. Vet parasitol. 2008; 25;152:53-59.
6. Fayer R. *Cryptosporidium*: a water-borne zoonotic parasite. Vet Parasitol. 2004;126: 37-56.
7. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010;124:80-89.
8. Rossignol JF, Maisonneuve H. Nitazoxanide in the treatment of *Taenia saginata* and *Hymenolepis nana* infections. Am J Trop Med Hyg. 1984;33: 511-512.
9. Schnyder M, Kohler L, Hemphill A, Deplazes P. Prophylactic and therapeutic efficacy of nitazoxanide against *Cryptosporidium parvum* in experimentally challenged neonatal calves. Vet parasitol. 2009; 160:149-154.
10. Hoste H, Torres-Acosta JF, MÁ AD, Brunet S, Sandoval-Castro C, Adote SH. Identification and validation of bioactive plants for the control of gastrointestinal nematodes in small ruminants. Tropical Biomed. 2008;25:56-72.
11. Basile A, Senatore F, Gargano R, Sorbo S, Del Pezzo M, Lavitola A, et al. Antibacterial and antioxidant activities in Sideritis italica (Miller) Greuter et Burdet essential oils. J Ethnopharmacol. 2006;107: 240-248.
12. Mostafa OM, Eid RA, Adly MA. Antischistosomal activity of ginger (Zingiber officinale) against *Schistosoma mansoni* harbored in C57 mice. Parasitol Res. 2011;109:395–403.
13. Abdel-Hafeez EH, Ahmad AK, Kamal AM, Abdellatif MZ, Abdelgelil NH. In vivo antiprotozoan effects of garlic (Allium sativum) and ginger (*Zingiber officinale*) extracts on experimentally infected mice with *Blastocystis* spp. Parasitol res. 2015; 114:3439-3444.
14. Abouelsoued DM, Shaapan RM, Elkhateeb RM, Elnattat WS, Hammam AM, Hammam AM. Therapeutic efficacy of ginger (*Zingiber officinale*), ginseng (*Panax ginseng*) and sage (*Salvia officinalis*) against *Cryptosporidium parvum* in experimentally infected mice. Egypt J Vet Sci. 2020;51:241-251.
15. Henriksen SA, Pohlenz JFL. Staining of *Cryptosporidia* by a modified Ziehl–Neelsen technique. Acta Vet Scand. 1981; 22:594-596.

16. Gabr NS, Ahmed AK, Belal US, Abd Rabou RA, Ahmed RF, Abdel-Hafeez EH. Molecular characterization of *Cryptosporidium* isolates from humans by nested polymerase chain reaction-restriction fragment length polymorphism (nPCR-RFLP) analysis in Egypt. Trop. Biomed. 2019;36:1-10.
17. Heelan JS, Ingersoll FW. Essentials of Human Parasitology. Delmar Thomson, Albany, New York, 2002.
18. Abouel-Nour MF, EL-Shewehy DI, Magdy M, Hamada SF, Morsy TA. The efficacy of three medicinal plants: garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum.* I-Immunological response. J Egypt Soc Parasitol. 2015;45:559-570.
19. Gaafar MR. Effect of solar disinfection on viability of intestinal Protozoa in drinking water.J Egypt Soc Parasitol.2007;37:65-86.
20. Schumacher JH, O'garra A, Shrader B, Van Kimmenade A, Bond MW, Mosmann TR, et al. The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme-linked immunosorbent. J immunol (Baltimore, Md.: 1950). 1988; 141:1576-1581.
21. Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J Physiol Pharmacol. 2013;57:51–62.
22. Mahady GB, Pendland SL, Yun GS, Lu ZZ, Stoia A. Ginger (*Zingiber officinale Roscoe*) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. Anticancer Res. 2003;23:3699-3702.
23. Ficker CE, Smith ML, Susiarti S, Leaman DJ, Irawati C, Arnason JT. Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (Indonesian Borneo). J Ethnopharmacology. 2003;85:289-293.
24. Iqbal MJ, Reddy OU, El-Zik KM, Pepper AE. A genetic bottleneck in the'evolution under domestication'of upland cotton Gossypium hirsutum L. examined using DNA fingerprinting. Theor Appl Genet. 2001;103:547-554.
25. Brantley RK, Williams KR, Silva TM, Sistrom M, Thielman NM, Ward H, et al. AIDS-associated diarrhea and wasting in Northeast Brazil is associated with subtherapeutic plasma levels of antiretroviral medications and with both bovine and human subtypes of *Cryptosporidium parvum*. Braz J Infect Dis. 2003;7:16-22.
26. Banerjee S, Mullick HI, Banerjee J, Ghosh A. Zingiber officinale:'a natural gold'. Int J Pharmaceutical Bio-Sci. 2011; 2:283-294.
27. Shahbazi P, Nematollahi A, Arshadi S, Farhang HH, Shahbazfar AA. The protective effect of Artemisia spicigera ethanolic extract against *Cryptosporidium parvum* infection in immunosuppressed mice. Iran J Parasitol. 2021;16(2):279.
28. Choi WH, Jiang MH, Chu JP. Antiparasitic effects of *Zingiber officinale* (ginger) extract against *Toxoplasma gondii*. J App Biomed. 2013;11(1):15–26.
29. Mahmoud A, Attia R, Safaa SA, Ibraheim Z. Ginger and cinnamon: can this household remedy treat giardiasis? Parasitological and histopathological studies. Iran J Parasitol. 2014;9:530.
30. Kniel KE, Sumner SS, Lindsay DS, Hackney CR, Pierson MD, Zajac AM, et al. Effect of organic acids and hydrogen peroxide on *Cryptosporidium parvum* viability in fruit juices. J food protect. 2003;66(9):1650-1657.
31. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol,[8]-gingerol,[10]-gingerol and [6]-shogaol. J Ethnopharmacol. 2010;127:515-520.
32. Fawzy E, Zalat RS, Rashed HE, Salama MA, Saleh AA, AbdelHamed EF. Effect of cinnamon and ginger methanolic extracts on murine intestinal cryptosporidiosis. *in-vivo* evaluation. J Egypt Soc Parasitol. 2019;49:689-698.
33. Elmahallawy EK, Elshopakey GE, Saleh AA, Agil A, El-Morsey A, El-Shewehy DM, et al., S-Methylcysteine (SMC) ameliorates intestinal, hepatic, and splenic damage induced by *Cryptosporidium parvum* infection via targeting inflammatory modulators and oxidative stress in swiss albino mice. Biomedicines. 2020; 8:423.
34. Tawfek NS, Abdalla AA, Erfan TA. Modulatory effect of ginger extract on albino rats induced by D-Galactosamine and lipopolysaccharide. Sci. 2017; 7:01-14.

35. Sheng Y, Wu T, Dai Y, Ji K, Zhong Y, Xue Y. The effect of 6-gingerol on inflammatory response and Th17/Treg balance in DSS-induced ulcerative colitis mice. Ann Transl Med. 2020; 8(7).
36. Xu N, Lei H, Li X, Wang Q, Liu M, Wang M. Protective effects of Ginger Essential Oil (GEO) against chemically-induced cutaneous inflammation. Food Sci Technol. 2019;39:371-377.
37. Tzipori S. Introduction. Cryptosporidiosis: current trends and challenges. Microb Infect. 2002;10:1045.
38. Harp JA, Jardon PH, Atwill ER, Zylstra MI, Checel ST, Goff JP, et al., Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. Am J Vet Research. 1996; 57:1586-1588.
39. Al-Masoudi HK. Antigiardial activity of *Zingiber officinale* in combination with honey in vivo. J Babylon Univ Pure Appl Sci. 2011;2:450-454.