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Anti-cryptosporidial activity of *Camellia sinensis* (green tea extract) in experimentally infected immunocompromised mice

Eman S. EL-WAKIL[1], Eman Ali MOHAMED[1], Eman Ahmed EL-WAKIL[2], Tarek S. ABOUSHOUSHA[3], Neimat Mousa AMER[1]

[1] **Department of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt**
[2] **Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt**
[3] **Department of Pathology, Theodor Bilharz Research Institute, Giza, Egypt**

Abstract. *Cryptosporidium parvum*, an Apicomplexan parasite, is an important cause of diarrheal disease, especially in immunodeficient hosts. Nevertheless, there is no entirely successful therapeutic agent against cryptosporidiosis to date. Hence, this study aims to test the potential prophylactic and therapeutic effect of *Camellia sinensis* (green tea extract) in dexamethasone immunosuppressed mice versus the nowadays used drug, Nitazoxanide (NTZ). Parasitological and molecular methods were used to characterize *Cryptosporidium* oocysts before infection. Fifty bred female Swiss Albino mice were divided into 5 groups; group I (GI)(GTP): immunosuppressed and prophylactically treated with green tea extract for 5 days prior to infection, group II (GII)(GTT): immunosuppressed, infected with *Cryptosporidium parvum* and treated with green tea extract, group III (GIII)(NT): immunosuppressed, infected and treated with NTZ, group IV (GIV)(PC): immunosuppressed and infected (Positive control), group V (GV)(NC): immunosuppressed and non-infected (Negative control). Furthermore, parasitological examination for oocysts in the stool, and histopathological examination for the small intestine and liver specimens were performed for the study groups. *Cryptosporidium* oocysts used for induction of infection proved to be *Cryptosporidium parvum* genotype 2. Moreover, a significant oocyst reduction in fecal samples correlated with an improvement of histopathological changes in the small intestinal and liver tissues in GI(GTP), GII (GTT) and GIII(NT) groups. Besides, the GII(GTT) group showed the best improvement in parasitological and histopathological parameters among the test groups.This study revealed that *Camellia sinensis* (green tea extract) has potential activity against cryptosporidiosis and could serve as a promising prophylactic and therapeutic anti-cryptosporidial agent.

Keywords: *Cryptosporidium parvum*; *Camellia sinensis* (green tea extract); Nitazoxanide; Histopathology

Address for correspondence: Eman S. El-Wakil, Department of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt; Email: drfaith@ymail.com

INTRODUCTION

Apicomplexan parasites of the genus *Cryptosporidium* infect the gastrointestinal tract of a wide range of host species, including humans (Innes *et al.* 2020). The majority of human infections are due to either *Cryptosporidium parvum* (*C. parvum*) or *Cryptosporidium hominis* (*C. hominis*) (Feng *et al.* 2018). Infections are spread by environmentally resistant spores that primarily contaminate drinking water and occasionally food sources. Infection with *Cryptosporidium* species may cause significant outbreaks of diarrhea that generally last less than two weeks in immunocompetent individuals. In immunosuppressed individuals, diarrhea may be copious and result in significant morbidity and mortality (Sparks *et al*. 2015).

No unrestrictedly effective therapies exist to treat cryptosporidiosis (Gargala 2008; Marzook and Sateriale 2020). Against this background, 100 anti-cryptosporidial drugs have been identified, but they usually have only temporary effects, and sometimes relapses happen (Abubakar *et al*. 2007).

Nitazoxanide (NTZ) is the only drug approved by Food and Drug Administration (FDA) in immunocompetent patients but has not demonstrated adequate results in children and immunodeficient patients, and there is no FDA-approved vaccine (Atia *et al*. 2016; Ashigbie *et al*. 2021).

These facts highlight the urgent need to search for alternatives (Spark *et al*. 2015). A widely used approach that has increasingly been used is to search for new drugs from natural resources (Kayser *et al*. 2003; Mammeri *et al*. 2018).

Camellia sinensis (common green tea) is a medicinal plant species widely distributed globally and commonly used in the folk medicine of different countries (Reygaert 2018). Green tea, the dried leaves of the *Camellia sinensis* plant, contains various biologically active compounds, including polyphenolic compounds (collectively termed catechins), amino acids, vitamins, and caffeine (Jigisha *et al.* 2012; Kochman *et al.* 2021).

Green tea constituents have been reported to possess anti-infective effects against bacteria, viruses and various fungi (Steinmann *et al.* 2013), anti-tumorigenic properties (Boehm *et al.* 2009), anti-inflammatory, anti-oxidative, and anti-proliferative actions (Reygaert 2018; Kochman *et al.* 2021).

Moreover, it was successfully tested as a novel anti-parasitic herbal treatment for *Entamoeba histolytica* (Shaker *et al.* 2018), malarial infection (Thipubon *et al.* 2015), *Trypanosoma cruzi* (Guida *et al.* 2007), and *Leishmania amazonensis* (Inacio *et al.* 2013).

Concerning the evidence regarding the medicinal aspects of *Camellia sinensis*, in our work, we have attempted validation of the probable protective and curative value of green tea extract versus nitazoxanide in prohibiting the fulminant outcomes in *Cryptosporidium*-infected immunocompromised mice.

MATERIALS AND METHODS

1. Animal source and handling

This study was conducted on laboratory-bred Swiss albino female mice (n = 50) weighing about 20-25g obtained from the *Schistosome* (Biological Supply Center in Theodor Bilharz Research Institute (TBRI), Giza, Egypt). Mice were kept in a well-ventilated plastic cage in conditioned rooms (27±2°C) with free access to *ad libitum* food and water and away from direct sunlight, ensuring good sanitary conditions. The study was performed at the Parasitology Department at TBRI.

2. Immunosuppression

Immunosuppression was performed by giving the animals synthetic corticosteroids, dexamethasone (dexazone, 0.5mg) [Kahira pharmaceuticals and chemical industries company, Shoubra, Cairo, Egypt] orally at a dose of 0.25 µg/g body weight/day for 14 successive days prior to inoculation with *Cryptosporidium* oocysts. The mice continued to receive dexamethasone at the same dose throughout the experiment (Tarazona *et al.* 1998).

3. Infection

Cryptosporidium oocysts from naturally infected diarrheic calves were obtained from Animal Reproduction Research Institute, Giza, Egypt.

3.1. Collection of fecal samples

The stool samples of infected calves were collected in sterile clean stool cups, ensuring that the specimens were not contaminated with water or urine. Stool samples were coproscopically examined by direct wet smear and iodine smear to exclude the presence of any other parasitic stage (ova, larva, trophozoite or cyst) (Garcia 2007).

3.2. Staining of fecal samples

The presence of oocysts was confirmed through staining by the modified Ziehl–Neelsen method (Henriksen and Pohlenz 1981) and immunofluorescence method (Hanscheid *et al.* 2008) prior to molecular identification.

3.3. Molecular identification of *C. parvum*

Molecular identification and genotyping of *C. parvum* were performed through nested PCR and PCR-RFLP.

3.3.1. Nested PCR-RFLP

DNA extraction

DNA extraction was performed from the stool specimens of the different study groups through QIAamp Fast DNA Stool Mini Kit (cat. no. 51604; Qiagen-Germany). The freeze/thaw cycle was allowed 5 times each for 5 minutes to rupture the oocyst wall. The DNA was isolated from the specimens by a fast spin-column technique, and the PCR inhibitors were removed from DNA through Inhibit EX Buffer, guided by the manufacturer's instructions.

DNA amplification by nested PCR

All primers specific to the *C. parvum* target genes used in this research were precisely illustrated according to previous reports. The first PCR primers were BCOWPF (5′-ACCGCTTCTCAACAACCATCTTGTCCT C-3′) and BCOWPR (5′-CGCACCTGT TCCCACTCAATGTAAACCC-3′); while the nested PCR primers were Cry-15(5′-GTAGATAATGGAAGAGATTGTG-3′) and Cry-9 (5′-GGACTGAAATACAGG CATTATCT TG-3′) (Spano *et al.* 1997 a,b; Yu *et al.* 2009). The anticipated product sizes were 769-bp and 553-bp, consecutively. Extracted DNA (2.5 µl) in 1x PCR buffer was mixed with $MgCl_2$ (1.5 mM), dNTP (250 µM), primers (10 pmoles) and Taq DNA polymerase (1.25 units) to reach a volume of 25 µl. Thirty cycles of denaturation were performed for the samples at 94 °C for 1 min, annealing at 65 °C for 1 min and extension at 72 °C for 10 min. The nested PCR reaction used 2.5 µl of the extended PCR product to amplify the 553-bp gene fragment (Spano *et al.* 1997b). Negative and positive controls were present in all PCR procedures.

3.3.2. Restriction fragment length polymorphism (RFLP)

A positive sample by nPCR was then subjected to digestion using RFLP with restriction enzyme cleavage Rsa I to detect *Cryptosporidium* genotypes. A mixture was produced, including 10 µl nested PCR product with 17 µl of nuclease-free water, 2 µl of green buffer and 1 µl RsaI enzyme. The mixture's ingredients were exposed to gentle mixing and spinning for a few seconds. Incubation of samples was achieved at 37 °C for 5 min. The results were checked by agarose gel electrophoresis and UV light trans-illumination to visualize the digested fragments. Genotyping was performed by the digestion pattern: Genotype 1 was considered if the Rsa I digestion resulted in 4 bands: 34 bp, 106 bp, 125 bp, and 285 bp, and genotype 2 if the Rsa I digestion resulted in 3 bands: 34 bp, 106 bp, and 410 bp.

3.4. Animal infection

After collecting stool samples, oocysts were purified according to (Arrowood and Donaldson 1996). Purified oocysts were kept in a 2.5 % potassium dichromate solution and stored at 4 °C until required. The infective inoculum was prepared (Reese *et al.* 1982), and the number of oocysts in the concentrated stock inoculum was counted to determine how much the fluid volume was the inoculum per mouse. Mice were orally infected with *Cryptosporidium* oocysts using oral-gastric gavage (day 0). Each mouse was infected with *Cryptosporidium* oocysts in a dose of about 3×10^3 oocysts/mouse (Benamrouz *et al.* 2012; El-Wakil *et al.* 2021). Fecal pellets were obtained from inoculated mice individually after infection and exposed to parasitological examination using the modified Ziehl–Neelsen method to detect *Cryptosporidium spp.* oocysts and to ensure that the mice were successfully infected.

4. Drugs preparation administration

4.1. Plant material and extraction

Dried leaves of *Camellia sinensis* (green tea) from the family *Theaceae* were purchased from a local herbal shop (Cairo, Egypt). The plant sample was identified, and a voucher specimen was deposited at the Medicinal Chemistry Department at TBRI, Giza, Egypt. Plant leaves were ground to a fine powder with an electric mill for the extraction process.

Five hundred grams of dried green tea were soaked in 85% methanol for one week in dark place to avoid the harmful effect of light on light sensitive phytochemicals; then, the extract was filtered through Whatman No.1 filter paper several times. After that, the filtrate was concentrated by a Buchi Rotatory evaporator at 60 °C to remove methanol completely. Finally, the crude dried methanol extract was kept in clean, dry and dark vial for further use (Ghareeb *et al.* 2018a).

4.2. Preliminary phytochemical screening

Conventional standard protocols were utilized for the detection of different chemical classes in the tested plant extract, including flavonoids, phenols, anthocyanins, tannins, terpenoids, coumarins, anthraquinones, and saponins (Trease and Evans 1983; 1989; Harborne 1993; Sofowora 1993; Edeoga *et al.* 2005; Ghareeb *et al.* 2014).

4.3. Animal groups

Mice were divided into 5 groups, 10 mice each:

Group I: immunosuppressed and was infected after a 5 days course of the methanol extract of green tea prophylactic administration in a dose of 150 mg/Kg body weight (Shaker *et al.* 2018).

Group II: immunosuppressed, infected and received the methanol extract of green tea in a dose of 150 mg/Kg body weight (Shaker *et al.* 2018). The extract was given for 5 successive days starting from the seventh day post-infection (dpi).

Group III: immunosuppressed, infected and received oral suspension of nitazoxanide, obtained in the form of (nanazoxid) (100 mg/5 ml suspension) manufactured and provided by [Medizen Pharmaceutical Industries for Utopia Pharmaceuticals], in a dose of 100 mg/Kg body weight (Li *et al.* 2003; El-Wakil *et al.* 2021). The drug was administered for 5 successive days starting from the seventh dpi.

Group IV: immunosuppressed and infected (Positive control).

Group V: immunosuppressed and non-infected (Negative control).

The doses were calculated by extrapolation of human therapeutic doses to animal doses according to the table of (Paget and Barnes 1965).

5. Drug assessment

5.1. Parasitological examination

After administration of drugs, fecal pellets were collected from infected mice on day 12 pi and subjected to parasitological examination using the modified Ziehl–Neelsen method to count the number of *Cryptosporidium* oocysts (Garcia 2007). The number of parasites was expressed per gram of feces (OPG) (Benamrouz *et al.* 2012).

The percentage of the efficacy of each drug was calculated using the following equation (Hosking *et al.* 1996):

$$\text{Efficacy (\%)} = \frac{(\text{mean OPG of infected untreated group} - \text{mean OPG of infected treated group}) \times 100}{\text{mean OPG of infected untreated group}}$$

5.2. Histopathological examination

The small intestine and liver sections were excised and submitted to histopathological examination. They were fixed in 10% formalin. After fixation, the tissues were processed for paraffin embedding. Histopathological sections of 4 µm thickness were stained with hematoxylin & eosin stain in the pathology lab of TBRI. They were examined microscopically to detect pathological changes and assess the cure rates after drug administration (Drury and Wallington 1980).

6. Statistical analysis of data

The data were analyzed using Microsoft Excel 2016 and the statistical package for social science IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). Continuous normally distributed variables were represented as mean±SD, with a 95% confidence interval. The ANOVA test was performed and followed with Dunnett T3 as a Post-Hoc test to compare the means of normally distributed variables between groups. A p-value was set at 0.05, P >0.05; non-significant, p-value <0.05; significant, and p-value <0.001; highly significant (Peat and Barton 2005).

7. Ethical consideration

This study was approved by the Research Ethics Committee of TBRI (PT: 585). All applied experiments on animals were conducted according to the guidelines of the National Institutes of Health (NIH, 1996) and its amendments for the care and use of laboratory animals.

RESULTS

1. *C. parvum* oocysts identification

When fecal samples were stained with the modified Ziehl–Neelsen staining method, the oocysts appeared as oval/round bodies, about 4–5 µm, with a pink to red to deep purple color (Fig.1a). In the immunofluorescence detection method, the oocysts appeared as ovoid or spherical brilliant apple/green structures with thick walls (Fig.1b).

Regarding the molecular identification of *C. parvum* oocyst, the sample used for animal infection was positive in PCR reaction, and the nested PCR product size was about 553-bp. The restriction enzyme Rsa I digestion of nPCR product targeting COWP gene revealed the presence of *Cryptosporidium parvum* genotype 2. The genotype digestion products of 34, 106, and 410 bp (Fig. 2).

2. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of several classes of secondary metabolites, including tannins, flavonoids, saponins, phenols, anthocyanins, and terpenoids (Table 1). Tannins, flavonoids, and phenol compounds predominate in the extract.

3. Oocyst shedding

In the present study, it was observed that prophylactic treatment with green tea (GI), therapeutic treatment with green tea (GII), and NTZ treatment (GIII) significantly reduced ($P < 0.001$) the mean *Cryptosporidium* oocysts shedding (50.8×10^3, 15.9×10^3, and 38.1×10^3 per group, respectively) relative to infected untreated controls (GIV) (91.2×10^3) with 44%, 83%, and 58% percentage of reduction, respectively. GII showed significant improvement ($P < 0.001$) more than GI and GIII. GI showed the least improvement, while GII showed the best response among the test groups with a marked reduction of the mean oocyst count in the stool (Table 2).

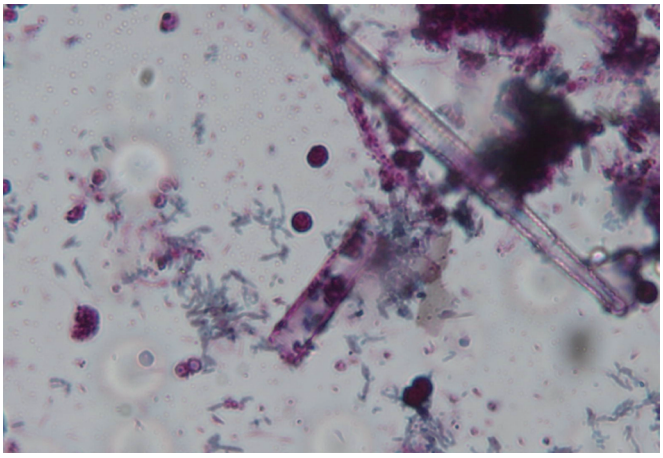
4. Histopathological examination of intestinal and liver sections of the study groups

GI:

Examination of small intestinal sections revealed partial improvement of the histopathological changes that follow cryptosporidiosis infection in villous broadening with a decrease of villous height to crypt length ratio. There was partial healing of intestinal mucosa with focally ulcerated mucosa and expansion of the villous core by mononuclear inflammatory cells. Sections examined from the liver revealed preserved hepatic lobular architecture and cloudy swelling of hepatocytes (Fig. 3).

GII:

Examination of sections from the small intestine revealed remarkable improvement of the histopathological changes following cryptosporidiosis infection, including restoration of the normal villous architecture with preserved villous height to crypt length ratio. The mucosal lining became intact with the healing of the surface epithelium and preserved the brush border of the villi. No oocysts could be detected. Examination



a

b

Fig. 1. a) *Cryptosporidium* oocyst (stained red to deep purple with the modified Ziehl–Neelsen method); **b)** immunofluorescence staining of *Cryptosporidium* oocyst (ovoid or spherical brilliant apple/green structure)

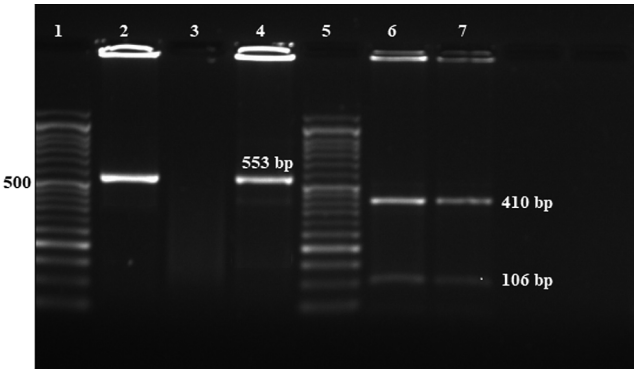


Fig. 2. Agarose gel electrophoresis showing: **Lane 1:** 50 bp DNA molecular weight marker, **Lane 2:** Positive control, **Lane 3:** Negative control, **Lane 4:** Positive sample of nested PCR products targeting COWP gene of *Cryptosporidium* at 553 bp, **Lane 5:** 50 bp DNA molecular weight marker, and **Lane 6:** RFLP products of the sample after digestion with *Rsa*I endonuclease (*C. parvum* genotype 2 digestion products at 410, 106, and 34 (too small to be detected) bp.

Table 1. Preliminary phytochemical screening of green tea extract

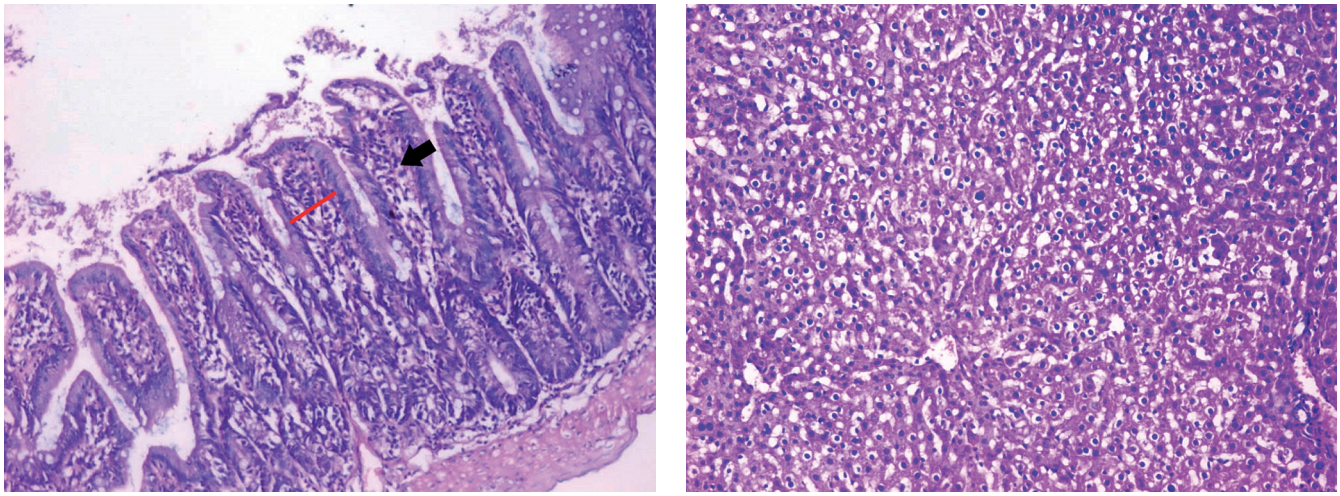
Chemical classes	**Green tea extract**
	Presence/ Absence of phytoconstituents
Flavonoids	+++
Tannins	+++
Terpenoids	++
Saponins	++
Anthocyanins	+
Phenols	+++
Coumarins	–
Anthraquinones	–

+: Low; ++: Moderate; +++: High; and –: Absence

Table 2. *Cryptosporidium* oocysts count/gram stool (Mean ± SD) $\times 10^3$ at 12^{th}-day post-infection (dpi)

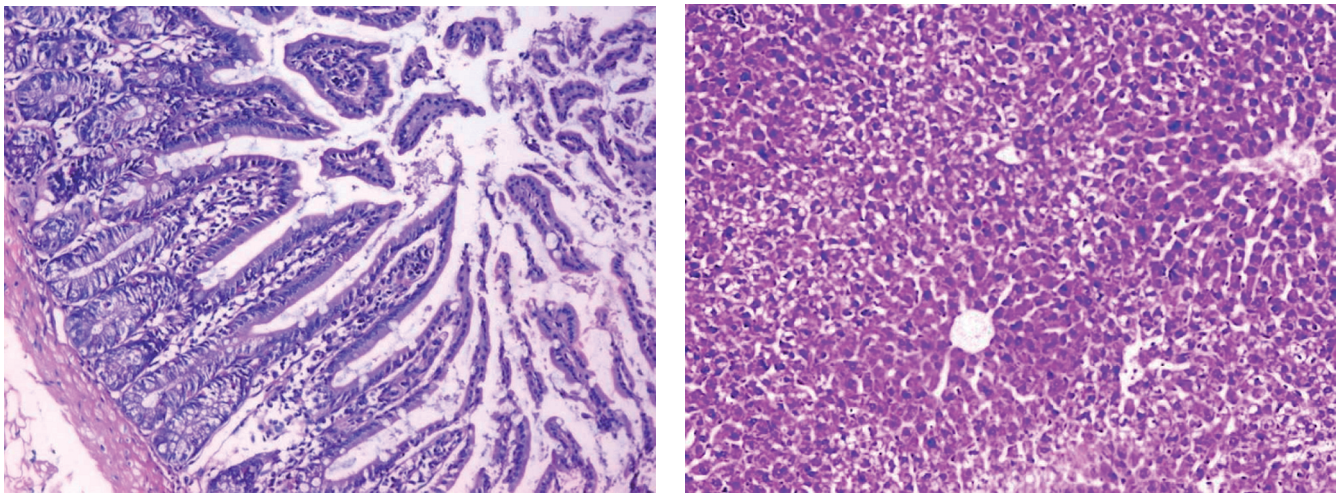
Mice groups		**GI: Green tea prophylactic treatment**	**GII: Green tea therapeutic treatment**	**GIII: NTZ treatment**	**GIV: (+ve Control)**	**GV: (-ve Control)**
Mean ± SD		**50.8 ± 2.8**	**15.9 ± 2.1**	**38.1 ± 4.1**	**91.2 ± 3.2**	**0**
% of Efficacy		**44%**	**83%**	**58%**	**–**	**–**
ANOVA		1011.3 (P-value = 0.001**)				
Post-Hoc test Dunnett T3	**GI**	–	0.001**	0.004**	0.001**	–
	GII	–	–	0.001**	0.001**	–
	GIII	–	–	–	0.001**	–
	GIV	–	–	–	–	–

*The mean difference is significant at the 0.05 level.



a **b**

Fig. 3. a) A section of the small intestine in GI revealed villous broadening (red line) with an expansion of the villous core by mononuclear inflammatory cells (black arrow) (H&E stain, X200); **b**) Sections examined from the liver in this group showed preserved hepatic lobular architecture and cloudy swelling of hepatocytes (H&E stain, X200).



a **b**

Fig. 4. a) A section of the small intestine in GII revealed returning of the normal villous pattern, normal mucosa, and goblet cells (H&E stain, X200). **b**) Sections examined from the liver in this group showed preserved hepatic lobular architecture (H&E stain, X200).

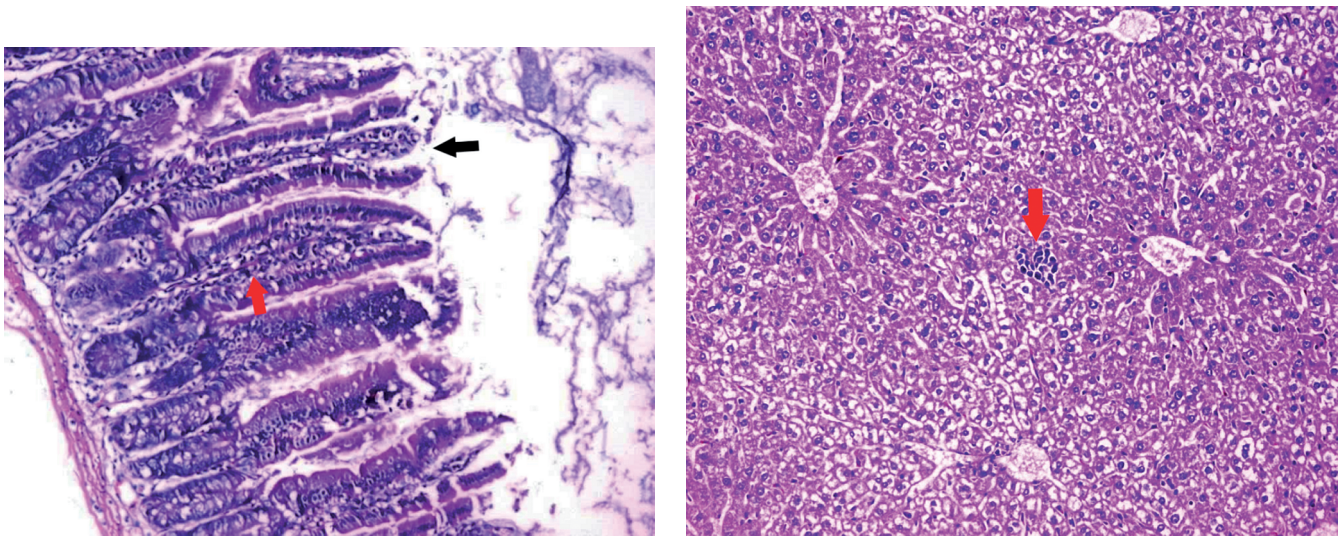
of liver sections showed preserved hepatic lobular architecture (Fig. 4).

GIII:

Examination of small intestinal sections revealed partial improvement in the histopathological changes, including partial villous blunting and broadening with a moderate decrease in villous height to crypt length ratio. There were ulcerations in the intestinal mucosa with partial healing, moderate goblet cell depletion and moderate inflammatory infiltration in the lamina propria. Sections examined from the liver revealed cloudy swelling of hepatocytes with focal mononuclear cellular infiltration (Fig. 5).

GIV:

Examination of sections from the small intestine revealed profound histopathological alteration in villi architecture consistent with the infection of cryptosporidiosis. These included loss of villous architecture with



a b

Fig. 5. a) A section of the small intestine in GIII revealed moderate villous broadening, infiltration by mononuclear inflammatory cells within the villous core (red arrow), focal degeneration of the villous tip regions (black arrow), and increased mucin production (H&E stain, X200). **b)** Sections examined from the liver in this group showed focal mononuclear cellular infiltration (red arrow) (H&E stain, X200).

villous atrophy, shortened broad villi, and a decreased ratio between villous height to crypt length. Mucosal ulceration and goblet cell depletion were detected together with infiltration of lamina propria with inflammatory cells that were mostly lymphocytes. *Cryptosporidium* stages were noticed along the brush border of the villi and in the intestinal lumen as rounded to oval bodies, purple-stained, and measuring 4–6µm. Examination of liver sections revealed hepatocellular degeneration and moderate mononuclear cellular infiltration (Fig. 6).

GV:

Examination of small intestinal sections revealed the normal architecture of small intestinal villi with average length and width with healthy mucosa. The brush border was well-defined, and goblet cells were of the average number. Examination of liver sections revealed preserved hepatic architecture with hepatocytes arranged in regular plates around the central vein with portal tracts showing normal structures (Fig. 7).

4. Discussion

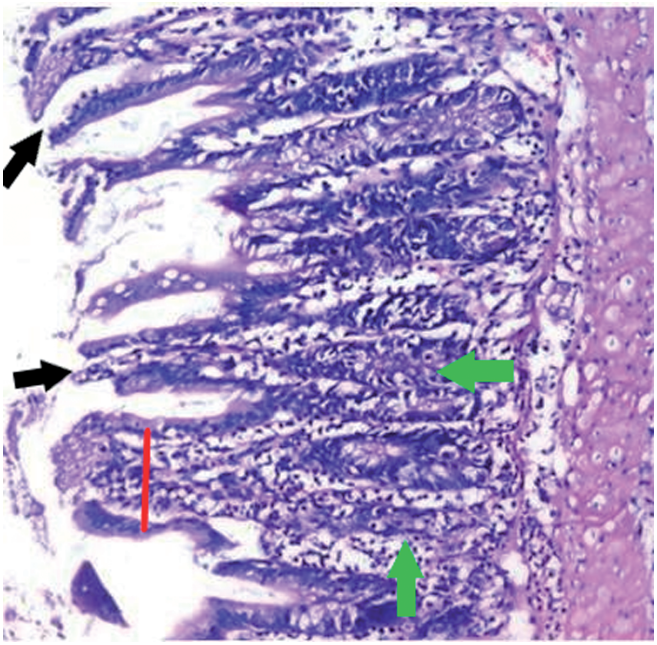
Cryptosporidiosis is a major and globally distributed diarrheal disease in humans and animals. *Cryptosporidium* oocysts are also one of the major environmental concerns, and despite their importance, fully effective drugs are not yet available (Zhu *et al.* 2021).

Acid-fast (AF)-modified Ziehl–Neelsen staining is one of the most common differential staining techniques used to diagnose *Cryptosporidium* oocysts (Arrowood 1997; Zaglool *et al.* 2013).

However, the detection limits of conventional microscopy for *Cryptosporidium* have been reported to be as low as 50 000 to 500 000 oocysts per gram of human feces (Weber *et al.*1991), resulting in low levels of infection or sporadic shedding possibly going unnoticed when conventional methods of detection are used. Immunofluorescent procedures have a high sensitivity. Oocysts appear apple green against a dark background in immunofluorescent microscopy. This fluorescence method, together with modified Ziehl-Neelsen (acid-fast) confirmatory staining, is a sensitive and specific approach for identifying *Cryptosporidium* oocysts in the stool (MacPherson and McQueen 1993; Caccio and Widmer 2014).

Detection of cryptosporidiosis using PCR-based methods is more sensitive than conventional methods for detecting oocysts in feces. Molecular methods can also identify the species/genotypes and subtypes of *Cryptosporidium* (Caccio *et al.* 2005; Ware *et al.* 2013).

Being an intracellular parasite may protect *Cryptosporidium* from drugs applied orally. Several drugs have been examined for their efficiency against cryptosporidiosis (Abdou *et al.* 2013). Only nitazoxanide is approved



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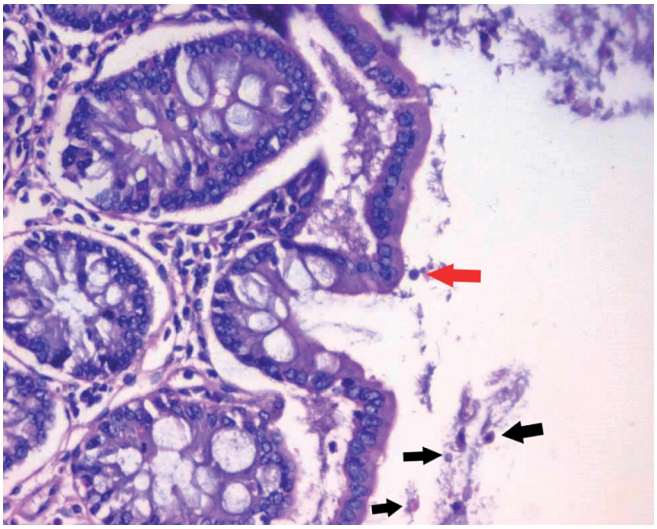

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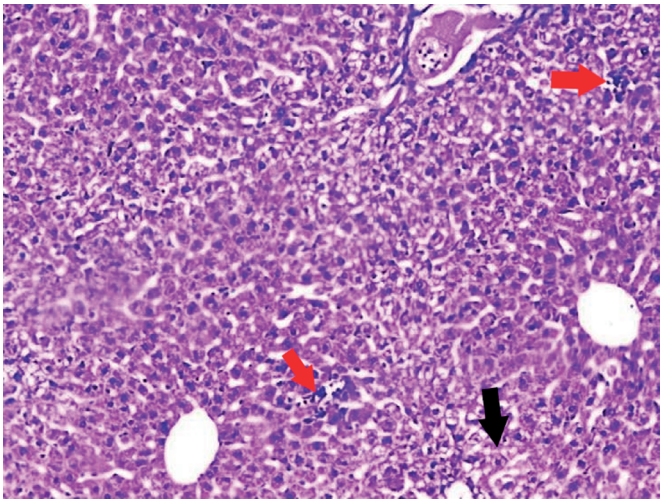

c

Fig. 6. a) A section of the small intestine in GIV revealed marked villous broadening (red line) with decreased villous height to crypt length ratio. There was dense infiltration by mononuclear inflammatory cells within the villous core (green arrows), degeneration of the villous tip-regions (black arrows), and increased mucin production (H&E stain, X200). **b**) Sections from the small intestine revealed many adherents (red arrows) and separate (black arrows) *Cryptosporium* stages, probably oocyst (H&E stain, X1000). **c**)Sections examined from the liver in this group showed hepatocellular degeneration (black arrow) and focal mononuclear cellular infiltration (red arrows) (H&E stain, X200).

by the FDA to treat cryptosporidiosis in immunocompetent individuals but not for immunocompromised individuals (Gargala 2008; Atia *et al.* 2016). It affects the parasite by blocking its metabolism-necessary anaerobic energy, enhancing its clearance. However, nitazoxanide is not fully effective in immunosuppressed individuals without an effective host immune response and has a transit effect with relapses in immunocompetent ones (Cabada and White 2010; Zhu *et al.* 2021).

Dexamethasone, a synthetic glucocorticoid, was used in the current work to induce chemical immunosuppression. This helps the infection be caught easily by mice, extends the infection duration, and supports the detection of anti-parasitic activities of potentially suitable drugs (Baishanbo *et al.* 2006; El-Wakil *et al.* 2021).

Considering the side effects and resistance to many anti-parasitic drugs, attention has moved toward plant extracts used in traditional medicine as possible new options for treatment (Gaafar 2012; Abdelmaksoud *et al.* 2020).

Tannins, flavonoids, and phenol compounds predominate in the green tea extract. The detected classes

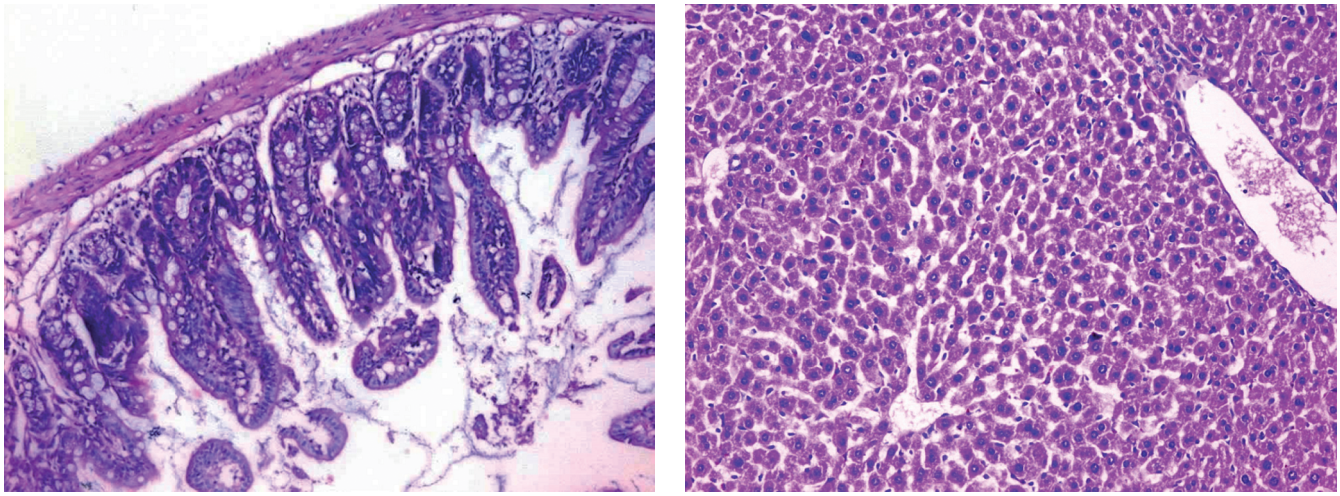

Fig. 7. a) A section of the small intestine in GV revealed normal villous architecture with a normal brush border (H&E stain, X200); **b**) Sections examined from the liver in this group showed preserved hepatic lobular architecture (H&E stain, X200).

were reported to possess a broad spectrum of biological activities like anti-parasitic (Júnior *et al.* 2016), hepatoprotective (Sobeh *et al.* 2018), anti-inflammatory (Ghareeb *et al.* 2018b), and hepato-renal protective potentials (Ghareeb *et al.* 2019).

In the present study, when used as a prophylactic agent, green tea reduced the mean *Cryptosporidium* oocyst count to 50.8± 2.8 opg compared to 91.2±3.2 opg compared to control groups resembling 44% percentage of efficacy.

Concerning chemoprophylaxis for cryptosporidiosis, there is a shortage of human data. Rifabutin, azithromycin, or clarithromycin were shown to reduce the occurrence of cryptosporidiosis in groups treated with these drugs (Holmberg *et al.* 1998; Fichtenbaum *et al.* 2000). Besides, the prophylactic role of mefloquine on cryptosporidiosis in experimentally infected immunocompromised mice was reported by El-Wakil *et al.* 2021.

In the present study, administration of green tea extract in GII after infection resulted in a highly significant reduction in shedding of oocysts with a percentage of reduction of 83% compared to the infected control group. Furthermore, the reduction achieved by green tea extract treatment was superior to NTZ (83% versus 58%).

In the same context, the therapeutic efficacy of green tea extract was demonstrated for many other parasites, including *Plasmodium berghei* in mice (Thipubon *et al.* 2015), with pleasant activity against *Entamoeba histolytica* in mice (Shaker *et al.* 2018), *Trypanosoma cruzi in vitro* and *in vivo* (Guida *et al.* 2007), *Trypanosoma brucei* in culture (Vigueira *et al.* 2012), *Leishmania amazonensis in vitro* and *in vivo* (Inacio *et al.* 2013), and *Babesia* spp. *in vitro* and *in vivo* (Aboulaila *et al.* 2010). The principal effect of catechins in green tea on various parasitic infections is decreased parasite growth and numbers. Other effects detected were fragmentation of parasitic DNA and reduced synthesis of parasite's fatty acids (Reygaert 2018).

Over the past two decades, there has been an increasing interest in catechins which are polyphenols present in green tea, as an alternative for pharmacological intervention in a spectrum of therapeutic issues. They may interact with different protein molecules or other biological targets that parasites possess (Kochman *et al.* 2021). Their anti-parasitic activities have been studied in deeper detail, and monomeric catechins exhibit a broad range of anti-protozoal activity targeting important enzymes (Shaker *et al.* 2018).

Regarding histopathological examination of intestinal and liver sections in the positive control group (GIV), there was a profound alteration in villi architecture of the small intestine consistent with *Cryptosporidium* infection in terms of loss of villous architecture and villous atrophy. *Cryptosporidium* stages were noticed along the brush border of the villi. Examination of the liver sections revealed hepatocellular degeneration and moderate mononuclear cellular infiltration (Fig. 6). These results agree with those of Waters and Harp (1996), who documented intestinal inflammatory changes like inflammatory infiltrate and villous atrophy due to *Cryptosporidium* infection. Besides, numerous

studies have detected similar histopathological changes in infected animals (Abu El Ezz *et al.* 2011; El-Sayed and Fathy 2019; El-Wakil *et al.* 2021).

Variable degree of improvement of histopathological changes in the small intestinal and liver tissues was detected following prophylactic treatment with green Tea (GI), therapeutic treatment with green Tea (GII), and NTZ treatment (GIII) groups, with the best results detected in GII treated with green tea. In line with our results, previous studies on immunosuppressed mice have documented that NTZ treatment is not fully effective, with limited efficacy in infected intestinal sections (Taha *et al.* 2017; Moawad *et al.* 2021).

CONCLUSION

Results of the present study indicated that the methanol extract of *Camellia sinensis* (green tea) has promising prophylactic and therapeutic effects against *C. parvum*. The methanol extract of green tea is cheap, easily obtained and could avoid the adverse reactions caused by chemotherapeutic drugs. Further studies are required to investigate the mechanism of action of *Camellia sinensis* in cryptosporidiosis and to develop other derivatives of *Camellia sinensis* with higher efficacy.

Ethical consideration: This study was approved by the Research Ethics Committee of TBRI (PT: 585). All applied experiments on animals were conducted according to the guidelines of the National Institutes of Health (NIH, 1996) and its amendments for the care and use of laboratory animals.

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Conflicts of interest: None.

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