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ORIGINAL ARTICLE

# Evaluation of the therapeutic efficacy of some essential oils in experimentally immunosuppressed mice infected with *Cryptosporidium parvum*

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**Abstract**
Cryptosporidiosis is a serious intestinal disease affecting mal-nourished children and immunocompromised individuals with severe fatal diarrhea. Our present work was done to evaluate the possible curative effects of different essential oils (Mint, Thyme, Chamomile and Basil) on *Cryptosporidium parvum* (*C. parvum*) in vivo compared with nitazoxanide (NTZ). Seventy immunosuppressed white Albino male mice were allocated in 7 groups as follows: group I infected and not treated (Positive control), group II (GII) treated with NTZ, group III (GIII) treated with Mint essential oil, group IV (GIV) treated with Thyme essential oil, group V (GV) treated with Chamomile essential oil, group VI (GVI) treated with Basil essential oil and group VII (GVII) naïve not infected mice (Negative control). Evaluation was done using parasitological, histopatholgical, serological as well as biochemical methods. All study groups revealed significant reduction ($P$ value $< 0.01$) in the mean number of *C. parvum* oocysts in stool. Results of GII were the best with 87.7% reduction in the oocysts count followed by GIII (77.9%), GIV (74.7%), GVI (68.2%) and lastly GV (67.2%). Improvement of the histopathological damage in the small intestine was shown in treated groups. All treated mice showed significant upregulation in the interferon gamma (IFN-γ) levels, significant reduction in the malondialdehyde (MDA) levels and increase in superoxide dismutase (SOD) levels ($P$ value $< 0.0001$). It is concluded that Mint, Thyme, Chamomile and Basil oils showed promising anti-cryptosporidial, anti-inflammatory and antioxidant functions.

**Keywords** *Cryptosporidium parvum* · Nitazoxanide · Mint · Chamomile · Thyme · Basil

## Introduction

Cryptosporidiosis is a zoonotic disease caused by the obligate intracellular intestinal protozoon *Cryptosporidium parvum* (*C. parvum*). It affects humans, ruminants and a diversity of many other animals (Craighead et al. 2021). Cryptosporidiosis can result in severe fatal diarrhea in mal-nourished children and immunocompromised individuals (El-Ashkar et al. 2022).

Innate immunity plays a great role to control cryptosporidiosis, which invades enterocytes. This depends mainly on the IFN-γ formation. The enterocytes produce interleukin-18 (IL-18) which synergizes with IL-12 to encourage the innate lymphoid cell (ILC) to produce IFN-γ. IFN-γ acts on enterocytes for early restriction of the parasite growth (Gullicksrud et al. 2022).

Nitazoxanide (NTZ) is the currently used drug in the cryptosporidiosis treatment that was approved by the Food and Drug Administration (FDA). Unfortunately, NTZ has unsatisfactory therapeutic effect in immunosuppressed patients and very low cure rates (56%) in mal-nourished children (Taha et al. 2017). Few other drugs are available for *C. parvum* treatment such as azithromycin, roxithromycin, paromomycin (Gargala 2008). However, drug toxicity, resistance, limited efficacy and restricted availability in developing countries were reported. Therefore, the urgent need for

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finding a novel, safe, effective and inexpensive treatment for these patients is still established (Abdelmaksoud et al. 2020). This need stimulated the scientists to investigate the natural medicinal herbs especially ones that had been known to be used traditionally for parasitic infections (Mendonça et al. 2021).

Mentha leaves, also known as mint or peppermint, are widely distributed worldwide with a diversity of uses. They are used in herbal teas, as food additives or as spices (Baser et al. 1999). Mint oil contains numerous nutrients and minerals like manganese, iron, magnesium, folate, calcium, potassium, copper, omega-3 fatty acids, Vitamin C and Vitamin A. Also it is rich in menthol, menthyl acetate, menthone, pulegone, piperitone, limonene, and carvone as volatile bioactive compounds (Kligler and Chaudhary 2007; McKay and Blumberg 2006).

Mint oil is known to have anti-inflammatory, anti-diarrheal, spasmolytic, antiemetic, anti-parasitic, antibacterial, anti-depressive, diaphoretic, analgesic, antioxidant, keratoprotective and hepatoprotective properties (İşcan et al. 2002; Kiran and Patra 2002; Farzaei et al. 2017). It also showed an effect in relieving non-ulcer dyspepsia and irritable bowel syndrome (IBS). Moreover, it showed antimicrobial properties on both gram positive and gram negative bacteria (Mahadevappa et al. 2014). Mint oil acts to strengthen the immune system and it can be used as a mosquito repellent (Iscan et al. 2002). It also showed antifoaming and choleretic effects that support its use in medicinal field (Mahadevappa et al. 2014).

Thyme (*Thymus vulgaris*) has been known to be used in traditional medication as anti-inflammatory, expectorant, antiseptic, antibacterial, anti-parasitic and antioxidant. These effects are mainly due to the functions of the thymol and carvacrol constituents (Burt 2004). Recent studies have showed its antifungal, antiviral, anti-leishmanial, and anticancer effects (Kowalczyk et al. 2020). Essential oils of thyme and carvacrol were tested against *C. parvum* in vitro with promising results (Gaur et al. 2018).

Chamomile (*Matricaria chamomilla*) is traditionally used in medicinal treatment for its analgesic and anti-inflammatory activities. It is widely consumed as a tea (Speisky et al. 2006). It is used to treat wounds, ulcers, eczema and gastrointestinal manifestations like diarrhea and vomiting (Díaz et al. 2014). Many flavonoids had been found in the chamomile composition like apigenin, luteolin, patuletin and quercetin. Other constituents include terpenoids, sequiterpenes and chamazulene (Raal et al. 2012).

Basil (*Ocimum basilicum*) essential oil main components are terpenes and phenylpropanoids. It also contains linalool, phenolic compounds, alcoholic compounds as well as aldehydes (Mahmoudi et al. 2020; Milenković et al. 2019). Many studies had reported the therapeutic properties of basil essential oil including antimicrobial, insect repelling, antifungal, anti-infammatory, antioxidant and anticancer activities (da Silva et al. 2022).

So, our present study was performed to evaluate the potential therapeutic effects of different essential oils (Mint, Thyme, Chamomile and Basil) on *C. parvum* in vivo compared with NTZ.

### Aim of the work

Evaluation of the potential curative and therapeutic role of Mint, Thyme, Chamomile and Basil essential oils in treating immunosuppressed mice experimentally infected with *C. parvum* oocysts in comparison with the reference drug NTZ.

## Materials and methods

### Animals

Seventy Swiss Albino laboratory-bred male mice were used in this study. Their age was 4–6 weeks old and they measured 20–25 g in weight. All experiments were performed in well—ventilated conditioned places away from direct sunlight, in plastic cages provided with clean beddings.

### Experimental design

Seventy mice were divided into seven groups (ten mice each):

Group I (Positive control) immunosuppressed, infected and not treated.

Group II (Drug control) immunosuppressed, infected and treated with NTZ.

Group III (Mint) immunosuppressed, infected and treated with Mint essential oil.

Group IV (Thyme) immunosuppressed, infected and treated with Thyme essential oil.

Group V (Chamomile) immunosuppressed, infected and treated with Chamomile essential oil.

Group VI (Basil) immunosuppressed, infected and treated with Basil essential oil.

Group VII (Negative control) immunosuppressed and non-infected.

### Induction of immunosuppression

Mice were immunosuppressed by administration of dexamethasone (Dexazone, Kahira Pharmaceuticals and Chemical Industries Company—Egypt). Each mouse received an oral dose of 0.25 ug/g/day of dexamethazone via esophageal tube. The drug was given for 14 days prior to induction of *C. parvum* infection. Mice received dexamethazone for the whole experiment period (Rehquel et al. 1998).

### C. parvum oocysts identification

*C. parvum* oocysts were purchased from the *Theodore Bilharz Research Institute (TBRI) in Giza, Egypt.* Samples were identified by PCR to identify *C. parvum*. DNA isolation was done by (QIA amp DNA Stool Mini Kit QIAGEN, Hilden, Germany) extraction kit. DNA was then amplified by quantification of 18S ribosomal gene of *Cryptosporidium* following the manufacturer instructions (Abdelhamed et al. 2019).

### Induction of Infection

Infection was performed via oral inoculation of oocysts using esophageal tube. Each mouse received 0.1 ml of the oocysts' inoculum containing $10^3$ *C. parvum* oocysts/ml dissolved in 200 µl of phosphate buffered saline (PBS) (Abdelmaksoud et al. 2020). Mice feces were collected daily and examined for *C. parvum* oocysts to insure mice infection. Mice were confirmed to be infected at the 4th day after inoculation. The treatment started at the 5th day post infection (p.i). All drugs were given orally using esophageal tube.

## Treatment

### Nitazoxanide (NTZ) (drug control)

Nanazoxid, Utopia Pharmaceutical Company, Egypt was used. It was obtained as 100 mg/5 ml suspension. NTZ was given at a dose of 10 mg/kg/day for successive 14 days (El-Sayed and Fathy 2019).

### Essential oils

Mint, Thyme, Chamomile and Basil essential oils were purchased in their commercial pharmaceutical form from a local market in Giza, Egypt. Mint essential oil was given at a dose of 20 mg/kg/day for successive 14 days (Salin et al. 2011). Thyme essential oil was given at a dose of 15 µg/kg/day for successive 14 days (Farrag et al. 2021). Chamomile essential oil was given at a dose of 1000 mg/kg/day for successive 14 days (Corpas-Lopez et al. 2015). Basil essential oil was given at a dose of 500 mg/kg/day for successive 14 days (Uraku et al. 2015).

### Mice scarification

Mice were sacrificed at 19th day p.i. They received intraperitoneal anesthetic (500 mg/kg thiopental)—anticoagulant (100 units/ml heparin) according to Liang et al. (1987). Blood samples were collected for assessment of IFN-γ levels in the sera. Parts of jejunum were removed to be subjected to biochemical analysis and ileal parts were dissected for histopathological examination in all groups.

### Parasitological evaluation

Stool pellets were collected and stained with Modified Zheil Nelsen (MZN) stain for parasitological examination (Henricksen and Pohlenz 1981). Samples were examined under the oil immersion lens (X 100). Examination was done for confirmation of the mice infection and for counting *C. parvum* oocysts.

### Histopathological evaluation

Ileal segments underwent dissection and fixation in formalin (10%) solution. After that, embedding in paraffin wax blocks was done. Sections were performed and stained with Hematoxylin and Eosin (H&E).

### Evaluation of IFN-γ levels

Serum samples were subjected to sandwich Enzyme-Linked Immunosorbent Assay (ELISA) using (Rat IFN Gamma ELISA Kit PicoKine™, Catalog number: EK0374) kit. The steps were done following the manufacturer's guidlines. The values of IFN-γ were measured from the standard curve. The detection ranged from 31.2 to 2000 pg/ml.

### Biochemical analysis

Samples from jejunum were perfused prior to dissection with PBS solution (pH 7.4) with 0.16 mg/ml heparin to remove excess RBCs. After that, tissues were homogenized in 5–10 ml cold buffer (100 mM potassium phosphate (pH 7.0) that contains 2 mM EDTA)/gram tissue. Then centrifuged at 4000 rpm for 15 min at 4 °C, collection and storage of supernatants at − 20 °C was done till use. A volume of 1.0 ml of supernatant was mixed with 0.5 ml ice-cold extraction reagent in a test tube, vortexed for 30 s and then centrifuge at 4000 rpm at 4 °C for 10 min. Then, the uppermost aqueous layer was collected and assayed colorimetrically. The superoxide dismutase (SOD) and malondialdehyde (MDA) levels were measured using (CAT. No. SD 25 21 and CAT. No. MD 25 29 respectively) kits. SOD activity measures were expressed in U/g tissue while MDA value was expressed in nmol/g tissue.

### Ethics statement

Our study protocol was approved and carried out according to the guidelines of the Laboratory Animal Centre for

Research Ethics Committee at Faculty of Medicine, Tanta University (code number: 35947/10/22).

### Statistical analysis

Data were expressed as mean and standard deviation (± SD). Student t test was performed using GraphPad software for assessment of the statistical significance among all groups. *P* value was considered significant at < 0.01.

## Results

### Parasitological evaluation

All study groups (GII, GIII, GIV, GV and GVI) revealed statistically highly significant decrease (*P* value < 0.01) in the *C. parvum* oocysts count compared to GI (Positive Control). The best result was shown with GII (treated with NTZ) with percentage reduction 87.7% followed by GIII, GIV, GVI and the least percentage reduction was shown in GV that was 67.2% (Table 1).

### Histopathological evaluation

#### GVII (negative control)

Ileal sections examination of the negative control group showed intestinal villi with preserved architecture (retained average villous/crypt length ratio with intact brush border) and normal mucin secretion Fig. 1a

#### GI (positive control)

Ileal sections examination of the positive control group (infected non-treated) revealed villous shortening and broadening. There were mucosal ulcerations as well as focal mucin depletion. Lamina propria showed inflammatory cellular infiltrate mainly lymphocytes, macrophages and plasma cells. Also, hyperplasia of lymphoid follicles was seen Fig. 1b and c

**Table 1** The results of *C. parvum* oocysts ($\times 10^3$) counting

| Group | Mean ± SD | % Reduction | *P* value |
|---|---|---|---|
| GI positive control | 25.7 ± 7.6 | 0 | < 0.01 |
| GII (NTZ)* | 3.2 ± 0.7 | 87.7 | |
| GIII (Mint Oil)* | 5.7 ± 2.0 | 77.9 | |
| GIV (Thyme oil)*$^N$ | 6.5 ± 0.9 | 74.7 | |
| GV (Chamomile oil)*$^N$ | 8.4 ± 1.2 | 67.2 | |
| GVI (Basil oil)*$^N$ | 8.1 ± 0.7 | 68.2 | |

*Statistically significant (*P* value < 0.01) compared to GI (Positive control), $^N$ statistically significant (*P* value < 0.01) compared to GII (NTZ treated)

#### GII (NTZ treated) and GIII (mint oil treated)

Ileal sections examination of the NTZ treated and Mint Oil treated groups revealed marked improvement of the histopathological damage resulted from *C. parvum* infection. The normal architecture of the villi was restored. Healing of the mucosa occurred with intact surface epithelium apart from minimal surface erosions. Lamina propria showed slight inflammatory cellular infiltration Fig. 1d and 2a

#### GIV (thyme oil treated) and GV (chamomile oil treated)

Ileal sections examination of the Thyme Oil treated and Chamomile Oil treated groups revealed partial improvement of the histopathological damage resulted from *C. parvum* infection. The villi were blunted. Partial healing of the mucosa occurred with focal ulcerations. Lamina propria showed mild inflammatory cellular infiltration Fig. 2b c and d

#### GVI (basil oil treated)

Ileal sections examination of the Basil Oil treated group revealed only slight improvement of the histopathological damage resulted from *C. parvum* infection. Small intestinal villi were blunt and broad. Intestinal mucosa showed partial healing. Lamina propria showed moderate inflammatory cellular infiltration Fig. 2e

### Serological evaluation

IFN-γ levels showed significant increase in *C. parvum*—infected mice (282.7 ± 5.8) compared to the negative control ones (211.5 ± 5.5) (*P* value < 0.0001). All treated mice showed significant upregulation in the IFN-γ with mean levels reaching 482.8 ± 7.7 (*P* value < 0.0001) Fig. 3

### Biochemical analysis evaluation

The levels of MDA showed increase in the infected mice with *C. parvum* oocysts compared to those non- infected (62.2 ± 2.5 vs. 20.1 ± 2). While the levels of SOD were decreased in the infected mice with *C. parvum* oocysts compared to those non- infected (133.2 ± 3.6 vs. 348.9 ± 9.5). All treated groups of infected mice significantly reduced the MDA levels and increased SOD level compared to the infection control group (*P* value < 0.0001) Fig. 4

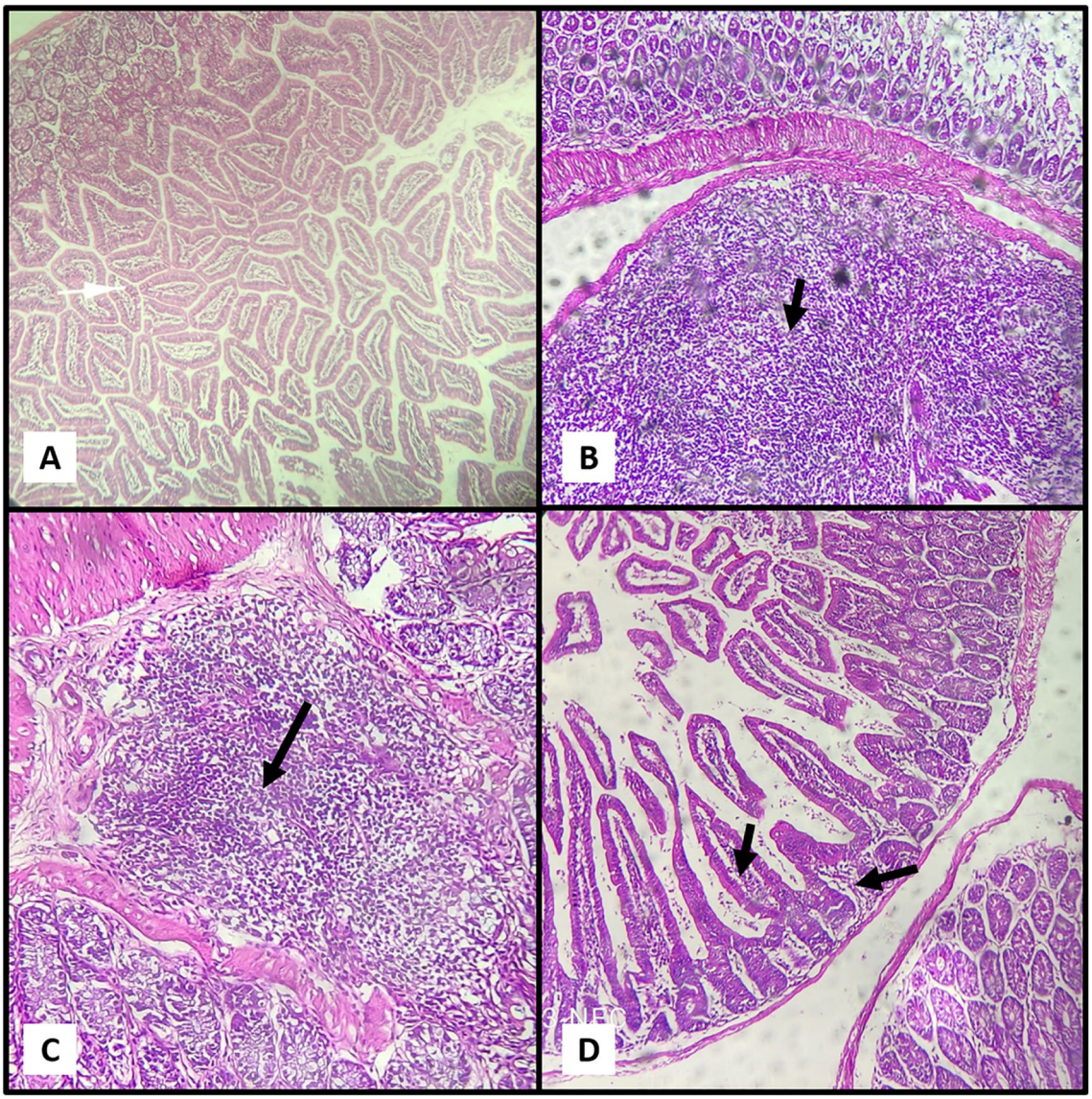


**Fig. 1** **a** Section of Ileum in GVII (Negative Control) showing normal villous architecture. **b**, **c** Sections of Ileum in GI (Positive Control) showing pathological changes with marked inflammatory infiltrate in the lamina propria 'black arrows'. **d** Section of Ileum in GII (NTZ) revealed healing of intestinal villi with slight inflammatory infiltrate 'black arrows' (X 10 power—H&E stain)

## Discussion

*C. parvum* causes severe fatal diarrhea in immunosuppressed individuals (Farid et al. 2022). Unfortunately, the available drugs for cryptosporidiosis treatment show many drawbacks with increasing resistance. So, the researchers started to search for a new alternative drug preferably from natural source to be less toxic and can be produced easily in adequate quantities (Mendonça et al. 2021).

Our results revealed that treatment with Mint, Thyme, Chamomile and Basil oils could significantly reduce *C. parvum* oocysts shedding in experimentally immunosuppressed infected mice with percentage reduction in oocysts number 77.9%, 74.7%, 67.2% and 68.2% respectively. However, the best percentage reduction was shown with NTZ (87.7%) and the mean number of shedded oocysts in the NTZ group was statistically significant compared to Thyme, Chamomile and Basil oil groups.

The groups treated with NTZ and Mint oil showed marked improvement in the histopathological damage of the intestinal villi following *C. parvum* infection. Partial healing of the intestinal damage was shown in the groups treated with Thyme and Chamomile oils while only slight improvement was seen in the group treated with Basil oil.

IFN-γ is the major cytokine that is upregulated in cryptosporidiosis infection to enhance innate as well as acquired

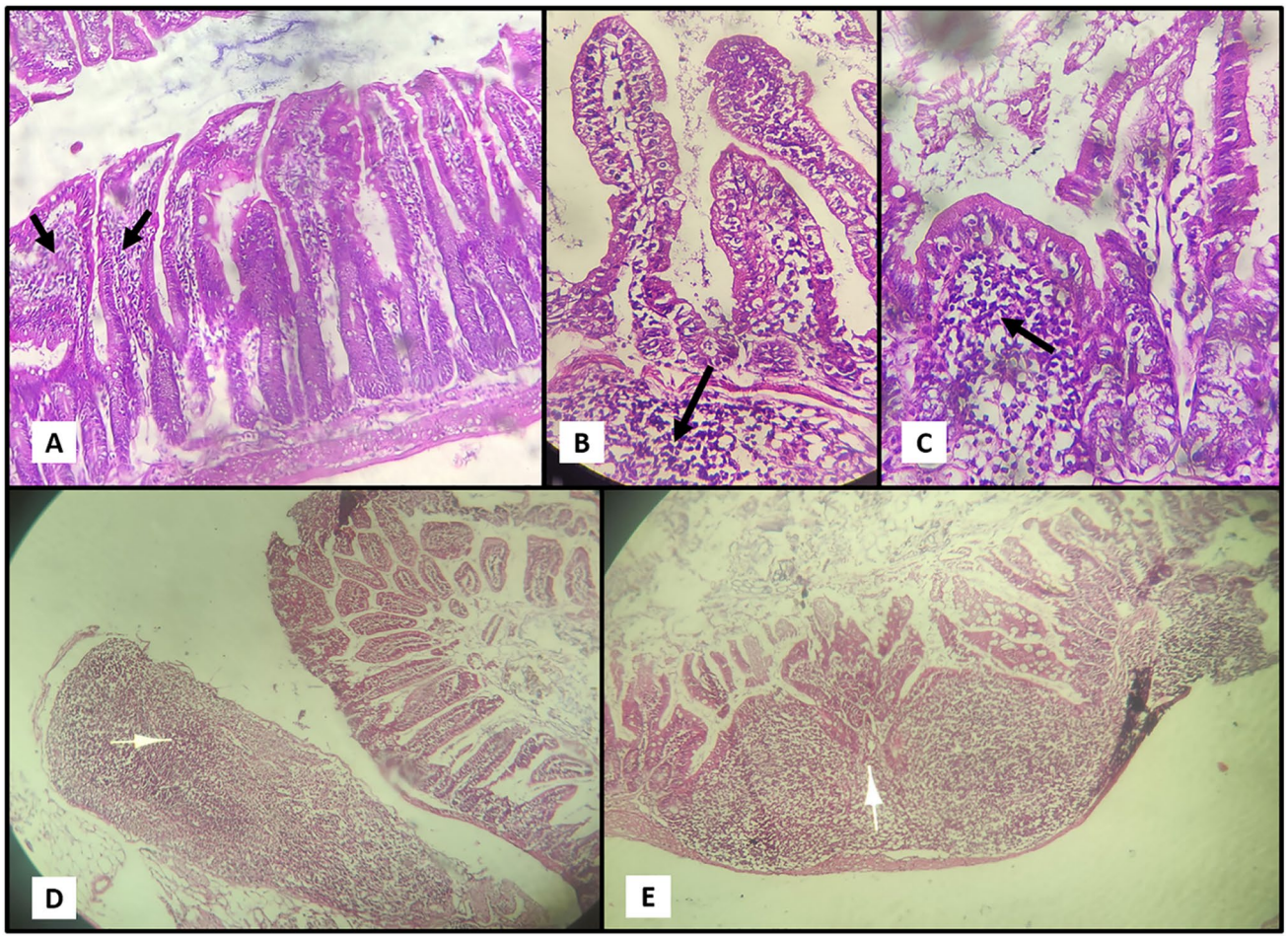


**Fig. 2** **a** Section of Ileum in GIII (Mint) revealing intestinal improving with slight inflammatory infiltrate 'black arrows'. **b** Sections of Ileum in GIV (Thyme) partial healing with mild inflammatory cellular infiltrate in the lamina propria 'black arrow'. **c** Section of Ileum in GV (Chamomile) revealing partial healing with some broad villi and mild inflammatory infiltrate in the lamina propria 'black arrow'. **d** Section of Ileum in GV (Chamomile) revealing inflammatory infiltrate 'white arrow'. e Section of Ileum in GVI (Basil) showed minimal healing of intestinal villi with moderate inflammatory infiltrate 'white arrow' (X 10 power—H&E stain)

immunity. Early *C. parvum* infection induces secretion of IFN-γ by natural killer (NK) cells, dendritic cells and macrophages (Borad and Ward 2010). In our study, all infected mice with *C. parvum* oocysts induced cellular inflammatory response in the intestinal tissues with significant upregulation of IFN-γ levels. This was in agreement with (Lean et al. 2002; Tessema et al. 2009; El-Sayed and Fathy 2019). However, the groups treated with Chamomile and Basil oils showed statistically significant decrease in IFN-γ levels compared to the group treated with NTZ.

Reactive oxygen species (ROS) production in adequate levels is important for maintaining the redox balance, however, overproducing ROS and other free radicals' initiates oxidative stress leading to cell destruction and death (Poljsak et al. 2013; Di Meo et al. 2016). Antioxidants help protecting humans and animals against infectious diseases by inhibiting and scavenging the free radicals. In our study, it was shown that the experimental infection with *C. parvum* resulted in increase in the MDA levels and decline levels of SOD. This was agreeing with (Bhagat et al. 2017). All treated groups of infected mice significantly decreased the MDA and elevated the SOD levels in comparison with the infection control group. However, significant improvement in MDA and SOD levels was observed in the NTZ group in comparison with the Thyme, Chamomile and Basil oils groups.

Generally, plant extracts are used for animal feeding as antioxidants to protect them from free radicals' harm effects (Liu et al. 2018). They act by binding to free radicals to scavenge them, chelating metals, donating atoms of hydrogen atoms and suppressing the action of pro-oxidative enzymes (Brown et al. 2019; Dorman et al. 2003).

In our study, Mint oil showed marked improvement in the small intestinal histopathological changes and elevation in the IFN-γ levels. This was agreeing with Hejna

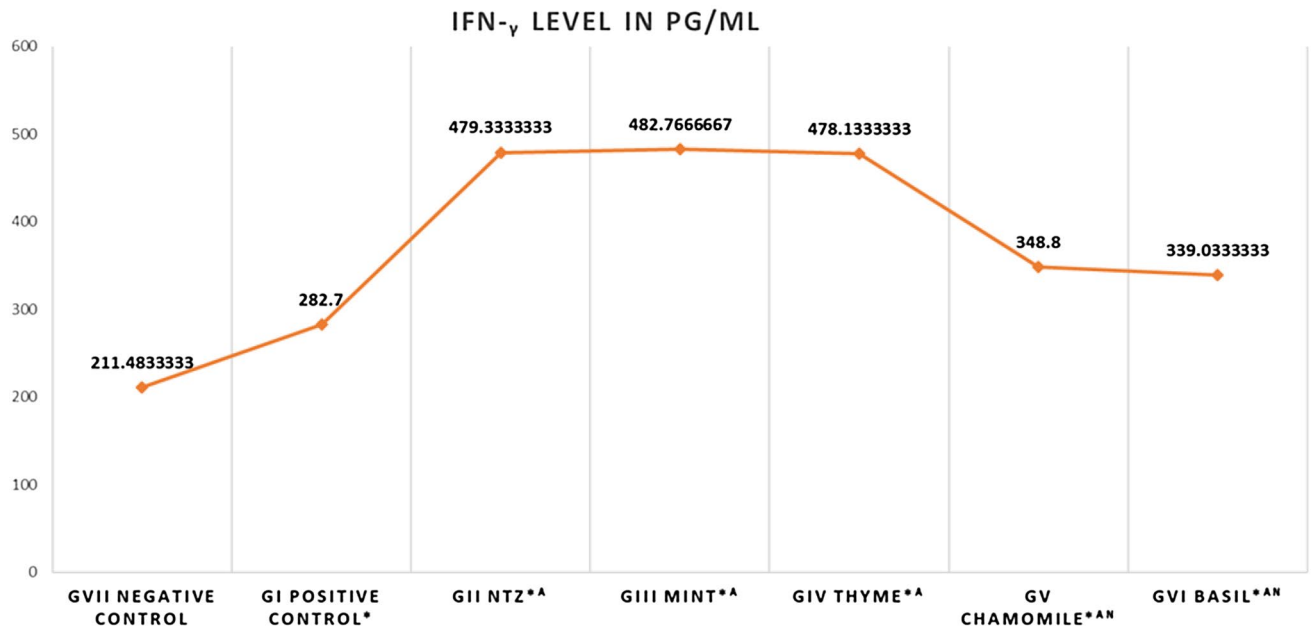


**Fig. 3** IFN-γ levels in all groups in pg/ml. *Statistically significant ($P$ value < 0.0001) compared to GVII (Negative control), [A] statistically significant ($P$ value < 0.0001) compared to GI (Positive control), [N] statistically significant ($P$ value < 0.0001) compared to GII (NTZ treated)

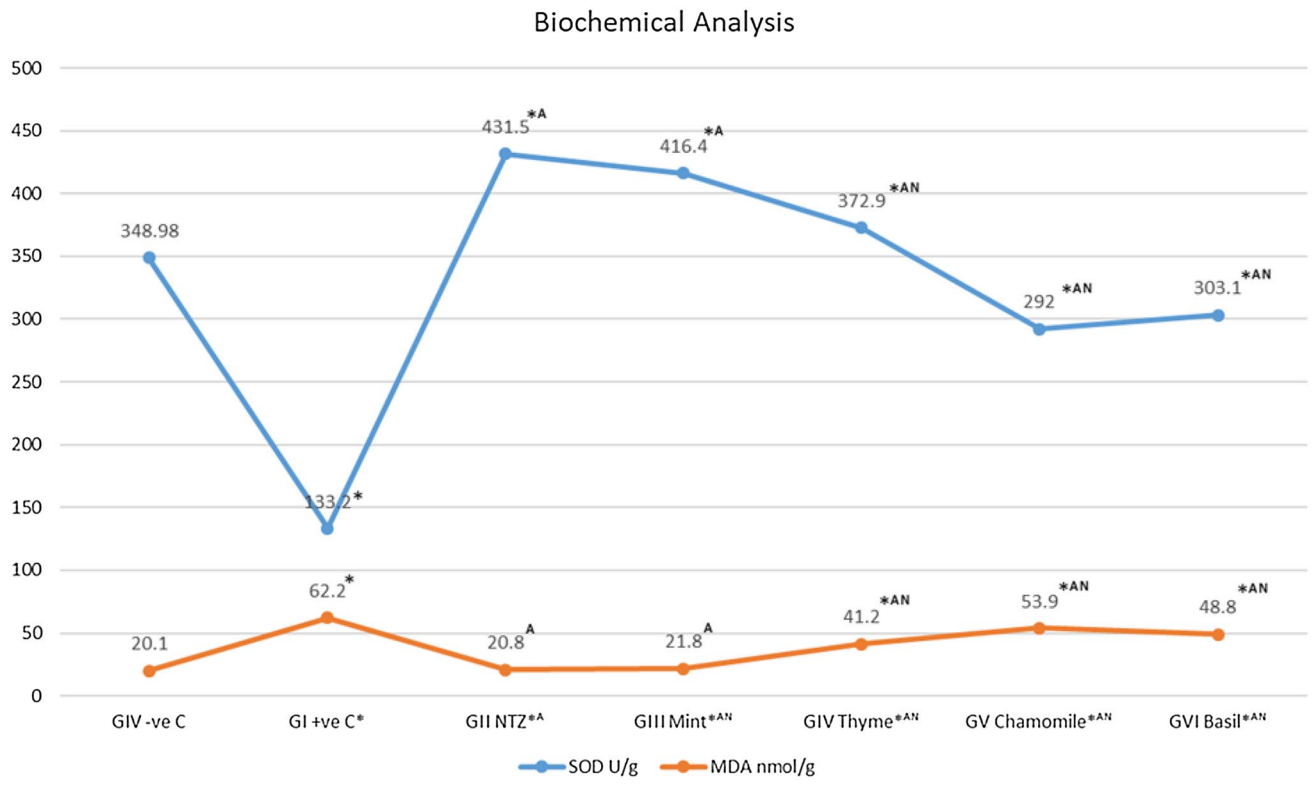


**Fig. 4** SOD (U/g tissue) and MDA (nmol/g tissue) levels in all groups. *Statistically significant ($P$ value < 0.0001) compared to GVII (Negative control), [A] statistically significant ($P$ value < 0.0001) compared to GI (Positive control), [N] statistically significant ($P$ value < 0.0001) compared to GII (NTZ treated)

et al. (2021), who revealed that Mint oils have the ability to inhibit pro-inflammatory cytokines production revealing high therapeutic potential and control of the inflammatory response. Also, in the present work, Mint oil succeeded to decrease the MDA levels and elevate the SOD levels in the small intestinal tissues. These effects can be explained by the phenolic and flavonoid constituents of Mint oil that can donate hydrogen or electrons to the free radicals inhibiting formation of hydroxyl peroxide and controlling oxidative stress damage (Djeridane et al. 2006; Krzyzanowska et al. 2011). This was in agreement with Teixeira et al. (2013); Nickavar et al. (2010); Wu et al. (2019); Hejna et al. (2021), who confirmed the antioxidant effects of Mint oil.

In our work, Thyme oil showed partial improving in the small intestinal histopathological damages and significant elevation in the IFN-γ levels. Also, Thyme oil succeeded to decrease the MDA levels and elevate the SOD levels in the small intestinal tissues. However, the anti-oxidant markers levels of the reference drug (NTZ) were significantly better than the levels obtained by Thyme oil treatment.

Thyme was reported to have anti-coccidial effects (Abbas et al. 2012; Remmal et al. 2011). Gaur et al. (2018) had tested the Thyme extract efficacy against *C. parvum* in vitro with satisfying results. Thyme was reported to have anti-Cryposporidial effects reducing their development and suppressing the parasites' invasion capacities. These effects owe to the thymol and carvacrol constituents of Thyme. They affect Calcium ions mediated transmitted signals. Also, they inhibit ATP synthesis and enzyme activity (Bessoff et al. 2013; Murphy et al. 2010). Moreover, Kara et al. (2022), had examined Thyme extract against *C. parvum* in rats and concluded that Thyme extract effectively treated as well as prevented prophylactically the *C. parvum* infection with no signs of toxicity.

In the present work, Chamomile oil showed partial improving in the small intestinal histopathological pathology and significant elevation in the IFN-γ levels. Also, Chamomile oil succeeded to decrease the MDA levels and elevate the SOD levels in the small intestinal tissues. However, the IFN-γ levels and the anti-oxidant markers levels of the reference drug (NTZ) were significantly better than the levels obtained by Chamomile oil treatment.

Previous studies confirmed the antioxidant and anti-parasitic properties of Chamomile (Lee and Shibamoto 2002). These effects are most probably due to the Chamomile components having anti-inflammatory properties like α-bisabolol, chamazulene and apigenin that act against pro-inflammatory mediators (Srivastava et al. 2009). Bisabolol and bisabolol oxide show anti-5-lipoxygenase effect (Braga et al. 2009). Chamomile extract was shown to inhibit the PGE2 release by inhibiting Cyclooxygenase-2 (COX-2) enzyme activity and decreasing the expression of COX-2 mRNA and proteins (Srivastava et al. 2009). Sabatke et al. (2022) reported that polysaccharides that are present in chamomile tea showed a synergistic effect with NTZ against *Giardia intestinalis*. It acts synergistically with NTZ to increase its efficacy and decrease its therapeutic dose. They contributed together and inhibited parasitic adhesion to intestinal cells.

Chamomile essential oil was tested against *Leishmania* promastigotes in vitro. It showed activation of apoptosis, membrane damage and decreased total ATP levels in the mitochondria (Hajaji et al. 2018).

In the present study, Basil oil showed only slight improvement in the small intestinal histopathological changes. It significantly elevated the IFN-γ levels in serum. Also, Basil oil succeeded to decrease MDA levels and elevate SOD levels in the small intestinal tissues. However, the IFN-γ levels and the anti-oxidant markers levels of the reference drug (NTZ) were significantly better than the levels obtained by Basil oil treatment.

Basil oil demonstrated anti-infammatory properties owing to its linalool, eugenol and limonene contents that act to inhibit COX-2 enzyme (Złotek et al. 2016).

Basil oil showed anti-oxidant activities that are explained by the synergistic effects between many constituents rather than be referred to a single compound (Tavallali et al. 2019). The mechanisms of action include sequestration of free radicals, acting as hydrogen donors and chelating metal ions. Moreover, Koroch et al. (2017) reported a methylation behavior with the highest anti-oxidant function was linked to linalool-eugenol. The great anti-oxidant potential of the Basil oil is related to the high proportion of composites that contain a phenol ring with an (OH) group.

Basil oil showed antibacterial activity owing to linalool and other components. Anti-Gram-positive bactericidal action was shown against *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* as well as anti-Gram-negative bactericidal activity was revealed against *Pseudomonas aeruginosa* and *Salmonella* spp. (Baldim et al. 2018). Linalool is capable of changing cell permeability due to its higher membrane fluidity facilitating other components entry enhancing its activity in a synergistic manner. Basil essential oil was stated by Stanojevic et al. (2019) to possess high bacterial inhibitory effect against *Salmonella enterica*, *Providencia stuartii*, *Staphylococci* and *Streptococci* compared to ciprofloxacin and gentamicin.

NTZ showed the best results in our study. It markedly succeeded in restoring the normal intestinal villous architecture following *C. parvum* infection. It also significantly elevated the IFN-γ levels in serum, decreased the MDA levels and elevated the SOD levels in the small intestinal tissues.

NTZ is a broad spectrum anti-parasitic drug. It has 2 main components tizoxanide as well as tizoxanide-glucuronide that were evidenced to decrease the *C. parvum* growth (Gargala et al. 2000). Excretion of NTZ in bile was proved

explaining its efficacy against *C. parvum* induced cholangitis in immunosuppressed patients. NTZ given for 3 days was reported to be effective in immunocompetent patients while only 59% of AIDS patients that were suffering from cryptosporidiosis showed a sustained clinical cure with maintained NTZ. However, 3000 mg/day NTZ and sustained administration for long duration were unsafe (Rossignol 2006).

## Conclusion

The present study showed that essential herbal oils represent a promising alternative for cryptosporidiosis treatment. Mint, Thyme, Basil and Chamomile oils reduced *C. parvum* oocysts shedding and relived the histopathological intestinal changes. The best results were seen with Mint oil followed by Thyme, Chamomile and Basil oils respectively. Furthermore, study oils succeeded to elevate the IFN-γ levels in serum, increase the SOD levels and decrease the MDA levels in intestinal tissues representing anti-inflammatory and antioxidant properties.

**Authors' contributions** All authors contributed to the study conception and design. Material preparation and experimental work was done by NMT and BM.E. Data collection and analysis were performed by RSZ. EK prepared histopathological figures and analysis. The first draft of the manuscript was written by NMT and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Availability of data and material** The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflicts of interest** Authors declare that there are no any conflicts of interest.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Ethical approval** Our study protocol was approved and carried out according to the guidelines of the Laboratory Animal Centre for Research Ethics Committee at Faculty of Medicine, Tanta University (code number: 35947/10/22).

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