Research Square

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The efficacy of the Origanum genus (Lamiaceae) essential oils on experimentally infected mice with Blastocystis spp.

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Abstract

Background

Blastocystis spp. is a common intestinal protozoan with a controversial role in gastrointestinal disease, and the variable efficacy of conventional therapies has prompted the search for alternative treatment options. This study evaluated the therapeutic effects of *Origanum* essential oil in experimentally infected mice compared with nitazoxanide (NTZ).

Methods

Forty male Swiss albino mice were allocated into four groups: negative control, infected untreated, NTZ-treated (100 mg/kg/day), and *Origanum* oil-treated (0.5 mL/kg/day). Mice were orally infected with 1×10^5 *Blastocystis* cysts and treated for seven days. Treatment efficacy was assessed by fecal cyst counts, intestinal nitric oxide (NO) and malondialdehyde (MDA) levels, and histopathological examination of intestinal tissues. Data was analyzed using one-way ANOVA.

Results

Infected untreated mice exhibited reduced activity and soft stools. Clinical improvement was observed in both treatment groups within 5–7 days, and no mortality occurred during the study. The infected untreated group showed the highest cyst burden ($6.2 \times 10^5 \pm 0.4 \times 10^5$ cysts/g stool), whereas NTZ and *Origanum* oil treatment significantly reduced cyst shedding by 85.6% and 91.3%, respectively, compared with infected controls ($p < 0.001$). No cysts were detected in the non-infected control group. Infection was associated with significant increases in intestinal NO and MDA levels, indicating oxidative stress and inflammation. Both treatments significantly reduced these markers ($p < 0.001$), with values in the *Origanum*-treated group approaching those of the negative control group. Histopathological examination revealed villous atrophy, epithelial desquamation, and inflammatory cell infiltration in infected untreated mice. NTZ treatment resulted in partial improvement of these lesions, whereas *Origanum* oil treatment was associated with marked restoration of villous architecture and minimal inflammatory changes.

Conclusions

Under this experimental model, *Origanum vulgare* essential oil improved parasitological, biochemical, and histopathological parameters in *Blastocystis*-infected mice. Further studies are needed to identify active constituents, optimize dosing, and confirm these findings in other models and clinical settings.

Introduction

Blastocystis spp. are anaerobic intestinal protozoa commonly found in humans and animals worldwide. Although long considered commensal, accumulating evidence suggests that certain *Blastocystis* subtypes are associated with gastrointestinal symptoms, including abdominal pain, diarrhea, flatulence, and irritable bowel syndrome–like manifestations [1, 2]. The pathogenesis of *Blastocystis* infection has been linked to disruption of intestinal epithelial integrity, increased mucosal permeability, and induction of oxidative and inflammatory damage [3, 4].

Currently, Nitazoxanide (NTZ) is one of the most effective drugs against *Blastocystis* spp. [5]. However, variable cure rates, drug resistance, and treatment relapse highlight the need for alternative or adjunct therapies [6].

In recent years, essential oils derived from medicinal plants have attracted attention due to their antimicrobial, antiparasitic, antioxidant, and anti-inflammatory properties [7]. Among them, *Origanum* species (family Lamiaceae), particularly *O. vulgare*, *O. majorana*, and *O. syriacum*, have demonstrated potent activity against a range of protozoa and helminthes [8, 9]. Their major constituents, carvacrol and thymol, are phenolic monoterpenes that disrupt microbial membranes, inhibit enzymatic activity, and modulate host immune responses [10, 11].

The current study aimed to evaluate the efficacy of *Origanum* essential oil in experimentally *Blastocystis*-infected mice, assessing both parasitological and histopathological outcomes, and comparing its effects with the conventional drug Nitazoxanide.

Materials and Methods

1. Experimental Animals

Forty male Swiss albino mice (20–25 g) were maintained under standard laboratory conditions with free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (Approval No. 442/10/2023) and conducted according to international guidelines for animal care.

2. Parasite Isolation and Inoculation:

Blastocystis cysts were isolated from stool samples of naturally infected patients and purified using sucrose flotation. *Blastocystis* cysts are maintained by repeated Sub-cultivation with LE media. The organism could be maintained for more than three months [12]

3. Extraction of Essential Oil (Oil)

3. Extraction of Essential Oil (*Origanum* Oil)

Fresh aerial parts of *Origanum* (leaves and flowering tops) were washed, shade-dried for 7–10 days, and ground into coarse powder. About 250 g of the material was hydro-distilled for 3 h using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate and stored in amber vials at 4°C. Oil yield (mL/100 g dry weight) was recorded. The extracted *Origanum* essential oil was characterized by its strong aromatic odor and pale-yellow color which was extracted following standard methods [10, 13].

4. Study Design

Mice were divided into four groups (n = 10 each):

Group I (Negative control)

Non-infected, untreated.

Group II (Infected untreated)

Received *Blastocystis* inoculum only

Group III (NTZ-treated)

Infected mice treated orally with Nitazoxanide (100 mg/kg/day) for 7 days. [5]

Group IV (*Origanum* oil–treated)

Infected mice treated orally with *Origanum* essential oil (0.5 mL/kg/day) for 7 days [14].

Each experimental mouse from the second group to fourth group was orally inoculated with 1×10^5 viable cysts suspended in 0.2 mL phosphate-buffered saline (PBS). On the seventh day post group III and group IV were treated as mentioned above.

5. Parasitological Assessment

Fecal samples were collected daily from each group. The number of *Blastocystis* cysts per gram of stool was quantified using a hemocytometer under ×400 magnification. Mean cyst reduction rates were calculated relative to the infected untreated group.

6. Biochemical determinations:

For assessment of antioxidant activity, intestinal tissues were homogenized and analyzed following El-Shenawy et al. (2008) [15]. Nitric oxide (NO) levels were determined as total nitrite/nitrate using the Griess reaction after nitrate reduction [16], while lipid peroxidation was measured as malondialdehyde (MDA) equivalents using the thiobarbituric acid method [17].

7. Histopathological Examination

At the end of the experiment, mice were euthanized, and small intestinal tissues were fixed in 10% buffered formalin, processed, and stained with hematoxylin and eosin (H&E) following [18] Sections were examined microscopically (×200–400) for villous atrophy, epithelial damage, inflammation, and parasite presence.

8. Statistical Analysis

Data was expressed as mean ± SD. Differences among groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. A *p*-value < 0.05 was considered statistically significant.

Results

1. Clinical Observations

All infected mice in Groups II–IV developed mild to moderate signs of illness during the first week post-infection, including reduced activity, ruffled fur, and soft stools. These signs were most pronounced in the infected untreated group (Group II). Mice treated with either *Origanum* essential oil or nitazoxanide (NTZ) showed improvement in general condition and appetite within 5–7 days of treatment. No mortality was recorded during the experimental period.

2. Parasitological Findings

Quantitative stool examination revealed a high mean parasite count in the infected untreated group (Group II), reaching $6.2 \times 10^5 \pm 0.4 \times 10^5$ cysts/g stool on day 14 post-infection.

Treatment with NTZ (Group III) significantly reduced *Blastocystis* cyst shedding by 85.6% compared with the infected untreated group ($p < 0.001$). Similarly, treatment with *Origanum* essential oil (Group IV) significantly reduced cyst shedding by 91.3% ($p < 0.001$) (Table 1). No *Blastocystis* forms were detected in the non-infected control group (Group I).

Table 1
Shedding of Blastocystis spp. cysts (×10⁵/g stool/day) in the stools of the different groups of mice

Group	Treatment	Mean ± SD (×10⁵ cysts/g stool/day)	% Reduction vs. Infected Control	p-value
Group I	Non-infected (Negative control)	0.00	—	—
Group II	Infected untreated (Positive control)	6.20 ± 0.40	—	—
Group III	Nitazoxanide (20 mg/kg/day)	0.90 ± 0.20	85.6%	< 0.001
Group IV	Origanum essential oil (20 mg/kg/day)	0.54 ± 0.10	91.3%	< 0.001
Data are expressed as mean ± standard deviation (SD) for six mice per group.				

3. Biochemical Determinations

Intestinal nitric oxide (NO) and malondialdehyde (MDA) levels were significantly higher in the infected untreated group (Group II) than in the non-infected control group (Group I). The mean NO and MDA levels in Group II were 52.8 ± 4.6 µmol/g and 15.2 ± 1.4 nmol/g, respectively (Table 2).

Table 2
Effect of *Origanum* essential oil and nitazoxanide on nitric oxide (NO) and malondialdehyde (MDA) levels in the intestinal tissue of *Blastocystis spp.*–infected mice

Group	Treatment	NO (µmol/g tissue)	MDA (nmol/g tissue)
Group I	Non-infected (Negative control)	24.1 ± 2.5	5.8 ± 0.7
Group II	Infected untreated (Positive control)	52.8 ± 4.6	15.2 ± 1.4
Group III	Nitazoxanide (20 mg/kg/day)	32.4 ± 3.2 ***	8.7 ± 0.9 ***
Group IV	Origanum essential oil (20 mg/kg/day)	28.6 ± 2.8 ***	6.9 ± 0.8 ***
Data are presented as mean ± standard deviation (SD) for six mice per group.			
Statistical analysis: One-way ANOVA followed by Tukey's post hoc test.			
*p < 0.001 compared with infected untreated control.			

Treatment with NTZ (Group III) significantly reduced NO and MDA levels compared with the infected untreated group ($p < 0.001$). Likewise, treatment with *Origanum* essential oil (Group IV) significantly reduced NO and MDA levels to 28.6 ± 2.8 µmol/g and 6.9 ± 0.8 nmol/g, respectively ($p < 0.001$) (Table 2).

4. Histopathological Examination

Histopathological examination of intestinal tissues from the infected untreated group (Group II) revealed severe villous atrophy, epithelial desquamation, brush-border loss, and dense inflammatory cell infiltration consisting of lymphocytes, plasma cells, and eosinophils within the lamina propria. Multiple vacuolated *Blastocystis* cysts were observed within the mucosa and crypts, accompanied by ulceration and necrosis (Fig. 1C–D).

Sections from the NTZ-treated group (Group III) showed partial restoration of villous architecture, a reduction in the number of observed cysts, and mild to moderate inflammatory cell infiltration within the lamina propria (Fig. 2A–B).

Intestinal sections from the *Origanum* oil-treated group (Group IV) showed restoration of mucosal architecture with intact villi, preserved epithelial lining, and minimal inflammatory cell infiltration. No *Blastocystis* cysts were observed in the examined sections (Fig. 2C–D).

The non-infected control group (Group I) showed normal intestinal architecture with intact villi and no evidence of inflammatory changes (Fig. 1A–B).

Histopathological lesion scores were significantly lower in both treated groups than in the infected untreated group ($p < 0.001$). The lowest mean lesion score was recorded in the *Origanum* oil-treated group.

Discussion

This study demonstrated that Origanum essential oil showed promising therapeutic effects against *Blastocystis* spp. infection in mice by significantly reducing parasite load and improving intestinal histopathology. The efficacy of Origanum essential oil was comparable to, and slightly higher than, that of nitazoxanide, achieving a cyst reduction rate of 91.3% compared with 85.6% for nitazoxanide. These findings are consistent with previous studies reporting that natural plant extracts, including Origanum species, markedly suppress *Blastocystis* proliferation [19, 20]. Additionally, Origanum majorana oil has been shown to effectively eliminate *Giardia lamblia* and *Entamoeba histolytica* in experimental models [21]. Origanum vulgare extracts also exhibit broad antiparasitic activity attributed to bioactive compounds such as carvacrol and thymol, which disrupt protozoal membranes and inhibit energy metabolism [22, 23]. These observations align with earlier in vitro studies demonstrating significant inhibitory effects of phenolic-rich Origanum extracts against *Blastocystis hominis* [19]. Collectively, these

findings support the potential use of Origanum essential oil as an effective natural alternative to nitazoxanide in the management of *Blastocystis* infections.

Conversely, some investigations have reported weaker antiparasitic effects of Origanum oils. Moderate and dose-dependent reductions in Blastocystis cyst counts following treatment with *O. vulgare* extracts have been documented [24]. Other studies indicated reduced efficacy of Origanum oil in chronic *Blastocystis* infections, possibly due to resistance of encysted stages or variability among parasite subtypes [25]. Additionally, comparative studies have shown that *O. majorana* extracts exerted lower anti-Blastocystis activity than other medicinal plants such as *Allium sativum*, *Matricaria chamomilla*, *Artemisia judaica*, and *Nigella sativa*, as well as nitazoxanide [26, 27]. Differences in Blastocystis subtypes have also been suggested as a contributing factor to variable treatment responses [28].

In the present study, *Blastocystis* infection induced a significant increase in intestinal oxidative stress markers, as evidenced by elevated nitric oxide (NO) and malondialdehyde (MDA) levels in the infected untreated group. These findings are consistent with previous reports linking Blastocystis pathogenicity to oxidative damage and excessive production of reactive oxygen species [29, 7]. Treatment with either nitazoxanide or Origanum vulgare essential oil significantly reduced these oxidative markers; however, Origanum oil demonstrated superior antioxidant and tissue-protective effects. Similar antioxidant properties of Origanum vulgare oil have been reported previously, including significant reductions in MDA levels and enhancement of antioxidant enzyme activity [30]. Origanum majorana extract has also been shown to maintain intestinal antioxidant balance and reduce lipid peroxidation during parasitic infections [21].

Furthermore, experimental studies have confirmed the potent antioxidant activity of phenolic compounds present in Origanum vulgare extracts [22]. Treatment with Origanum and Thymus essential oils has been shown to reduce NO and MDA levels while improving intestinal histopathology in infected animals [20]. These antioxidant and anti-inflammatory effects are largely attributed to phenolic monoterpenes that stabilize cell membranes and inhibit oxidative chain reactions [31, 23].

Nevertheless, inconsistent antioxidant effects of Origanum preparations have been reported, with some studies indicating that significant reductions in oxidative stress markers occur only at higher doses, and that prolonged *Blastocystis* infection may limit the antioxidant response [32, 25]. Overall, most available evidence supports the present findings that Origanum vulgare essential oil alleviates oxidative stress, reduces intestinal NO and MDA levels, and promotes mucosal healing, although dosage, treatment duration, and infection chronicity may influence the degree of protection achieved.

Histopathological examination in this study confirmed severe intestinal damage induced by *Blastocystis* infection, including villous atrophy, epithelial desquamation, goblet cell depletion, and inflammatory cell infiltration. These findings agree with previous reports demonstrating that *Blastocystis* secretes cysteine proteases and other virulence factors that degrade tight-junction proteins such as occludin and ZO-1, resulting in increased intestinal permeability [33, 4]. Similar mucosal destruction has been observed in

experimentally infected animal models [3]. Other studies have emphasized the role of oxidative stress and immune-mediated mechanisms in Blastocystis-induced epithelial damage [1, 2].

Treatment with nitazoxanide and Origanum vulgare essential oil markedly improved these histopathological alterations, restoring villous architecture and reducing inflammatory infiltration. Comparable improvements in intestinal structure following Origanum treatment have been reported previously [21, 20, 22, 25]. The protective effect of Origanum vulgare oil has also been attributed to its ability to enhance antioxidant enzyme activity and inhibit lipid peroxidation [30].

However, incomplete mucosal restoration following treatment has been described in some studies, with persistence of mild inflammation and villous atrophy [3]. Extended treatment duration has been suggested as necessary for complete recovery [32], and strain variability among Blastocystis subtypes may further influence tissue damage and therapeutic outcomes [28].

Conclusion

In this experimental mouse model, *Origanum vulgare* essential oil was associated with significant improvements in parasitological, biochemical, and histopathological parameters of *Blastocystis* infection. Treatment reduced cyst shedding, decreased oxidative stress markers, and improved intestinal histological architecture. Nitazoxanide also produced significant improvements in the measured outcomes. The observed effects of *Origanum* essential oil may be related to the combined antiparasitic, antioxidant, and anti-inflammatory properties of its bioactive constituents.

These findings suggest a potential therapeutic value of *Origanum* essential oil against *Blastocystis* infection under experimental conditions. However, further studies are required to confirm these effects, standardize formulations, identify active compounds, and evaluate safety and efficacy in different models and clinical settings.

Summary of Findings

This study evaluated the therapeutic efficacy of Origanum vulgare essential oil compared with nitazoxanide (NTZ) in experimentally induced Blastocystis infection in mice. Infected untreated mice showed severe mucosal atrophy, epithelial desquamation, and inflammation. NTZ treatment significantly reduced cyst shedding and improved intestinal structure but achieved only partial mucosal recovery. In contrast, Origanum essential oil markedly reduced cyst counts, restored villous architecture, and normalized oxidative and inflammatory markers, indicating near-complete healing. The oil’s superior efficacy is attributed to its bioactive components, carvacrol and thymol, which exert synergistic antiparasitic, antioxidant, and anti-inflammatory effects. These findings highlight Origanum essential oil as a promising, natural alternative to synthetic antiparasitic drugs. Further studies are recommended to isolate its active compounds, optimize dosing, and evaluate long-term safety and efficacy.

Limitations of the Study

This study has several limitations that should be considered when interpreting the findings. First, the experiment was performed using a single animal model (male Swiss albino mice), which, although widely used in parasitological research, may not fully reproduce the complexity of *Blastocystis* infection in humans, including host immune variability, gut microbiota differences, and long-term disease progression.

Second, the study evaluated only one fixed dose and one treatment duration for both nitazoxanide and *Origanum vulgare* essential oil. Therefore, dose–response relationships, optimal therapeutic windows, and potential toxicity at higher or repeated doses were not investigated. This limits the ability to define the most effective and safest dosing regimen.

Third, only a single *Blastocystis* isolate was used for experimental infection. Given the well-documented genetic diversity of *Blastocystis* spp. and the known variability in virulence among subtypes, the results may not be generalizable to other isolates or subtypes that could respond differently to treatment.

Fourth, although the study assessed important biochemical markers of oxidative stress (NO and MDA) and detailed histopathological changes, it did not explore the underlying molecular or immunological mechanisms, such as cytokine profiles, gene expression changes, or signaling pathways involved in parasite clearance and tissue repair.

Fifth, the follow-up period was relatively short, and therefore the study did not evaluate long-term outcomes, including possible reinfection, parasite recrudescence, or sustained tissue recovery after cessation of therapy.

Sixth, the *Origanum vulgare* essential oil was used in crude form without full chemical characterization or quantification of its active constituents. As a result, variability in composition (e.g., carvacrol and thymol content) may influence reproducibility and limits comparison with standardized pharmaceutical preparations.

Finally, interactions between the treatments and gut microbiota were not assessed, although such interactions may play an important role in both *Blastocystis* pathogenicity and treatment response.

Overall, these limitations highlight the need for further standardized, mechanistic, and long-term studies to better define the therapeutic potential of *Origanum vulgare* essential oil.

Abbreviations

NTZ
Nitazoxanide
NO
nitric oxide
MDA
malondialdehyde

Spp.
species
H&E
haematoxylin and eosin

Declarations

Funding:

We received no funds.

Author Contribution

Author ContributionsE.H.A.-H. conceived and designed the study, supervised the experimental work, and revised the manuscript critically.M.M.A.-F., M.M.S., and R.A.M.A.R. performed the animal experiments and parasitological investigations.M.M.S.A.N. contributed to data collection, statistical analysis, and interpretation of results.S.M.H. conducted the public health analysis and contributed to the discussion section.R.A.M.A.R. assisted in laboratory procedures and prepared the figures and tables.E.H.A.-H. and M.M.A.-F. wrote the initial draft of the manuscript.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request

Ethical approval

All experimental procedures were approved by the Institutional Animal Ethics Committee (**Approval No. 442/10/2023**) and conducted according to international guidelines for animal care.

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Figures

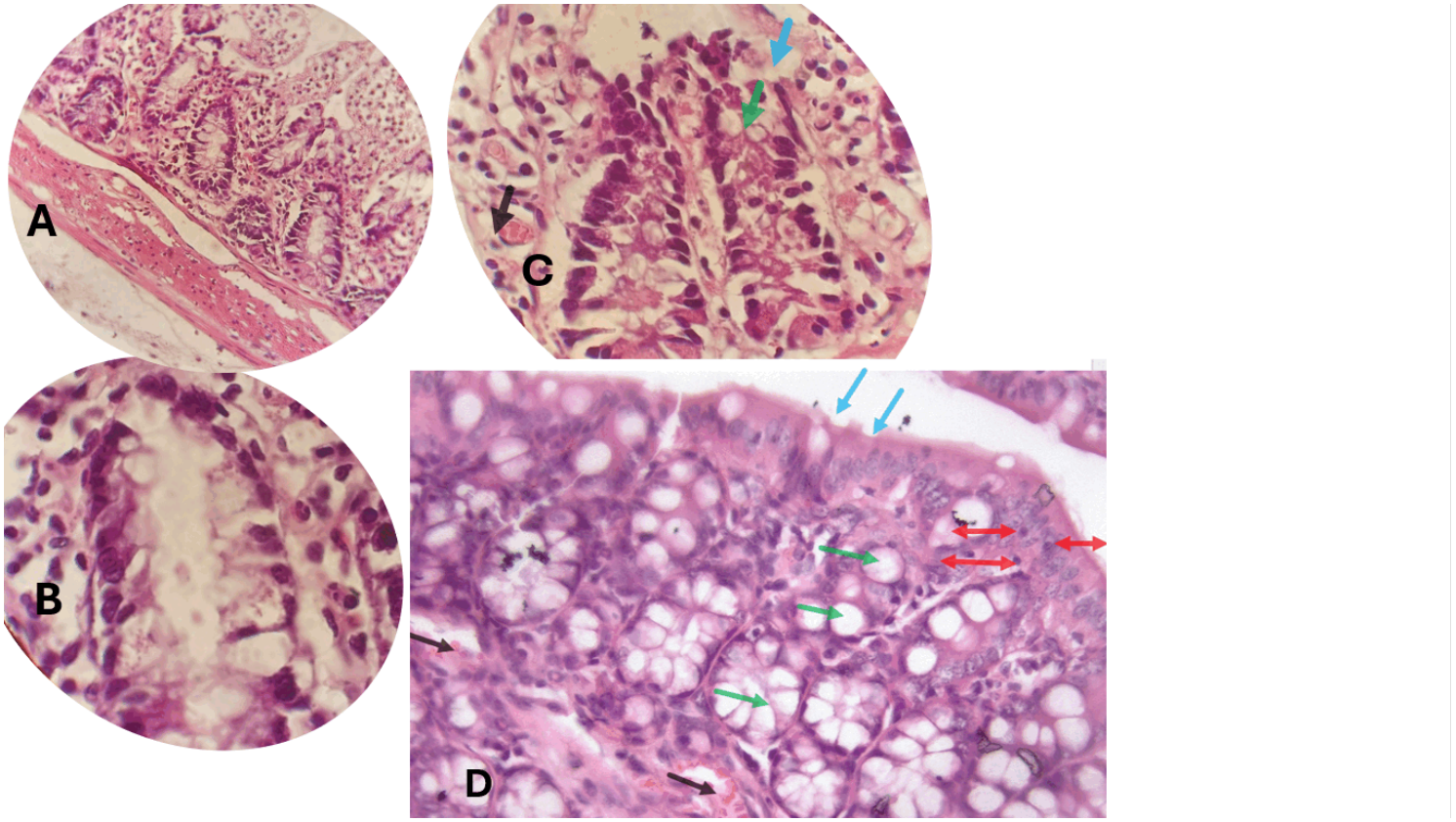

Figure 1

Histopathological changes in the intestinal mucosa of control and Blastocystis-infected mice (H&E stain).
(A–B) Non-infected control group showing normal intestinal architecture with intact villi, preserved epithelial lining, well-organized crypts of Lieberkühn, and absence of inflammatory infiltration or parasitic forms (H&E, ×40 and ×100). (C–D) Infected untreated group showing marked villous atrophy, epithelial desquamation, crypt distortion (blue arrows), dense inflammatory cell infiltration within the lamina propria, numerous vacuolated *Blastocystis*cysts and trophic forms (red arrows), and areas of mucosal necrosis and ulceration (black arrows) (H&E, ×40 and ×200).

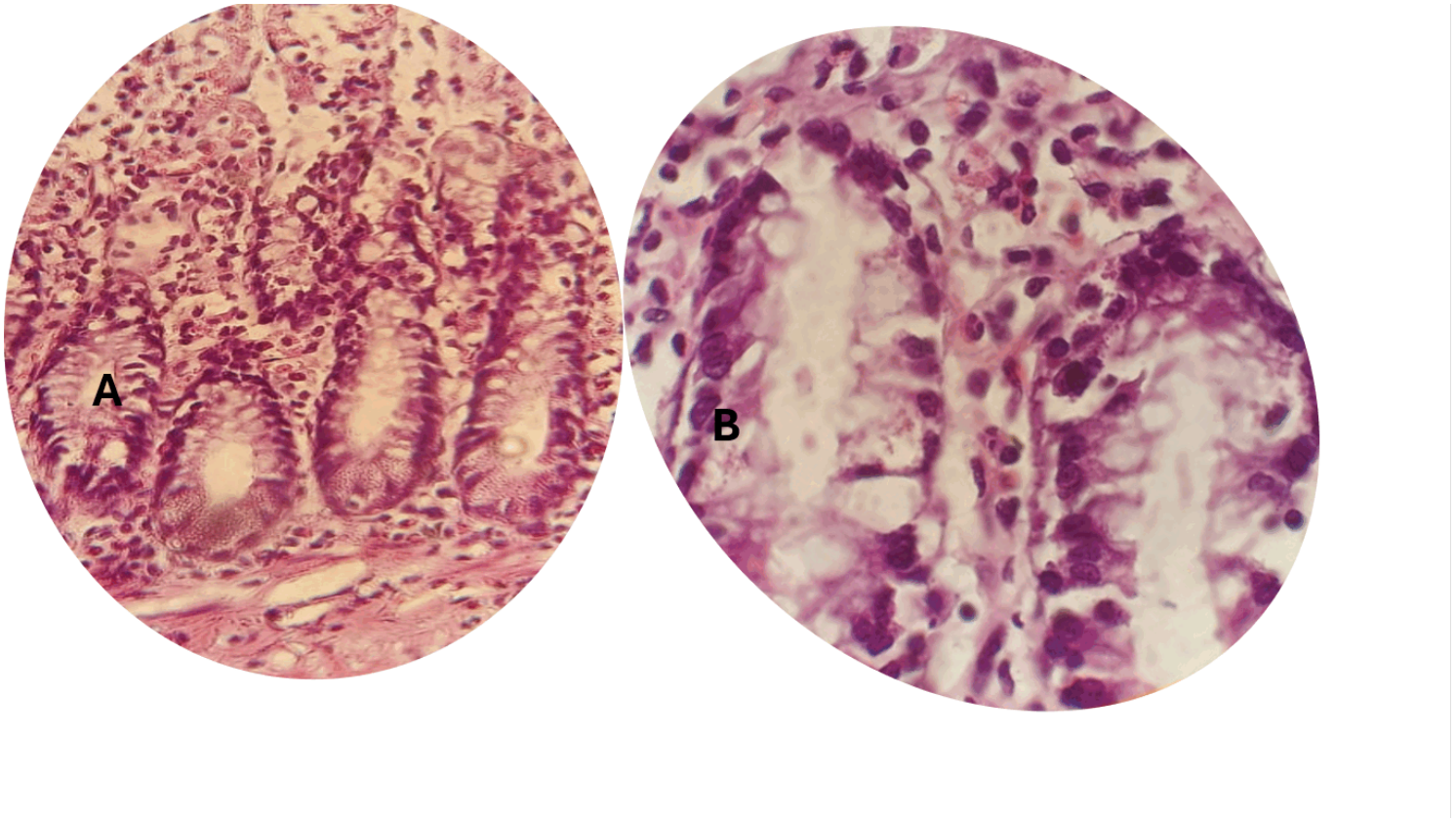

Figure 2

Histopathological changes in the intestinal mucosa following treatment with Origanum essential oil and nitazoxanide (H&E stain).
(A–B) Origanum essential oil-treated group showing restoration of intestinal architecture with intact elongated villi, preserved epithelial lining, well-organized crypts, and minimal inflammatory cell infiltration (H&E, ×40 and ×100). (C–D) Nitazoxanide-treated group showing partial restoration of villous architecture, reduced epithelial damage, decreased parasitic forms, and moderate inflammatory cell infiltration within the lamina propria (H&E, ×40 and ×100).