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## Original Article

# Therapeutic Effect of Chitosan Nanoparticles and Metronidazole in Treatment of Experimentally Giardiasis Infected Hamsters

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***Abstract***

***Background:*** The present study aimed to assess the therapeutic effect of chitosan nanoparticles and metronidazole against *Giardia lamblia* as well as evaluate the efficacy of loading metronidazole on chitosan nanoparticles.

***Methods:*** This study was carried out at medical Parasitology Department, Faculty of Medicine, Zagazig University and Theodor Bilharz Research institute (TBRI) from February 2019 to February 2020 on 45 hamsters. They were divided into 5 groups 9 hamsters each: Group A non-infected hamsters, Group B infected control group, Group C, D and E infected with *G. lamblia* and treated with Chitosan nanoparticles (CsNPs), metronidazole (MTZ) and metronidazole-loaded chitosan nanoparticles (MTZ-CsNPs) respectively.

***Results:*** The highest percentage of reduction in the *Giardia* cyst and trophozoite counts were in group that received MTZ-CsNPs (94.69%, 94.29%). Lower percentages of reduction were recorded for MTZ treated group (90.15%, 89.52%) and CsNPs treated group (63.64%, 75.24%). Histopathological examination showed marked healing of intestinal mucosa after treatment with MTZ-CsNPs.

***Conclusion:*** CsNPs showed a therapeutic effect against *Giardia* infection in hamsters. Loading of metronidazole on chitosan nanoparticles enhanced therapeutic effect of both CsNPs as well as metronidazole.

## Introduction

*Giardia lamblia* is considered the most common human enteropathogenic protozoan that affect about 200 million people annually, as well as a large number of animal hosts, inhabits the duodenum and jejunum, and transmits faeco-orally (1).

The clinical manifestations of giardiasis ranges from asymptomatic carriers to both acute and chronic diarrhea with or without other gastrointestinal manifestations (2).

*G. lamblia* is considered the most common protozoan pathogen of traveler´s diarrhea together with *Entamoeba histolytica* and *Cryptosporidium* species (3).

Up till now, Metronidazole is the treatments of choice of giardiasis given as a single dose. However, it is mutagenic and showed some carcinogenic activity, so, albendazole and nitazoxanide are alternative agents (4).

Persistence of manifestations after medical treatment of giardiasis are common, even without continued infection but indicates an increase in resistance to these synthetic pharmaceuticals (3). For these reasons, there is an increasing interest to search for new, safe and effective natural drugs (5).

Chitosan is a natural nontoxic biopolymer derived by the deacetylation of chitin; chitosan attracted great interest due to its wide range of antimicrobial and antifungal activity and reduced toxicity to mammalian cells (6). Nanobiotechnology have shown significant progress in parasitic infections treatment, this is based on the unrivaled properties of nanoparticles including chitosan nanoparticles shown outstanding inhibitory effects against parasitic infections, through inhibition and lysis of cell wall, protein synthesis inhibition, alternation of cell membrane, nucleic acid synthesis inhibition and antimetabolite activity (7). Chitosan Nano form agents could be used as a safe, effective alternative therapy for giardiasis (8).

This study was conducted to assess the therapeutic effect of chitosan nanoparticles, metronidazole and metronidazole-loaded chitosan nanoparticles against *G. lamblia* in experimentally infected hamsters.

## Materials and Methods

### *Experimental animal*

This study was carried out at medical Parasitology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt and Theodor Bilharz Research Institute, Giza, Egypt from Feb 2019 to Feb 2020 on 45 healthy laboratory bred male Syrian hamsters (*Mesocricetus auratus*), with a weight range of 100-110 gr.

Animals were kept on standard diet containing 24% protein, 4% fat and about 4-5% fiber, water ad-libidum and under a temperature of 240C and the study was approved by the Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC).

### *Ethical consideration*

Hamsters used in this study were maintained according to the research protocols following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals and as approved by ethics committee of the Faculty of Medicine, Zagazig University.

### *Giardia cyst collection and infection induction*

Fresh stool samples containing at least 3-5 *G. lamblia* cysts per high power field and free from other parasites, were obtained from patients attending the outpatient clinic of Pediatric Department of Zagazig University Hospital.

Stool samples were processed to obtain the infecting dose 10,000 cysts/ ml. Each hamster except those of group A, was orally infected with 1ml of *G. lamblia* cyst suspension containing 10,000 cysts, using esophageal tube (9).

### Experimental design

Hamsters were divided into five groups, 9 hamsters each:

- Group A (CN): normal control.
- Group B (CI): infected without receiving treatment.
- Group C (CsNPs): infected and treated with Chitosan nanoparticles suspension in a dose of (50µg/hamster/day) for 7 consecutive days (6).
- Group D (MTZ): infected and treated with full dose of metronidazole (Snaofi-Aventis Pharmaceutical, Cairo, Egypt), suspension form of 125 mg/ml and given orally in a dose of 7ml/ hamster/day) for 7 consecutive days (9,10).
- Group E (MTZ-CsNPs): infected and treated with metronidazole-loaded chitosan nanoparticles, at half dose (3.5 mg/ hamster/day) for 7 consecutive days (9).

Three weeks after infection, groups (C, D and E) were given the corresponding drugs.

### CsNPs Preparation

CsNPs were prepared by using ionic gelation technique. Chitosan (Cs) was dissolved in 1% acetic acid solution with sonication, until the solution appeared transparent. Sodium hydroxide was added to raise PH to become 4.6-4.8. Tripolyphosphate (TPP) at concentration 1 mg/ml was prepared by dissolving of TPP in distilled water. The acidic chitosan solution (0.3%) was added to an equal volume of TPP solution, with the help of magnetic stirring at room temperature, then the formation of CsNPs were achieved spontaneously. Nanoparticles were purified by centrifugation at 10.000 rpm for 30 min at 4 °C. Followed by removal of supernatant. Furthermore, CsNPs were rinsed via distilled water for removal of any sodium hydroxide. These nanoparticles were stored at 4-8 °C till used (11).

### Characterization

The size measurements of nanoparticles and zeta potential were performed on freshly prepared samples using photon correlation spectroscopy and laser Doppler with an average size of 20 ± 5 nm and zeta potential ranged from +42.8 mV.

### Copro-parasitological examination

To insure hamsters' infection, hamsters' fecal specimens were collected and a copro-parasitological examination was done using direct smear examination (unstained and Lugol's iodine stained smear) to detect *G. lamblia* cyst in their stool three weeks post-infection (10).

Two weeks after drug administration, Stool samples were collected for *Giardia* cyst counting using (MIF) (10).

### Animal scarification

Hamsters were sacrificed two weeks after drug administration. Euthanasia was carried out by intraperitoneal anesthesia using thiopental sodium (10). Intestinal contents of each sacrificed hamster were subjected to direct parasitological examination to count *G. lamblia* trophozoites number in five successive fields per animal (9).

### Histopathological assessment

Three segments one cm length each were cut at a distance of 5, 15 and 25 cm from the gastroduodenal junction, stained with hematoxylin-eosin (H&E), examined to assess the histopathological changes that occurred during infection and detect mucosal healing after drug administration (12).

### Statistical analysis

Data collected, were tabulated and analyzed using SPSS ver. 20.0 (Chicago, IL, USA), quantitative data was represented by mean ± SD. ANOVA test was used to test statistical significance differences.

Reduction percentages ( R**%)** (13)

$$R\% = \frac{C-T}{C}$$

Where C means: Mean of control group results. T means: Mean of treated group results.

## Results

### *Giardia cyst count*

Quantitative assessment of the intensity of infection of *Giardia* cyst per gram stool showed a statistically significant difference among the different groups ($P$<0.001) (Table 1).

**Table 1:** *Giardia* cyst count per gram stool and percentage reduction among the studied groups

| ***Variable*** | ***Giardia cyst count*** | | |
|---|---|---|---|
| | Mean ± SD | Range | Percentage of reduction |
| Group B ( CI) | 13200 ± 1200 | 11400-15000 | |
| Group C ( CsNPs) | 4800 ± 1073.3 | 3600-6600 | 63.64 |
| Group D ( MTZ) | 1300 ± 420.3 | 600-2400 | 90.15 |
| Group E ( MTZ-CsNPs) | 700 ± 244.9 | 600-1200 | 94.69 |
| F | 253.07 | | |
| *P* | < 0.001** | | |

### *Giardia trophozoite count*

Therapeutic effect of different drug regimen on the count of *Giardia* trophozoites in small intestinal content showed statistically significant difference among groups ($P$<0.001) (Table 2).

**Table 2:** Trophozoite count per gram stool and percentage reduction among the studied groups

| ***Variable*** | ***Giardia trophozoites count*** | | |
|---|---|---|---|
| | Mean ± SD | Range | Percentage of reduction |
| Group B ( CI ) | 262.5±11.1 | 250-282.5 | |
| Group C ( CsNPs) | 65±7.74 | 52.5-72.5 | 75.24 |
| Group D ( MTZ) | 27.5±5.2 | 22.5-35 | 89.52 |
| Group E ( MTZ-CsNPs) | 15.0±4.47 | 10-22.5 | 94.29 |
| F | 1372.4 | | |
| *P* | < 0.001** | | |

### *Histopathological assessment*

H&E sections of small intestine, obtained from hamsters in group A, revealed intact villi with average length and width and well-defined brush border (Fig. 1a,b).

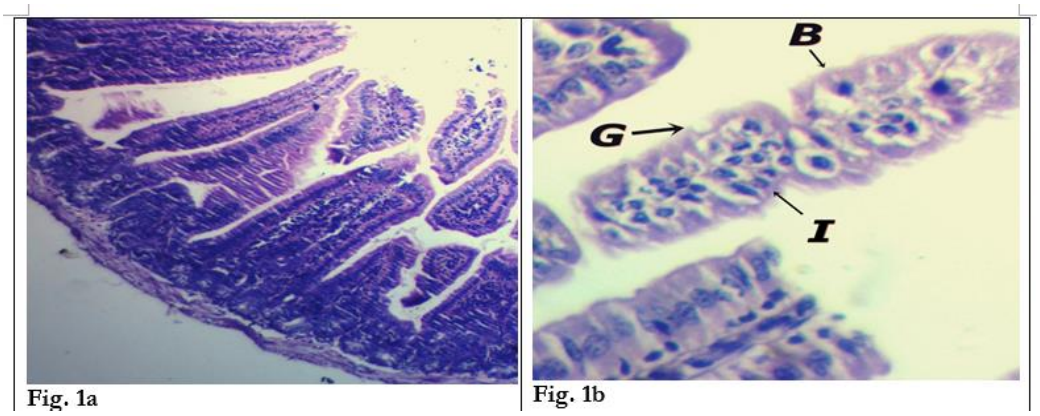


**Fig. 1a:** Intestinal cut section H&E stained showing normal villous architecture in group A (100x); **b:** Intestinal cut section H&E stained showing intact villous brush border (arrow B), average number of goblet cells (arrow G) and inflammatory cells (arrow I) in lamina propria in group A (400x).

Comparing to group A, hamsters of group B showed villous shortening and degeneration, decreased ratio of villous height to crypt length and diffuse loss of brush border and mucosal ulceration (Fig. 2a,b,c).

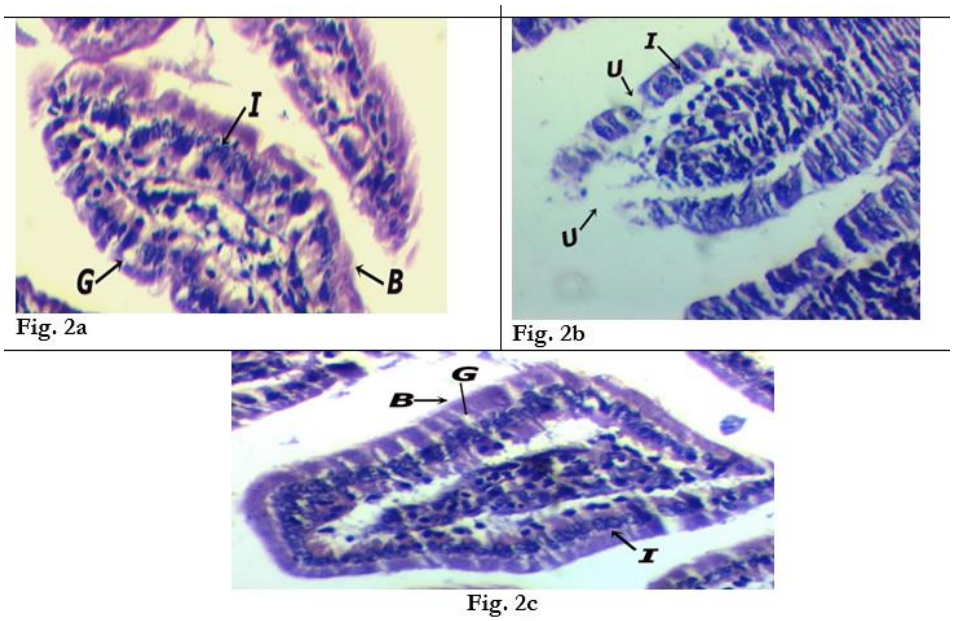


**Fig. 2a:** Intestinal cut section H&E stained showing shortened broad villi in group B (100x); **b:** Intestinal cut section H&E stained showing marked inflammatory cells infiltration (arrow I) in lamina propria in group B (400x). **c:** Intestinal cut section H&E stained showing ulceration of intestinal epithelium (arrow U) & inflammatory reaction in group B (400x).

Histopathological improvement appeared in small intestinal sections from hamsters of groups C, D and E, compared to group B. Groups C and D showed partial healing with mild villous shortening and not marked decrease in the ratio of villous height to crypt length (Fig. 3a,b).

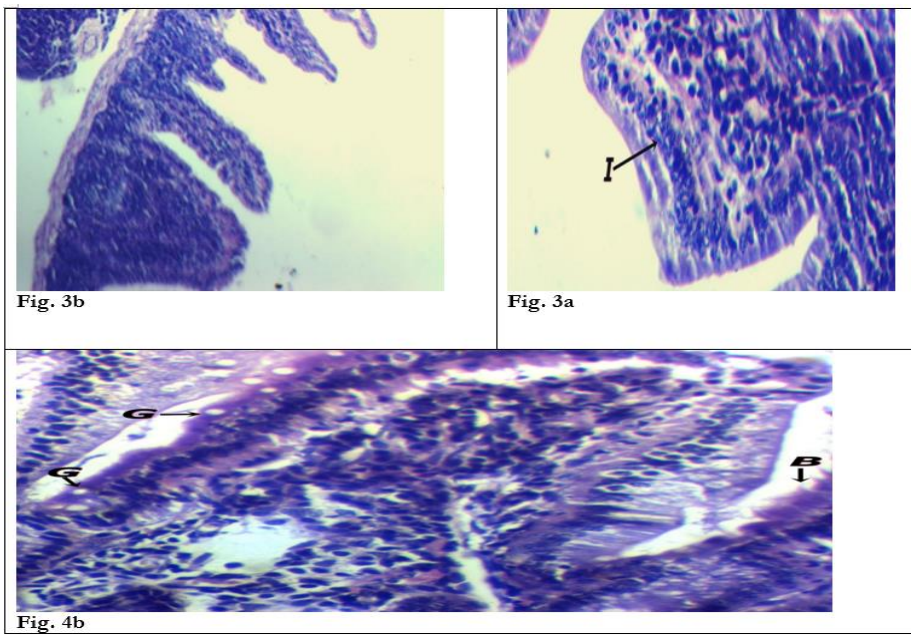


**Fig. 3a:** Intestinal cut section H&E stained showing partial healing of intestinal villi (arrow B), mild inflammatory cells infiltration (arrow I) and mild goblet cells depletion (arrow G) in group C (400x); **b:** Intestinal cut section H&E stained showing partial villous healing (arrow B), mild goblet cell depletion (arrow G) & mild inflammatory reaction (arrow I) in group D (400x).

Group E showed marked healing intestinal mucosa, absence of mucosal ulceration, preservation of brush border, average villous architecture and preserved ratio of villous height to crypt length (Fig. 4a,b).

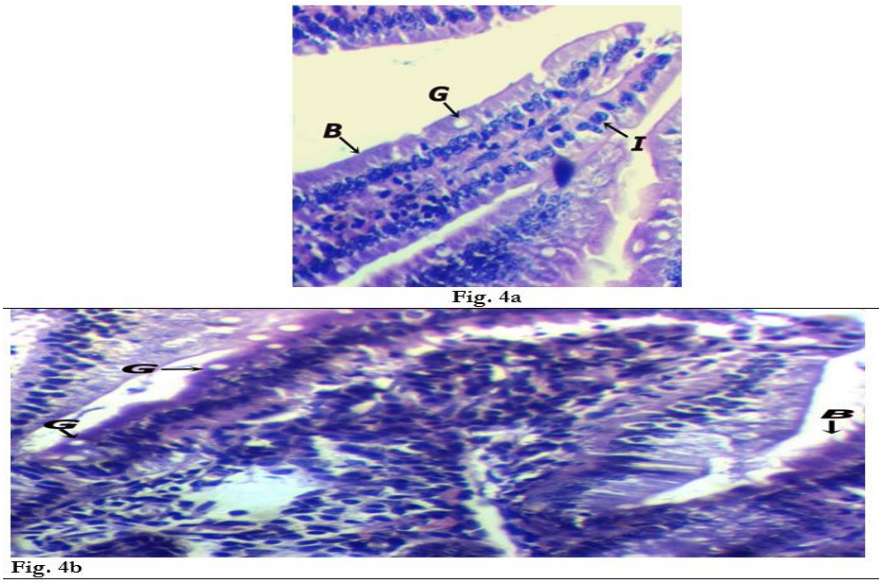


**Fig. 4a,b:** Intestinal cut section H&E stained showing marked improvement of intestinal brush border (arrow B) with patchy inflammation (arrow I) and minimal depletion of goblet cells (arrow G) in group E (400x)

Histopathological examination of the small intestinal sections revealed a statistically significant difference ($P$<0.001) in goblet cell count/H.P.F in small intestinal sections of hamsters among the studied groups (Table 3).

**Table 3:** Percentage of goblet cells among the studied groups

| *Variable* | *Goblet cell count/ H.P.F* | | *Percentage of goblet cells* |
|---|---|---|---|
| | Mean ± SD | Range | |
| Group A (CN) | 10.46±1.57 | 8.25-12.5 | |
| Group B ( CI) | 3.08±1.24 | 2-5.25 | 29.45 |
| Group C ( CsNPs) | 6.25±1.09 | 4.75-7.75 | 59.75 |
| Group D ( MTZ) | 4.75±1.14 | 3.75-6.75 | 45.41 |
| Group E ( MTZ-CsNPs) | 8.83±1.63 | 6.25-10.75 | 84.42 |
| F | 29.33 | | |
| *P* | < 0.001** | | |

There was a statistically significant difference ($P$<0.001) in the inflammatory cell counts/H.P.F in small intestinal sections of hamsters (Table 4).

**Table 4:** Inflammatory cell count and percentage of reduction in treated groups compared to control infected group

| *Variable* | *Inflammatory cell count/ H.P.F* | | *Percentage of reduction* |
|---|---|---|---|
| | Mean ± SD | Range | |
| Group A (CN) | 30.25±3.17 | 25.5-34.25 | - |
| Group B ( CI) | 88.21±4.28 | 81.25-93.75 | - |
| Group C ( CsNPs) | 45.46±3.02 | 42-49.75 | 49.04 |
| Group D ( MTZ) | 51.71±1.36 | 49.75-53.25 | 41.38 |
| Group E ( MTZ-CsNPs) | 37.29±2.37 | 34-40.5 | 57.73 |
| F | 338.94 | | |
| *P* | < 0.001** | | |

## Discussion

*G. lamblia*, one of the most common protozoa, which infects broad range of population worldwide (14). Age appears to be an important risk factor for susceptibility to giardiasis being more reported in infants and young children (15). Its presentations range from asymptomatic cyst passer, to both acute and chronic diarrhea with or without other gastro-

intestinal manifestations (16). *G. lamblia* resistance to routine therapy is growing day by day. Therefore, great efforts are performed for development of new, alternative agents against giardiasis (17).

Chitosan act as antimicrobial drug through interaction with the cell surface anionic molecules (18). Moreover, it can open tight junctions, because of its muco-adhesion property and have a rapidly modifiable pH responsive solubility (19). Furthermore, the use of nanoparticles improves the bioavailability and decreases the side effects of therapy (20).

In this study, CsNPs showed (63.64%) reduction percentage of *Giardia* cyst count in stool. CsNPs have a higher antimicrobial activity than chitosan (8). However, chitosan nanoparticles could only hinder the growth of microorganisms rather than killing them (21).

MTZ treated hamsters showed (90.15%) reduction percentage of *Giardia* cysts in stool. The reduction in number of *Giardia* cysts was (93.7%) comparing to the infected control group (9).

Metronidazole had more efficacy on giardiasis in different studies among human beings (22). Besides, metronidazole can act against *G. lamblia* infection through DNA damage (5). It may also inhibit oxygen consumption by the parasite (23).

On the contrary, a study was performed to assess metronidazole susceptibility of 11 clinical isolates of *G. lamblia,* he found that all isolates were resistant to metronidazole (24).

The possible causes of *Giardia* treatment failure may be: reinfection, inadequate dose, impaired immunity, drug resistance, sequestration in the biliary system and could occur due to formation of antioxidant network to protect oxygen-sensitive metallo-enzymes (25).

The (MTZ-CsNPs) treated group showed *Giardia* cyst count (700±244.9) with the highest reduction percentage (94.69%). Nanoparticles achieved successful control of metronidazole release over 24 hours (26). CsNPs produced an improvement in absorption and bioavailability of albendazole in treatment of *Echinococcus multilocularis* (27). Moreover, loading of bee venom on CsNPs enhanced its efficacy against amoebiasis (28). In addition, MTZ-CsNPs high efficacy could be illustrated as chitosan nanoparticles can lead drug for better delivery (29). Furthermore, it can produce controlled drug release, which enhances stability, solubility, efficacy and reduction of drug toxicity (30).

Group E (MTZ-CsNPs) showed the highest reduction percentage of trophozoite count (94.29%), however in (MTZ) treated group the percentage of reduction was (89.52%).

The percentage reduction in *Giardia* trophozoite count in chitosan nanoparticles treated group was (75.25%). Trophozoite reduction (79.6%) was reported and suggested that CsNPs were effective against *Giardia* trophozoites due to the mucoadhesive properties that prolonged the time of action and reduced elimination in GIT (6).

Different concentrations of nano-chitosan showed significant activity against *G. lamblia* cysts and trophozoites (31).the percentage reduction in *Giardia* trophozoite count of metronidazole treated group was (89.52%). Ammar *et al.* (2014), also achieved reduction of (87.06%) in trophozoite count (10).

On the contrary, metronidazole failure was reported, and pyruvate ferredoxin oxidoreductase (PFOR) and its cofactor ferredoxin had been essential for *Giardia* resistance against nitroimidazoles (32).

In our study, group E (MTZ-CsNPs) showed the highest percentage of reduction in trophozoite count /gm stool (94.29%).

Loading of drugs on chitosan, nanoparticles increased the loaded drug efficacy against the target organism (31). Moreover, the role of CsNPs was declared in increasing the efficacy of spiramycin loaded on it, against acute toxoplasma infection (33).

Histopathological changes were improved in groups C, D and E in comparison to infected control group B. Besides, the use of metroni-

dazole-loaded chitosan nanoparticles in group E, showed the best evidence of cure compared to metronidazole or CsNPs alone.

Histopathological sections of the control infected group, revealed goblet cells depletion (3.08±1.24), villous shortening and degeneration and diffuse loss of brush border. Besides, mucosal ulceration and inflammatory cellular infiltration (88.21±4.28) of lamina propria. Patients with giardiasis also showed reduction of mucosal surface areas in comparison with controls (34). Besides, other studies reported similar changes in intestinal sections obtained from *Giardia* infected control hamsters (9, 10, 2).

However, upper gastrointestinal endoscopic biopsies were normal in cases that revealed *Giardia* trophozoites in another study. This indicated the low virulence of some *Giardia* species and the role of host immunity in reduction of parasitic pathological mechanisms (35).

The (CsNPs) treated group revealed partial healing of mucosa with mild villous shortening and mild improvement of goblet cell count (6.25±1.09) In addition, reduction of inflammatory cellular infiltration (49.04%), comparing to infected control group. CsNPs could increase intestinal villous height and decrease crypt depth (36). Moreover, CsNPs showed significant effect against local GIT diseases and intestinal disinfection (37).

Chitosan nanoparticles improved GIT mucosal epithelial cells necrosis caused by toxic agents (38). CsNPs had anti-inflammatory effects (39). In addition, chitosan nanoparticles produced cellular oxidative stress through increasing reactive oxygen compounds, associated with cytotoxicity to various cells including mucosal epithelium (40).

The small intestinal mucosa of metronidazole treated group showed partial healing with mild improvement in goblet cells count (4.75±1.14), mild shortening of villi and reduction of inflammatory cellular count. Other researchers reported partial healing of small intestinal after metronidazole administration in *G. lamblia* infected hamsters (9, 10).

Besides, metronidazole appeared to have anti-inflammatory, anti-oxidant and immunomodulatory effect especially in intestinal lumen (41). Moreover, MTZ has anti-oxidant effect and increase intestinal mucosal thickness in rats (42).

On the other hand, metronidazole produced cytotoxicity to gastrointestinal cells with mucosal injury and imbalance (43). Patients showed persistence of symptoms and microscopic duodenal inflammation, after metronidazole treated *Giardia* infection (44).

The combined (MTZ-CsNPs) treated group showed high degree of healing, absence of mucosal ulceration, preservation of brush border, average villous architecture, goblet cell count/H.P.F was (8.83±1.63) and (57.73%) reduction in inflammatory cell count.

(MTZ-CsNPs) had non-cytotoxic behavior in cell culture and produced non-significant changes in cell morphology (45). Chitosan nanocomposites showed better properties and increasing solubility of drugs forming stable complexes for safe delivery to the specific site (46).

## Conclusion

Chitosan nanoparticles showed a therapeutic effect against *Giardia* infection in hamsters with a significant reduction in numbers of Giardia cyst in stool and trophozoite in their intestinal contents. Loading of metronidazole on chitosan nanoparticles enhanced the therapeutic effect of both CsNPs as well as metronidazole. Moreover, histopathological findings revealed the role of MTZ-CsNPs in healing of intestinal pathological changes induced by Giardia infection.

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## Conflict of interest

Authors have nothing to disclose.

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