Original Article

Therapeutic efficacy of *Moringa oleifera* loaded chitosan nanoparticles in *Giardia lamblia* experimentally infected mice

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ABSTRACT

Background: Giardiasis treatment is a challenge as traditional medical treatment exhibits drug resistance with multiple side effects. Hence ongoing trials for exploring anti-giardiasis alternatives are being achieved utilizing chitosan nanoparticles (CsNPs) and medicinal plants.
Objective: To investigate the therapeutic efficacy of *Moringa oleifera* leaves extract (MOE) and *M. oleifera* extract-loaded CsNPs (MOE-Cs NPS) against experimental giardiasis compared to Metronidazole (MTZ).
Material and Methods: Eighty male Swiss albino mice were divided into two equal groups: group A (non-infected), and group B (infected). Each group was divided into four subgroups. While mice of subgroups A1, and B1 represent negative and positive giardiasis controls; those of A2, A3, A4, and B2, B3, B4 were treated with MOE, MOE-CsNPs, MTZ, respectively. Treatments began on the 3rd day post infection (dpi) and lasted for seven days, and scarification was done three days after the end of treatments. Evaluation parameters included stool cyst load, histopathological intestinal changes, and cyst ultrastructural changes by scanning electron microscopy (SEM). Hepatic and renal toxicity were biochemically assessed.
Results: The marked improvement in cyst shedding was recorded in subgroup B3. *Giardia* cyst reduction rate was (86.01%) with significant efficacy ($P \leq 0.001$) compared to B2 and B4 subgroups (76.89% and 79.76%) respectively. Results of SEM demonstrated that the MOE-CsNPs-treated group had the most noticeable cyst disfigurement alterations, with no harmful effects on hepato-renal functions.
Conclusion: Combined MOE and CsNPs therapy is a novel potential natural product for reducing the number of *Giardia* cysts shedding in stool and improving the pathological changes in treating giardiasis.
Keywords: chitosan nanoparticles, *G. lamblia*, mice, *Moringa*; treatment.

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INTRODUCTION

Giardiasis is one of the most frequent intestinal protozoal illnesses in humans caused by *G. lamblia*. It is a flagellated protozoan that infects both human and animals' small intestines[1]. It infects approximately 280 million individuals worldwide every year. In developing and developed countries, prevalence rates were estimated to be 20-30% and 2-5%, respectively[2]. Since 2004, giardiasis has been a part of the WHO's 'Neglected Diseases Initiative[3]. It spreads by fecal-oral ingestion of cysts or indirectly through the consumption of contaminated food or water[4].

Human giardiasis has a wide range of clinical symptoms, from asymptomatic infection to acute or chronic diarrhea, malabsorption, weight loss, and retarded growth, particularly in young and immunocompromised individuals[5]. In giardiasis, trophozoites are frequently present on the villus surface or between the intestinal villi causing mucosal inflammation, intraepithelial inflammatory cell infiltration, and villus architectural alterations[6]. An experimental animal model of giardiasis showed increased colon mucus production with enterocyte apoptosis altering the intestinal mucosal barrier[4].

Because there is no vaccine for giardiasis, medication therapy is the only way to manage the condition. Metronidazole and Nitroimidazole, both of them are administered as a single dose, are the drugs of choice for giardiasis. However, they have many side effects such as nausea, metallic taste, hepatic injury, and carcinogenic possibility, as well as continuous growing resistance to these drugs[7]. Several medications, including albendazole, nitazoxanide, and paromomycin, have been used to treat giardiasis; however, these drugs can't be administered safely in infants, pregnant, or lactating women[8]. Moreover, the US 'Food and Drug Administration' didn't certify MTZ due to its severe side effects, including carcinogenic risks in mice and rats[9]. As a result, finding more efficient, natural, and safe substitute medications is a top focus to overcome these issues.

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Medicinal plants such as *M. oleifera* have great medical value. Vitamins, flavonoids, essential amino acids, polyphenols, and phenolic acids are among the beneficial substances found in *M. oleifera*[10]. *Moringa* leaves extract showed pharmacological characteristics such as anti-oxidant, immune-modulatory, anti-inflammatory, anti-diarrheal[11], anti-helminthic and anti-protozoal effects[12]. Moringa also demonstrated ovicidal action on *Fasciola* eggs[13].

Nanoparticles are now employed in many areas of nanomedicine, including diagnostics, bioimaging, drug delivery, and vaccine development. Nanotechnology is starting to produce novel alternative medications for parasitic infections[14]. Biodegradable nanoparticles are among the most significant categories that have lately gained popularity for use in this sector, including nano-cellulose, nano-chitosan, and nano-poly-lactic-co-glycolic acid (PLGA). Chitosan is a naturally occurring polymer derived mostly from crustacean shells by deacetylation of chitin recovered from the shells[15]. It has several biological pharmacological actions, including bacteriostatic, antioxidant, immunomodulatory, and anticancer properties[16]. In fact, CsNPs are prepared using a variety of methods including the emulsion approach, ionic gelation method, reverse micellar method, and self-assembling method[17]. Action of CsNPs showed remarkable inhibitory effects against parasitic infections *via* cell wall inhibition and lysis, inhibition of protein synthesis, cell membrane modifications, nucleic acid synthesis inhibition, and antimetabolite action[18]. Chitosan nano-form agents have the potential to be a safe and effective alternative therapy for giardiasis[19]; in addition to possessing effective antifungal, antibacterial, and antiprotozoal agents[20]. These CsNPs have also been shown to be anti-*Cryptosporidium*[20], anti-*Plasmodium*[21], anti-*Leishmania*[22], anti-*Trypanosoma*[23], and anti-*T. gondii*[24]. Furthermore, CsNPs suppressed protozoan development *in vitro* of *P. falciparum, G. lamblia,* and *T. vaginalis*[25]. The present study was designed to investigate the therapeutic effects of MOE and MOE-CsNPs against experimental giardiasis compared to MTZ in mice infected with *G. lamblia*.

MATERIAL AND METHODS

This experimental study was conducted at Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt during the period from May 2023 to October 2023.

Study design: Mice were divided into group A (non-infected treated control group), and group B (infected treated experimental group). Each group was divided into 4 subgroups; non treated (1), and treated (2,3,4) with MOE, MOE-CsNPs and MTZ, respectively. Treatments began on the 3rd dpi and lasted for 7 d. All animals were sacrificed 3 d after the end of treatments for parasitological, biochemical, and histopathological assessments as well as SEM evaluation.

Preparation of *G. lamblia* cyst inoculum used for infection: Cysts were isolated from the stools of diarrheic patients visiting Alexandria University Hospital's Outpatient Clinic. The stools were collected in sterile, clean stool cups. Samples that have at least 3-5 *G. lamblia* cysts in a wet mount smear, and that were free of other parasites, were utilized for inoculum preparation. *Giardia* cysts were concentrated three times using the sedimentation technique with normal saline and centrifugation for 3 min at 2000 rpm. The cyst count was calculated in one milliliter of stool sample. The needed infectious dosage of 10,000 cysts/ml was determined by averaging three counts[26].

Preparation of MOE: The MO leaves were purchased from local market in Egypt. Leaves were dried, grounded into powder, sieved, and dissolved in the least possible quantity of methanol, and an emulsifying agent (1% Tween 80).

Preparation of MOE-CsNPs: Chitosan NPs were commercially obtained from 'Nanotech for Photo Electronics Company', Egypt (http://www.nanotecheg.com/). It was dissolved in 1% acetic acid to prepare a solution with a concentration of 1 mg/ml. An emulsion was then made by dropwise addition of a methanol-containing extract to Cs solution using a magnetic stirrer, followed by 2 min homogenization of high-speed (1000 rpm). To prepare the NPs, the tripolyphosphate (TTP) aqueous solution (1 mg/ml) was added dropwise to the previously created emulsion while stirring gently (300 rpm for 30 min). For optimal ionic gelation, the MOE-CsNPs suspension was kept at room temperature overnight. To estimate loading efficiency, a portion of the NPs was separated by centrifugation at 20000 rpm for 15 min[27].

Physiochemical characterization of CsNPs and MOE: Morphological properties of CsNPs were studied by transmission electron microscope (TEM) (JEOL-JSM, 2100 PLUS, Japan) at the Faculty of Science, Alexandria University (Fig. 1). Fourier Transform Infrared Spectroscopy (FTIR) was measured by zeta sizer (Fig. 2), and Zeta Potential at the Faculty of Pharmacy, Alexandria University (Fig. 3). Efficiency of MOE-CsNPs was measured using a Spectrophotometer at the Institute of Graduate Studies and Research, Alexandria University[27].

Experimental animals: The present study included 80 male laboratory-bred Swiss albino mice, aged 3–4 w old, weighing 15–20 gm each. The mice were maintained in well-ventilated laboratory cages and were kept in the animal house of the Institute of Graduate Studies and Research, Alexandria University. The mice were divided into two main groups with eight subgroups (each of 10 mice) as shown in the following table. Before starting

the experiment, stool samples were collected for three successive days to ensure that the mice were parasite-free. Stool smears were swabbed immediately after applying D'Antoni's iodine[2].

Group A (non-infected subgroups)	Group B (infected subgroups)
A1: Non-treated.	**B1:** Non-treated.
A2: Treated with MOE.	**B2:** Treated with MOE.
A3: Treated with MOE-CsNPs.	**B3:** Treated with MOE-CsNPs.
A4: Treated with MTZ.	**B4:** Treated with MTZ.

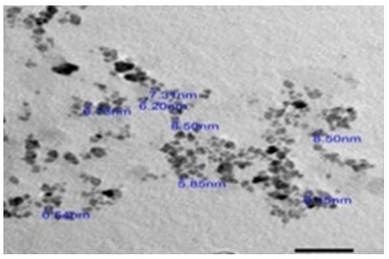

Fig. 1. The TEM image of MOE-loaded CsNPs their shape and size. The scale bar indicates 50 nm.

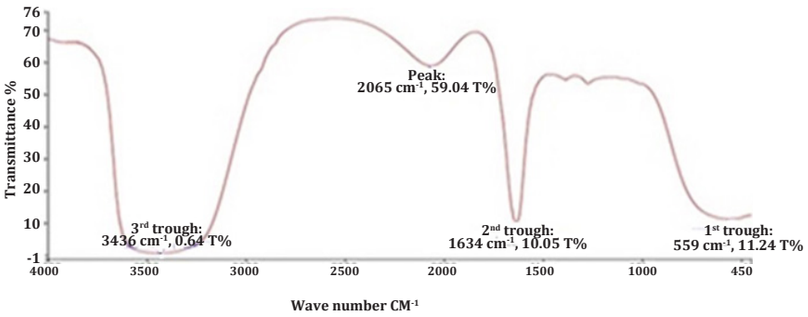

Fig. 2. Characterization of MOE-loaded chitosan NPs by using FTIR.

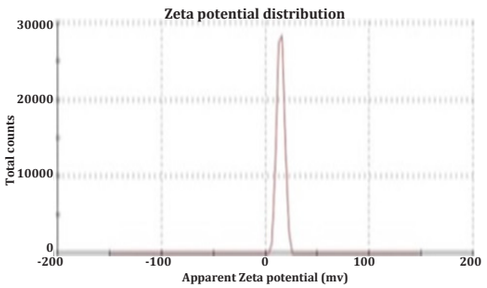

Fig. 3. Characterization of MOE-loaded CsNPs by using a Zeta potential.

Mice infection and sacrifice: All mice in group B were infected by intra-esophageal inoculation of one ml of *G. lamblia* cyst suspension containing 10,000 cysts/mouse[26]. Treatments began on the third dpi and lasted for seven days. All animals were sacrificed 3 days after the end of treatments[28].

Drug regimens: Treatments began on the third dpi and lasted for seven days. The MTZ suspension (Sanofi-Aventis Pharmaceutical, Cairo, Egypt, 125 mg/ml) was administered orally in a dose of 7 mg/mouse/day[29]; MOE extract was administered alone in a dose of 300 mg/kg/day, and MOE-CsNP was administered in a dose of 300 mg/kg/day[30].

Parasitological examination: To confirm mice infection, stool examination was performed on three successive days one week after infection using unstained wet mount and stained smears with Lugol's iodine, and trichrome stain[31] (Fig. 4).

Cyst count: Two days after the last dose of treatment, ~one gram stool was taken before sacrifice from each mouse. The mean number of cysts/HPF was calculated in the iodine-stained smears using a hemocytometer[32].

Histopathological examination: Specimens of 2–5 cm from the proximal part of the duodenum and jejunum of each mouse were fixed in 10% formalin, processed to paraffin blocks, and sectioned slides (4 µm) were stained using hematoxylin and eosin (H&E). Sections were examined microscopically using both low and high power to determine the histopathological changes and to detect the degree of mucosal healing due to drug treatment[33].

Scanning electron microscopic studies (SEM): Specimens from the duodenum and proximal jejunum of infected treated mice were processed immediately[34]. For 2 h, the specimens were kept in cold glutaraldehyde, and then fixed in 2% osmium tetroxide for 2 h before dehydration in escalating degrees of ethanol and drying with liquid CO_2. The samples were mounted on stainless steel holders and sputter-coated with a thin

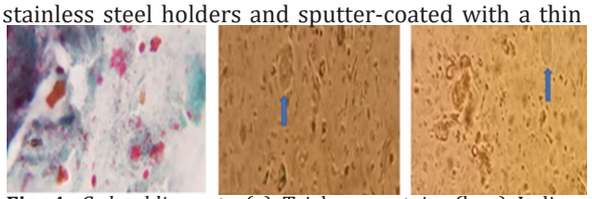

Fig. 4. *G. lamblia* cysts (a) Trichrome stain, (b, c) Iodine-stained smear of mice stools (X100).

layer of gold, and the shape and surface topography of collected cysts and small intestine villi were determined using SEM JEOL (JSM, 6360LA) at the Faculty of Science, Alexandria University, Egypt.

Biochemical study: Blood (~3 ml) was collected in heparinized tubes from sacrificed mice from all groups and were kindly tested for blood urea nitrogen, creatinine, glutamic oxaloacetic transaminase (SGOT), and glutamic pyruvate transaminase (SGPT) using a biochemical blood analyzer.

Statistical analysis: Collected data were tabulated and analyzed by SPSS version 20.0 on IBM compatible computer. Quantitative values of the measured parameters were expressed as mean ± standard deviation (SD). One way ANOVA test, pairwise comparison between each 2 groups was performed using Post Hoc Test (Tukey). Results were considered significant at $P \leq 0.05$.

Ethical considerations: A standard procedure was established in accordance with the World Health Organization's 'Good Laboratory Practice' guidelines. Animal experiments were conducted following the animal care committee of Menoufia University's Faculty of Medicine's ethical animal rules and regulations, as well as the globally recognized standards for experimental animal usage and care. According to academic research and safe disposal guidelines, all of the slaughtered animals' remains were destroyed using safe disposal techniques. The current study was approved by the Research and Ethics Committee of Menoufia University's Faculty of Medicine (code number: 5/2023 PARA6). The experiment complied with the ARRIVE guidelines and was carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

RESULTS

Cyst count: The effectiveness of MOE, either alone or loaded on CsNPs, was compared to MTZ in this study. Stool examination of *G. lamblia*-treated infected mice compared to infected non-treated mice demonstrated a reduction in the number of *G. lamblia* cysts in treated groups with varying percentages (Table 1). The significant highest cyst reduction rate of 86.01% was recorded for the MOE-CNsNPs treated group, followed by 79.76% for MTZ group and 76.89% for MOE group.

Table 1. Cyst count/gm stool and % of reduction among the studied groups.

Subgroup	Cyst count (Mean±SD)	Reduction rate (%)	Statistical analysis
B1	14400^{b}±400	-	
B2	3328.7^{a} ± 323.3	76.89	F=113.120
B3	2011.9^{a}±453.2	86.01	P<0.001*
B4	2914.3^{a}±862.6	79.76	

*: Significant ($P \leq 0.05$).

Histopathological results: Histopathological examination of sections from control negative group A1 (non-infected non-treated mice) showed normal structure with average length and width of villi without any pathological changes in the mucosa or the lamina propria (Fig. 5A). Sections of all mice (100%) of positive control group B1 (infected non treated) showed presence of cysts, strong inflammation, strong villus destruction and high grade of dysplasia (Fig. 5 B, C). Among the treated groups, MOE-treated group (B2) showed absence of cysts or dysplasia, mild inflammation, and mild villous destruction (100%) (Fig. 5D). All mice (100%) of MOE-CsNPs treated group (B3) showed normal villous architecture, absence of cysts, inflammation, or dysplasia with statistically significant difference in favor of MOE-CsNPs treated group (P<0.0001), (Fig. 5E). The MTZ treated group (B4) showed mild presence of cysts, moderate degree of inflammation, moderate villous destruction, and low grade of dysplasia (Fig. 5F).

Results of SEM: The ultrastructure of *G. lamblia* cyst embedded in the small intestinal mucosa of the infected non-treated group (B1), was damaged with swelling, shortness, fusion, and disruption of the microvillus structure (Fig. 6 a, b). The brush border was either distorted or lost leaving an eroded surface area.

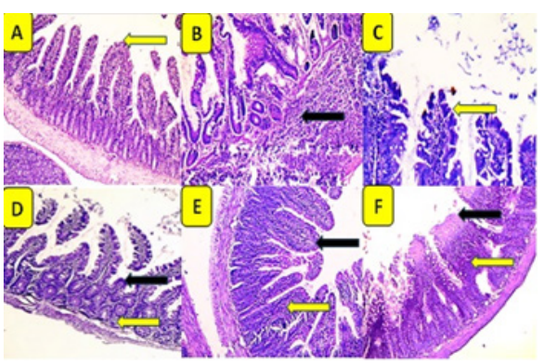

Fig. 5. Cross sections from studied groups. Control negative (A1) shows normal villous architecture (yellow arrow) with preserved goblet cells and normal crypts **(A)** (H&E x200). Control positive (B1) shows inflammatory cellular infiltrate observed mainly in the core of the villi and extending into the submucosa (black arrow), edema, and destruction of intestinal villi with depletion of goblet cells **(B)** (H&E x400). It also shows high-grade dysplasia manifested by great pleomorphism, absence of mucin, and frequent mitosis (yellow arrow) **(C)** (H&E x400). Infected treated with MOE (B2) shows mild to moderate inflammation with inflammatory infiltrates in the core of villi (yellow arrow) with intact mucosa, mild blunting and shortening of villi (black arrow) **(D)** (H&E x200). Infected treated with MOE-CsNPs (B3) shows mild inflammation in the core of villi (yellow arrow) with intact mucosa, intestinal villi, and crypts (black arrow) **(E)** (H&E x200). Infected and treated with MTZ (B4) shows marked inflammation with inflammatory infiltrate in the core of villi (yellow arrow), marked edema, blunting, and shortening of villi (black arrow) **(F)** (H&E x200).

Duodenal mucosa showed linear circular impressions at the sites of parasite attachment with a displacement of the microvilli around the cyst. The cyst appeared ovoid with an outer filamentous layer. While in MOE treated group (B2) the cyst outermost layer appeared mildly shrunken with dimpling, roughness, and corruption of the outer surface (Fig. 6 c, d). For MOE-CsNP (B3), marked shrinkage and corrugation (Fig. 6 e, f); flattening and irregularities (Fig. 6 g, h) were noted in MTZ treated group (B4).

Biochemical results: Liver biomarker enzymes (ALT and AST) were slightly elevated in both MOE and MOE-CsNPs treated mice either infected or noninfected groups. Meanwhile, enzyme elevation in MTZ treated mice either infected or noninfected was highly elevated but with no statistically significant difference for ALT and AST respectively. There was no statistically significant difference for renal parameters between all treated groups whether infected or non-infected (Table 2).

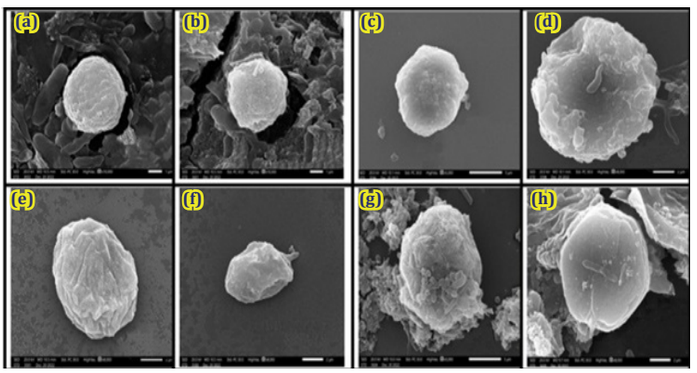

Fig. 6. SEM ultrastructure of *G. lamblia* cyst embedded in the small intestinal mucosa of different infected groups. The infected non-treated mice show damaged small intestinal mucosa with swelling, shortness, fusion, and disruption of the microvillus structure. The brush border is either distorted or lost leaving an eroded surface area. Duodenal mucosa showed linear circular impressions at the sites of attachment with a displacement of the microvilli around the cyst. The cyst appeared ovoid with an outer filamentous layer (**a, b:** X10000). In MOE treated mice, the cyst outermost layer appeared mildly shrunken with dimpling, roughness, and corruption of the outer surface (**c:** X8000, **d:** X 5000). In MOE-CsNPs treated mice, marked shrinkage and corrugation were noted (**e, f:** X8000), while flattening and irregularities were noted in MTZ treated mice (**g:** X5000, **h:** X8000).

Table 2. Clinical biochemistry parameters in the study groups.

	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	Urea (mg/dl)
Non-infected group (A)				
A1	48.7 ± 7.56	116.7 ± 15.3	0.61 ± 0.01	50.56± 8.83
A2	53.8 ± 4.62	127.3 ± 27.7	0.59 ± 0.08	52.84± 6.03
A3	50.1 ± 7.86	118.3 ± 28.5	0.54 ± 0.01	51.08±6.42
A4	61.1 ± 6.32	125.7 ± 24.7	0.58 ± 0.02	56.11±5.34
Infected group (B)				
B1	54.22 ± 3.20	134.7 ± 21.3	0.63 ± 0.02	55.16±2.73
B2	68.51 ± 4.73	128.1 ± 14.6	0.55 ± 0.02	51.38±3.53
B3	65.73 ± 5.38	131.8 ± 19.4	0.52 ± 0.07	53.14±7.51
B4	86.52 ± 7.6	149.3 ± 11.2	0.61 ± 0.05	54.50±8.20
Statistical analysis				
F	5.573	8.231	3.053	5.241
***P* value**	0.21	0.14	0.43	0.32

DISCUSSION

Giardiasis is a serious global health concern caused by *G. lamblia*. It is now one of the top ten parasitic infections affecting human health[4]. Resistance to standard therapy is increasing in *G. lamblia*, and the number of cases is likely to rise. As a result, considerable effort has been made to identify new, alternative anti-giardiasis drugs[9]. Reinfection, inadequate dose, poor immunity, drug resistance, biliary sequestration, and the formation of an antioxidant network to protect oxygen-sensitive metalloenzymes are all likely causes of *Giardia* therapeutic failure. However, most of the drugs used have severe negative side effects and are thus contraindicated[35]. The WHO advised using herbs and natural foods as one method for eradicating *Giardia* cysts and trophozoites. The usage of medicinal plants has increased in recent years due to their low risk of adverse effects[2]. The use of NPs increases bioavailability and reduces therapeutic adverse effects[36]. Chitosan acts as an antimicrobial agent by interacting with anionic molecules on the cell surface. Furthermore, due to its muco-adhesion characteristic and rapidly modifiable pH-responsive solubility, it can open tight junctions enhancing drug delivery[37]. So, the present study evaluated the therapeutic effect of MOE-CsNPs against experimental giardiasis in comparison to MTZ in mice infected with *G. lamblia*.

Our results showed that the number of fecal *G. lamblia* cysts was significantly decreased with 86.01% reduction in the MOE-CsNPs treated group, while the MTZ treated group had a decrease rate of 79.76% and the MOE-treated group's reduction rate was 76.89%. The effect of MOE in reducing cyst count was in accordance with Saad El-Din *et al.*[38] who noticed that

treating infected mice with MOE and the combined group with PZQ dramatically reduced the number of Schistosoma mansoni worms and the number of ova/g tissue and eliminated the parasites. Besides, Hammi *et al*.[39] reported that MOE may constitute novel promising molecules for the development of new leishmaniasis combination therapies due to the action of the flavonoid-rich MOE. The researchers attributed its strong antileishmanial capacities to its phenolic components.

The high efficacy of MOE-CsNPs may be attributed to the better drug-delivery contribution by chitosan nanoparticles. The combined therapy also produces controlled drug release, which improves drug stability, solubility, effectiveness, and reduces drug toxicity. It was also shown that its mucoadhesive characteristics prolonged the period of action and decreased clearance in GIT. El-Gendy *et al*.[9] found similar results with the group treated by MTZ-CsNPs producing the greatest reduction in *G. lamblia* cyst counts (94.69%). The reduction rate was 90.15% in MTZ treated group and 63.64% in CsNPs treated group. Furthermore, in an *in vitro* study, Yarahmadi *et al*.[19] proved the efficacy of chitosan in inactivating *G. lamblia* cysts. Increasing the chitosan concentration and exposure duration reduced the viability of the cysts. In another approach, Said *et al*.[40] found that the combination of silver, chitosan, and curcumin nanoparticles is effective and safe in the treatment of intestinal giardiasis. Because chitosan prolongs contact duration and delays evacuation, this combination may be effective in the treatment of diarrhea patients. Chitosan NPs were shown to reduce the number of *Giardia* cysts and viability by 52.3% and 58.2%, respectively[1]. Moreover, Yousef *et al*.[41] showed that in a chitosan NPs treated group the largest percentage reduction in both cyst and trophozoite counts in hamsters were 80.59% and 76.55%, respectively. They showed also that Tomex®-treated hamsters had significantly lower cyst and trophozoite counts of 53.35% and 53.51%, respectively, in infected intestines as compared to control-infected intestines.

For further evaluation, we investigated the histopathological alterations in the intestine. The proximal part of the duodenum and jejunum were chosen to evaluate the course of infection since both sections of the small intestine have the highest burden of intestinal giardiasis in both immunocompetent and immunosuppressed mice. This was allocated to the supportive biochemical conditions in these parts for the parasite, as well as the presence of specific receptors[42]. In comparison to the non-infected control group (A1), histopathological examination of the infected non-treated group (B1) revealed significant pathological alterations in the intestinal mucosa. Similarly, microvillus alterations in giardiasis were described as villous shortening and degeneration, a reduced ratio of villous height to crypt length, and diffuse loss of the brush border with epithelial barrier dysfunction[36,43]. This was attributed to the presence of several host factors as well as trophozoite interaction with the intestinal epithelium[44]. Solaymani-Mohammad[42] attributed the histopathological effect to disruption of intestinal epithelial 'tight junction' proteins which is considered a milestone in the pathological alterations associated with *Giardia* infection *in vitro* and *in vivo*. Furthermore, in our study, in comparison with the treated groups (B2, B3, B4), the infected non-treated group (B1) had a higher number of goblet cells. Goblet cells' function is crucial in giardiasis. The significant alterations in goblet cells and mucins in the small intestine imply that mucus may play a role in *Giardia* clearance or invasion[36].

Our study found that the pathological alterations in infected groups treated with MOE were better than with MTZ alone. However, improvements were more noticeable in mice administered MOE-CsNPs, with full repair and normal villous architecture, indicating that MTZ alone induces partial healing of the intestinal villi. Besides, our findings support prior research reporting combination therapy more effective than MOE or MTZ alone. According to Baz *et al*.[36], gold NPs appeared to have a greater improvement effect on intestinal wall histology than MTZ and showed the high effectiveness of gold NPs alone or in combination for the elimination of *G. lamblia*. The MTZ results were consistent with several previous researches[45,46].

Our results regarding SEM examination of the duodenum and proximal jejunum of the infected non-treated group revealed brush border damage. When we utilized the MOE-CsNPs, all ultrastructural mucosal abnormalities found in the infected non-treated B1 group began to return to normal. These findings were consistent with the findings of Baz *et al*.[36] who stated that when gold NPs were utilized, the ultrastructural mucosal abnormalities found in the *Giardia*-infected non-treated mice began to revert to normal, and the healing of intestinal mucosa improved.

Furthermore, Yousef *et al*.[41] revealed that trophozoites in *Giardia*-infected hamsters treated with chitosan NPs showed significant ultrastructural alterations. Using SEM, Saad *et al*.[47] discovered that oocysts of Cryptosporidium spp. treated with silver and copper oxide nanoparticles suffered significant structural alterations that resulted in oocyst viability loss.

Our results of the biochemical blood parameters tested for assessment of drug toxicity revealed that Infection with *Giardia* causes a slight increase in circulating levels of the liver enzymes ALT and AST but kidney function was more or less within average. The group treated with MTZ showed marked elevation in liver function in comparison with the group treated with MOE. This result was in accordance with Almanzor *et al*.[48] who found out that MOE therapy markedly

lowered serum ALT, AST, and ALP activity in S. mansoni-infected mice. Abdel Fattah *et al*.[49] attributed MOE protective effects against hepatic damage to the phenolic composites in its ingredients.

In conclusion, the current data highlights that combining MOE and CsNPs therapy is a novel potential natural method for reducing the number of *Giardia* cyst shedding in stool and improving the pathological changes in the gut after giardiasis. Future studies should concentrate on MOE and CsNPs' pharmacological potential and anti-*Giardia* action.

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Conflict of Interest: The authors declare no conflict of interest.

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