Research Article

Efficacy of Chamomile Extract Loaded Chitosan Nanoparticles as a Therapeutic Agent against *Entamoeba histolytica*

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Abstract | The objective of this study was to determine the antiparasitic activity of chitosan nanoparticles loaded with chamomile extract against *Entamoeba histolytica* infection. Chitosan nanoparticles were prepared using the sol–gel technique with minor modifications and were characterized by atomic force microscopy (AFM) and Fourier-transform infrared spectroscopy (FTIR). Acute infection was induced in mice by the oral administration of 10^3 viable cysts. After infection, the animals received either single or combined treatment with chitosan nanoparticles and chamomile extract for two weeks. The results showed that histological alterations were significantly reduced in all treated groups compared with the infected untreated group. Notably, intestinal pathological changes were mildest in the group treated with chitosan nanoparticles loaded with chamomile extract, indicating enhanced therapeutic efficacy of the combined formulation. The results exhibited that chitosan nanoparticles loaded with chamomile extract demonstrated significant antiparasitic activity against *E. histolytica* infection in mice and may represent a promising alternative therapeutic approach for amoebiasis.

Keywords | *Entamoeba histolytica*, Chamomile extract, Chitosan nanoparticles, Small intestine

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INTRODUCTION

A common cause of health problems in developing countries is the protozoan parasite *Entamoeba histolytica* (*E. histolytica*), the causative agent of amoebic dysentery. Intestinal infections caused by *E. histolytica* can lead to bloody and mucus-containing diarrhea, with acute amoebic dysentery being the most prevalent clinical manifestation. *E. histolytica* has two life cycle stages: trophozoites and cysts. In the large intestine, trophozoites may exist asymptomatically; however, they can invade the intestinal mucosa and disseminate via the bloodstream, resulting in amoebic colitis. Severe complications include intense abdominal pain, amoebic liver abscess, pleuropulmonary abscess, and extraintestinal lesions, particularly in the liver (Kantor et al., 2018). The most commonly used antimicrobial agents for the treatment of amebiasis are 5-nitroheterocyclic drugs, especially metronidazole and, more recently, nitazoxanide (Farthing, 2005).

Herbal plants have recently gained increasing attention for the treatment of intestinal disorders, including infectious diseases (Kadhim and Hasan, 2025; Sandri

et al., 2026). *Matricaria chamomilla* (MC), commonly known as German chamomile, is one of the most widely used medicinal herbs. It is distributed in various regions, including Asia, Australia, and South America. Two species *Chamaemelum nobile* and *Matricaria chamomilla* are commonly used in herbal medicine. Chamomile contains several bioactive constituents, including α-bisabolol, terpenoids, polysaccharides, essential oils, minerals, fatty acids, flavonoids, and phenolic compounds. It exhibits numerous pharmacological properties, such as antibacterial, anti-inflammatory, antifungal, anti-ulcer, antispasmodic, antiviral, and sedative effects. Internally, chamomile is used to aid digestion and treat diarrhea, nausea, urinary tract disorders, and dysmenorrhea. Externally, it is applied to promote wound healing and treat skin rashes, infections (such as shingles and boils), sore throat, hemorrhoids, and eye inflammation (Ali et al., 2013).

Chitosan, a polysaccharide partially deacetylated from chitin, has been widely utilized in medical applications for many years (Hadi *et al.*, 2024). It is an important material for nanoparticle synthesis due to its biodegradability and non-toxic nature. Numerous studies have investigated chitosan nanoparticles (NPs) for the treatment of *Giardia lamblia* infection (Said *et al.*, 2012). Additionally, chitosan NPs have been shown to enhance the efficacy of ivermectin as an antifilarial therapy (Ali *et al.*, 2013).

The present study focuses on evaluating the ability of chamomile extract and chitosan nanoparticles to promote regeneration of small intestinal tissue following *E. histolytica* infection in a mouse model.

MATERIALS AND METHODS

Plant extraction

Flowers of *M. chamomilla* were obtained locally from various locations in Kirkuk. The dried flowers were ground into a fine powder. Fifty grams of the powdered plant material were mixed with 500 mL of distilled water, and the mixture was concentrated to dryness using a rotary evaporator. The resulting extract was stored at 4 °C until use (Hajjaj *et al.*, 2013).

Synthesis of chitosan nanoparticles

Nanomaterials were prepared using a chemical approach via the sol–gel process (Jasim et al., 2021). Chitosan was dissolved in deionized distilled water and subjected to ultrasonic treatment for 30 minutes. The pH of the solution was adjusted to 12 using 1 N NaOH, and the mixture was stirred with a magnetic stirrer for one hour at room temperature. The pH was then adjusted to 4 using 1 N HCl and stirred for an additional hour at ambient temperature. Finally, the pH was adjusted to 7 using 1 N HCl and stirred for 60 minutes at room temperature. The solution was subsequently centrifuged, and the precipitate was collected for further use.

Characterization of prepared nanoparticles

Atomic force microscopy (AFM) was used to generate two- and three-dimensional surface topography of the nanoparticles (Lyles *et al.*, 2013). The Fourier-transform infrared (FT-IR) spectra of the chitosan nanoparticles were analyzed after drying the samples overnight in an incubator (Augustine *et al.*, 2005).

Collection of stool sample

Diarrheal stool samples were collected from infected individuals during the study. The specimens were examined microscopically using a direct wet saline smear. Fresh samples were preserved in 2.5% potassium dichromate solution at 4 °C until use. The fecal material was inoculated, suspended, and subsequently centrifuged. The sediment was washed three times and resuspended in phosphate-buffered saline (PBS, pH 7.4) containing the antibacterial agents penicillin and streptomycin. Approximately 10^3 viable cysts were orally administered to mice to induce acute infection (Hamad, 2021).

Experimental design

Twenty-five male Swiss white mice, aged 4–6 weeks and weighing 18–22 g, were obtained from the Iraqi Center for Cancer Research. The animals were housed in plastic cages, provided with a standard diet from the same source, and given sterile water in special bottles under controlled temperature and ventilation conditions. The mice were divided into five groups, each containing five animals.

The first four groups were orally inoculated with 0.1 mL of prepared *Entamoeba* inoculum using a micropipette, and fecal samples were examined daily to confirm the presence of the parasite. The fifth group was not infected and served as a healthy control to compare with infected tissues.

- Group 1 (Positive control): Mice received 0.1 mL of *Entamoeba* inoculum orally.
- Group 2: Mice received chamomile extract at a dose of 50 mg/kg orally for 14 consecutive days, administered once daily.
- Group 3: Mice received chitosan nanoparticles at a dose of 50 mg/kg orally for 14 consecutive days, administered once daily.
- Group 4: Mice received chitosan nanoparticles loaded with chamomile extract orally for 14 consecutive days, administered once daily.

Histopathological examination

Histological samples of the small intestine were collected

from both treated and untreated mice at the end of the experiment and placed in sterile containers containing 10% formalin for preservation. The tissues were then processed for histological examination using the hematoxylin and eosin (H & E) staining method. Briefly, samples were dehydrated in a graded series of alcohols, cleared in xylene, embedded in paraffin, and sectioned at 5 µm thickness. The sections were mounted on glass slides, stained with hematoxylin to visualize nuclei, counterstained with eosin to highlight cytoplasm and extracellular matrix, and examined under a light microscope to assess intestinal tissue morphology and pathological changes.

RESULT AND DISCUSSIONS

Characterization of chitosan nanoparticles

Atomic force microscopy

Atomic Force Microscopy (AFM) was used to examine the surface morphology and topography of the nanoparticles (Figure 1). This technique provides two- and three-dimensional images of nanoparticle surfaces at the atomic level (Fadhil et al., 2015), allowing estimation of the average particle diameter at the nanoscale. Chitosan nanoparticles prepared using the sol–gel method were analyzed using AFM (Table 1). Careful surface analysis is required, as factors such as contamination can influence the results (Figure 2).

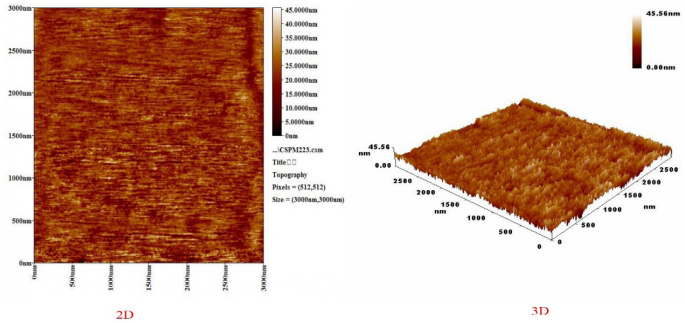

Figure 1: Atomic force microscopy (AFM) images of chitosan nanoparticles synthesized using the sol–gel method, showing 2D and 3D surface topography.

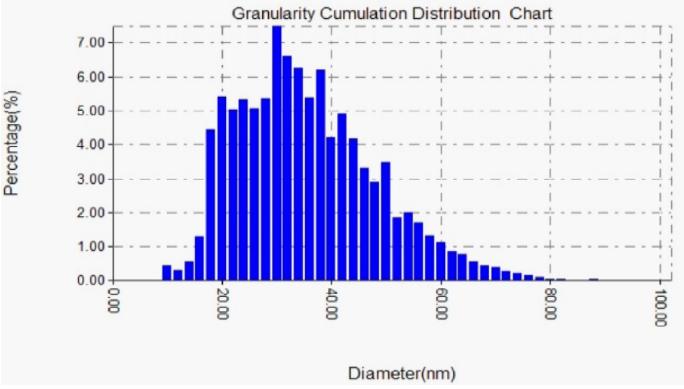

Figure 2: Average particle size of chitosan nanoparticles as determined by atomic force microscopy (AFM).

Table 1: Estimated size of chitosan nanoparticles.

Granularity Cumulation Distribution Report

Sample: Sample Name	**Code:**Sample Code
Line No.:lineno	**Grain No.:**7696
Instrument:SPM	**Date:**2011-01-01
Avg. Diameter:34.63 nm	**<=10% Diameter:18.00 nm**
<=50% Diameter:32.00 nm	**<=90% Diameter:52.00 nm**

Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)
10.00	0.45	0.45	38.00	6.20	65.01	66.00	0.56	98.19
12.00	0.30	0.75	40.00	4.21	69.22	68.00	0.45	98.65
14.00	0.56	1.31	42.00	4.91	74.13	70.00	0.39	99.04
16.00	1.29	2.60	44.00	4.17	78.30	72.00	0.29	99.32
18.00	4.43	7.03	46.00	3.31	81.61	74.00	0.21	99.53
20.00	5.39	12.42	48.00	2.88	84.50	76.00	0.16	99.69
22.00	5.02	17.44	50.00	3.47	87.97	78.00	0.09	99.78
24.00	5.33	22.77	52.00	1.86	89.83	80.00	0.05	99.83
26.00	5.04	27.81	54.00	2.00	91.83	82.00	0.05	99.88
28.00	5.34	33.15	56.00	1.70	93.53	84.00	0.03	99.91
30.00	7.46	40.61	58.00	1.33	94.85	88.00	0.04	99.95
32.00	6.59	47.19	60.00	1.13	95.98	90.00	0.03	99.97
34.00	6.24	53.43	62.00	0.87	96.86	94.00	0.01	99.99
36.00	5.38	58.81	64.00	0.78	97.64	102.00	0.01	100.00

Fourier transform infrared spectroscopy

Fourier-Transform Infrared Spectroscopy (FTIR) was used to identify the main functional groups involved in the synthesis and capping of the nanoparticles. Spectra were recorded in the range of 400–4000 cm^{-1} to detect chemical bonds and functional groups in the compounds. The FTIR spectra of chamomile extract and chitosan nanoparticles loaded with chamomile extract are presented in Table 2 and Figure 3.

Table 2: FTIR spectral analysis of (A) chamomile (*Matricaria chamomilla*) extract and (B) chitosan nanoparticles loaded with chamomile extract.

A

	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
Chamomile extract	2978.21-2904.06	O-H stretch	Alcohol
	1597.40	N-H bend C=C stretch	Amine, Alkene
	1400.74	C-H bend	Alkane
	1251.49	C-O stretch	Alcohols, Carboxylic Acids, Esters, Ethers
	1054.71	C-O stretch	Alcohols, Carboxylic Acids, Esters, Ethers
	614.43-438.50	C-Br stretch	Metal Oxide

B

	Frequency of Absorption (cm^{-1})	Bonds	Compound class of Functional Groups
Chitosan NPs with chamomile extract	3334.19	C=C stretch	Alkene
	2983.12-2905.06	O-H stretch	Alcohol
	1635.99	N-H bend C=C stretch	Amine, Alkene
	1452.55-1400.69	C-H bend	Alkane
	1250.69	C-O stretch	Alcohols, Carboxylic Acids, Esters, Ethers
	1056.53	C-O stretch	Alcohols, Carboxylic Acids, Esters, Ethers
	588.52	C-Br stretch	Alkyl Halides

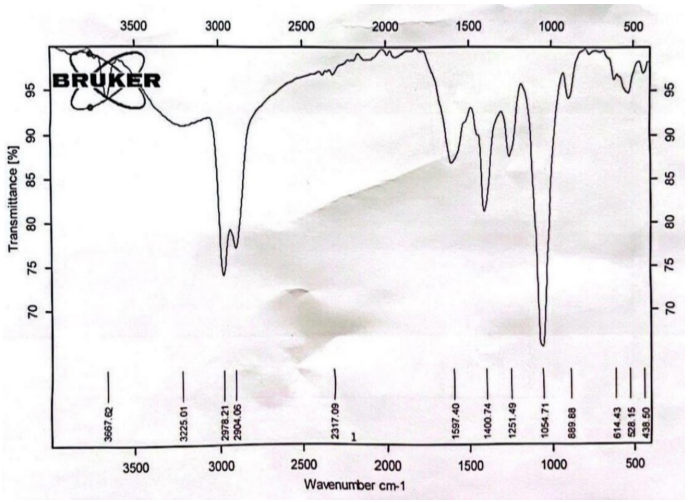

Figure 3: Fourier-transform infrared (FTIR) of chamomile (*Matricaria chamomilla*) extract.

For the chitosan nanoparticles with chamomile extract, peaks at 2978.21–2904.06 cm^{-1} corresponded to O–H stretching, indicating the presence of alcohol groups. A broad peak at 1597.40 cm^{-1} was attributed to N–H bending and C=C stretching, representing amine and alkene groups. Peaks at 1400.74 cm^{-1} were associated with C–H bending of alkanes, while peaks at 1251.49 cm^{-1} and 1054.71 cm^{-1} indicated C–O stretching, characteristic of alcohols, carboxylic acids, esters, and ethers. Peaks observed at 614.43–438.50 cm^{-1} corresponded to C–Br stretching, representing alkyl halides.

Similarly, the FTIR spectrum of chamomile extract showed a strong peak at 3334.19 cm^{-1}, corresponding to C=C stretching of alkenes. Peaks at 2983.12–2905.06 cm^{-1} indicated O–H stretching of alcohol molecules, while a broad peak at 1635.99 cm^{-1} represented N–H bending and C=C stretching (amines and alkenes). Peaks at 1452.55–1400.69 cm^{-1} were associated with C–H bending of alkanes, and peaks at 1250.69 cm^{-1} and 1056.53 cm^{-1} corresponded to C–O stretching of alcohols, carboxylic acids, esters, and ethers. A peak at 588.52 cm^{-1} was observed for C–Br stretching, indicating alkyl halides.

These results confirm the presence of characteristic functional groups, demonstrating the successful incorporation of chamomile extract into the chitosan nanoparticles (Figure 4).

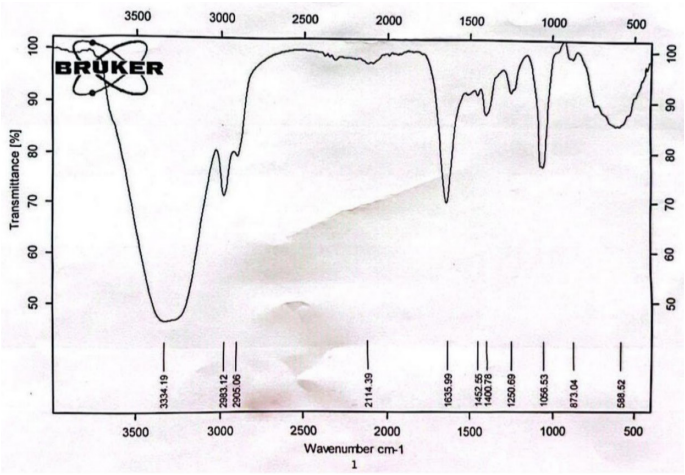

Figure 4: Fourier-transform infrared (FTIR) spectrum of chamomile extract loaded onto chitosan nanoparticles.

Parasitological results

On day four post-infection, mice in the experimental groups began to excrete *Entamoeba* cysts in their stool. Several clinical signs were observed starting four days before the end of the experiment, including hair loss and reduced activity.

As shown in Figure 5, histological examination revealed that mice treated with chamomile extract showed partial improvement in intestinal morphology, including moderate to mild villous thickening and slight mucoid degeneration, compared with the infected untreated group. These findings are consistent with Khayyal *et al.* (2019), who reported protective effects of chamomile extract against tissue damage. Chamomile extract has been shown to preserve most histological structures by modulating inflammatory markers, oxidative stress, and apoptosis (Sabatke *et al.*, 2022). Polysaccharides in chamomile, when combined with antiparasitic drugs, may inhibit parasite attachment to intestinal cells and enhance drug efficacy, as reported in studies on *Giardia lamblia* treatment.

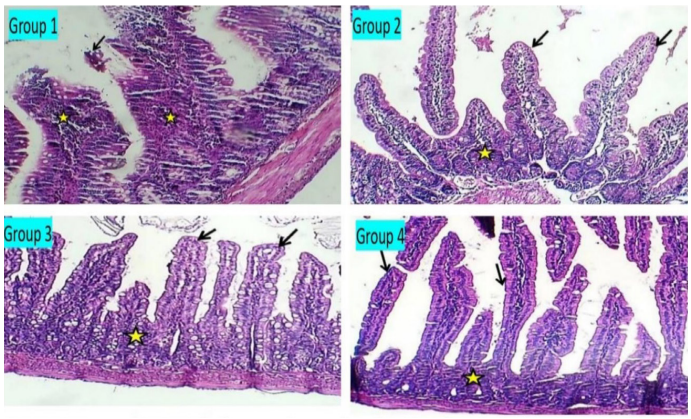

Figure 5: Histological examination of the duodenum (H & E stain, 100×). Group 1: Section showing enteritis with mucosal thickening, sloughing of lining cells (black arrow), and leukocyte infiltration in the tissue core (asterisk). Group 2: Section showing normal villi with intact enterocytes (black arrow) and normal intestinal glands (asterisk). Group 3: Section showing normal mucosal villi (black arrow) and normal intestinal glands (asterisk). Group 4: Section showing normal mucosal villi (black arrow) and normal intestinal glands (asterisk).

Mice treated with chitosan nanoparticles displayed mild mucosal healing, normal villous thickness, and preserved cytoarchitecture of villus cells. These results align with Ahmed et al. (2016), who demonstrated that chitosan nanoparticles exert substantial effects on local gastrointestinal disorders and intestinal disinfection, and Wardani *et al.* (2018), who observed improved epithelial cell recovery in the gastrointestinal tract following nanoparticle treatment. However, Hu *et al.* (2011) reported that chitosan nanoparticles could induce cellular oxidative stress, increasing reactive oxygen species and causing cytotoxicity in mucosal epithelial cells.

Notably, mice treated with chitosan nanoparticles loaded with chamomile extract exhibited greater histological improvement than those treated with chamomile extract alone. Sections of the small intestine from this group showed restoration of normal duodenal mucosa, normal villous thickness, and intact villus cell cytoarchitecture.

This enhanced effect is supported by previous studies, such as El-Gendy *et al*. (2021), who reported that loading metronidazole onto chitosan nanoparticles improved the therapeutic efficacy of both chitosan nanoparticles and the drug in hamsters experimentally infected with *Giardia*.

Overall, these results indicate that the combination of chitosan nanoparticles and chamomile extract provides superior protection and regenerative effects on intestinal tissue following *E. histolytica* infection compared with either treatment alone.

CONCLUSION

In summary, the combined therapy was more effective than the single treatments, as mice treated with chamomile extract loaded onto chitosan nanoparticles exhibited pronounced repair of the intestinal mucosa.

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NOVELTY STATEMENT

Our study has shown that chitosan nanoparticles loaded with chamomile extract have high therapeutic and preventive efficacy against amoebic pathogens in rats.

AUTHOR'S CONTRIBUTION

NAJ,SAA:.Writing and Methodology.HHK, LAY:.Writing, Statistics, Analysis.AFH:.Corresponding Author and Reviewer Responses.

Generative AI and AI-assisted technology statement

The authors pledge not to use artificial intelligence tools in the article.

Ethical approval

Ethical approval to conduct this study and to use animals in the experiments was obtained from the Biotechnology Research Center, Al-Nahrain University.

Conflict of interest

The authors have declared no conflict of interest.

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