Therapeutic efficacy of *Zingiber officinale* loaded chitosan nanoparticles in intestinal murine trichinellosis

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Abstract

The purpose of this research was to compare the impacts of *Zingiber officinale* extract and its chitosan(CS) nanoparticles(NPs) on the alterations of oxidative stress, histopathological, and parasitological factors in mice infected with *Trichinella spiralis* to albendazole treatment as well as groups of controls. The current study included 64 mice, 8 of them were negative controls and 56 of them were infected. The extracts have been administered orally to experimentally infected mice at a dose of 100 mg/kg/day, 50mg/kg/day beginning 24 hours after infection and continued for 14 days on parasite load, intestinal histology, skeletal muscle, heart and oxidative damage. With the exception of the healthy control group, all groups were infected orally with *T. spiralis* larvae at a dose of 200 larvae per mouse. On the 35th day following infection, all groups were sacrificed. The results demonstrated a significant reduction in the mean number of *T. spiralis* adult worms and larvae count was observed in all treated groups (74.82%, 62.47%, 48.71%, 30.30% & 24.47%) & (71.80%, 59.26%, 50.39, 37.59% & 27.41%) respectively. The group of mice that received *Zingiber officinale* NPs exhibited the greatest reduction in mature worm and larvae counts, at 100 and 50 Mg/kg, respectively. For the group treated by *Zingiber officinale* NPs 100 mg/kg, it showed better rank in adult worms and larvae count compared to other infected and treated groups and albendazole administered infected group. This was further demonstrated by decreased parasite load, histological changes, and oxidative stress as compared to the control and albendazole-treated infected groups. SOD,GSH concentrations have been investigated in sera and showed that all treatment of infected mice with *Zingiber officinale* extract 50 mg/kg and its chitosan nanoparticles, 100 mg/kg considerably improved (P>0.01) all parameter levels in the sera, bringing them back to the original values of the negative control. Finally, we can infer that the usage of *Zingiber officinale* NPs may have therapeutic promise in the treatment of trichinellosis.

Keywords: *Trichinella spiralis*, Albendazole; Chitosan nanoparticles; *Zingiber officinale* rhizome.

1. Introduction

The most prevalent way *Trichinella spiralis* was passed on to humans was through the ingestion of meat that is raw or undercooked derived from sick pigs or game [1].Trichinellosis is a potentially fatal zoonotic parasite infection prevalent all over the world [2].Trichinellosis is caused by cells that cause inflammation. Furthermore associated, it is with a high level of cytokines that induce inflammation in the suffering hosts [3].*Trichinella spiralis* infects a variety of hosts, including humans [4]. *T. spiralis* has parenteral and enteral phases in its life cycle. The enteral or intestinal phase is characterised by gastrointestinal sign as an example diarrhoea and pain in the stomach. Periorbital edoema, myalgia, and muscle weakness are symptoms of the parenteral or muscular phase [5]. Treatment of *T. spiralis* is also debatable due to the resistance of the muscle tissue-encysted larvae [6]. The hidden larvae are extremely difficult to eliminate [7]. In the acute phase, migrating larvae may cause myocarditis, thromboembolic illness, and encephalitis [8,9]. Trichinellosis is routinely treated with benzimidazoles such as mebendazole and albendazole. These medicines, however, are not completely effective against *T. spiralis* larvae [10]. Ginger has the capacity to boost digestive juices while also combating pollutants.
These effects may aid in parasite removal [11]. The isolated chemicals contain functional and pharmacological qualities such as antihyperglycemic, antibacterial, anticarcinogenic, anti-inflammatory, antilipemic, anticancer, and antimutagenic activities[12 and 13]. Zingiber officinale, also known as ginger, is a plant in the Zingiberaceae family. It was utilised as a spice and flavouring agent all throughout the world. It additionally contains antioxidant properties, that stimulates the immune system and allows the human body to naturally fight illnesses.[11].The ultra structural study by [14] revealed degenerative effects Zingiber officinale extract on both adults and larvae in addition to obvious improvement of the histopathological changes in the small intestine and muscles. Aromatic plants, specifically their essential oils or components, have long been known for their ability to combat a wide range of species, including bacteria, fungi, viruses, protozoa, insects and parasites [15,16, and 17]. Zingiber officinale has also been developed to improve its functionality in the form of nano particles as a medication delivery with many advantages that it requires to boost the prevention and treatment of inflammatory bowel disease [18]. Chitosan nanoparticles (CS NPs) have been used in cryptosporidium therapy as a drug delivery method [19]. Previous studies have used NPs as vehicles to deliver drugs in order to improve therapeutic effectiveness [20]. Diseases can affect hosts that are healthy also stimulating the immune system of the afflicted host.In response, the immune system produces damaging oxidants known as free radical species [21]. Reactive oxygen species (ROS) have been associated to chronic inflammation, age-related diseases, and cancer [22]. Because free radicals are involved in several disease pathways are affected by this. Most antioxidants inside cells have an enzymatic and not enzymatic effect on ROS generation [23]. Superoxide dismutases (SODs), Catalase (CAT), reductase of glutathione (GR), and peroxidases of glutathione (GPx) are antioxidant enzymes found in the complex innate immune system [24]. Chemicals that are not enzymes, such as glutathione (GSH)[25] are also essential. By conjugating ROS and detoxifying lipid peroxidation products, nanoparticles of chitosan (CS NPs) have been used as a drug delivery approach for cryptosporidium treatment [26]. The general goal of this work is to look into the effects of Zingiber officinale extract and their (CS NPs) on oxidative stress, histopathological and parasitological measures in trichinellosis-infected mice in experimental designs.

2. Materials and procedures

Ojarud’s group method was used to prepare an extract of Zingiber officinale (ginger). With some adjustments, it was used. Zingiber officinale plant portion (roots) which was purchased at the local supermarket in Egypt's Quisna Governorate. Every one dried Zingiber officinale roots was pulverized into a fine powder using a mechanical grinder. In a beaker, 200g of powder was dissolved in 600ml of 95% ethanol before being placed in a water bath for 2 hours at 50°C before filtering using filter paper (Whatmanno.1). It was washed several times and dried at room temperature until it dries completely[27]. The basic extract was weighed and then kept in a dark bottle at 4 degrees Celsius, this study used sixty-four laboratory-bred male parasitic free BALB/c mice (25-30 g) aged between six and eight weeks. The animals were received from Theodore Bilharz Research Centre (TBRI, Giza in Egypt) and were cared for according to institutional and national requirements. Inoculum preparation: The diaphragms of seriously diseased pigs were cut into tiny pieces, chopped, and digested in 1% pepsin-hydrochloride. Larvae were collected by sedimentation after an overnight period of incubation at 37 °C, washed many times in physiological saline (0.85%), and the total number of larvae per ml was recorded. [28, 29]. Mice were fasted for 12 hours before infection and then administered infective larvae (200 orally) through oral route using a tuberculin syringe with blunt tip, as described in [30]. Chitosan was dissolved in 1% acetic acid solutions and left at room temperature for 20-24 hours with magnetic stirring until the desired clarity was obtained. Chitosan concentrations in the range of 0.05 to 0.5% w/v were synthesised. To prevent particle aggregation, the solutions of chitosan were treated with the surfactant tween 80 [0.5% (v/v)], and the pH of the chitosan liquids was adjusted to 4.6–4.8 with 1N (Na OH) by dissolving 10mg of TPP in 10 ml of deionized water and diluting it to the following concentrations: 0.25, 0.50, 0.75, 1, 1.5, and 2 mg/ml. A 0.1% sodium tripolyphosphate solution was created [31]. A 0.22 micron Millipore filter was used to filter all solutions. TPP solution was injected dropwise
with a syringe into chitosan solution while magnetic stirring at 2.5:1 (v/v) (chitosan: TPP) at 800 rpm at room temperature. Visual examination of the materials resulted in three classifications: clear solution, opalescent suspension, and aggregates. Very tiny particles are represented by the opalescent suspension zone.

Extract-loaded CS NPs were made by the dropwise adding of solution of CS to an aqueous solution of sodium TPP (having extract at concentration of 100 mg/ml) with constant stirring, followed by sonication. The separation of extract-loaded NPs from aqueous suspension was done by centrifugation at 20,000 ×g for 30 min at 14 °C.

The resulting chitosan particle aggregation was centrifuged at 12000 g for 30 minutes. Water has been used to resuscitate the pellet. The chitosan nanoparticles dispersion was dried in the freezer before further use or analysis. CH nanoparticle characterization: The chitosan nanoparticles that were created were characterised using the procedure outlined below. To confirm the production of nanoparticles, the solution was scanned with TEM (Transmission electron microscopy): The Quanta 400 ESEM/EDAX (FEI) was used to analyse the size and shape of dried CH nanoparticles. A tiny amount of vacuum dried chitosan nanoparticles samples were held on a TEM stub for 6 minutes using double-sided sticky tape and sputtered at 50mA. Following that, the sample-containing stub was placed in the Transmission electron microscopy (TEM) Room. The photomicrograph was taken at an acceleration voltage of 20 KV.

The encapsulation effectiveness (AE) and capacity for loading of nanoparticles were estimated as follows:

% \( AE = \frac{(A-B)}{A} \times 100 \)

% \( LC = \frac{(A-B)}{C} \times 100 \)

Where A is the total amount of extract, B is the free volume of extract, and C is the total weight of nanoparticles

Dose of albendazole: International Pharmaceutical Industries Co.A single tablet (100mg) was mixed in 50ml of filtered water and administered orally three consecutive days at the dosage of 50mg/kg/day [5].

Animal study design groups: The sixty-four male laboratory mice were divided into eight groups (8 mice per cage). GI: Non-infected and untreated, GII: = infected but untreated, GIII: infected and treated with albendazole drug, GIV: infected and treated by CSNPs; GV: infected and treated by extract 50 mg/kg; GVI: infected and treated by extract 100 mg/kg; and GVIII: infected and treated by extract 100 mg/kg-uploaded CSNPs.

Mice that were infected (GIII - GVIII) were treated orally beginning on the first day post infection (pi) with albendazole (ABZ) medication (GIII) or CSNPs (GIV) and extracts were tested twice daily (GV to GVIII) for 14 days. The infected mice were slaughtered by neck dislocation on the 7th and 30th days after in fecion (fig. 1).

Fig.1: Experimental layout flow chart Parasite count estimation.

3. Parasitological examination:

On the seventh day, a total of four mice from each infected group (positive and treated infected groups) were slaughtered to evaluate the effects of the extracts and their NPs on worm adults (gastrointestinal phase) [32]. Small intestines were removed from both untreated and untreated infected animals, opened, washed, and incubated in 10 mL saline at 37 C for 2 hours to allow worms to depart the intestine and enter the storage vessel. The fluid was cleaned several times until it was pure. The fluid was then collected and centrifuged for 5 minutes at 1,500rpm. The excess fluid was drained, and the sediment was reconstituted in a few drops of saline
before being inspected under the dissecting microscope drop by drop for counting the adults [33,34]. The effectiveness of the medication group or both tested extract groups was estimated using the equation [35].

The efficacy% = A - B multiplied by 100A
Where:

A denotes the number of larvae or worms taken from animal control.

B = The total number of worms or larvae taken from animals who were treated.

Total larval in muscles: On the 35th day, mice were slaughtered, and muscle larvae counts in whole corpses were determined [36]. In brief, mouse corpses were weighed, diced, and digested in 200 mL of distilled water containing 1% acid pepsin hydrochloride [37]. Following a two hour incubation at 37°C with constant stirring by an electromagnetic stirrer, the encysted larvae were collected by sedimentation and washed multiple times in filtered water. A McMaster count chamber was used to count the quantity of larvae. [38].

Biochemical analysis: Superoxide Dismutase 3 (SOD3) and -glutathione (-GSH) levels had been determined using ELISA kits obtained from (Wuhan Fine Biotech Co., China). The collection and Storage: The complete blood samples was collected at room temperature for 2 hours before being centrifuged at 1000g for a period of 20 minutes. The serum was taken right away, and the assay was carried out. Nonpyrogenic, nonendotoxin, and disposable blood collection vials.

4. The ELISA measurement principle is as follows:

These kits used the Competitive-ELISA detection method. The target has already been coated prior to use on the microtiter plates. During the procedure, every target in the sample or reference competes for target-specific sites on the Biotinylated Detection Antibody with a fixed amount of target on the solid phase supporter. The plate was cleaned of excess conjugated and unbound sample or standard, and each microplate well was incubated with Streptavidin-Biotin Complex (SABC). Then a solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added to each well. The enzyme-substrate activity is halted, and the colour change is measured spectrophotometrically at 450nm. The target concentration in the specimens is then determined through the comparison of their OD to the standard curve.

Superoxide Dismutase 3 (SOD3): The enzyme activity of SOD3 was determined using an ELISA kit (Wuhan Fine Biotech Co., China). Its function as a key antioxidant enzyme in serum samples was measured using a kit. In summary, xanthine oxidase reduced 2-(4-iodophenol)-3-(4-nitrophenol)-5-phenyltetrazolium chlo-ride to create a red formazan product. SOD3 prevented this reduction, and the generated coloured complex absorbance was measured at 450nm. What are -glutathione S-transferases (a-GST) It, like GSH, was determined using a colorimetric approach using 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) according to the kit’s procedure. In a nutshell, DTNB reacts with reduced thiol(-SH) groups to produce complexes. The absorbance at 450 nm was measured to estimate the -GST levels [39].

4. Histopathological study:

Histopathological study:

The connection of the proximal and distal thirds was removed. The muscle specimens were collected from the intestine, skeletal muscle and heart of mice slaughtered on the 35th dpi, as well as one

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*Egypt. J. Chem. 67, No. 8 (2024)*
centimetre from the jejunum, or digestive tract, of mice killed on the 7th dp [40].

These specimens have been embedded in 10% buffered formalin, split into 1 cm thick slices, dehydrated with alcohol, washed with xylol, and immersed in the wax of paraffin before being processed into paraffin blocks. Using a Reichert Rotary microtome, serial paraffin sections of 5m thickness were cut and dyed with Harries Hematoxylin and Eosin [41]. Every result is reported statistically as the average standard error (SE). The results were statistically analysed using one way analysis of variance (ANOVA) in the SPSS 15.0 programme, IBM Company (NY, USA), and a value of p<0.05 was considered statistically significant [42].

5. Results:

The results of CSNPS morphology analysis by SEM is shown in (fig. 2) A. As seen, the majority of particles were spherical and had smooth surface with a homogeneous polydispersity. The mean diameter obtained by SEM is smaller and the mean particle size was 60 nm.

B - The mean zeta potential of the synthesized NPs were showing good stability with 52.3mV at 25.1°C with a conductivity of 0.127mS /cm. The mean particle size distribution of the NPs was ranged from 20 – 110 nm the majority of particle size was 50 – 80 nm.

At day 35 following infection, there had been a significant drop in overall larval numbers in the infected treated group (27 ± 3.74, 40.25 ± 3.40, 55 ± 5.4874.75 ± 5.85 and 81 ± 6.32) ingroups (GVI, GVIII, GVII, GV and GIV) as compared with infected untreated group GII (107.25 ± 6.80) (p< 0.001). The most effective reduction of larval count was found in groups (GVI and GVIII) which received Zingiber officinale extract loaded CS NPs compared with Zingiber officinale extract groups (GV and GVII) (p< 0.001) (Table 1). Resistance infection rate in adult worms and larvae count was highest reduction ingroup treatment with Zingiber officinale extract 50mg/Kg loaded CSNPS(GVI), inhibition (74.82%and71.80%) followed by Zingiber officinale extract 100mg/Kg loaded CSNPS (G VIII), inhibition (62.47 and 59.26%), compared to groups of Zingiber officinale extract 100mg/kg loaded CSNPS which gave (48.71 and 50.39%, 30.30 and 37.59% growth inhibition) while CS NPs group showed 24.4% and 27.41% growth inhibition. It was found no resistance infection rate in adult count and larvae count in treatment with albendazol treated group.

Table 1. Effects of extracts and their NPs against adult worms and encysted larvae

Non – significant, p. value > 0.05, * Significant p value < 0.05, ** Highly significant p value < 0.01, *** Very highly significant p value < 0.001, P1: Comparison with G II, P2: Comparison with
G III, P3: Comparison with G IV, P4: Comparison with G V, P5: Comparison with G VI, P6: Comparison with G VII.

6. Biochemical measurements:

ELISA technique was utilized to investigate some factors in mice's sera. SOD3, α-GSH levels were tested, and illustrated in (Fig.3).

Treatment of infected mice with extract and extract-loaded CS NPs were significantly improved (P<0.01) compared to the infected mice group (GII), SOD level reporting the highest significant increase (P<0.001) in sera of GVIII and GVI and had close to the original levels of negative control (Fig. 3). Also GSH level reporting the highest significant increase (p<0.01) in sera of GVI and GVIII and had close to the original levels of negative control (Fig. 4).

**Fig.3 and 4:** SOD (A) and GSH (B) Serum levels after treatments compared positive and negative controls (GII and GI). Non - significant p. value > 0.05, significant p value < 0.05, highly significant p value < 0.01, very highly significant p value < 0.001


6. Histopathological alterations:

Histopathological alterations in separate experimental groups' small intestine sections (H&E at high power view X (200, 400) Figs. 5 (A-O). Non-infected and non-treated (negative control) (GI) small intestine structure, with no histological alterations Fig. 5 (A). Positive control (GII) The gut wall was visible mild intra-luminal infestation by viable parasites and partially necrotic villi Fig. 5 (B, C). Albendazole drug (GIII) intestinal wall showed no parasitic infestation, and average villi Fig. 5 (D, E). Infected, treated by CSNPs (GIV) intestinal wall showed few scattered intra-mucosal calcified parasites Fig. 5 (F, G). Infected,
treated by extract 50 mg/kg (GV) intestinal wall showed mild intra-mucosal calcified parasites, and partially necrotic villi Fig.5(H,I). Infected, treated by extract 50 mg/kg extract-loaded CSNP (GVI) intestinal wall showed mild intra-mucosal calcified parasites, and partially necrotic villi Fig.5(J,K). Infected, treated by extract 100 mg/kg (GVII) intestinal wall showed mild intra-luminal and intra-mucosal calcified parasites, and partially necrotic villi Fig.5(L,M). Infected, treated by extract 100 mg/kg-loaded CSNPs (GVIII) intestinal wall showed mild intra-luminal and intra-mucosal calcified parasites, and partially necrotic villi Fig.5(N,O).

Fig.5(A). Non-infected and non-treated (negative control) (GI) showing average villi composed of connective tissue core (black arrow) covered by tall columnar epithelial cells (blue arrow) with average goblet cells (red arrow), positive control (GII) B: intestinal lumen showing wall mild intra-luminal infestation by viable parasites (black arrow) with necrotic intestinal villi (red arrow) C: another view showing mild intra-luminal infestation by viable parasites (black arrow) with necrotic intestinal villi (red arrow), albendazole drug (GIII) D: intestinal wall showing no parasitic infestation (black arrow), and average villi (blue arrow), E: another view showing no parasitic infestation (black arrow), and average villi (blue arrow), infected, treated by CSNPs (GIV). F: view of intestinal wall showing few scattered intra-mucosal calcified (black arrow). G: another view showing few scattered intra-mucosal calcified parasites (black arrow), infected, treated by extract 50 mg/kg (GV). H: intestinal wall showing mild intra-mucosal infestation by calcified parasites (black arrow). I: another view showing mild intra-mucosal infestation by calcified parasites (black arrow), with partially necrotic villi (red arrow), infected, treated by extract 50 mg/kg-loaded CSNP (GVI). J: intestinal wall showing mild intra-luminal infestation by calcified parasites (black arrow), K: another view showing mild intra-luminal infestation by calcified parasites (black arrow), with partially necrotic villi (red arrow), infected, treated by extract 100 mg/kg (GVII). L: high power view of intestinal wall showing mild intra-mucosal infestation by calcified parasites (black arrow). M: another view showing mild intra-luminal infestation by calcified parasites (black arrow), with partially necrotic villi (red arrow), infected, treated by extract 100 mg/kg-loaded CSNPs (GVIII). N: intestinal wall showing mild intra-luminal and intra-mucosal infestation by calcified parasites (black arrow). O: another view showing mild intra-mucosal infestation by calcified parasites (black arrow), with partially necrotic villi (red arrow).

Fig. 5: (H&EX 200B, D, F, H, J, L and N) &(H&E X 400 C, E, G, I, K, M and O).

Histopathological changes in the skeletal muscle sections of different experimental groups (H&E at high power view X (200 ×, 400 ×) Figs.6 (A-O) Non-infected and non-treated (negative control) (GI) exhibited typical muscle cells with no inflammatory response Fig. 6(A). Positive control (GII) muscles of the skeletal system showed high parasite infection with moderate inflammatory infiltration, and muscle fibres that have become partly necrotic Fig.6(B,C). Albendazole drug (GIII) skeletal muscles showed no parasitic infestation,
and scattered partially necrotic muscle fibers Fig. 6 (D,E). Infected, treated by CSNPs (GIV) muscles of the skeletal system showed heavy infestation by viable parasites with marked inflammatory infiltrate, and partially necrotic muscle fibers Fig. 6 (F,G). Infected, treated by extract 50 mg/kg (GV) muscles of the skeletal system showed average heavy infestation by viable parasites with mild inflammatory infiltrate, and partially necrotic muscle fibers Fig. 6 (H,I). Infected, treated by extract 100 mg/kg -loaded CSNP (GVII) skeletal muscle showed mild infestation by viable parasites with mild inflammatory infiltrate, and partially necrotic muscle fibers Fig. 6 (J,K). Infected, treated by extract 100 mg/kg (GVIII) muscles of the skeletal system revealed severe parasite infestation, a moderate inflammatory infiltration, and partially necrotic muscle fibres. Fig. 6 (L,M). Infected, treated by extract 100 mg/kg-loaded CSNPs (GVII) skeletal muscle showed mild intra-luminal and intra-mucosal calcified parasites, and partially necrotic villi Fig. 6 (N,O).

![Image of muscle samples](image-url)

Fig. 6 (A). Non-infected and non-treated (negative control) (GI) skeletal muscle showing average muscle fibers with distinct borders (black arrow) and peripherally located nuclei (blue arrow), and average interstitium with average blood vessels (red arrow), positive control (GII). B: skeletal muscle showing heavy infestation by viable parasites (black arrow) with partially necrotic muscle fibers (red arrow), C: another view showing heavy infestation by viable parasite (black arrow) with mild inflammatory infiltrate (blue arrow), and partially necrotic muscle fibers (red arrow), albendazole drug (GIII). D: skeletal muscle showing no parasitic infestation (black arrow), and scattered partially necrotic muscle fibers (blue arrow), E: view showing no parasitic infestation (black arrow), and scattered partially necrotic muscle fibers (blue arrow), infected, treated by CSNPs (GIV). F: skeletal muscle showing heavy infestation by viable parasites (black arrow), partially necrotic muscle fibers (red arrow), and marked inflammatory infiltrate (blue arrow), G: another view showing heavy infestation by viable parasites (black arrow), partially necrotic muscle fibers (red arrow), and marked inflammatory infiltrate (blue arrow), infected, treated by extract 50 mg/kg (GV). H: skeletal muscle showing heavy infestation by viable parasites (black arrow), partially necrotic muscle fibers (blue arrow), infected, treated by extract 50 mg/kg -loaded CSNP (GVI) skeletal muscle showed mild infestation by viable parasites (black arrow), partially necrotic muscle fibers (blue arrow), and mild inflammatory infiltrate (red arrow), infected, treated by extract 50 mg/kg extract-loaded CSNP (GVI). J: skeletal muscle showing mild infestation by viable parasites (black arrow), partially necrotic muscle fibers (blue arrow), and mild inflammatory infiltrate (red arrow) (H&E X 400), infected, treated by extract 100 mg/kg (GVII). L: skeletal muscle showing heavy infestation by viable parasites (black arrow), partially necrotic muscle fibers (red arrow), and mild inflammatory infiltrate (red arrow) (H&E X 200). M: another view showing heavy infestation by viable parasites (black arrow), partially necrotic muscle fibers (blue arrow), and mild inflammatory infiltrate (red arrow), and infected, treated by extract 100 mg/kg-loaded CSNPs.
Histopathological alterations in distinct experimental groups’ heart portions (H&E at high power view X (200 ×, 400 ×) Figs. 7(A-O). Non-infected and non-treated (negative control) (GI) showed normal heart A wall displaying live cardiac muscle fibres with clear cell boundaries, with a centre oval. average blood vessels and no histopathological changes were observed Fig. 7(A). Positive control (GII) heart showed scattered calcified ova with mildly necrotic muscle fibers, and mildly congested blood vessels Fig. 7(B,C). Albendazole drug (GIII) heart showed no parasitic infestation, mildly congested blood vessels, and scattered partially necrotic muscle fibers Fig. 7(D,E). Infected, treated by CSNPs (GIV) heart showed scattered calcified ova with partially necrotic muscle fibers, and mildly congested blood vessels Fig. 7(F,G). Infected, treated by extract 50 mg/kg (GV) heart showed average blood vessels (black arrow) with partially necrotic muscle fibers Fig. 7(H,I). Infected, treated by extract 100 mg/kg (GVII) heart showed partial infestation by viable parasites with partially necrotic muscle fibers Fig. 7(J,K). Infected, treated by extract 100 mg/kg-loaded CSNP (GVIII) heart showed heavy infestation by viable parasites with partially necrotic muscle fibers Fig. 7(L,M). Infected, treated by extract 50 mg/kg-loaded CSNP (GVIII) heart showing heavy infestation by viable parasites (black arrow) with partially necrotic muscle fibers.
(blue arrow), K: another view demonstrating significant parasite infestation (black arrow) with partially necrotic muscle fibres (blue arrow), infected, treated by extract 100mg/kg (GVII). L: heart wall showing partially muscular fibres (black arrow), M: another view showing partially necrotic muscle fibres (black arrow) and infected, treated by extract 100mg/kg-loaded CSNPs (GVIII). N: heart wall showing mildly congested blood vessels (black arrow) with partially necrotic muscular fibres (blue arrow) and O: another view showing partially necrotic muscular fibres (blue arrow).

Fig. 7: (H&EX 200B, D, F, H, J, L and N)&(H&E X 400 C, E, G, I, K, M and O).

7. Discussion
Trichinellosis was a prevalent zoonose for which anthelmintics such as albendazole were commonly used to treat human trichinellosis, despite the fact that it was not completely effective [43]. Albendazole medication is safe for both adults and children; however, it is not suggested for pregnant women or children under the age of two [44, 45]. There is a growing desire for reliable and efficient drugs, especially those derived from herbs, which are less harmful and have few adverse effects [46]. (Zingiber officinale var. rubrum) extract has been found to have a therapeutic impact by enhancing DNA repair, antioxidants, and lowering lipid peroxidase [47]. The goal of this study was to evaluate the effect of Zingiber officinale extract-loaded CH NPs versus various stages of T. spiralis in mice to that of albendazole, a commonly used commercial medicine, and a positive control. The current investigation discovered a substantial decrease in the average count of adult warms and larvae in all treated groups. The current study found that GVI, which received Zingiber officinale extract loaded CS NPs (50mg/kg), the average number of adultworms and larvae has decreased significantly (71.80% and 74.82%), followed by GVIII, which received Zingiber officinale extract loaded CS NPs (100mg/kg) (59.26% and 62.47%) reduction, while GVII received Zingiber officinale extract (100mg/kg), GV obtained Zingiber officinale extract (50mg/kg) and had the lowest effectiveness (Table 1). These results agreed with [48, 49], T. spiralis worms were completely eradicated in albino rats after treatment with albendazole, and disagreed with [50]. In all treated groups, the average number of adult worms and larvae decreased. However, it was discovered that albendazole had a reduced efficiency against encysted larvae with a reduction rate of 65.2% [51]. The current study found a highly significant (p<0.001) in all treated groups, the average number of adult worms and larvae decreased when compared to the positive control. The current findings revealed a substantial p<0.001 decrease in terms of average count of larvae and worms throughout all treatments groups with Z. officinale extract loaded CHNPs (50 and 100 mg/kg), followed by Z. Officinale extract at doses of (100 and 50 mg/kg) in comparison to the positive group. The chitosan results were consistent with [52]. Who discovered that chitin and chitosan MPs induced an immune response in BALB/c mice infected with L. major, the findings coincided with [53], who stated that chitosan microcrystals appear to be the most effective choices for improving absorption through the mouth of the active pharmaceutical substance. Furthermore, [54] revealed that microencapsulated formulations (based on chitosan particles of varying concentrations) were devised to improve the solubility rate of albendazole in mice infected with T. spiralis during the parasite cycle’s intestinal phase. However, the results were lower than those obtained by [55].
chitosan particles reduced both egg counts and adult and in mice infected with Hymenolepis nana (77% for eggs and 95% for adults). The current findings opposed the findings of [20], who discovered no significant decrease in parasite number in infected individuals treated with chitosan nanoparticles in the course of treatment of murinetoxoplasmosis compared to controls. Also, [14]. T. spiralis adult worms and larvae were identified in Z. officinale (64.5%, 68%), whereas Strongyloides ransomi, the treatment with (25g/kg body weight) ginger showed the highest FECR (92.6%), [56]. In addition, antiprotein properties of Z. officinale (ginger) extract against Giardia lamblia trophozoites were discovered[57]. T. spiralis larvae showed resistance to infection in Curcuma and Pomelo-treated groups (70.9% & 70.9%, respectively). T. spiralis adult worms, on the other hand, showed resistance to infection in Curcuma and Pomelo-treated groups (94.13% and 80.2%, respectively)[58]. Zingiber officinale's parasiticidal impact may be related to its anticholinergic effect [59], as acetylcholine is a neurotransmitter in nematode muscles[60]. Furthermore, its antiparasitic action could be attributed to its powerful proteolytic enzyme "zingibain" [61]. Many parasitic and protozoan illnesses are caused by lipid peroxidation, which produces free radical species [62,63]. In biological systems, preventing lipid peroxidation is critical. All oxygen-metabolizing cells have cellular antioxidant defence systems to prevent the damaging effects of [64]. In aerobic organisms, superoxide dismutase is an important physiological antioxidant defence system. By detoxifying hydrogen peroxide, this enzyme avoids the production of the hydroxyl radical[65]. SOD, GPx, and TAS are enzymes called antioxidants which aid our bodies fight free radicals[66]. GPx and SOD are two of the more essential antioxidant defensive molecules, preventing the creation of new free radical species by converting existing free radicals into less damaging molecules prior to their reaction, or by suppressing free radical synthesis from other molecules.

GSH is a non-protein thiol source that are able to act as a substrate for glutathione peroxidase and have been shown to be a functional anti-oxidative molecule in a variety of tissues [67]. GSH shields the cell from the harmful effects of both endogenous and external oxidants [68]. The present investigation agrees with [58], since there was a significant rise in SOD3 and GSH concentrations in the serum of every group treated were compared to a positive control. According to the research, there was a substantial rise in SOD3 and GSH levels in serum of mice infected with T. spiralis who were treated with Curcuma and Pomelo NPs extract compared to the control group. In previous investigations, it was discovered that NPs increased GSH concentrations in serum in mice[69,70]. The decrease in erythrocyte catalase, SOD, and GSHPx levels may be related to parasite infection and malnutrition, which resulted in increased oxidative stress and likely caused erythrocyte hemolysis [71]. The reduction in SOD activity seen in our investigation could be attributable to parasitemia and oxidative stress [63]. Level of SOD and GSH in untreated infected mice were much higher than in infected treated animals. Following treatment of the tested extract, serum SOD levels increased significantly in the 100 and 50mg/kg CS NPs. On the 35th day pi, extract 50 and 100mg/kg CS NPs showed a considerable rise in GSH and returned to normal. However, animals treated with 50 mg/g extract and 100 mg/kg loaded chitosan nanoparticles had the greatest decrease in inflammation and larval deposition, as well as the highest SOD and GSH levels of all treatment groups.
The obtained results revealed that the extracts loaded CSNPs had a greater immunological effect that could considerably boost SOD antioxidant towards normal condition. Although the current investigation found that SOD levels in the serum of T. spiralis infected and treated mice were higher, [72,73]. SOD levels were observed to be decreased in the serum of Toxoplasma infected mice. An overabundance of radicals in the tissue was thought to be the primary reason of reduced enzyme function [58]. They discovered that SOD3, concentrations in Kumquat--loaded chitosan nanoparticles treated mice increased significantly in comparison to the positive control. The NPs can pass through capillaries and be absorbed by cells, allowing them to permeate tissues. Trichinella larvae induce a severe inflammatory reaction after piercing skeletal muscle tissue, which is responsible for the associated myopathy [74]. Histopathological examinations of the small intestine, skeletal muscle, and heart of distinct experimental group sections demonstrated an improvement in histopathological alterations with both treatments, including Reduced in varying degrees of histopathological abnormalities, including inflammatory cellular infiltration, necrotic villi, calcified ova, necrotic muscle fibres, enhanced regenerative muscles, and larval deposition are all examples. MBZ was more effective than the other extracts examined. Many prior investigations have validated these histological findings, indicating that certain trichinicidal drugs are more effective when given at an early stage [75,76]. The tested extract caused moderate inflammation in the majority of infected mice, but the treated animals with Zingiber officinale extract loaded CSNPs group100mg/Kg. and Zingiberofficinaleextract50mg/Kg demonstrated the highest reduction in inflammation and larvae accumulation of any treatment group, which agrees with AbouRayia’s team [5]. This could be due to the fact that CS-based microcrystals seems to be the best option for better oral absorption [47]. Controlling inflammation inhibited the release of systemic cytokines from activated immune cells, which improved symptoms such astissue edema, fever and vasculitis. [70]. In addition, Abdel Latif’s findings were consistent with the most recent CS discoveries [72]. CS enhanced intestinal morphometric measurements and in mice infected with H.nana, They returned the alterations to about normal length and width, in addition to the crypts. 8. The abbreviations:

- ANOVA: Analysis of variance
- CAT: Catalase
- CS: Chitosan
- E: Eosin
- GPSS: peroxidases of glutathione
- GSH: Glutathione
- H: Hematoxylin
- NPs: Nanoparticles
- Pi: Post infection
- ROS: Reactive oxygen species
- SABC: Streptavidin-biotin complex
- SE: Standard error
- SEM: Scanning electron microscopy
- SOD3: Superoxide dismutase 3
- TBRI: Theodore bilharz research centre
- TEM: Transmission electron microscopy
- TMB: Tetramethylbenzidine
- TPP: Tripolyphosphate

Conclusion

Zingiber officinal eextract loaded CSNPS and Zingiberofficinale extract groups are well tolerated and safe and have anti-inflammatory and therapeutic effects on T. spiralis infection. These extracts could be promising treatments for trichinellosis.

Financial assistance. This study got no explicit funding from any commercial or not for profit source. No conflict of interest exists.
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