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Original article

Histological changes in the liver of mice infected with *Schistosoma mansoni* and treated with chitosan nanoparticles loaded with mefloquine

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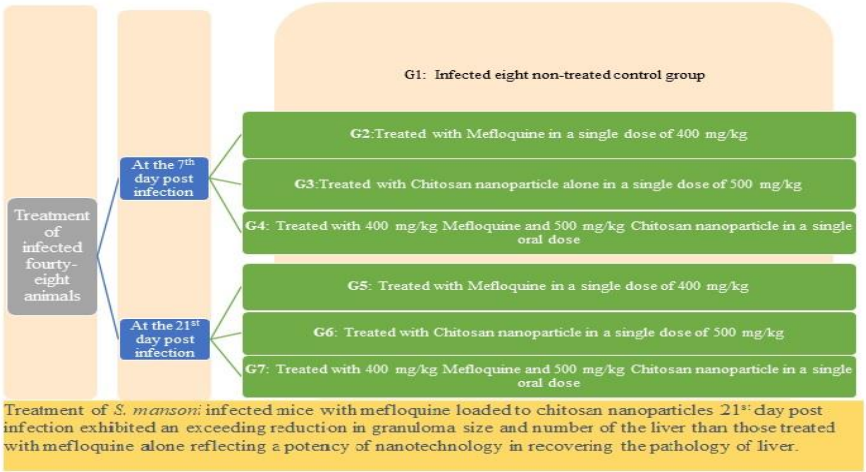
ABSTRACT

The antimalarial drug mefloquine (Mef) has shown an evident antischistosomicidal activity. The most preferable way of drug administration is through the oral route through which the total absorption of the drug is limited due to enzymatic degradation and the physiological barrier PH. Thus, the current investigation was conducted to assess the potential impact of Mef and chitosan nanoparticles (CS-NPs), which is known for its importance as a drug delivery structure, on the histological variations of liver that were induced in mice infected with *Schistosoma mansoni.* Fifty-six mice were grouped into seven separate collections with eight mice in each group; **G1** (positive non-treated control mice). At the 7th day post infection (PI), the groups were divided as follows: **G2** (Mef dosage 400 mg/kg), **G3** (CS-NPs dosage 500 mg/kg), **G4** (Mef dosage 400 mg/kg with CS-NPs dosage 500 mg/kg). At the 21st day PI, the groups from **G5-G7** were treated with the same previous doses respectively. The whole animals were managed to be sacrificed on the 56th day PI. Treatment with 400 mg/kg Mef at days 7 and 21 PI has reduced the granuloma diameter by 24.85% and 69.65% respectively, while Mef loaded on CS-NPs at days 7 and 21 PI showed a reduction in granuloma diameter by 57.18% and 100% respectively. Meanwhile, Mef loaded on CS-NPs treatment has reduced the number of granulomas in the liver. In conclusion, treatment of Mef loaded on CS-NPs is more effective than free Mef especially when administered 21 days PI.

Graphical abstract



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1. Introduction

Schistosomiasis, an intravascular parasitic disease prevalent worldwide, is acquired by the trematode flatworm *Schistosoma* with estimations of 237 million individuals worldwide infected; another 600–779 million are in danger of being subjected to contagion [1, 2, 3].

Even though praziquantel (PZQ) is still used officially as an effective and safe antischistosomal medication, there are reasons why new drugs against schistosomiasis must be discovered and developed [4,5].

So, its extensive use in World Health Organization (WHO)-recommended mass drug administration, which is a serious means for the evolution of PZQ-resistant schistosome strains [6], its restricted spectrum of action where a second dosage must be administered directly after a short period to remove parasites that have matured since it does not perfectly eliminate the premature stages of *Schistosoma* [7], and the low water solubility that affects its absorption, necessitating higher doses - and eventually more risks - to achieve the desired effect [8].

Since schistosomiasis and malaria are co-endemic in many parts of Africa, it was suggested to test the antimalarial drug mefloquine (Mef) for its antischistosomal possibilities [9]. Recently, Mef has been presented with substantial potency in countering schistosomes, exhibiting a decrease in the worm load percentage [10, 11, 12]. In vitro, the 4-quinolinemethanol derivative or arylaminoalcohol mefloquine (Lariam, Mephaquin, or Mefliam) appears less successful in mature forms than in immature stages [13]. Scanning electron microscopic findings indicated widespread tegumental damage in mature and immature stages subjected to Mef including enlargement, retraction and wrinkling [14]. Since nanoparticles can deliver toxic components or pharmaceuticals to the target pathogen without hurting or damaging the host tissues by enhancing medication pharmacokinetics [15], it was recommended to test the synergistic effect of Mef and chitosan nanoparticles (CS-NPs) on the liver of mice infected with *Schistosoma mansoni*.

2. Materials and Methods

Experimentation was accomplished at Theodor Bilharz Research Institute (TBRI) where the treatment and handling of mice conformed with the international principles of research ethics adopted by the institute.

2.1. Animals

Fifty-six female pathogen-free mice, C57BL/ 6 strain, had similar ages of 42-56 days old with approximately 18-20 grams weight were used in this study. The mice were maintained in cages of a nonporous, non-opaque plastic material which is suitable for readily viewing mice and can be easily sanitized. The cages were kept in an air-conditioned animal room where temperatures ranged between 20–25ºC, and the mice were supplied with a standard pelleted diet.

The mice were randomly separated into seven groups each of which had eight mice. 0.1 ml of *S. mansoni* cercarial suspension, attained from TBRI, was pipetted in a small petri-dish where the numbers of cercariae were determined. Infection of mice was done subcutaneously with one hundred *S. mansoni* cercariae per mouse [16] which were provided by the SBSP, TBRI.

2.2. Drugs and dose

2-2-1 Mefloquine (Mef):

(Mephaquin) tablet (Mepha Ltd., Aesch Basel, Switzerland) lot 0850074) was used in a carrier of 3% (v/v) Ethanol and 7% (v/v) Tween 80[10].

2-2-2 Chitosan nanoparticles (CS-NPs):

A 93% deacetylated chitosan was obtained from Sigma Aldrich, USA (Batch no. 419419). Construction of CS-NPs was through using the method of ionotropic gelation [17].

2.3. Research plan

- Animals were divided as follows:

G1: Infected untreated control mice.

On the 7th day of PI, the groups were divided as follows:

G2: Animals were given a 400 mg/kg dosage of Mef.

G3: Animals were given a 500 mg/kg dosage of CS-NPs.

G4: Animals were given 400 mg/kg Mef with 500 mg/kg CS-NPs.

On the 21st day of PI, the groups were divided as follows:

G5: Animals were given a 400 mg/kg dosage of Mef.

G6: Animals were given a 500 mg/kg dosage of CS-NPs.

G7: Animals were given 400 mg/kg Mef with 500 mg/kg CS-NPs.

2.4. Histopathological examinations

Specimens of the hepatic tissue of all mice were settled in buffered formalin for twenty-four hours for fixation followed by washing under flowing water. After washing, they were dipped in ethyl alcohol in increasing concentrations, and then cleared and purified in xylene and inserted in paraffin wax. Each block of paraffin was segmented into sections of 4-6 μm and then 5 parts had been removed for Haematoxylin & Eosin [18], and Masson's trichrome staining [19].

With an ocular micrometer, the mean diameter of *Schistosoma* granulomas was measured in microns in each hepatic section and the size was measured by obtaining the lesion in two right- angled to one another diameters: firstly, the largest of which has been taken where rotation 90^{o} of the ocular micrometer was done and the largest diameter vertical to the early one has been obtained. The 10 largest granuloma diameters in each hepatic section were taken for the calculation of the average granuloma diameter for each mouse group [20].

3. Results

The antischistosomal efficacy of the antimalarial drug Mef alone or in combination with CS-NPs on granuloma is summarized in Tables 1 and 2.

In all mice groups, granulomas consisted of ova with miracidia which were surrounded by plasma cells and

lymphocytes where worms were ringed with cellular permeation in portal tracts.

As demonstrated in Table 1, there was an increase in the ratio of degenerated eggs and a decrease of intact eggs` ratios in treated mice with a complete absence of granulomas and ova in the groups of Mef loaded to CS-NPs 21 days PI.

Table 2 shows that the treated group with Mef loaded on CS-NPs has the greatest decline in granuloma count at day 21 when compared with the afflicted untreated group, Mef or CS-NPs treated mice. On days 7 and 21, it was displayed an expressing decrease in granuloma count in Mef mice comparable to the afflicted non-treated mice. An insignificant decline in granuloma count in the CS-NPs mice group was demonstrated at day 7 when compared with the afflicted mice group and significantly relative to the control group at 21 days PI.

The results showed that treatment with Mef loaded on CS-NPs has the greatest decline in hepatic granuloma diameter at day 21 comparable to the afflicted untreated group, Mef or CS-NPs treated mice. The lowest reductions in hepatic granuloma diameter were in mice with CS-NPs at days 7 and 21 when compared with the afflicted untreated group.

Liver sections in mice from G1 to G6 showed the portal tracts including numerous granulomas where the worms are enclosed and bounded by the cellular infiltration as shown in **Fig. 1** to **Fig. 6**. The granuloma is composed of an intact ovum which has a miracidium bounded by macrophages, eosinophils, neutrophils and histocytes. Liver section in mice from the group Mef loaded on CS-NPs at day 21 PI (G7) showing no hepatic granuloma as shown in **Fig. 7**.

4. Discussion

Schistosomiasis is a devastating parasitic sickness rising due to the flatworm *Schistosoma* that influences nearly 240 million annually which is treated as one of the three greatly important human diseases in the Caribbean, Asia, Africa and South America [21]. Over 200,000 individuals die due to schistosomiasis globally each year, and in 2021, roughly 251,4 million people required treatment for prevention [22].

The primary therapy for adult *schistosoma* worms and their juvenile lesser forms is still praziquantel (PZQ); nevertheless, it cannot prevent reinfection and has certain undesirable side effects, allergic reactions, and hypersensitivity reactions [23]. In addition, due to the emergence of resistant parasites in the programs conducted for controlling schistosomiasis through PZQ medication, there is a persistent need for evolving new drugs [24].

There were programs of studies that have shown that several quinoline antimalarial medications have antischistosomal characteristics and features, where the highest reduction of *S. mansoni* worm's rates have been recorded in Mef [14]. According to the investigators, Mef which is used for the prevention of malaria has also shown satisfactory results against *S. haematobium* [25].

Nanoparticles are heavy particles that range in size from 10 to 100 nm and can be made in a variety of ways. Nanostructures, which are considered nanomachines, were shown in several studies to aid oral drugs and genetic elements in reaching the targeted sites of the organism, increasing the drug bioavailability and solubility, and decreasing its toxicity [26, 27].

Some of the characteristics that set nanoparticles apart from conventional drug delivery systems involve their outstanding stability and specificity, ability to use multiple administration routes, and capability for controlled release of drugs [28].

Recent research on the antiparasitic properties of nanoparticles *in vivo* and *in vitro* has produced encouraging findings about the use of nanoparticles in the management of parasitic illnesses [29, 30, 31, 32, 33].

Chitosan, a naturally occurring mucoadhesive, cationic biocompatible polymer which is formed by the extraction of acetate moiety of chitin, is a diffusion booster that facilitates drug conveyance through intercellular and paracellular routes by loosening the epithelium`s tight connections, so lowering the quantities of drug used and their adverse effects [34, 35].

The present investigation is directed to estimate the performance of medication transmitting procedure through nanotechnology in encountering schistosomiasis with the aid of chitosan nanoparticles with Mef to assess and analyze the effectiveness of Mef either alone or in combination with chitosan for use as a therapy in curing the disease. This evaluation is carried out by histological investigations in the liver of mice.

Histopathological alterations in the infected hepatic tissue due to schistosomiasis originate from *Schistosoma* ova which are tucked into the presinusoidal gaps of the liver stimulating around these eggs a granulomatous inflammatory reaction [36]. The formation of granuloma as an immune response serves a defensive purpose [37]. Treatment with effective schistosomicidal medications destroys the worms and hence their egg production is halted. In the meantime, these medicines may influence the host`s immune system`s reaction to ova [38].

Mef prohibits haem crystallization to a darkened brown colour that is named haemozoin [39]. This represents a considerably important action of detoxification in *Schistosoma* that performs a type of antioxidant prophylactic defense role [40].

In the present study, examination of the hepatic histopathology in mice dealt with Mef loaded on CS-NPs at day 21 exhibited the greatest percentage in granuloma diameter and count decrease (100%, 100%) respectively where the histopathological alterations were quite little and the structure of the liver was nearly natural while Mef at day 21, the granuloma diameter and count decrease ratios were (69.65%, 88.2%) respectively.

Mice dealt with Mef loaded to CS-NPs at day 7 displayed ratios of decrease in granuloma diameter and count (57.18%, 73.1%) respectively that are greater than the decrease ratios of granuloma diameter and count in Mef dealt mice at day 7 (24.85%, 67.9%) respectively. In accordance with the present findings, **El-Lakkany et al. (2011)** reported that Mef therapeutic has diminished the granuloma diameter reaching 26.26% [41]. The reduction in the diameter of granuloma may be clarified briefly by granulomatous hypersensitivity alterations that result from a considerable lack of the

tissue reaction to soluble antigens of ova that was generated as a consequence of Mef influence on ova count.
Treatment with free CS-NPs at 21 days PI showed a significant relative reduction ($P<0.05$) in liver granuloma number (31.6%) and a moderately significant difference ($P<0.01$) in liver granuloma diameter (10.61%). In free CS-NPs dealt mice at day 7, the percentage of granuloma count decrease was insignificant (12.8%). In accordance with these results, **Elawamy et al. (2019)** demonstrated a reduction in granuloma number and diameter, so validating the function of chitosan nanoparticles in mitigating the harm done to the liver [42].

Decreased granuloma numbers may be referred to the mature stages removal through the use of drugs that cause a low number of deposited ova which results in prompting the immunopathology [43].
It was concluded that Mef loaded on CS-NPs is exceedingly efficient than Mef alone as regards of the hepatic granuloma formation in *S. mansoni*. This may be due to the increasing bioavailability of CS-NPs enclosed medications in the host and improved absorptive consumption through the *S. mansoni* membrane.

5. Conclusion

Based on the output of the present study, mefloquine loaded with chitosan nanoparticles established a potency in recovering the pathology of the liver. Using mefloquine loaded to chitosan nanoparticles in the treatment of *S. mansoni* is preferable and more effective than mefloquine alone since the histopathological examinations of the liver showed an exceeding decrement of granuloma in both number and diameter during the treatment by Mef loaded to CS-NPs three weeks PI than in those treated with Mef three weeks PI.

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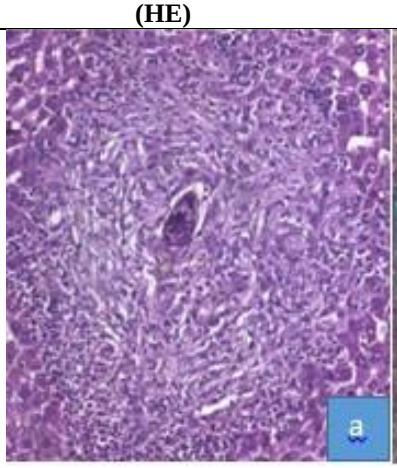
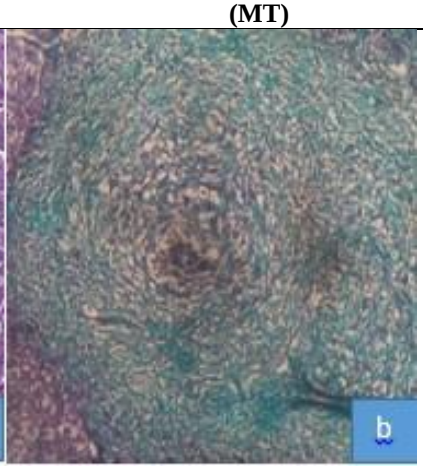
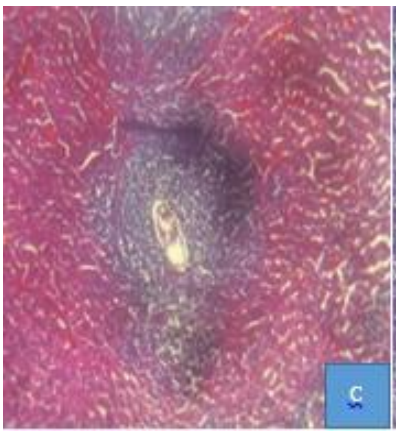
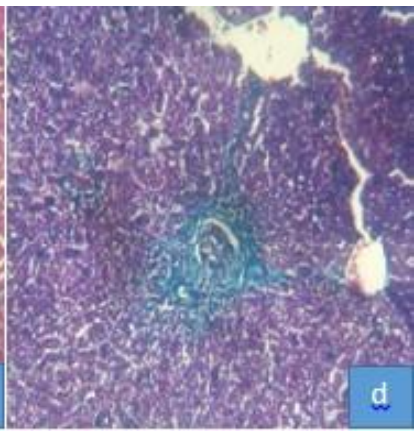
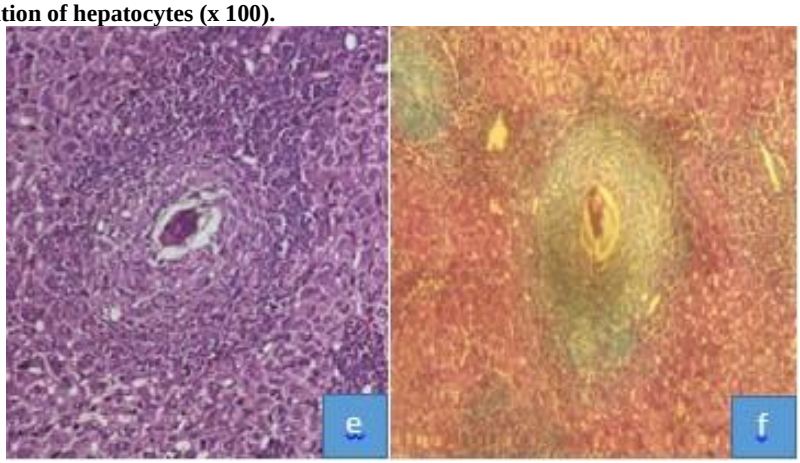
Declaration of conflict of interest: There are no conflicts to declare.

Animal groups	Granuloma diameter	Haematoxylin & Eosin staining (HE)	Masson`s Trichrome staining (MT)
Control infected (G1) fig. 1	359.12+12.9	 **a)Mice hepatic section (G1) displaying a considerable granuloma consisting of a tissue filled with eosinophil, and an egg with clearly visible undegenerated miracidium (x 100).**	 **b) Mice hepatic section from the positive control group (G1) showing large fibro cellular granuloma exhibiting ovum and many epithelioid cells with fibrous tissue(x 100).**
Mef 7 days PI. (G2) fig. 2	269.87+22.37	 **c) Liver section in mice from the group Mef after 7 days (G2) showing a granuloma with decreased diameter and mild hydropic de-**	 **d) Liver section in mice from the group Mef after 7 days (G2) showing a granuloma with decreased diameter (x 100).**

generation of hepatocytes (x 100).

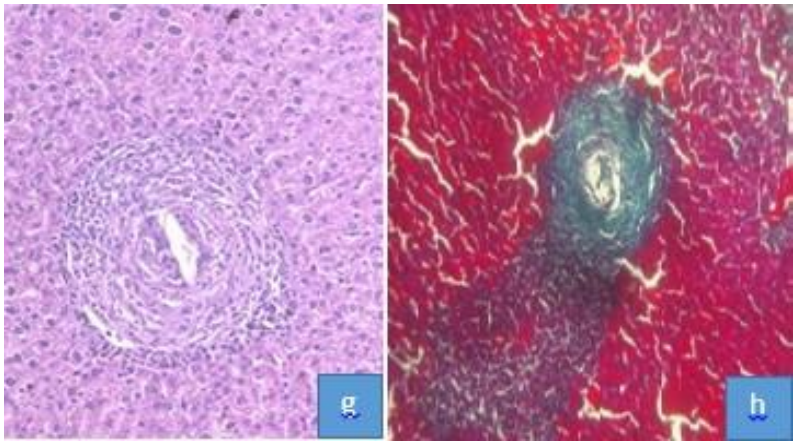
CS-NPs 7 days PI. (G3) fig. 3 319.1+15.52



e) Liver section in mice from the group of CS-NPs 7 days (G3) showing a tiny fibrocellular granuloma displaying a noticeably diminished miracidium, and the outline of granuloma is consistent and obviously distinguishable out of the adjacent cells (x 100).

f) Liver section in mice from the group of CS-NPs 7 days (G3) showing large fibrocellular granuloma surrounding intact ovum and encircled by scattered lymphocytes (x 100).

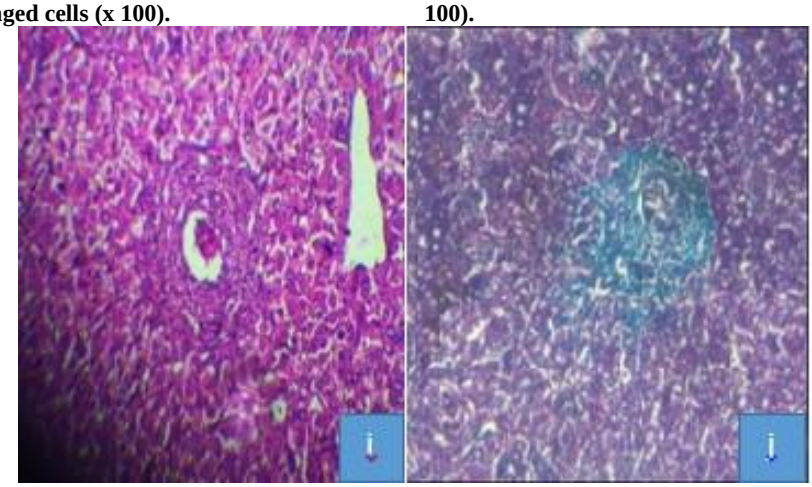
Mef loaded to CS-NPs 7 days PI. (G4) fig. 4 153.77+35.14



g) Liver section in mice from the group of Mef + CS-NPs 7 days (G4) illustrating a fibro cellular granuloma containing inflammation which is displayed as closely arranged cells (x 100).

h) Mice hepatic section from the group of Mef + CS-NPs 7 days (G4) showing a small demarcated granuloma with less inflammatory cells and dead ovum in the center (x 100).

Mef 21 days PI. (G5) fig. 5 109+23.27



i) Liver section in mice from the group Mef after 21 days (G5) showing a tiny fibrocellular granuloma displaying a noticeably diminished response. The outline of granuloma is consistent and distinguishable out of the adjacent cells (x 100).

j) Liver section in mice from the group Mef after 21 days (G5) displaying a diminutive fibro cellular granuloma in addition to the beginning of a degenerative process of the egg (x 100).

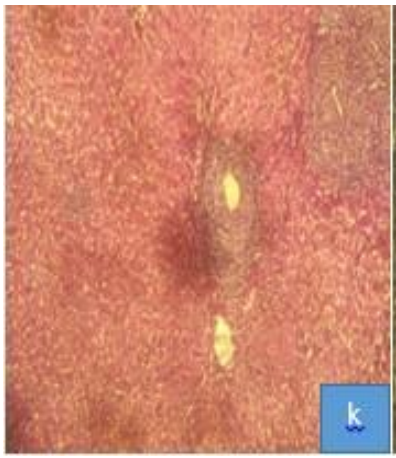
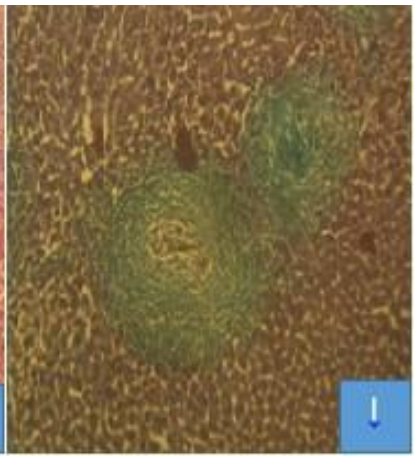
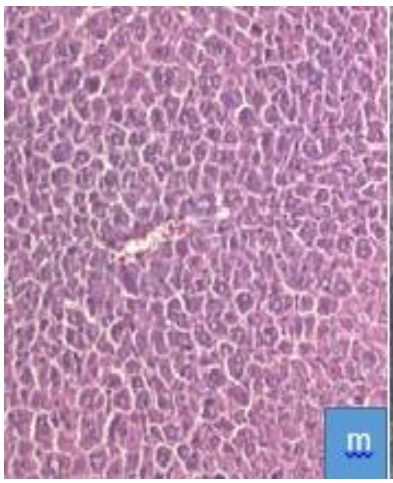
CS-NPs 21 days PI. (G6) fig. 6	221+25.47	 **k) Liver section in mice from the group Mef + CS-NPs after 21 days (G6) showing a small well-demarcated granuloma with less inflammatory cells and a degenerated ovum in the center (x 100).**	 **l) Liver section in mice from the group CS-NPs only after 21 days from infection (G6) showing more than one fibrocellular granuloma each of which consists of intact ovum and encircled congested blood vessels (x 100).**
Mef loaded to CS-NPs 21 days PI. (G7) fig. 7	0	 **m) Liver section in mice from the group Mef after 21 days (G7) showing no hepatic granuloma (x 100).**	 **n) Liver section in mice from the group Mef after 21 days (G7) showing no granuloma with hydropic degeneration of the hepatocytes (x 100).**

Table (1): The types of granuloma and the form of ova during treatment with Mef † ± CS-NPs‡ 7 and 21 days PI§.

Infected animal groups	**Granuloma sort**			**Ova condition %**	
	cellular %	**fibrocellular %**	**fibrous %**	**entirely intact**	**degenerated**
(G1) controls	22	76	2	89	11
(G2) Mef 7 days PI.	30	70	0	52	48
(G3) CS-NPs 7 days PI.	24	76	0	78	22
(G4) Mef loaded to CS-NPs 7 days PI.	35	65	0	45	55
(G5) Mef 21 days PI.	21	79	0	30	70
(G6) CS-NPs 21 days PI.	25	75	0	70	30
(G7) Mef loaded to CS-NPs 21 days PI.	0	0	0	0	0

† Mef stands for mefloquine
‡ CS-NPs stands for chitosan nanoparticle
§ PI stands for post-infection

Table (2): The granuloma diminution in count and diameter during treatment with Mef ± CS-NPs at days 7 and 21.

Infected animal group	Granuloma diameter in µm (M ± S.D)	Reduction %	No. of granulomas in successive power fields (10x10) (M ± S.D)	Reduction %
(G1) controls	359.12 ± 12.9	_	10.62 ± 2.91	_
(G2) Mef 7 days PI.	269.87 ± 22.37***	24.85	3.41 ± 1.4***	67.9
(G3) CS-NPs 7 days PI.	319.1 ± 15.52***	11.14	9.26 ± 0.14	12.8
(G4) Mef loaded to CS-NPs 7 days PI.	153.77 ± 35.14***	57.18	2.86 ± 1.07***	73.1
(G5) Mef 21 days PI.	109 ± 23.27***	69.65	1.25 ± 0.18***	88.2
(G6) CS-NPs 21 days PI.	221 ± 25.47**	38.46	7.26 ± 2.11*	31.6
(G7) Mef loaded to CS-NPs 21 days PI.	0 ± 0***	100	0 ± 0***	100

***** Referring to strongly significant difference (P < 0.001) in comparison to positive non-treated mice (G1).**
**** Referring to medium significant difference (P < 0.01) in comparison to positive non-treated mice (G1).**
*** Referring to relatively significant (P < 0.05) in comparison to positive non-treated mice (G1).**

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