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Parasitological and Immunological Study of the Effect of Chitosan Nanoparticles and Paromomycin on Giardiasis

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Abstract

Giardia lamblia is one of the most common protozoan causes of giardiasis and a common health problem in developing countries worldwide. Infected male albino rats with giardiasis were used in this study to assess the parasitological and immunological response to the efficiency of chitosan nanoparticles loaded with Paromomycin. After rats were orally inoculated with 10^5 viable cysts/rat for acute infection, the experimental group was infected and treated for eight days with either paromomycin alone or in combination with chitosan nanoparticles. *Giardia* cysts in the stool it is counted. Enzyme-linked immunosorbent assay (ELISA) kits were used to determine the immunological characteristics of gamma interferon (INF-γ) and tumor necrosis factor-alpha (TNF-α) cytokine response to antigens in serum samples after treatment. Examination showed that all treated infected rats had significantly fewer *Giardia* cysts than non-treated rats. In conclusion, the highest significant therapeutic effect was achieved by combining paromomycin with chitosan nanoparticles, as evidenced by the lower parasite numbers and amelioration of the immune status after treatment.

Keywords: ELISA, cytokines, chitosan nanoparticles, paromomycin, *Giardia*

1. INTRODUCTION

Giardiasis, which affects hundreds of millions of people, especially in developing countries, is caused by the flagellated parasite *Giardia lamblia*, also known as *Giardia intestinalis*. It colonizes and reproduces in the small intestine. Giardiasis is a part of the WHO's neglected diseases initiative [1]. Many outbreaks, especially those involving calves, are responsible for raw water contamination [2]. Giardiasis cannot be prevented by a human vaccination that is both effective and licensed, hence, the only treatment available is medication. Antimicrobial drug therapy is necessary for giardiasis, and 5-nitro heterocyclic drugs, especially metronidazole and nitazoxanide, are most frequently used [3]. Paromomycin (PM) is an aminoglycoside antibiotic with broad-spectrum antiprotozoal activity, known as aminocidin (injection) or gabromycin. Giardiasis is treated with oral doses of 30 mg/kg/day for at least 5 days in adults, adolescents, and children. Due to its apparent safety in that situation, it is usually the recommended treatment of giardiasis, at least during the first trimester of pregnancy [4]. Currently, more attention is focusing on natural active components from plants (herbal medicines and natural herbal extracts), animals, and other organisms that can prevent and treat many diseases, such as uses of Garlic (Allium sativum) as an antigiardial activity on white mice [5, 6]. Chitosan (CS), a polysaccharide (Chitin partially or completely deacetylated), has been used in medicine for the past 20 years. It is an essential factor in the synthesis of nanoparticles because it is non-toxic and biodegradable [7]. Because of their excellent drug delivery properties and confirmed efficacy in the composite nanoparticle form, chitosan nanoparticles are an alternative for loading [8]. To treat *Giardia lamblia* infection, chitosan was tested for the first time as an antiparasitic agent [9]. Chitosan nanoparticles (CsNps) have been the subject of numerous research studies for parasite infections. CsNps also enhanced the effectiveness of the anti-filarial medication ivermectin [10]. Additionally, acute *Entamoeba histolytica* with chitosan nanoparticles loaded with paromomycin was examined in the study of [11]. Previous studies have shown that Th1 and Th2 immune responses, characterized by the production of TNF-α and IFN-γ, are essential for protection against *Giardia* infection and have also demonstrated that the balance between these cytokines and the timing of their product was critical in the immune response to *Giardia lamblia* [12]. The present study aimed to investigate the anti-*Giardia lamblia* activity of chitosan and

paromomycin nanoparticles and chitosan nanoparticles loaded with paromomycin in experimentally infected rats.

2. Material and Methods

2.1 Sample Stool Collection

Diarrheal stool samples were taken from sick patients referred to several private laboratories. The samples were clear of other parasites and were analyzed microscopically using a direct wet saline smear. Before use, fresh samples are kept in a 2.5% potassium dichromate solution at 4 °C. The feces were suspended during inoculation, centrifuged, and the sediment cleaned three times before being suspended in phosphate saline (pH 7.4) containing antimicrobial penicillin and streptomycin. Rats were inoculated with about 10^5 viable cysts for an acute infection [13].

2.2 Drug

2.2.1 Paromomycin

Paromomycin is manufactured and purchased by a German company (PFIZER PHARMA PFE GmbH). Paromomycin was given orally once daily at 25-35 mg/kg/body weight for eight days.

2.2.2 Preparation of Chitosan Nanoparticles

Chitosan with deacetylated grade (93%) was manufactured and purchased from [Sigma-Aldrich-USA]. According to ionic coagulation and Nanoparticles, it was prepared [14].

Nanochitosan was orally given to rats at 20 mg/kg/body weight starting from the first day to the eighth day post-infection.

2.3 Experimental animal

The study was conducted on 40 male albino rats weighing 250±10 gm., at the biotechnology center at Al-Nahrain University. The animals were placed in plastic cages, fed a private provender obtained from the same place, and provided sterile water for drinking. Animals were divided into five groups; each group contains 8 rats as follows:

Group 1: Animals were given 0.1 ml of normal saline orally (control group).
Group 2: Animals were inoculated with 10^5 viable cysts (the infected control group).
Group 3: Infected and treated with chitosan nanoparticles.
Group 4: Infected and treated with paromomycin.
Group 5: Infected treated with chitosan nanoparticles loaded with paromomycin.
The program started from the first day to the eighth-day post-infection.

2.4 Animal scarification

All rats were quickly decapitated to complete the process of animal sacrifice. Each rat's blood was collected into clean tubes and centrifuged at 3000 rpm for five minutes. In the study of immunological parameters, the clear, non-hemolyzed supernatant serum was transferred to a clean tube and stored at -20 °C.

2.5 Immunological Research

Measurement of serum levels of gamma Interferon (INF-γ): Rat IFN gamma ELISA kit (ab2394425) (USA) uses our proprietary Simple Step ELISA® technology. Quantitate IFN gamma with 1.1pg/ml sensitivity. The manufacturer's recommendations for conducting the assay were followed.

Tumor Necrosis Factor (TNF-α) measurements: Rat TNF Alpha ELISA kit (ab2394425) (USA) uses our proprietary Simple Step ELISA® technology. Quantitate TNF Alpha with 1.1pg/ml sensitivity. The manufacturer's recommendations for conducting the assay were followed.

2.6 Analytical Statistics

In this study, analysis of variance (ANOVA) was utilized to compare means in a significant way. P< 0.05 was considered significant, and P< 0.01 was highly significant. Following is an evaluation of the reduction:

$$100\times \frac{\text{mean of infected control} - \text{mean of treated groups}}{\text{Mean of infected control}}$$

3. RESULTS

3.1 Giardia Cysts Count

In the studied infected groups, rats began to shed the cysts with feces on the third day after infection. Average number of G. *lamblia* cysts from the infected group of rats on the eight days after therapy, statistical analysis showed significant differences between all treated groups compared to the affected control group, P <0.05. The mean number of cysts was (11705 ±2976) in the infected untreated control group. Eight days after treatment with nanochitosan, the mean number of cysts output became (4015±499). The percent reduction in several G. *lamblia* cysts was (65.7%), which is statistically significant (P<0.05), while in group treatment by paromomycin, the mean number of cysts was (998±252), the percent reduction in the number of G. *lamblia* cysts was (91.4%), which give statistically significant (P<0.001). Combined therapy gives a better results than single therapy; in group treatment paromomycin with nanochitosan, the mean number of cysts was (326±310). The percentage was in the number of G. *lamblia* cysts (97.2%), which is statistically significant (P <0.001) (Table 1).

Table 1: Percentage reduction in the number of *Giardia* cysts per gram of stool between groups

Animals Group	Mean ± SD.	%Reduction in the number of cysts
Infected non treated	11705	0.0
Infected treated with nanochitosan	4015±499	65.7
Infected treated with paromomycin	998±252**	91.4
Infected treated with paromo mycin + Nanochitosan	326±310 **	97.2

Data are expressed as mean ± SD. * P< 0.05, ** P< 0. 01

3.2 Measurement of Immunological Parameters

3.2.1 Interferon Gamma (INF-γ)

Table (2) shows the INF-γ concentration in the infected control group's sera was (277.35± 4.12pg/ml) compared to the control group (179.72±2.43). Compared to the standard control group, all INF-γ levels increased both during the infection and after treatment. When compared to the infected group, the levels of INF- γ in the rat's groups treated with nanochitosan and paromomycin were respectively (394.51±3.21 pg. /ml) and (303.18±3.92 pg./ml); however, the group treated with nanochitosan - loaded paromomycin had the highest significant level (498.44±2.71pg/ml).

Table 2: The mean levels of INF-γ (pg/ml) in all studied groups

Animal Group	The mean level of INF-γ ± SD. (pg/ml)	% Elevation to infected control
Normal control	179.72±2.43	-
Infected Control	277.35± 4.12	-

Infected treated with Nanochitosan	394.51±3.21*	5.8%↑
Infected treated with paromomycin	303.18 ±3.92*	4.2%↑
Infected treated with Nanochitosan+ paromomycin	498.44±2.71**	40.1%↑

Data are expressed as mean ± SD. * P< 0.05, ** P< 0. 01

3.2.2 Tumor Necrosis Factor Alpha (TNF-α)

According to Table 3, the serum TNF-α mean levels in the infected control group was 2522.26 ± 20.26 pg/ml, differed from that of the control group, mean level TNF-α was 794.00 ±4.46 pg/ml.

TNF-α levels were found to be decreased in all treated groups compared to the infected control group.

Table 3: The mean levels of TNF-α (pg. /ml) in all studied groups

Animal Groups	Mean level of TNF-α ± SD. (pg./ml)	% Elevation to infected control
Normal Control	794.00 ±4.46	-
Infected Control	2522.26 ± 20.26	-
Infected treated with Nanochitosan	2311.27 ±42.00*	16.2% ↓
Infected treated with paromomycin	2441.61± 7.26*	13.4% ↓
Infected treated with Nanochitosan+ paromomycin	1715.41±21.03**	41.6% ↓

Data are expressed as mean ± SD. * P< 0.05, ** P< 0. 01 highly Significant

4. DISCUSSION

Giardiasis is help in finding a safe and effective treatment. Chitosan functions as an antibacterial drug by interacting with anionic molecules on the surface of cells [15]. Also, it can open tight junctions due to its mucosal adhesion capabilities, extending its presence at drug absorption sites and increasing drug penetration [16, 17].

The percentage of *Giardia lamblia* cysts in the stool was reduced by 65.7% in the current experiment using CsNPs. This result was in line with the study of [9], which showed that CsNPs treatment reduced the number of *Giardia* cysts in treated infected rats by 68.2%. A study [18] also discovered that *Giardia* cyst counts in hamster feces were decreased by 63.6 percent by chitosan nanoparticles. Because of its hydrophilic nature and heavy molecular weight, paromomycin is hard to absorb. This poorly absorbed aminoglycoside is being investigated as a potential treatment for giardiasis during pregnancy due to its 60–70% effectiveness against the disease [19]. In our study, giardiasis was treated with oral paromomycin for eight days, and as a result, 91.4% fewer cysts were excreted. Another study [20] demonstrated the efficacy of different doses of paromomycin against *G. lamblia* in lambs, showing a reduction of cyst secretion by up to 96.6% after treatment, which proves that paromomycin also has potential therapeutic effects in the treatment of *G. lamblia* in calves [21]. Natural and synthetic polymers are promising systems for carrying nanomedicines, particularly in oral drug administration with poor diffusion [22]. Chitosan nanoparticles are an excellent choice for loading because they offer desirable drug delivery capabilities since the polymer must meet biocompatibility and low toxicity to be used in drug delivery applications [20]. The percentage reduction in cyst secretion was 97.2% in the current study, and CsNPs combined with paromomycin reduced cyst secretion better than either compound alone. A similar study [23] showed the highest reduction in *Giardia* cyst count in the group rats that received chitosan nanoparticles loaded with paromomycin 100% after four days of treatment. This combination may increase the bioavailability of paromomycin, improve its retention time, and maintain its effectiveness against *Giardia* [17]. A related study [18] on *Giardia lamblia* that evaluated the efficacy of metronidazole loading onto Chitosan nanoparticles showed that the group receiving the loading drug experienced a high percentage reduction in the number of *Giardia* cysts of 94%. Researchers

employed mannosylated chitosan nanoparticles coated with paromomycin to treat leishmaniasis in the study of [24], which primarily targeted the amastigote stage of the parasite.

Like other components of innate and adaptive immune responses, cytokines play a role in cellular differentiation, repair of inflammation, and disease defense [25]. A murine study suggested by [26] demonstrated that *Giardia*-infected mice had elevated levels of both IFN-γ and TNF-α.

Compared to non-infected rats, infected group rats in the current study exhibited an increase in INF- γ secretion, suggesting they had the potential to control the infection, which was correlated with the number of cysts present in the experiment. This result is consistent with that revealed by [27], who found a significant association between enhanced IFN-γ production and parasite virulence. However, the highest level of IFN-γ was obtained after administration of cesium nanoparticles, either alone or in combination with, due to improved immunity; this was particularly obvious in the group treated by CS NPs with paromomycin.

Tumor necrosis factor alpha (TNFα) is a proinflammatory cytokine released during *Giardia* infection [28, 29]. TNF-α deficient mice showed delayed elimination of the *Giardia* parasite [30], and a human study showed that patients with giardiasis had elevated serum levels of TNF-α [31], suggesting that this cytokine is involved in immune response during infection.

In this study, the *Giardia*-infected control group's TNF-α concentration significantly increased compared to a normal control group. This outcome is consistent with that of [32], who reported that acute infection was associated with the highest levels of TNF-α, suggesting that it contributes to the pathogenesis of acute giardiasis. Furthermore, a comparable study revealed that TNF-α was found to be increased during *Leishmania* infection [29]. Pro-inflammatory cytokines play a significant role during the disease course and may be responsible for lipid peroxidation-induced tissue damage [33]. Another author [34] reported that increased tumor necrosis factor-alpha by activated macrophages can lead to enhanced oxygen radicals and lipid peroxide production.

According to our results, TNF-α levels were significantly decreased in all groups receiving CSNPs, although this was particularly evident in the group receiving paromomycin-combined CS NPs. Since chitosan has been proposed as an immunostimulant, the improvement in immune response can be explained by some of its properties, such as its anti-inflammatory and antibacterial effects [35].

5. Conclusion

Our present study shows that the anti-giardia properties of combination therapy with paromomycin-loaded chitosan nanoparticles achieve better results than single therapy (P 0.01). Therefore, the combined use of these compounds has promising potential for treatment and improving immune status after treatment.

Ethical consideration

The experiment was conducted as a general rule, and the center was responsible for the bio-supply program for animal ethics at the Biotechnology Research Centre at Al-Nahrain University, Iraq.

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