Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Silver Nanoparticles and Lactoferrin Ameliorate Immunocompromised Murine Cryptosporidiosis

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Abstract

Cryptosporidium parvum (*C. parvum*) is an intestinal parasite that infects immunosuppressed and immunocompetent people. Cryptosporidiosis is the second largest cause of diarrhea and mortality in children after rotavirus. Nitazoxanide (NTZ) has limited activity in immunocompromised individuals thus, it is increasingly necessary for evaluating new potential drugs against Cryptosporidium in immunosuppressed patients. The current research aimed to investigate the efficiency of Lactoferrin (LF) and Silver Nanoparticles (AgNps), separately, against *C. Parvum* infection in immunosuppressed mice in comparison with nitazoxanide and to study the effect of their combination on parasitological and histopathological changes. Oocyst output showed that shedding in mice treated with LF, AgNps, and NTZ combination was lower than NTZ treated one. Histopathological examinations of the infected mice treated by NTZ, LF, and AgNps in the intestine showed a regular villous pattern with no evidence of inflammatory changes. The section in the lung tissue showed no apparent pathological changes. Also, the toxicity in mice treated with LF, AgNps, and NTZ combination was lower than NTZ NTZ-treated one. The combined treatment of NTZ, LF, and AgNps resulted in the highest decrease in *Cryptosporidium* oocyst numbers compared to the other treatments tested. Lactoferrin is used as an adjuvant alongside other similar medications to treat cryptosporidiosis.

Keywords: Cryptosporidiosis, Nitazoxanide, Lactoferrin, Silver Nanoparticles, and immunosuppressed.

Introduction

Cryptosporidium sp. are apicomplexan parasites that colonize the gastrointestinal and respiratory epithelium's brush borders. Cryptosporidiosis was originally thought to be exclusively an infectious disease affecting young animals such as lambs and calves. Still, it is recognized as a major cause of diarrhea, cholangiopathy, and enterocolitis in humans [1]. Cryptosporidiosis in normal children, adults, and young animals is often a short-term illness marked by watery diarrhea, malnutrition, and decreased body weight. In 2019, approximately 1.5 million people died from diarrheal diseases, with fifty percent of them being children [2]. *Cryptosporidium* is a substantial cause of mild to severe diarrhea in children under two, especially infants, and comes second behind rotavirus [3]. Nitazoxanide is a Thiazolide antiparasitic agent with a wide-ranging antiviral effect being developed to treat influenza and other viral respiratory diseases. It is the sole FDA-approved medication for treating *Cryptosporidium.* [1]. In recent years, multiple studies have proven silver nanoparticles extremely harmful to bacteria and fungi [4,5]. This toxic effect is commonly connected with ion release and the production of oxidative stress [5]. Additionally, Camel's milk includes significant levels of Lactoferrin, which has recently been demonstrated to have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer effects [6]. Given the lack of efficient treatment against cryptosporidiosis, particularly in immunodeficient people. The purpose of the current research was to compare the effectiveness of lactoferrin, silver nanoparticles, and their combination with NTZ to that of NTZ treatment alone. This was achieved by assessing parasitological and histological characteristics in experimentally infected immunosuppressed mice.

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(Received 25 January 2025, accepted 28 March 2025)
DOI: 10.21608/ejvs.2025.355323.2614

Material and Methods

Experimental animals

The study was conducted at the Parasitology Department of Theodor Bilharz Research Institute (TBRI) in Giza, Egypt. It was performed on 70 male Swiss albino mice (CD1), which were six weeks old and weighed 18 and 20 grams. They were kept in cages with adequate air flow through perforated coverings that were cleaned every day, and they were given adequate nourishment and water. During the study, mice were maintained in an air-conditioned room at a temperature of 21 degrees Celsius. Food comprising 24% protein, 4% fat, and around 4-5% fiber was given out, as well as specialized water bottles. Additionally, pathogens were avoided. The mice were kept in this habitat for a week before the studies to verify that they had adapted to the surroundings. The Institutional Animal Care and Use Committee (SU-IACUC), Suez University, Egypt (261223) authorized all experimental methods, which were carried out following global standards for handling and utilization of animals used for research.

Drugs

a) Nitazoxanide (100 mg/5ml suspension) produced and supplied by [Medizen Pharmaceutical Industries for Utopia Pharmaceuticals, Egypt] was used. A daily oral dose of nitazoxanide suspension (200 mg/kg body weight) was supplied through an esophageal tube. Beginning ten days following infection and continuing for 5 days, the doses were given in grams depending on the weight of the mice (every mouse weighs between 18 and 20 grams) [7].

b) Dexamethasone was produced and supplied by [Kahira Pharmaceuticals and Chemical Industries Company, Shoubra-Cairo, Egypt]. Mice received 0.25 mg/kg of dexamethasone orally for 14 days [7].

c) Nanosilver (2 mg/Kg mice), tiny silver particles with a 10 to 30nm length range. (Nanotech Egypt-October City). Chemical reduction is the most commonly utilized process for producing stable, colloidal suspensions of silver nanoparticles in organic or water-based solvents. Typically used reducing agents include borohydride, citrate, and elemental hydrogen [8].

d) Lactoferrin: 1.8 ~ 2 g/kg, from camel's milk, lactoferrin was produced. Proteins from ammonium sulfate whey precipitate were adsorbed to the matrix to perform hydrophobic interaction chromatography [9].

The parasite

The veterinary clinics of Cairo University's Faculty of Veterinary Medicine provided the *Cryptosporidium* oocysts used to infect the mice in this investigation. Sterile, clean fecal cups were used to collect the fecal samples of infected diarrhoeic calves; the specimens shouldn't have been polluted with urine or water.

Pre-weaned calves are dominated by *C. parvum* [10]. Furthermore, as *C. parvum* species may infect both humans and animals, the findings of any experiment including this protozoan would demonstrate that it applies to both hosts.

Microscopic examination of faecal sample

All faecal samples were subjected to the modified Ziehl-Neelsen staining technique as described by [14].

Ziehl-Neelsen method for acid-fast staining:

(A). Carbol fuchsin stain:

(B). Decolorizing solvent:

(C) Methylene blue:

- Smears of feces were made on a glass slide, allowed to air dry at room temperature, and then fixed for five minutes with methanol.
- After 10 to 12 minutes of carbol fuchsin staining, fixed smears were rinsed with tap water.
- After one minute of 3% acid alcohol decolorization, smears were counterstained for 30 seconds with a solution of methylene blue chloride.
- After being rinsed with tap water, smear slides were allowed to air dry. A slide has been covered and examined using a 100x objective lens to identify and count *C. parvum* oocysts. The background is bright blue, and the oocysts are purple.

Stool samples were taken from mice infected with *Cryptosporidium parvum* collected from the feces of infected calves with diarrhea in the veterinary clinics of the Faculty of Veterinary Medicine, Cairo University. Infected cattle feces was collected in containers prepared for stool sampling. It had been taken into consideration to maintain the samples free of water and urine. The fecal samples were then transferred to the parasitology department at Theodor Bilharz Research Institute (TBRI).

Immunosuppression

Before being infected with *Cryptosporidium* oocysts, the immune system was inhibited for two weeks with the artificial corticosteroid dexamethasone (Dexazone) given orally through an esophageal tube at a dose of 0.25 mg/kg [7,11]. All animals were given the same dose of dexamethasone throughout the trial.

Experimental design

Seventy mice, six-week-old male Swiss albino mice (CD1) weighing 18-20 grams were utilized, and they were divided into seven main groups:

1) Ten immunosuppressed control mice were not infected with the parasite.
2) Ten immunosuppressed control mice were infected with the parasite (10000 oocysts/ mouse).
3) Ten immunosuppressed mice were infected and treated with nitazoxanide (200 mg/Kg) [7].
4) Ten immunosuppressed mice were treated with lactoferrin (1.8 ~ 2 g/kg).
5) Ten immunosuppressed mice were treated with silver nanoparticles (2 mg/Kg).
6) Ten infected immunosuppressed mice treated with lactoferrin loaded on nanosilver.
7) Ten infected immunosuppressed mice treated with lactoferrin loaded with nanosilver and also treated with nitazoxanide.

Parasitological assessment

Mice inoculation

Each mouse received around 3×10^3 *Cryptosporidium* oocysts to initiate infection [12].

Collection of stool samples

After transferring every single mouse to a separate clean box for 30- 60 minutes, fecal samples were collected, weighed, then dissolved in specified quantities of formol saline 10%, and passed through clean gauze, producing a clear film. Using a micropipette, 50 µl of every sample was extracted and counted for *Cryptosporidium* oocysts [13]. The oocyst count per gram of stool was then determined [12].

Microscopic examination of stool:

All fecal samples were examined via microscopy by the direct wet smear and concentration techniques and then stained using modified Ziehl-Nielsen acid-fast stain (MZN) as suggested by [14].

Animal scarification

After 23 days of infection, mice were injected intraperitoneally with an anesthetic-anticoagulant solution (500 mg/kg thiopental and 100 units/ml heparin), then all mice were scarified by performing a quick decapitation. Portions of the small intestine and lungs were removed from each mouse and histopathologically examined in all groups.

Histopathological assessment

To investigate the histological features of different tissues, 1 cm-long sections from every mouse's small intestine and lung were obtained and promptly placed in 10% buffered formalin.

Efficacy of selected drugs [15]

The efficacy of chosen drugs against *Cryptosporidium* oocysts was computed as follows:

$$\text{Efficacy (\%)} = \frac{\textbf{Total oocysts before treatment} - \text{Total oocysts after treatment}}{\text{Total oocysts before treatment}} \times 100$$

Evaluation of toxic effects of nanoparticles

To evaluate toxicity levels in the liver and kidney of all tested groups, glutathione (GSH) and lipid peroxide (malondialdehyde) (MDA) were measured using a colorimetric technique [16,17].

Statistical Analysis

The data was represented by SPSS (version 16.0) using standard deviation (SD) and mean values. In addition to categorical variables being given frequencies and percentages, continuous variables were shown. Utilizing a student's *t*-test, the groups were compared. With the use of linked table data, the degree of significance (*P*-value) was determined. The following was the expression for the level of significance:

P>0.05 non-significant, P< 0.05 Significant, P<0.01 Highly significant, *P*< 0.001 Very highly significant.

Results

Parasitological results of stool examination

The oocyst shedding with Lactoferrin, Silver Nano, and NTZ combination had the lowest shedding rate (5.33±0.577) compared to the high shedding rate in the positive control (40.33±2.517). Compared to other treated groups, the Lactoferrin, NTZ, and AgNps treated groups had the highest percentage (86.78%) of oocyst shedding reduction. (Table 1).

Histopathological results

Histopathological examinations of the normal group (negative control) showed a normal villous pattern of the intestinal mucosa (Fig 2 A) and no pathological change in lung tissue (Fig 3 A). The Positive infected control group showed a distorted villous pattern in the intestine with a broad base (yellow line) and adherent crypto spores to the villous tip (black arrow) (Fig.2B&C). A section of the lung tissue of the positive group of mice shows moderately congested lung alveoli (black arrow) (Fig.3B). The Infected mice treated with Lactoferrin showed a focal distortion of the villous pattern in the intestine (black arrow), mildly infiltrated by mononuclear inflammatory cells (yellow arrow) (Fig. 2D). Lung tissue showed no apparent histopathological changes (Fig.3C). The infected mice treated with Silver Nanoparticles in the intestine showed a mildly irregular villous pattern

(yellow arrow) with no evidence of inflammatory changes (Fig.2E). Section in the lung tissue showed no apparent pathological changes (Fig.3D). The Infected mice treated by NTZ in the intestine showed a mildly broadened villous tip (black arrow) with focal inflammatory changes (yellow arrow) (Fig.2F). A section of the lung tissue showed no apparent pathological changes (Fig.3E). The Infected mice treated with Lactoferrin and Silver Nanoparticles in the intestine showed a regular villous pattern, mildly infiltrated by mononuclear inflammatory cells (yellow arrows) (Fig.2G). A section of lung tissue showed no apparent histopathological changes (Fig.3F). The Infected mice treated by NTZ, Lactoferrin, and Silver Nanoparticles in the intestine showed a regular villous pattern with no evidence of inflammatory changes (Fig.2H). A section in the lung tissue showed no apparent pathological changes (Fig.3G).

Evaluation of toxic effects of nanoparticles

Assessment of glutathione (GSH)

Following the evaluation of the yellow product at 405 nm, the glutathione (GSH) concentration was estimated in mmol/g. Table (2) shows that the normal (negative control) group of mice had a GSH concentration of (14.850±0.5891), while the infected (positive control) group had a reduction of (6.867±0.3011) mmol/g, which was extremely statistically significant ($p<0.05$). Mice receiving Lactoferrin and Silver Nano showed a high level of GSH (13.850±0.1517) mmol/g.

Assessment of Malondialdehyde (MDA)

Following detecting the pink product at 534 nm, the malondialdehyde (MDA) concentration was estimated in mmol/g. Table (2) shows the levels of hepatic MDA in immunosuppressed groups. The MDA level in the infected (positive control) group was (5.250±0.5577) mmol/g, while the normal (negative control) group's level was (2.067±0.2251) mmol/g. MDA levels were observed to be lower in all treated groups than those of infected control. The group receiving Lactoferrin and Silver Nano had the largest decrease in the MDA level (3.017±0.1835) mmol/g.

Discussion

Cryptosporidium is a parasitic intracellular organism that affects the epithelial lining of the luminal surfaces of the gastrointestinal and respiratory systems in a wide range of hosts [18]. *C. parvum* is a protozoan parasite that infects humans as well as many domestic and wild animals [19].

In the current study, both Lactoferrin and Silver Nanoparticles exhibited an antiprotozoal impact against cryptosporidiosis, as evidenced by decreased oocyst shedding but not total oocyst eradication. When compared to the control group, the group that got combined NTZ, Lactoferrin, and Silver Nanoparticles treatment had the greatest reduction in fecal oocysts. In contrast, the control group showed the smallest decline. This observation lends support to the hypothesis that NTZ therapy is insufficient in infected immunocompromised hosts [20].

The current findings further demonstrated that Lactoferrin and Silver Nanoparticles may increase the efficacy of NTZ. Zheng [21] established the contribution of silver nanoparticles in *Cryptosporidium* infections, indicating that nano-Ag reduced *C. parvum* numbers and vitality in several water specimens in a variable but effective manner. These effects are most likely caused by its cytotoxic and cell-suppressive actions, which are aided by AgNPs' well-known antibacterial properties.

Our findings were consistent with those of Cameron [22], who found that AgNPs decreased the ability to survive *C. parvum* oocysts in a dose-dependent manner using the excystation assay and shell/sporozoite ratios. Similarly, Saad [23] discovered that *C. parvum* oocysts extracted from human and animal wastes remained inert following treatment with NPs. The oocyst's being inert was explained by NP-induced alterations in the structure of its wall. Su [24] exposed oocysts to 100 µg/ml of AgNPs and 63.5 µg/ml of Ag ions (100 µg/ml of AgNO3) over four hours. They also found that Ag ions were inefficient at inactivating oocysts, showing that the effect of AgNP action was dependent on the NP's inherent properties. This supports our findings that AgNPs are more harmful to oocysts than NTZ.

In this respect, two theories have been evolved to clarify the damaging impact of NPs on *C. parvum* oocysts: first, it works on the parasites' surface, as believed by Choi and Hu [25], who proposed that NPs, via ROS generation, might disrupt parasite surface lipophosphoglycan and glycoprotein molecules, which are essential to infection, causing parasitic infection reduction. Second, the nanoparticles may bind to DNA molecules and disrupt the helical structure by cross-linking inside and among nucleic acid strands [26]. Furthermore, NPs within eukaryotic cells can disturb biochemical processes [27]. Chang [28] found that because NPs are smaller in size, they can readily pass into the cell via the pores found within the cell membrane and are expected to have negative impacts on eukaryotic cells, including protozoa.

Paredes [29] studied lactoferrin's anti-cryptosporidia activity at various phases of parasite development. Lactoferrin, at physiological quantities, destroyed the sporozoites, which are necessary for infection, but had no impact on oocyst survival or parasite intracellular development [29]. This is consistent with our results.

Nitazoxanide (NTZ) and other nitrothiazole salicylamide compounds exhibit extensive

antiparasitic activity [30]. These findings are consistent with those of Gargala [31], who discovered that NTZ dramatically reduced the period of diarrhea and mortality in both adults and malnourished children. Rossignol [32] reported that the efficiency of NTZ varied according to the degree of immunosuppression. Nitazoxanide works by blocking pyruvate: ferredoxin oxidoreductase (PFOR), an enzyme required for the metabolism of some anaerobic bacteria and parasites [33]. The PFOR in *Cryptosporidium* has an unusual structure in which the enzyme comprises a C-terminal cytochrome P450 protein; hence, Nitazoxanide action via PFOR is questionable [34].

The histological examination of ileal sections (infected control positive) indicated significant pathological alterations in the intestinal mucosa when compared to the non-infected control group. Similarly, Abdelhamed [35] and Moawad [36] found that *cryptosporidium* infection causes dysplastic alterations in the intestines in mice. Soufy [37] identified the histopathological consequence as brush boundary displacement, resulting in villi shortening and widening and villous atrophy, most likely due to the pathogen's release of toxins that destroy epithelial cells. This is consistent with our results.

These results are equivalent to those of Waters and Harp [38], who observed intestinal inflammatory alterations such as inflammatory infiltration and villous shrinkage in response to *Cryptosporidium* infection. Furthermore, several investigations have found similar histological abnormalities in infected animals [39, 40, 41]. Previous investigations carried out in immunosuppressed mice indicated only partial effectiveness for single NTZ therapy in infected intestinal sections [36,42,43]. Our findings reflect prior studies that demonstrated favorable results with combination treatment vs utilizing NTZ separately [35,36,44].

The liver could be regarded as a site of nanoparticle accumulation in mice; hence, a toxicological analysis was conducted on this organ. Monir Doudi and Mahbubeh Setorki [45] discovered that when three groups of adult male Wister rats were treated with an intraperitoneal injection of Ag NPs at doses of 5, 10, and 100 ppm for seven days, serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic-pyruvic Transaminase (SGPT) levels were not significantly higher at various intervals than in the control group. Nonetheless, histological investigation revealed partial destruction of liver tissue.

Mahsa [46] studied the impact of Ag NPs on the liver at varied doses for twenty-eight days and found that silver nanoparticles had no significant influence on liver transferases, while they raised liver caspase-3 activity. Conversely, Heydrnejad [47] discovered that the experimental groups had greater levels of liver enzymes than the control group.

Shahin [48] found a dose-dependent increase in liver enzymes (at concentrations of 5, 10, 20, and 40 ppm of Ag NPs) but no histopathologic alterations in the liver. As a result, differences between studies might be related to the structural heterogeneity of silver nanoparticles, the path of distribution, and the time of the therapy [49,50].

The form of nanoparticles has a considerable impact on their toxicological effects [51,52]. The various forms of particles can cause varied biological reactions and correspond with the degree of tissue or cell damage. Several toxicity studies suggested that crystalline or fiber-like particles can produce more harm to cells while also being more likely to remain in tissues or organs [53,54]. Nanocrystalline silver particles are highly cytotoxic to cultured keratinocytes when subjected to different forms of silver, as confirmed by preventing cell division and changes in cellular morphology of keratinocytes and fibroblasts [55,56]. These findings show that our colloidal AgNPs caused no acute toxic effects in the treated mice, following Pattwat **M**. [57] who found that colloidal AgNPs did not produce any pathological changes in any epidermal or dermal layer.

According to our research, therapy with either lactoferrin alone or nano-silver causes MDA to drop and the GSH antioxidant to rise. This demonstrates the effectiveness of this treatment in immunosuppressed groups.

Hepatic GSH levels in immunosuppressed groups were observed to be higher with Lactoferrin and Silver Nano combination. Chen, H.A. [58] reported similar findings, showing that LF decreased the production of MDA and markedly enhanced the activity of antioxidant molecules, such as GSH.

Malondialdehyde (MDA) levels are the most useful biological measure for identifying lipid peroxidation in oxidative stress research [59,60]. Hepatic MDA levels in immunosuppressed groups were observed to be lower in all groups receiving treatment as compared to the infected control group. The group treated with Lactoferrin and Silver Nano showed the largest reduction in MDA levels (3.017±0.1835) mmol/g. The findings correlate with those of Farid [61], who indicated that LF therapy reduced MDA levels in the hepatic tissue of rats; this is consistent with our results.

The LF antioxidant effect has been noted in several chemical and biological contexts. According to Shinmoto [62], LF reduces lipid peroxidation by lowering the reduction of H_2O_2 to OH via the Fenton reaction by sequestering iron. Raghuveer [63] observed that incorporating LF in the dietary regimen of premature infants also reduced iron-induced

oxidation products. Britigan [64] and Satu e-Gracia [65] discovered that LF exhibited antioxidant properties due to the iron linked to the protein could not operate as a catalyst for the production of the hydroxyl radical, which works as an oxidative stress detoxicant.

Conclusion

The current study proved that the combined treatment of NTZ, Lactoferrin, and Silver Nanoparticles showed the greatest decline rate in *Cryptosporidium* oocyst number compared to the other treatments tested. Moreover, it significantly boosted intestine histological characteristics. Lactoferrin is used as an adjuvant alongside other similar medications to treat immunocompromised murine cryptosporidiosis.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Institutional Animal Care and Use Committee (SU-IACUC), Suez University, Egypt (261223), authorized all experimental methods, which were carried out following global standards for handling and utilization of animals used for research

TABLE 1. Cryptosporidium oocyst mean number and percentage reduction in stool

Groups	Number of oocyst /HPF (mean +SD)	The percentage of reduction in the number of oocysts
Positive control	40.33+2.52	
Lactoferrin	25.33+1.53***	**37.19**
Silver Nano	36.64+2.08	**9.15**
Nitazoxanide	16.33+1.53***	**59.51**
Lactoferrin Nano	19+2***	**52.89**
Lactoferrin +NTZ Nano	5.33+0.52***	**86.78**

* P< 0.05*= significant.

TABLE 2. Mean and standard deviation (*P* value) of Malondialdehyde (MDA) and hepatic Glutathione (GSH) of immunosuppressed groups

Groups	GSH	MDA
Negative control	14.85±0.59	2.07±0.23
Positive control	6.87±0.30*** (29.53)	5.25±0.56*** (12.27)
Lactoferrin	10.2±0.33*** (16.85)	4.27±0.60*** (8.39)
Silver Nano	11.03±0.39*** (13.23)	3.75±0.23*** (12.65)
Nitazoxanide	12.3±0.48*** (8.21)	3.05±0.71** (3.22)
Lactoferrin Nano	13.85±0.15** (4.02)	3.02±0.18*** (7.97)
Lactoferrin +NTZ Nano	9.72±0.19*** (20.27)	4.8±0.15*** (24.35)

* P< 0.05*= significant

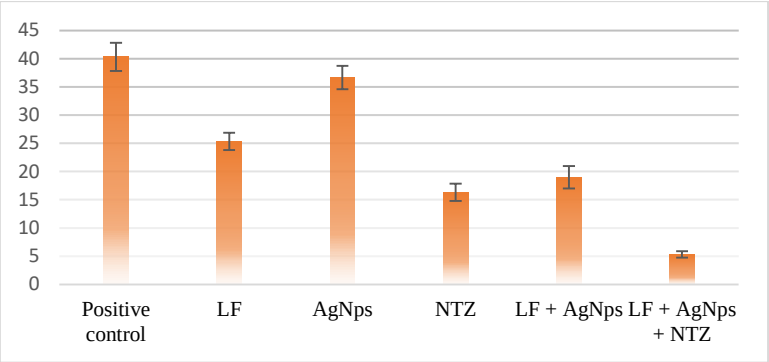

Fig. 1. Cryptosporidium oocyst mean number and percentage reduction in stool [66]

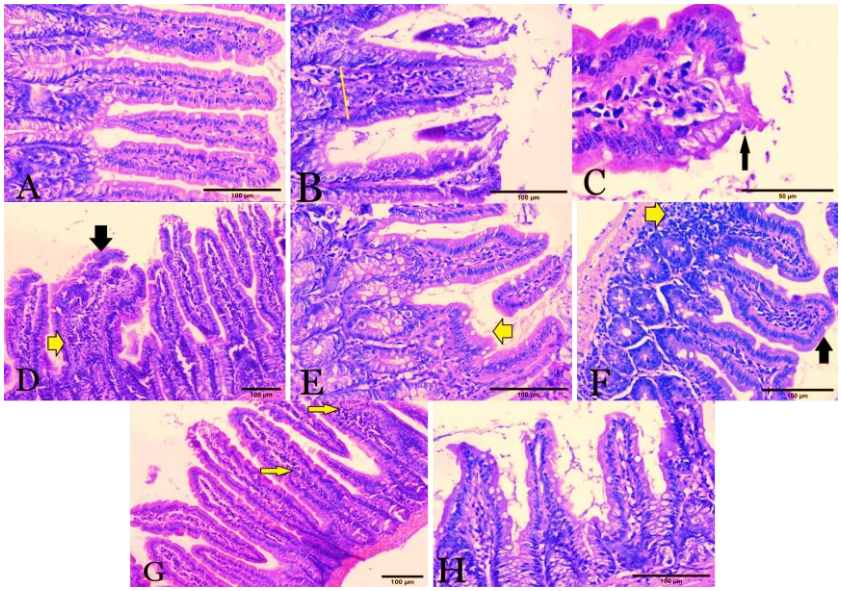

Fig. 2. The intestinal mucosa. A: (Normal group of mice (control negative). B: (Positive infected control group). C: (Positive infected control group). D: (Infected group treated by LF) E: (Infected group treated by AgNps) F: (Infected group treated by NTZ). G: (Infected group treated by LF+ AgNps). H: (Infected group treated by LF+ AgNps+NTZ).

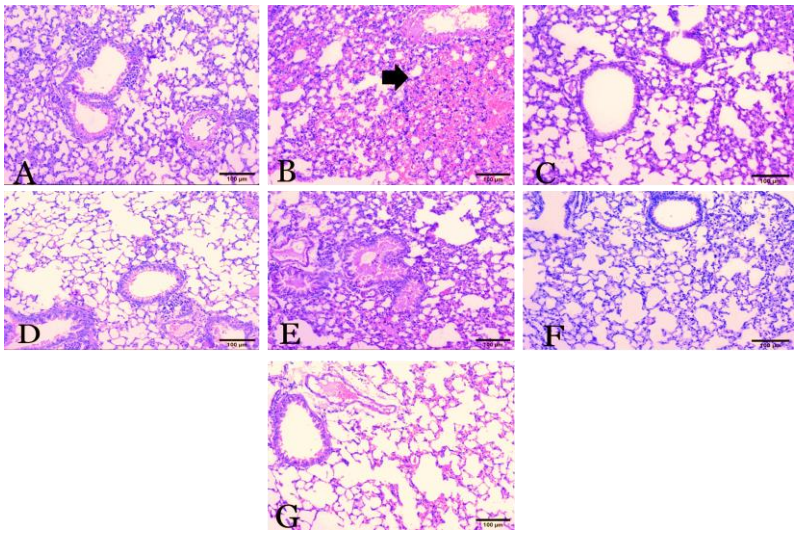

Fig. 3. In lung tissue. A: (Normal group of mice (control negative). B: (Positive infected control group). C: (Infected group treated by LF). D: (Infected group treated by AgNps). E: (Infected group treated by NTZ). F: (Infected group treated by LF+ AgNps). G: (Infected group treated by LF+ AgNps+NTZ).

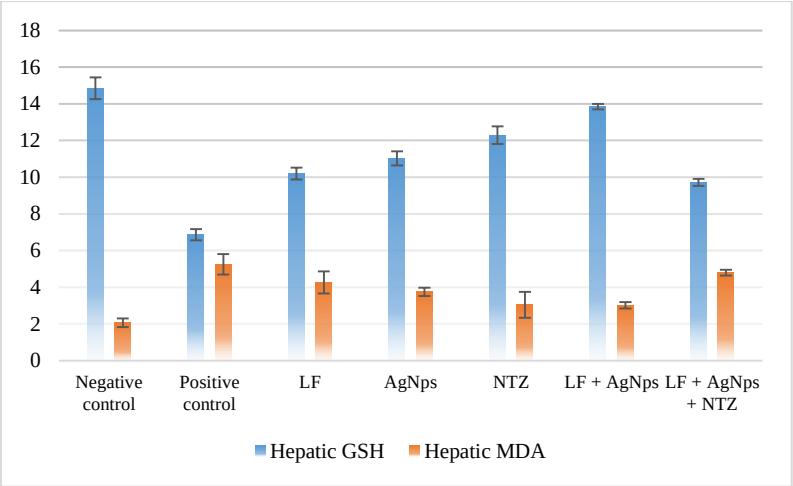

Fig. 4. Mean and standard deviation of Malondialdehyde (MDA) and hepatic Glutathione (GSH) of immunosuppressed groups

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فاعلية جسيمات الفضة النانوية واللاكتوفيرين في علاج الفئران المثبطة مناعيا المصابة

بداء *الكريبتوسبوريديوم*

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الملخص

الكريبتوسبوريديوم بارفوم (*C. parvum*) طفيلي معوي يصيب الأشخاص ذوي المناعة المثبطة والأشخاص ذوي المناعة الطبيعية. يُعد داء الكريبتوسبوريديوم ثاني أكبر سبب للإسهال والوفيات لدى الأطفال بعد فيروس الروتا. يتميز النيتازوكسانيد (NTZ) بنشاط محدود لدى الأشخاص ذوي المناعة الضعيفة، لذا تزداد الحاجة لتقييم أدوية جديدة ضد *الكريبتوسبوريديوم* لدى المرضى ذوي المناعة الضعيفة. يهدف البحث الحالي إلى دراسة فعالية اللاكتوفيرين (LF) وجسيمات الفضة النانوية (AgNps)، كل على حدة، ضد عدوى *الكريبتوسبوريديوم بارفوم* لدى الفئران ذات المناعة الضعيفة مقارنةً بالنيتازوكسانيد، ودراسة تأثير مزجهما على التغيرات الطفيلية والنسيجية المرضية. أظهرت النتائج ان إنتاج الكيسات البيضية لدى الفئران المعالجة بمزيج اللاكتوفيرين وجسيمات الفضة النانوية و النيتازوكسانيد كان أقل منه لدى الفئران المعالجة بالنيتازوكسانيد فقط. أظهرت الفحوصات النسيجية المرضية في الامعاء للفئران المصابة التي عولجت بـالنيتازوكسانيد واللاكتوفيرين وجسيمات الفضة النانوية نمطًا زغابيًا منتظمًا دون إي دليل على حدوث تغيرات التهابية. ولم يُظهر مقطع أنسجة الرئة أي تغيرات مرضية ظاهرة. كما كانت السمية في الفئران المعالجة بمزيج بـالنيتازوكسانيد واللاكتوفيرين وجسيمات الفضة النانوية أقل من تلك المعالجة بـالنيتازوكسانيد فقط. وقد أدى العلاج المشترك بـالنيتازوكسانيد واللاكتوفيرين وجسيمات الفضة النانوية إلى أعلى انخفاض في أعداد أكياس الكريبتوسبوريديوم مقارنةً بالعلاجات الأخرى المختبرة. ويُستخدم اللاكتوفيرين كعامل مساعد إلى جانب أدوية أخرى مماثلة لعلاج داء *الكريبتوسبوريديوم*.

الكلمات الدالة: داء الكريبتوسبوريديوم ، نيتازوكسانيد ، لاكتوفيرين ، جسيمات الفضة النانوية ، مثبط مناعيا.