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THERAPEUTIC EFFICACY OF LACTOFERRIN LOADED ON NANO-PARTICLES IN *TRICHINELLA SPIRALIS* INFECTION IN MICE

By

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Abstract

Trichinellosis is a worldwide risky parasitic zoonosis caused by a nematode of the genus *Trichinella*. Anthelmintic such as albendazole and mebendazole commonly used to treat trichinellosis are badly water soluble and exhibit unfavorable bioavailability after oral administration increasing need for effective and safe drug. This study evaluated the therapeutic efficacy of lactoferrin loaded on silver nanoparticles in treating *T. spiralis* infection in mice by parasitological, immunological, and histopathological parameters. Forty albino mice were classified into 8 groups of 5 mice. G1: Non-infected non-treated (normal), (G2): Infected non-treated (infected control). G3: Infected and treated with albendazole, G4: Infected and treated with lactoferrin, G5: Infected and treated with silver nanoparticles, G6: Infected and treated with lactoferrin loaded on silver nanoparticles, G7: Infected and treated with albendazole in combination with lactoferrin, & G8: Infected and treated with albendazole in combination with lactoferrin loaded on silver nanoparticles.

The results revealed a significant decline in all treated mice with the highest reduction rate of *T. spiralis* adult count in G8 treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles (98.59%), followed by G7 treated with albendazole and lacto-ferrin (95.77%), G3 treated with albendazole (90.38%), G6 treated with lactoferrin loaded on silver nanoparticles (84.27%), G4 treated with lactoferrin (59.86%), and lowest reduction rate (22.3%) was in G5 treated with nanoparticles. Also, there was a significant decline in TNF- α & IL-10 levels and an improvement of the intestinal histopathological changes in mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles.

Keywords: *Trichinella spiralis*, Lactoferrin, Silver nanoparticles, Histopathology, TNF, IL-10.

Introduction

Trichinosis is a globally food-borne parasitic disease (Thanchomnang *et al,* 2021), and considered an emerging or re-emerging infectious disease in several regions due to changing diets and cooking habits (Yang *et al,* 2020), categorized among top of ten food-borne parasites (Abdelrahman *et al,* 2020). Globally, 10,000 people were estimated to be *Trichinella* spp infected annually (Allam *et al,* 2021). Human infections occur by accidental ingestion of raw or undercooked pork containing infectious *T. spiralis* encysted larvae (Yang *et al,* 2020). Adults reside in the intestinal mucosa and last for 10–20 days in mice and rats or 4–6 weeks in humans**.** but larvae encapsulated in muscle fibers remain for months to years (Sun *et al,* 2015). *Trichinella spiralis* life cycle consists of two phases, enteral and muscular (Abou Rayia *et al,* 2017). Enteral one is manifested by abdominal symptoms, gastroenteritis with diarrhea and abdominal pain (Wilson *et al,* 2015), and usually misdiagnosed as botulism (El-Bahnasawy *et al,* 2014). Muscular phase is manifested by periorbital edema, myalgia, & muscle weakness (Yu and Qi, 2015). Low infection intensity remained asymptomatic but, heavy one caused severe muscle pain and complications (Nassef *et al,* 2018). Trichinellosis led to risky inflammatory reactions in the heart, lungs, and CNS up to death (Elmehy *et al,* 2021).

Anthelmintic such as albendazole and mebendazole are poor water soluble and exhibit unfavorable bioavailability after oral administration (Nasser *et al,* 2018). They were

contraindicated in pregnancy and children <3 years; others are carcinogenic (Salama *et al*, 2021).

Lactoferrin is an iron-binding glycoprotein found in most body fluids, high concentrations in mammalian milk and in bovine milk and isolated from human milk that killed certain protozoa, such as *Toxoplasma*, *Entamoeba*, & *Giardia* **(**Chang *et al*, 2020). The anti-inflammatory treatment during trichinellosis by steroids has many side effects that limit as they mainly suppress the immune response increasing parasite burden and survival in host tissue (Balaha *et al*, 2020). But, the anti-inflammatory and immunomodulatory roles of lactoferrin have increasing scientific interest (Chang *et al*, 2020). Nanomedicine improved oral delivery by bioavailability enhancement with minimize adverse effect (Hassan *et al*, 2021). Their excellent antibacterial action, high surface-to-volume ratio, and good chemical stability, silver nanoparticles have gained a lot of popularity among nanomaterials. (Majumdar and Kar, 2023), and also proved to be safe antiparasitic agent **(**Dkhil *et al*, 2023**)**.

This study aimed to evaluate the therapeutic efficacy of lactoferrin loaded on silver nanoparticles in the treatment of the intestinal phase of *Trichinella spiralis* infection in mice by parasitological, immunological, and histopathological assessment.

Materials and Methods

This experimental study was carried out at Department of Parasitology, Faculty of Medicine, Benha University, & Biological Unit, Theodor Bilharz Research Institute (TBRI), from February 2022 to November 2023.

Parasite: *Trichinella spiralis* strain of was obtained from laboratory-bred infected albino mice in TBRI.

Isolation and preparation of inoculum: Infected mice were sacrificed five weeks post-infection (PI), and dissected, and their muscles were digested in 1% pepsin and 1% HCl in 200ml distilled water. Mixture was incub ated at 37°C for two hours with continuous agitation by an electric stirrer. The digested product was passed through a 50-mesh/inch sieve to remove coarse particles. Encysted larvae were collected on a 200-mesh/inch sieve, washed twice with tap water, and then suspended in 150ml of tap water in a conical flask. The supernatant fluid was discarded and sediment larvae were counted, by taking three samples, each of 20µm from the sediment, spread on a slide and counting larvae under a binocular microscope (Nada *et al*, 2018). Mice were infected orally with 300larvae/mouse (Hassan *et al*, 2021).

Drugs: 1-Albendazole suspension was obtained from the Sigma Pharmaceutical Co., Egypt, and was administered at a dose of 50mg/kg. 2- Lactoferrin was prepared at TBRI, Giza, Egypt, and was administered in a dose of 50mg/kg, which was prepared by hydrophobic interaction chromatogramphy from milk acid (Yoshida, 1989). 3- Silver nanoparticles were prepared at TBRI, Giza and was given in a dose of 50mg/kg (El-Melegy *et al*, 2019), prepared by using chemical reduction method (Solomon *et al*, 2007).

Experimental design: Forty laboratory bred adult Swiss Albino mice weighed 20-30 gm were classified into 8 groups of 5 mice each: G1: Neither infected nor treated (negative normal), G2: Infected non-treated (positive control), G3: Infected and treated with albendazole in a dose of 50 mg/kg, G4: Infected and lactoferrin in treated in a dose of 50mg/ kg, G5: Infected and silver nanoparticles treated in a dose of 50mg/kg, G6: Infected and lactoferrin loaded on silver nanoparticles treated in a dose of 50mg/kg, G7: Infected and albendazole treated in a dose of 50mg/kg in combination with lactoferrin in a dose of 50mg/kg, and G8: Infected and albendazole treated in a dose of 50mg/kg in combination with lactoferrin loaded on silver nanoparticles in a dose of 50mg/kg.

Treatment started on the 3rd day PI for three sequential days. Mice were sacrificed by cervical dislocation on the 7th day PI to evaluate drugs effect on *Trichinella* adults.

The drugs' efficacy was assessed: I- Parasitological by counting of *T. spiralis* adults in

sacrificed mice. Mice were opened longitudinally intestine was dissected out and, cut into small pieces, washed, incubated in 10ml normal saline at 37℃ for 2hr to allow them to be free in the container, and washed several times till it became clear. Fluid was collected, centrifuged at 1,500rpm for 5 min., supernatant was decanted; sediment was reconstituted in a few saline drops and examined drop by drop under dissecting microscope to count adults (Basyoni and El-Sabaa, 2013).

II- Immunological by measuring systemic TNF-α & IL-10 levels in blood samples taken from a retro-orbital vein from mice of on 7th day PI (El-Melegy *et al*, 2019). Sera were separated by centrifugation and TNF-α level and IL-10 level was determined by ELISA (Kits USA & Canada, R&D Systems, Inc. and Abcam respectively).

III- Histopathological by examining serial sections of small intestine of all mice sacrificed (1cm at junction of first 1/3 and second 2/3), fixed in 10% formalin, dehydrated, cleared, embedded in paraffin blocks, sectioned 5µ thickness, stained in hematoxylin and eosin (Shalaby *et al*, 2010).

Ethical consideration: The protocol was approved by the Research Ethical Committee, Faculty of Medicine, Benha University (No. MD 5-2-2022), that followed Helsinki Declaration Guidelines (2008).

Statistical analysis: Data were tabulated and analyzed using program SPSS (Statistical Package for Social Science) version 20. Descriptive data were calculated as mean and ±SD). ANOVA test compared the mean of more than two groups of data where *P* value <0.05 was considered significant (*) and P value <0.01 was considered highly significant (**). Reduction rate (R) was calculated between infected treated and infected non-treated mice as follows: R = [(A-B)/A] ×100 % Where A= mean number recovered from infected non treated mice and B= mean number recovered from infected treated ones ((Feldman *et al*, 2003).

Results

Details were given in tables (1, 2 & 3) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12).

Table 1: Comparison between different groups regarding *Trichinella spiralis* adult count (n=5).

Groups	Mean ±SD	R	P2
G1: Negative control	0.0 ±0.0	No	
G2: Positive control	85.20 ±6.65	0%	
G3: Albendazole	8.20 ±1.92	90.38%	<0.001**
G4: Lactoferrin	34.20 ±4.76	59.86%	<0.001**
G5: Silver nanoparticles	66.20 ±3.56	22.3%	<0.001**
G6: Lactoferrin loaded on silver nanoparticles	13.40 ±2.07	84.27%	<0.001**
G7: Albendazole and Lactoferrin)	3.60 ±1.52	95.77%	<0.001**
G8: Albendazole and Lactoferrin loaded on silver nanoparticles	1.20 ±0.84	98.59%	<0.001**
ANOVA	470.16		
P value	<0.001**		

P = significance between different groups, . P2= significance between G2 & and other infected treated groups.
**= highly significant (P <0.01).

Table 2: Comparison between different groups as to TNF- α (Pg/ml) level.

Groups	Mean ±SD	R	P1	P2
G1	6.8±0.90			
G2	47.40±4.16		<0.001**	
G3	16.03±1.01	66.18%	<0.001**	<0.001**
G4	27.60±1.71	41.77%	<0.001**	<0.001**
G5	23.77±0.60	49.85%	<0.001**	<0.001**
G6	21.07±1.03	55.55%	<0.001**	<0.001**
G7	10.93±2.49	76.94%	0.019*	<0.001**
G8	10.83±0.40	77.15%	0.021*	<0.001**
ANOVA	135.36			
P value	<0.001**			

Table 3: Comparison between different groups as to IL-10 (Pg/ml) level.

Groups	Mean ± SD	R	P1	P2
G1	10.67 ± 0.55			
G2	147.03 ± 5.51		<0.001**	
G3	30.77 ± 1.95	79.07%	<0.001**	<0.001**
G4	130.10 ± 2.41	11.51%	<0.001**	<0.001**
G5	126.47 ± 6.14	13.98%	<0.001**	<0.001**
G6	94.17 ± 3.77	35.95%	<0.001**	<0.001**
G7	14.80 ± 1.31	89.93%	0.16	<0.001**
G8	13.23 ± 0.42	91.0%	0.373	<0.001**
ANOVA	896.96			
P value	<0.001**			

Parasitological showed a significant decrease in adult count in all treated mice compared to positive control (P2<0.001**). Mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles gave lowest value (1.20±0.84) as compared to positive control (85.20±6.65). Mice treated with silver nanoparticles gave highest value (66.20±3.56) as compared with positive control one (85.20± 6.65). The highest reduction rate (98.59%) was in mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles, followed by mice treated with albendazole and lactoferrin (95.77%), mice treated with albendazole (90.38%), mice treated with lacto-ferrin on silver nanoparticles (84.27%), mice treated with lactoferrin (59.86%), and least one (22.3%) was in mice treated with silver nanoparticles.

Immunologically showed that TNF-α level significantly decreased in treated mice as compared positive control (P2<0.001**). Mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles gave least value (10.83±0.40) as compared to positive control (47.40 ±4.16). Mice treated with lactoferrin gave highest value (27.60±1.71) as compared to positive control (47.40±4.16). IL-10 level showed a significant reduction in all treated mice compared positive control (P2<0.001**). Mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles gave lowest value (13.23±0.42) as compared to positive control (147.03±5.51). Mice treated with lactoferrin gave highest value (130.10± 2.41) as compared to the positive control (147.03±5.51).

Histopathology showed normal intestinal architecture, while sections of positive control showed some scattered adults, severely distorted villous pattern and sever inflammatory cellular infiltrate. Intestine of albendazole treated mice showed occasional adults, mostly preserved villous pattern, and moderate inflammatory cellular infiltrate. But, intestine of lactoferrin treated mice showed a mostly preserved villous pattern, mild inflammatory cellular infiltrate, and intestine of silver nanoparticles treated mice showed occasional degenerated adults, moderate distorted villous pattern, and mild inflammation. Intestine of lactoferrin loaded on silver nanoparticles treated mice showed mostly preserved villous pattern, and mild inflammation. Treatment with albendazole in combination with lactoferrin was better than albendazole as intestine of the former treated mice showed mostly preserved villous pattern and mild inflammation. The best result was with combination of albendazole and lactoferrin loaded on silver nanoparticles that showed mostly preserved villous pattern, and few inflammations.

Discussion

In the current study, there was a significant reduction in the recorded *T. spiralis* adult count in all infected treated mice as compared positive control mice.

The present study showed that adult count in mice treated with albendazole had a high reduction rate (90.38%). This agreed with Nassef *et al.* (2018), who found that albendazole-treated in murine trichinellosis gave a reduction of 90.2%. Also, it agreed with Nada *et al.* (2018) and Fahmy and Diab (2021),

they reported a reduction of 83.6% and 88.7%, respectively in albendazole-treated mice.

In the present study, *T. spiralis* adult count in mice treated with lactoferrin had a reduction of (59.86%). This agreed with Mossallam. (2009), they reported that lactoferrin reduced tachyzoites, without parasite burdens in mice with acute toxoplasmosis. Also, it agreed with León-Sicairos *et al.* (2012*)*, they reported that orally administered of bovine lactoferrin in mice amoebiasis and 20mg lactoferrin/kg orally administered daily for a week eliminated infection in 63% of trophozoites in cecum, and others (37%) showed a decrease in trophozoite numbers.

In the present study, adult count in mice treated with silver nanoparticles had a reduction of (22.3%)**.** This agreed with Taha *et al.* (2022), who reported that in vitro exposure of adults to silver nanoparticles, mortality was 100%, treated with 12.0ppm for 12hr.

In the current study, adult count in lacto ferrin loaded on silver nanoparticles-treated mice had a reduction of (84.27%) that was higher than in those treated with lactoferrin alone. This agreed with Anand *et al*. (2015), who reported that nano-formulation increa sed activity of lactoferrin protein against *Toxoplasma gondii* infected mice, as compared to native lactoferrin degraded by intestine.

The current study showed that *T. spiralis* adult count in mice treated with a combination of albendazole and lactoferrin had a reduction of (95.77%) that was higher than in those treated with albendazole alone due to synergistic action. This agreed with Salama *et al.* (2021), who reported a reduction of 93.5% in albendazole and prednisolone treated in *T. spiralis* infected mice. Also, adult count in mice treated with a combination of albendazole and lactoferrin loaded on silver nanoparticles gave maximum reduction of (98.59%). This agreed with Nassef *et al.* (2018), who reported that the highest *T. spiralis* reduction of (99.1%) was in mice treated by nanoparticles loaded with a full dose of albendazole.

The immune response against *T. spiralis* depends on T-helper cells stimulating cells of both Th1 & Th2 with an initial predominance of Th1 type and response of Th2 to cause protection and parasite expulsion (Ilic *et al,* 2012). This was done by secretion of cytokines (IL4, IL5, IL10, & IL13), and IgE (Bruschi and Chiumiento, 2012). The IL4 and IL13 activity causes TNF-α and INF-γ, release by activating intestinal mucosal mast cells resulting in local inflammation caused by TNF-α enhanced *T. spiralis* enteropathy development (Balaha *et al,* 2020). In helminthes-induced immunoregulation increased synthesis of IL-10 recurred feature of host's immunological response (Ilic *et al,* 2021**)**. IL-10 plays a crucial role in *Trichinella* immune response and immunoregulation by intestinal immune system **(**Bruschi and Dupouy-Camet, 2014).

In the present study, there was a significant elevation in TNF-α level in positive control mice as compared to negative ones, but treated mice showed a significant reduction in TNF-α level as emulated by infection. The TNF- α level in mice treated with albendazole was reduced (16.03±1.01). This agreed with Jari and Yousif (2020), who reported a significant decrease in TNF-α level (13.7±-0.6) in albendazole treated mice as compared to hydatidosis infected ones. Also, TNF- α level in mice treated with lactoferrin was reduced (27.60±1.71). This agreed with Siqueiros-Cendón *et al.* (2014), who reported that lactoferrin csuppressed pro-inflammatory cytokines preventing excessive inflammatory responses. Also, it agreed with Yanagisawa *et al.* (2022), who found that bovine lactoferrin markedly prevented rheumatoid arthritis pathogenicity by suppressing TNF-α production. Also, the present TNF-α level in mice treated with silver nanoparticles was reduced (23.77±0.60). This agreed with Wong *et al.* (2009), who found that silver nanoparticles decreased TNF-α formation of lipopolysaccharides stimulated macrophages by anti-inflammatory properties of silver nanoparticles by decreasing inflamma-

tion in peritoneal adhesions without side effects. Also, this agreed with Abdullatif *et al.* (2021), who reported that silver nano-particles inhibited TNF-α formation. Besides, Jari and Yousif, (2020), reported that TNF-α level in silver nanoparticles treated mice hydatidosis was reduced (12.3±1.6).

The present study showed that TNF-α level in mice lactoferrin loaded on silver nanoparticles treated was reduced (21.07±1.03) indicating more reduction in TNF-α level than in those treated only with lactoferrin. This agreed with Jari and Yousif (2020), who reported that nanocomposites were smart therapeutic carried chemical drugs to biological target capacity to absorb and thigh control of treatment release in inflammation areas protecting tissue damaged.

In the present study, the TNF- α level in a combination of albendazole and lactoferrin treated mice was reduced (10.93±2.49) indicating more reduction in TNF-α level than in those albendazole only treated. This agreed with Salama *et al.* (2021), who found that albendazole and prednisolone treatment significantly reduced TNF-α (51.5%) in murine trichinellosis.

In the present study, least TNF-α decrease in level was in mice treated with combination of albendazole and lactoferrin loaded on silver nanoparticles (10.83±0.40). This agreed with Jari and Yousif (2020) , who reported that the least decrease in TNF-α level was in albendazole and silver nanoparticles treated hydatidosis infected mice.

In the present study, there was significant elevation in IL-10 level in positive control mice as compared to negative ones. This agreed with Ding *et al. (*2017), reporting that Th2 cytokine IL-10 was markedly up-regulated in intestinal phase with a mixed systemic Th1/Th2 response in infected mice, with predominating Th2 response, with a significant reduction in IL-10 levels in all infected treated mice as compared to positive control. This agreed with Jari and Yousif (2020), who reported that echinococcosis treatment reduced IL-10level, and agreed with Amri *et al.* (2009), who reported high levels of cytokines IL-10 in patients who didn't respond to treatment.

In the present study, in albendazole treated mice IL-10 level was reduced (30.77±1.95). This agreed with Soliman *et al.* (2023), who showed that in *T. spiralis* infected mice, IL-10 level in albendazole treated mice was reduced (60.40±14.98). Also, IL-10 level in lactoferrin treated mice was reduced (130.1± 2.41) and IL-10 level in silver nanoparticles treated mice was reduced (126.47±6.14). This agreed with Margaroni *et al.* (2017), who showed a decrease in IL-10 level in visceral leishmaniasis infected mice treated with nanoparticles. But, it disagreed with Mohamed *et al.* (2019), who found a significant increase IL-10 level within 3 weeks in *L. donovani* infected mice were treated with silver nanoparticles compared to Pentostam.

The present study showed that IL-10 level in lactoferrin loaded on silver nanoparticles treated mice was reduced (94.17±3.77) with more reduction in IL-10 level than in those lactoferrin treated with only, which agreed with Halder *et al*. (2018), who found that IL-10 was decreased in *Leishmania donovani*-infected macrophages by lactoferrin-modified betulinic acid-loaded nanoparticles.

In the present study, IL-10 level in combination of albendazole and lactoferrin treated mice was reduced (14.80±1.31) than in mice treated with albendazole only due to synergistic action. This agreed with Yun *et al.* (2018), who found level of IL-10 in sera decreased in albendazole and IFN-α-treated mice than untreated in cystic echinococcosis ones. But, it disagreed with Palomares-Alonso *et al.* (2020), who found that IL-10 level didn't significantly change in albendazole and dexamethasone treated mice compared to cysticercosis infected ones.

In the present study, the least decrease in IL-10 level was in combination of albendazole and lactoferrin loaded on silver nanoparticles treated mice (13.23±0.42). This agreed with Jari and Yousif (2020), who found least decrease in IL-10 level was in albenda-

zole and silver nanoparticles as compared to hydatidosis treated mice.

In the current study, intestine of positive control mice showed some scattered adults, sever distorted villous pattern and inflammatory cellular infiltrate. But intestine of albendazole treated mice occasional showed adults preserved villous pattern with moderate inflammatory cellular infiltrate. This agreed with Balaha *et al.* (2020), who showed that small intestine of infected albendazole treated mice with moderately elongated villi, moderate edema and inflammatory cellular infiltrate. Also, it agreed with Fahmy and Diab (2021)**,** who found that infected mice treated with albenazole had mild inflammatory infiltrates, primarily in villi core with seemingly undamaged intestinal epithelial lining and finger-like villi. The present study showed that intestine of lactoferrin treated mice mostly preserved villous pattern, mild inflammatory cellular infiltrate due to antiinflammatory properties of lactoferrin and reduction of proinflammatory by TNF-α. This agreed with Liu *et al.* (2020), who reported that lactoferrin down regulated inflammation and up-regulated proliferation in intestinal epithelium in necrotizing enterocolitis injury. Also, it agreed with Liu *et al.* (2019), who reported that lactoferrin reduced inflammation and induced cell proliferation by potential pharmacological interventions. The intestine of silver nanoparticles treated mice sometimes showed degenerated adults, moderate distorted villous pattern and mild inflammatory cellular infiltrate. This agreed with Hassan *et al.* (2021), who found that mice given nano-particles didn't significantly differed in intestinal inflammatory changes as positive control.

In the present study, intestine of lactoferrin loaded on silver nanoparticles treated mice showed preserved villous pattern, and mild inflammation. This agreed with Gupta *et al.* (2015), who reported that after two days up to six days post-*Salmonella* treatment, the small intestine, liver, spleen, and brain in nanoformulated lactoferrin-treated mice were normal.

In the present study, intestine of mice treated with combination of albendazole and lactoferrin preserved villous pattern with mild inflammation. This agreed with Balaha *et al.* (2020), who found that albendazole and resiniferatoxin infected treated mice showed mild inflammation with normal intestinal epithelium. The present study showed that intestine of a combination of albendazole and lactoferrin loaded on silver nanoparticle treated mice gave the least inflammation intensity and mostly preserved villous pattern. This agreed with Hassan *et al. (*2021), who found that infected mice given ivermectin with nitazoxanide loaded on nanoparticles showed a marked reduction in inflammatory infiltrates with a mild increase in goblet cells and infection clearance.

Conclusion

Lactoferrin had a good effect on *Trichinella spiralis* infected mice as a promising treatment, especially when loaded on silver nanoparticles that improved its efficacy by increasedits delivery to tissues.

Also, lactoferrin treatment (either alone or loaded on silver nanoparticles) in combination with albendazole improved its efficacy as a good *T. spiralis* treatment.

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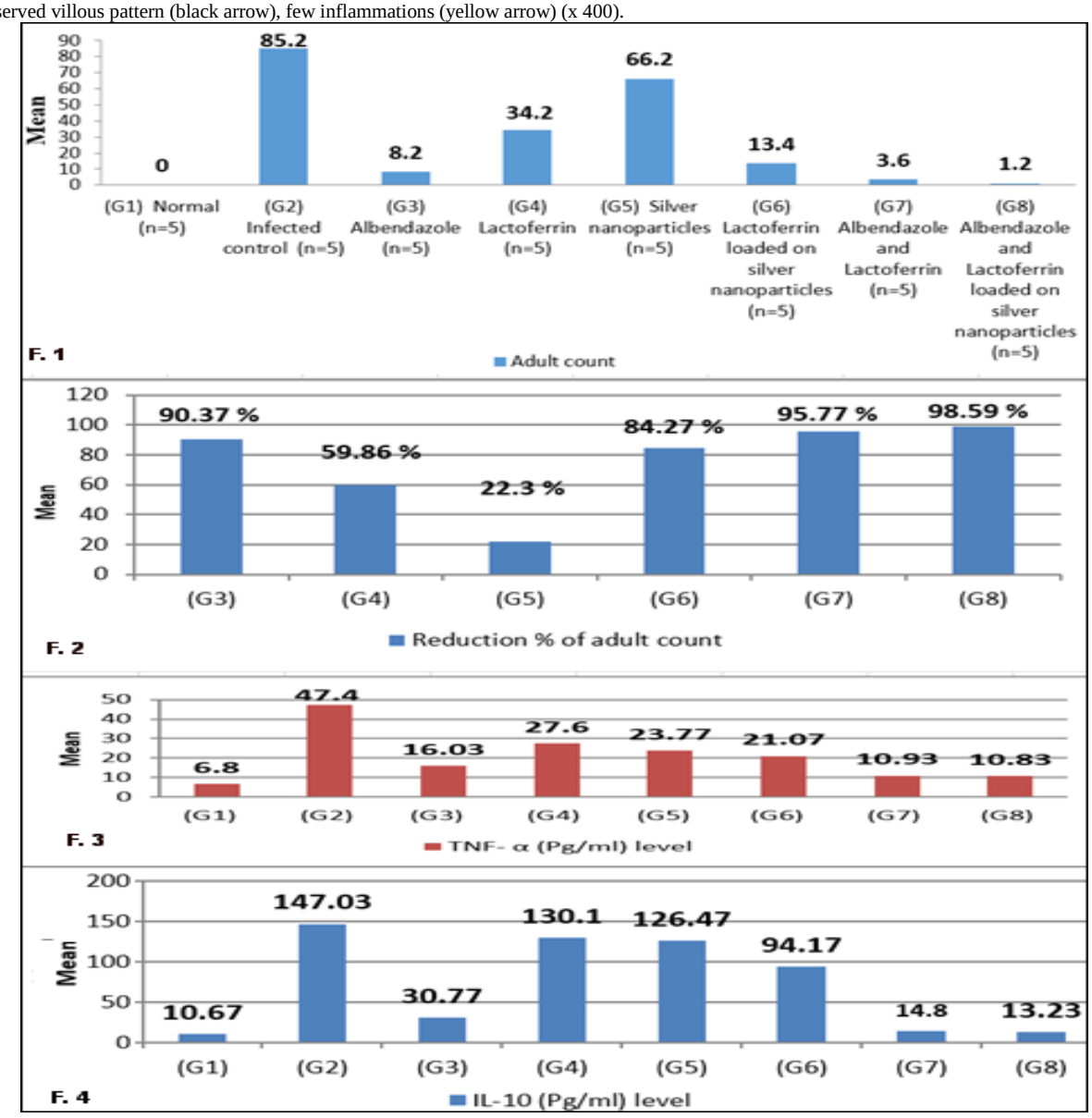
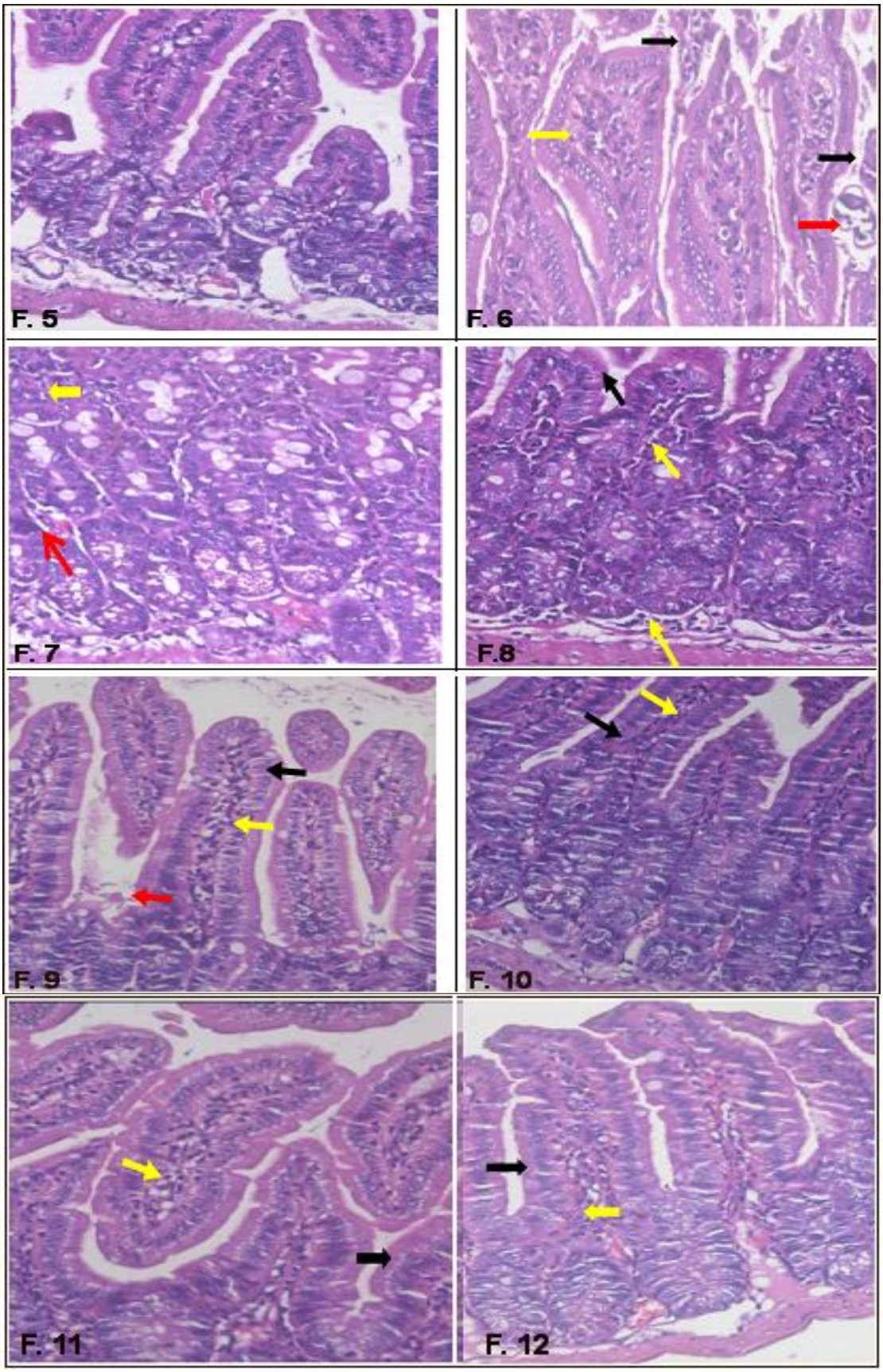
Explanation of figures

Fig. 1: Comparison between different groups regarding *T. spiralis* adult count.
Fig. 2: Comparison between different groups regarding reduction rate of *T. spiralis* adult count.
Fig. 3: Comparison between different groups regarding TNF- α(Pg/ml) level.
Fig. 4: Comparison between different groups regarding IL-10 (Pg/ml) level.
Fig. 5: Sections in negative control intestine stained with H&E, showed normal intestinal architecture (x 400).
Fig. 6: Sections in positive control intestine stained with H&E, showing some *T. spiralis* adults sections (red arrow) and a distorted villous pattern (black arrow), dense inflammation (yellow arrow) (x 400).
Fig. 7: Sections in albendazole treated mice intestine stained with H&E, showing occasional *T. spiralis* adults sections (red arrow) and a mostly preserved villous pattern, moderate inflammation (yellow arrow) (x 400).
Fig. 8: Sections in lactoferrin treated intestine mice stained with H&E, showed mostly preserved villous pattern (black arrow), and mild inflammation (yellow arrow) (x 400).
Fig. 9: Sections in silver nanoparticles treated intestine mice stained with H&E, showed occasional degenerated *T. spiralis* adults sections (red arrow) moderate distorted villous pattern (black arrow), mild inflammation (yellow arrow) (x 400).
Fig. 10: Sections in lactoferrin loaded on silver nanoparticles treated intestine mice stained with H&E, showed mostly preserved villous pattern (black arrow), mild inflammation (yellow arrow) (x 400).
Fig. 11: Sections in albendazole and lactoferrin treated intestine mice stained with H&E, showed mostly preserved villous pattern (black arrow), mild inflammation (yellow arrow) (x 400).
Fig. 12: Sections in albendazole and lactoferrin loaded on silver nanoparticles treated intestine mice stained with H&E, showed mostly preserved villous pattern (black arrow), few inflammations (yellow arrow) (x 400).

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