

Evaluation the levels of some immune parameters in rats infected with a parasite *Enterobius vermicularis* that treated with gold nanoparticles , neem extract, and albendazole

¹ Sinai Najy Muhsin, ² Ashraf Jamal Mahmoud, ³ Iktifaa Abdel Hamid

1, 2, 3 Biology Department, College Of Education For women, Tikrit university, Tikrit, Iraq

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Corresponding Author:

Name:

Sinai Najy Muhsin Saleh

E-mail:

sennaasena38@tu.edu.iq*

Tel: [07702302627](tel:07702302627)

ABSTRACT

Background and Objective: *Enterobius vermicularis* also known as pinworm, is the most common a parasitic worm infections in the world, with most cases among children. The current study aimed to demonstrate the levels of some immunological parameters in rats infected with pinworm *Enterobius vermicularis* and the effect of treatment with two concentrations of gold particles (1.5% and 2%), neem extract, neem extract with gold particles and albendazole treatment on those parameters. **Methods:** A total of 640 stool samples were collected from patients attending Tikrit Hospital and private laboratories in Tikrit city, for the period from January to October 2024. The rats were divided into five groups included: Negative control: uninfected, positive control: Infected without treatment, positive control: Infected with treatment, Immunized (injected with 1 ml of attenuated egg vaccine) infected and treated and negative control rats (treatment group only). **Results:** The different concentrations of gold nanoparticles, the loaded nanocomposite, neem extract, and medical treatment demonstrated effect on the levels of immune parameters in the treated groups compared to the infected group without treatment. In infected rats without treatment, a significant increase was found in the concentrations of transforming growth factor beta with a mean value 371.8 pg/ml and interleukin-8 with a mean value 288.8 pg/ml at a significant level ($P \leq 0.01$), while a significant decrease was found in the concentrations of tumor necrosis factor alpha with a mean value 18.3 pg/ml and interleukin-12 with a mean value 9.65 pg/ml at a significant level ($P \leq 0.01$). Meanwhile, histamine levels among the groups were within the normal range at a significant level ($P \leq 0.05$). No significant changes were observed in those parameters of other treated groups , which remain within normal values. **Conclusion:** Infection of laboratory rats with *Enterobius vermicularis* revealed a significant increases in the both concentrations of transforming growth factor beta and interleukin-8 at a significant level ($P \leq 0.01$), while a significant decreases were found in the concentrations of tumor necrosis factor alpha and interleukin-12 at a significant level ($P \leq 0.01$). Meanwhile, histamine levels among the groups were within the normal range at a significant level ($P \leq 0.05$).

Introduction

E. vermicularis (pinworm), also known as seatworm, is a nematode that parasitizes the large intestine of humans, especially children. It is the most common nematode, particularly in tropical and temperate regions, and can impact the economic and social development of the population. An estimated 200 million people are infected annually. Pinworms are most prevalent in crowded institutions such as kindergartens, schools, hospitals, and orphanages [1].

Humans become infected through direct contact with an infected person, inhaling airborne eggs during hot weather, consuming contaminated food and water, or being bitten by insects. The infection is usually asymptomatic. However, prolonged infection can cause anal itching, abdominal discomfort, insomnia, nocturia, inflammation of the fallopian tubes, vaginitis in females due to the worms' nocturnal migration, and other symptoms and signs that affect the health and development of children [2]. The type II immune response is activated in coordination with type II helper T cells (TH2) during pinworm infection with elevated levels of eosinophil cationic protein (ECP, a basic protein located in the primary structure of the eosinophil matrix) as well as IgE antibody (specific for worm antigens) [3, 4].

The development of environmentally friendly technologies is of great importance for expanding their use in biological applications. This includes the manufacture of nanoparticles, including gold nanoparticles (AuNPs), with sizes, chemical compositions, and shapes that enable their use in medical fields. These particles efficiently couple with antibodies to detect targeted biological molecules, and can therefore be used in technologies for detecting and diagnosing diseases such as cancer. They can also transport therapeutic agents to cells. Ultimately, these particles play a major role in medical treatment, and there is significant interest in using these particles in drug modification processes to reduce their side effects while simultaneously delivering high doses to targeted tissues [5].

Although many studies confirm that humans are the only host for *Enterobius vermicularis*, numerous studies indicated that animals can be infected with *Enterobius vermicularis*, either naturally or experimentally, a study conducted by [6] demonstrated a fatal infection with the human threadworm, *Enterobius vermicularis*, in chimpanzee kept in a zoo. Also, There is a study by [7] aimed to identifying *E. vermicularis* egg

antigens as a first step towards identifying diagnostic targets for detection in infected individuals, using pinworm eggs to prepare antigen strips and vaccinating them subcutaneously (with weekly booster doses) in Wistar rats to develop antibodies. Another study aimed to experimentally investigate the potential therapeutic effects of melatonin against the *Enterobius vermicularis* parasite in mice [8].

During infection with intestinal parasitic worms, the host initiates a type II response, coordinated by TH2 cells, to expel the worms. Type II reactions in the intestine lead to increased peristaltic movements, accelerated intestinal epithelial cell turnover, and goblet cell hyperplasia, leading to increased mucus production—effects collectively referred to as the weep-and-sweep response. (“Shedding” refers to the process by which the intestinal contents are diluted by increased water and mucus secretion, and, in parallel, increased smooth muscle activity to “clear” the intestinal contents [9]. The weep-and-sweep response is mediated primarily by the characteristic T helper 2 (TH2) cytokines IL-4, IL-5, and IL-13, and by inflammatory mediators: histamine and prostaglandins secreted by mast cells, for example. In addition, type 2 responses result in eosinophilia, and most IgE antibodies immediately bind to high-affinity IgE receptors on mast cells and basophils [10].

Neem plant contains active antiparasitic components such as phenolics, alkaloids, flavonoids, saponins, and tannins. Alkaloids paralyze the central nervous system of many parasitic worms. Phenolic compounds have oxidative and reduction properties that negatively affect free radicals, while flavonoids have a parasiticide effect. Saponins have a parasiticide effect by affecting the permeability and disintegration of cell membranes. Tannins paralyze parasites [11,12].

Materials and Methods

1: Nematode egg isolation: Worm eggs were isolated from positive samples. Ethics approval for the study was obtained from the ethics review committee of the college of dentistry, University of Tikrit. Floating eggs were obtained in the upper layer of the tubes due to the high specific gravity of zinc sulfate, which is the commonly used solution for detecting the presence of cysts of protozoa or worm eggs. Eggs were also suspended according to the method of [13]. The female worm was cut and transferred to a 0.1N NaOH solution. The solution was placed on a magnetic

stirrer for 10 minutes. The solution was placed in tubes and centrifuged at 3000 rpm for 5 minutes, then it was poured and distilled water was added to the precipitate. The sedimentation and washing process was repeated several times to remove the effect of the solution. The egg extract was then incubated in a 0.1N H₂SO₄ solution at 4°C.

2: Worm egg count: Egg counts were performed by taking a drop from the floating layer and placing it on a glass slide (counting chamber). The number of eggs was calculated in two squares and multiplied by 100, thus obtaining the number of eggs per gram [14].

3: Radiation: The vaccine was prepared in the laboratories of the Physics Department, College of Education for Pure Sciences, Tikrit University, where the solution containing the pinworm eggs was exposed to gamma rays of 200 and 250 Gray. Laboratory rats were injected with 1 ml of the weakened eggs intramuscularly using a sterile plastic syringe. Preparation of the aqueous extract of neem: The extract was prepared using the method of [15, 16].

4: Preparation of the loaded nanocomposite: The loaded composite was prepared by adding 5 ml of the aqueous extract of neem leaves to 45 ml of a solution of gold chloride (HAuCl₄.3H₂O). The mixture was stirred using a magnetic stirrer at 80°C for 30 minutes until the color changed to dark green [17].

5: Gold nanoparticles (GNPs): Gold nanoparticles (GNPs) were prepared by chemical synthesis. Citrate-coated GNPs with a diameter of 20 nm were prepared by reducing 1 mmol of HAuCl₄ using sodium citrate (1%) according to [18, 19]. As follows:

- A. 10 ml of HAuCl₄ was heated to near boiling temperature.
- B. 1 ml of citrate solution was quickly added.
- C. The solution was stirred continuously with a magnetic stirrer. The yellow color of the gold ions

began to fade to colorless, then a light blue color appeared, followed by a dark blue color, and then the color turned red after 15 minutes of heating.

D. The mixing continued until the solution reached room temperature.

E. The dark red mixture was filtered through a 0.22 μm Millipore membrane filter. The gold nanoparticles were then stored in a refrigerator at 4°C until use.

6: Immunological Tests: Immunological tests were conducted at the Central Laboratories Department at Tikrit University. The enzyme-linked immunosorbent assay (ELISA) technique was performed using SUNLONG biotech ELISA kit /China, to detect the following immunological tests included in the current study on all sera of the samples under study, as follows:

- A. Histamine concentration
- B. Tumor necrosis factor alpha (TNF-α) concentration
- C- Interleukin-12 (IL-12) concentration
- D- Transforming growth factor beta (TGF-β) concentration
- E. Interleukin-8 (IL-8) concentration

7: Experimental Infection in lab. Rats: In the current study, male laboratory rats were used, obtained from the Animal House - College of Veterinary Medicine, Tikrit University. Their ages ranged from 4-6 weeks, and their weights reached 150-200 grams. The rats were placed in plastic cages equipped with metal covers, with the floor of the cages covered with sawdust. The animals were carefully cared for, including cage cleanliness, daily sawdust replacement, and adequate conditions, including temperature and ventilation, were provided during the study period.

8: Study Design: The white rats were divided into different groups according to the study experiment, these groups included:

Groups	Number	Volume/ml	Treated material
Negative control: Uninfected	5	1 ml	0.9% saline
Positive control: Infected without treatment	5	-	without treatment
Positive control: Infected with treatment	12	1 ml	1.5% concentration of gold nanoparticles
	12	1 ml	2% concentration of gold nanoparticles
	12	1 ml	neem extract with gold nanoparticles
	12	1 ml	neem extract
	12	1 ml	albendazole treatment

Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	12	1 ml	1.5% concentration of gold nanoparticles
	12	1 ml	2% concentration of gold nanoparticles
	12	1 ml	neem extract with gold nanoparticles
	12	1 ml	neem extract
	12	1 ml	albendazole treatment
Negative control rats (treatment group only)	12	1 ml	1.5% concentration of gold nanoparticles
	12	1 ml	2% concentration of gold nanoparticles
	12	1 ml	neem extract with gold nanoparticles
	12	1 ml	neem extract
	12	1 ml	albendazole treatment

9: Analysis Statistical analysis: The results were statistically analyzed using Minitap, Ver17, and the ANOVA test was applied, where the arithmetic means were compared using Duncun multiple range test at probability levels ($p \leq 0.01$) and ($p \leq 0.05$) to determine the significant differences in the infection with threadworms under study and its relationship to the immune parameters when compared with the control groups.

Results

For histamine, the mean concentration was as follows: in uninfected negative control group was 12.26 ng/ml, in a positive infected control

group (without treatment) was 15.55 ng/ml, in a positive infected and treated group: the highest rate was 16.45 ng/ml for 1.5% concentration of gold nanoparticles treatment and the lowest rate was 13.79 ng/ml for albendazole treatment. In the immunized, infected and treated group: the highest rate was 14.40 ng/ml for 1.5% concentration of gold nanoparticles treatment and the lowest rate was 12.44 ng/ml for neem extract and in a negative control group (treatment group only): the highest rate was 14.33 ng/ml for 1.5% concentration of gold nanoparticles treatment and the lowest rate was 11.07 ng/ml for albendazole treatment. Table (1).

Table (1): Show the level of histamine concentration in the different groups

Groups		M±S.D
		Histamine ng/ml
Negative control: Uninfected		12.26±4.30 Cd
Positive control: Infected without treatment		15.55 ±2.39 Ab
Positive control: Infected with treatment	1.5% gold nanoparticles	16.45±3.17 A
	2% gold nanoparticles	13.98±4.14 bcd
	Neem extract with gold nanoparticles	15.25±2.74 Ab
	Neem extract	14.21±2.73 Abc
	Albendazole treatment	13.79±2.14 Bcd
Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	1.5% gold nanoparticles	14.40±4.07 Abc
	2% gold nanoparticles	13.41±3.04 Bcd
	Neem extract with gold nanoparticles	12.51±4.51 Cd
	Neem extract	12.44±4.53 cb
	Albendazole treatment	13.33±3.25 Bcd
Negative control rats (treatment group only)	1.5% gold nanoparticles	14.33±2.06 Abc
	2% gold nanoparticles	13.10±3.51 Bcd
	Neem extract with gold nanoparticles	12.27±5.22 Cd
	Neem extract	11.63±5.20 D
	Albendazole treatment	11.07±3.78 D
Statistical analysis		F-Value=2.60* p-value=0.05

The ** symbol indicates the presence of significant differences at the $P \leq 0.01$ level, the * symbol indicates the presence of significant differences at the $P \leq 0.05$ level, similar letters indicate no significant differences, different letters indicate the presence of significant differences.

In Tumor necrosis factor alpha (TNF- α), the mean concentration was as follows: uninfected negative control group was 166.5 pg/ml , in a positive infected control group(without treatment) was 18.3 pg/ml, in a positive infected and treated group: the highest rate was 172.6 pg/ml for neem extract with gold nanoparticles treatment and the lowest rate was 101.0 pg/ml for 1.5% concentration of gold nanoparticles treatment, in

the immunized, infected and treated group: the highest rate was 155.8 pg/ml for 1.5% concentration of gold nanoparticles treatment and the lowest rate was 141.5 pg/ml for albendazole treatment and in a negative control group (treatment group only): the highest rate was 140.6 pg/ml for 2% gold nanoparticles treatment and the lowest rate was 122.7 pg/ml for Albendazole treatment. Table (2).

Table (2): Level of TNF- α concentration in the different groups

Groups		M \pm S.D	
		TNF- α pg/ml	
Negative control: Uninfected		166.5 \pm 23.7	Ab
Positive control: Infected without treatment		18.3 \pm 3.919	H
Positive control: Infected with treatment	1.5% concentration of gold nanoparticles	101.0 \pm 26.2	G
	2% concentration of gold nanoparticles	158.2 \pm 34.4	Abc
	Neem extract with gold nanoparticles	172.6 \pm 31.0	A
	Neem extract	169.5 \pm 31.4	Ab
	Albendazole treatment	146.2 \pm 25.5	Cd
Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	1.5% concentration of gold nanoparticles	155.8 \pm 34.4	Abc
	2% concentration of gold nanoparticles	145.4 \pm 33.71	Cd
	Neem extract with gold nanoparticles	151.3 \pm 25.1	Bcd
	Neem extract	144.3 \pm 29.8	Cd
	Albendazole treatment	141.5 \pm 50.2	B
Negative control rats (treatment group only)	1.5% concentration of gold nanoparticles	133.5 \pm 34.22	Ef
	2% concentration of gold nanoparticles	140.6 \pm 33.55	D
	Neem extract with gold nanoparticles	139.8 \pm 31.26	De
	Neem extract	134.6 \pm 23.99	Ef
	Albendazole treatment	122.7 \pm 38.2	F
Statistical analysis		F-Value= 4.80**	P-Value=0.01

For Interleukin-12 (IL-12), the mean concentration was as follows: uninfected negative control group was 66.10 pg/ml , in a positive infected control group(without treatment) was 9.65 pg/ml, in a positive infected and treated group: the highest rate was 74.22 pg/ml for 2% concentration of gold nanoparticles treatment and the lowest rate was 30.99 pg/ml for 1.5% concentration of gold nanoparticles treatment, in the immunized, infected and treated group: the

highest rate was 65.86 pg/ml for Albendazole treatment and the lowest rate was 60.82 pg/ml for 1.5% concentration of gold nanoparticles treatment and in a negative control group (treatment group only): the highest rate was 73.03 pg/ml for 2% concentration of gold nanoparticles treatment and the lowest rate was 58.09 pg/ml for 1.5% concentration of gold nanoparticles. Table (3).

Table (3): Level of IL-12 concentration in the different groups.

Groups		M \pm S.D	
		IL-12 pg/ml	
Negative control: Uninfected		66.10 \pm 14.9	Ab
Positive control: Infected without treatment		9.65 \pm 2.54	D
Positive control:	1.5% concentration of gold nanoparticles	30.99 \pm 6.80	C
	2% concentration of gold nanoparticles	74.22 \pm 7.91	A

Infected with treatment	Neem extract with gold nanoparticles	68.68±7.97	Ab
	Neem extract	69.17±8.20	Ab
	Albendazole treatment	67.92±14.64	A
Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	1.5% concentration of gold nanoparticles	60.82±18.04	B
	2% concentration of gold nanoparticles	61.78±15.29	B
	Neem extract with gold nanoparticles	64.42±16.19	Ab
	Neem extract	64.44±13.63	Ab
	Albendazole treatment	65.86±11.49	Ab
Negative control rats (treatment group only)	1.5% concentration of gold nanoparticles	58.09±11.81	B
	2% concentration of gold nanoparticles	73.03±6.82	A
	Neem extract with gold nanoparticles	68.01±10.75	Ab
	Neem extract	64.07±12.58	Ab
	Albendazole treatment	65.18±14.47	Ab
Statistical analysis		F-Value=9.27**	P-Value=0.01

For transforming growth factor beta (TGF-β), the mean concentration was as follows: uninfected negative control group was 172.9 pg/ml , in a positive infected control group(without treatment) was 371.8 pg/ml, in a positive infected and treated group: the highest rate was 204.2 pg/ml for 1.5% concentration of gold nanoparticles treatment and the lowest rate was 142.1pg/ml for 2% concentration of gold nanoparticles treatment, in the immunized, infected and treated group: the

highest rate was 167.7 pg/ml for neem extract with gold nanoparticles treatment and the lowest rate was 135.9 pg/ml for 1.5% concentration of gold nanoparticles treatment and in a negative control group (treatment group only): the highest rate was 139.0 pg/ml for neem extract treatment and the lowest rate was 109.5 pg/ml for 1.5% concentration of gold nanoparticles treatment. Table (4).

Table (4): Shows the differences in level of TGF-b concentration between the different groups.

Groups		M±S.D	
		TGF-b pg/ml	
Negative control: Uninfected		172.9±29.9	C
Positive control: Infected without treatment		371.8±19.69	A
Positive control: Infected with treatment	1.5% concentration of gold nanoparticles	204.2±38.4	B
	2% concentration of gold nanoparticles	142.1±22.2	de
	Neem extract with gold nanoparticles	163.2±31.59	C
	Neem extract	158.7±31.16	Cd
	Albendazole treatment	137.9±32.57	E
Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	1.5% concentration of gold nanoparticles	135.9±29.3	E
	2% concentration of gold nanoparticles	147.4±24.9	De
	Neem extract with gold nanoparticles	167.7±32.88	C
	Neem extract	162.3±34.31	C
	Albendazole treatment	143.5±29.0	De
Negative control rats (treatment group only)	1.5% concentration of gold nanoparticles	109.5±29.1	G
	2% concentration of gold nanoparticles	118.2±31.5	Fg
	Neem extract with gold nanoparticles	137.5±28.7	E
	Neem extract	139.0±26.30	E
	Albendazole treatment	131.4±25.5	Ef
Statistical analysis		P-Value= 0.01	F-Value= 11.71**

Regarding interleukin-8 (IL-8), the mean concentration was as follows: the mean concentration was as follows: uninfected negative control group was 139.7 pg/ml , in a positive infected control group(without treatment) was 288.8 pg/ml, in a positive infected and treated

group: the highest rate was 150.0 pg/ml for neem extract with gold nanoparticles treatment and the lowest rate was 100.8 pg/ml for 2% concentration of gold nanoparticles treatment, in the immunized, infected and treated group: the highest rate was

148.1 pg/ml for neem extract with gold nanoparticles treatment and the lowest rate was 120.9 pg/ml for 2% concentration of gold nanoparticles treatment and in a negative control group (treatment group only): the highest rate was 135.9 pg/ml for Albendazole treatment and the

lowest rate was 96.1 pg/ml for 1.5% concentration of gold nanoparticles treatment. Table (5).

Table (5): Shows level of IL-8 concentration in the different groups.

Groups		M±S.D
		IL-8 pg/ml
Negative control: Uninfected		139.7 ±30.4 Bc
Positive control: Infected without treatment		288.8±15.28 A
Positive control: Infected with treatment	1.5% concentration of gold nanoparticles	124.3±31.50 C
	2% concentration of gold nanoparticles	100.8±38.2 D
	Neem extract with gold nanoparticles	150.0±38.7 B
	Neem extract	148.5±38.9 B
	Albendazole treatment	131.2±32.25 C
Immunized (injected with 1 ml of attenuated egg vaccine) Infected and treated	1.5% concentration of gold nanoparticles	123.4±20.34 C
	2% concentration of gold nanoparticles	120.9±36.3 C
	Neem extract with gold nanoparticles	148.1±38.2 B
	Neem extract	141.0±31.82 B
	Albendazole treatment	127.8±24.7 C
Negative control rats (treatment group only)	1.5% concentration of gold nanoparticles	96.1±15.31 D
	2% concentration of gold nanoparticles	124.5±31.63 C
	Neem extract with gold nanoparticles	130.6±31.16 C
	Neem extract	131.6±25.42 C
	Albendazole treatment	135.9±40.9 Cd
Statistical analysis		F-Value= 7.37 ** P-Value= 0.01

Discussion

Although the study recorded significant differences in histamine concentration at the significance level ($P \leq 0.05$) among the groups, the concentrations still within the normal range, this result is consistent with previous study by [20] that injected an extract of *Ascaris* worm into the peritoneum of guinea pigs, which did not lead to the release of histamine into the peritoneal fluid, as well as another study by [21] that concluded that mast cells and their components were not a major factor in the elimination of the nematode *Nippostrongylus brasiliensis* in rats infected with it. While this result did not agree with the study, by [22]. Mast cells store a number of inflammatory mediators including histamine and proteases that are released through a process called degranulation into tissues. The release process depends on several factors including the pH of the affected part of the intestine, the incubation time, and the worm load in the intestine [20].

As for the levels both of tumor necrosis factor alpha and Interleukin-12, this study demonstrated that the levels of TNF- α and IL-12 were lower in the group of infected rats without treatment

compared to the rest of the groups, this result agreed with both [23,24], the later indicated that Soluble products from *Trichuris suis* (*Trichella spiralis*) inhibit TNF- α and IL-12 secretion, inducing significant symptomatic

suppression in experimental autoimmune encephalomyelitis in mice.

A study conducted by [25] demonstrated the effects of the nematode *Litomosoides sigmodontis* (a rodent-infecting worm) on the inflammatory responses of mice injected with sublethal doses of lipopolysaccharide (LPS) (antigens present in the outer membrane of Gram-negative bacteria that increase IL-12 levels). Mice infected with adult worms showed lower levels of pro-inflammatory cytokines in the peripheral blood compared to controls. He interpreted these results as indicating that adult worms could attenuate the innate immune response by reducing the inflammatory response induced by LPS. Possible mechanisms for this phenomenon include the induction of higher frequencies of regulatory T cells, the development of alternatively activated macrophages, and reduced antigen presentation. All of these mechanisms are known to result from

helminth infection, and they all have the potential to reduce pro-inflammatory immune responses to LPS. Another possible explanation for the protective effects of adult worms is the release of helminth products that directly inhibit the LPS-induced immune response. Previous studies have demonstrated that parasitic worms can secrete products that reduce TLR responses to LPS. This may be because adult worms release immunomodulatory substances to protect the larval stages from destruction, especially since the presence of larvae in infected mice has been shown to increase levels of TNF- α and interleukin-12.

In addition, the study showed that the concentration rate of the transforming growth factor beta was high in the group of infected rats without treatment compared to the rest of the groups, This result is agreement with a study by [26] that demonstrated the immunomodulatory effects of excretory and secretory products (ESPs) of the gastrointestinal nematode *Haemonchus contortus* in vivo and vitro, goats were infected with different doses of ESPs produced by L3 larvae. The results indicated a significant increase in the expression of TGF- β signaling compared to the control group. Following deworming, the expression of the signaling regulator was significantly reduced. Furthermore, this result is also consistent with a study by [27] that infection with gastrointestinal nematodes is associated with increased epithelial TGF- β activity in vivo.

With reference to the level of TGF- β in both Positive control (Infected with treatment), immunized (Infected and treated) groups with different concentrations of gold particles, it may be due to the effect of these particles as a distinct medical technology in enhancing the delivery of drugs to the infected tissues and thus reducing the damage to those tissues and improving them through the effects of these particles on the body's response to treatment. As for the infected rats treated with albendazole which is considered a standard treatment for eliminating threadworms and reducing their effects on the inflammatory response.

Regarding interleukin-8, The present study found that the concentration of IL-8 was higher in the group of infected rats without treatment compared to the rest of the groups, This result agrees with the finding of a study by [28] which included the effect of concurrent parasitic infection (amoebiasis, filariasis, necatoriasis) and antiparasitic treatment on cytokine and chemokine responses in patients infected with single and

multiple parasites, inflammatory chemokines, including interleukin-8, were produced, after antiparasitic treatment, IL-8 secretion was significantly reduced. the result of this study also concurrent with a study by [29], Through his study, he determined the expression patterns of several cytokines, including interleukin-8 concentration and antioxidant genes, from an experimental infection with *Haemonchus contortus* (a nematode that infects ruminants) in Pelibuey lambs, where he showed an increase in the level of interleukin-8 concentration.

The decrease in interleukin-8 levels in both Positive control (Infected with treatment), immunized(Infected and treated) groups with different concentrations of gold particles and neem extract to within normal levels, may be due to the fact that gold nanoparticles can affect the immune system by reducing cytokine secretion. The particles may play a significant role in reducing inflammatory activity by stimulating or inhibiting immune cells from secreting cytokines, as well as due to the effect of albendazole treatment in eliminating the parasite and reducing the parasite load, which is reflected in the inflammatory response.

Conclusion

Infection of laboratory rats with *Enterobius vermicularis* demonstrated a significant increase in the concentrations of transforming growth factor beta and interleukin-8 at a significant level ($P \leq 0.01$), while a significant decrease was found in the concentrations of tumor necrosis factor alpha and interleukin-12 at a significant level ($P \leq 0.01$). Meanwhile, histamine levels among the groups were within the normal range at a significant level ($P \leq 0.05$).

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Declaration of interests

the authors declare no competing interests.

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Publication consent

Data and material availability

All data analyzed and generated in this study are included in this published research.

Author contribution

All authors participated in the study design and conception, data analysis, data collection, performance of the results, and assent to the final version.

References

- [1] Jukic, M., Nizeteo, P., Matas, J., and Pogorelic, Z. 2023. Trends and predictors of pediatric negative appendectomy rates: A single-centre retrospective study. *Children.*, 10(5), 39-50.
- [2] Pogorelic, Z., Beara, V., Jukic, M., Rashwan, H., Susnjar, T. 2022. A new approach to laparoscopic appendectomy in children-clipless/sutureless Harmonic scalpel laparoscopic appendectomy. *Langenbeck's Archives of Surgery*, 407(2), 779-787.
- [3] Ozturk, B.O., and Eroglu, f. 2024. Comparison of miRNA profiles in the immune response of pediatric acute appendicitis and pediatric enterobiasis patients caused by *Enterobius vermicularis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 118(7), 458-464.
- [4] Mohy, A.A., Al-Hadraawy, S.K., and ALhadrawi, K.K. 2022. Immunohistopathological Study for Patients with Appendicitis due to *Enterobius vermicularis* worm. *Egyptian Journal of Hospital Medicine*, 88(1), 3576-3581.
- [5] Raj, S., Sasidharan, S., Tripathi, T., and Saudagar, P. 2022. Biofunctionalized Chrysin-conjugated gold nanoparticles neutralize *Leishmania* parasites with high efficacy. *International Journal of Biological Macromolecules*, 2(5), 19- 211.
- [6] Murata, K., Nakano, T., Hasegawa, H., and Noda, A. 2002. Fatal infection with human pinworm, *Enterobius vermicularis*, in a captive chimpanzee. *Journal of Medical Primatology*, 31(2), 8-104.
- [7] De Kostha, N.S., Pathirana, S., Handunnetti, S., and Gunawardena, s.2022. Characterization of antigens of *Enterobius vermicularis* (pinworm) eggs. *ournal of Medical Primatology*, 12, 4-14.
- [8] Shlash, S.A., Hasnawi, N.M., Abu Gulel, H.A.K., and Shlashprofessor, S.A. 2022. Influence of melatonin in the treatment of experimental *Enterobius vermicularis* infection. *Indian Journal of Forensic Medicine & Toxicology*, 15(1), 314-317.
- [9] Alexander Perniss, A., and Bankova, L.G. 2024. Weep and sweep and the brom: tuft cell acetylcholine limits the worm. *Trends in Parasitology*, 40(8), 60-84.
- [10] Classon, C, 2020. Effects of intestinal worms on skin immunity and control of co-infection, Karolinska institutet, Stockholm, Sweden. Pp 27.
- [11] Thagfan, F.A., Al-Megrin, W.A., Al-Quraishy, S., and Dkhil, M.A.M. 2020. Mulberry extract as an ecofriendly anticoccidial agent: in vitro and in vivo application. *Brazilian Journal of Veterinary Parasitology*, 29(4), 1-9.
- [12] Hawsah, M.A., AL-Otaibi, T., ALojayri , G., AL-SHaebi , E.M., Dkhil, M.A., Elkhadragy , M.F., AL-Quraishy , S., and Gaber, R.A. 2023. In vitro studies for the antiparasitic activities of *Azadirachta indica* extract. *Food Scien. Journal of Technology*, 43(11), 1-9.
- [13] Faibarín, D. 1957. Physiological of *A. lumbricoides*. Experiment and techniques in parasitology. free man Company, Sanfrancisco. PP 20-23.
- [14] Ghazal, A.M. 1972. Experimental epidemiology of dwarf tapeworm *Hymenolepis nana* in the mouse. Ph.D. thesis , Univ. Bristol.
- [15] Jenkins, M.C., Castle, M.D., and Danforth, H.D. 1991. Protctive immunization against the intestinal parasite *Eimeria acervulina* with recombinant coccial intigen. *Poultry Science*, 70, 539-547.
- [16] Harborne, J.B., 1984. Phytochemical method. A guida to modern techniques of plant analysis. 2nd ed., ChaPman and Hail Ltd , London, NewYork.
- [17] Morsi, M.A., Oraby, A.H., Elshahawy, A.G., and Abd El-Hady, R.M. 2019. Preparation, structural analysis, morphological investigation and electrical properties of gold nanoparticles filled polyvinyl alcohol/carboxymethyl cellulose blend. *Journal of Materials Research and Technology*, 8, 5996-6010.
- [18] Herizchi, R., Abbasi, E., Milani, M., & Akbarzadeh, A. 2016. Current methods for synthesis of gold nanoparticles. *Artificial cells, nanomedicine and biotechnology*, 44(2), 596-602.
- [19] Leng, W., Pati, P., & Vikesland, P. J. 2015. Room temperature seed mediated growth of gold nanoparticles: mechanistic investigations and life

cycle assesment. *Environment. Science and Technology of Advanced Materials: Methods*, 2(5), 440- 453.

[20] Diamant, B. 1961. Histamine Release Elicited by Extracts from *Ascaris Suis* – Influence of Oxygen Lack and Glucose. *Acta Physiologica Scandinavica*, 52(1), 8-22.

[21] Keller, R. 1971. *Nippostrongylus brasiliensis* in the rat: Failure to relate intestinal histamine and mast cell levels with worm expulsion. *Journal of Parasitology*, 63(3), 473-481.

[22] Darmawi, U.B., Hambal, M., Tiuria, R., Frengkia, R., and Priosoeryanto, B.P. 2013. Mucosal Mast Cells Response in the Jejunum of *Ascaridia galli*-infected laying hens. *Media Peternakan*, 6(11), 113-119.

[23] Geiger, S.M., Massara, C.L., Peter, J.P., Omar, T.S., Carvalho, S., and Correa-Oliveira, R. 2003. cellular responses and cytokine profiles in *Ascaris lumbricoides* and *Trichuris trichiura* infected patients. *Journal of Immunology Research*, 24(11), 499-509.

[24] Kuijk, L.M., Klaver, E.J., Kooij, G., Van der pol, S.M.A., Heijnen, P., Bruijns, S.C.M., Kringel, H., Pinelli, E., Kraal, G., De vries, H.E., Dijkstra, C.D., Bouma, G., and Van die, I. 2012. Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis & differentially modulate human dendritic cell activation. *Molecular Immunology*, 51(2), 210-218.

[25] Hubner, M.P., Pasche, B., Kalaydjiev, S., Soboslay, P.T., Lengeling, A., Schulz-Key, H., Mitre, E., and Hoffmann, W.H. 2008. *Microfilariae of the Filarial Nematode Litomosoides sigmodontis* Exacerbate the Course of Lipopolysaccharide-Induced Sepsis in Mice. *Infection and Immunity*, 76(4), 1668-1676.

[26] Memon, M.A., Naqvi, M.A., Xin, H., Meng, L., Hasan, M.W., Haseeb, M., Lakho, S.A., Aimulajiang, K., Bu, Y., Xu, L., Song, X., Li, X., and Yan, R. 2020. Immunomodulatory dynamics of excretory and secretory products on Th9 immune response during *Haemonchus contortus* infection in goat. *PLOS Neglect. Tropical Diseases*, 14(4), 1-17.

[27] Knight, P.A., Wright, S.H., Brown, J.K., Sheppard, D., Huang, X., Thornton, E.M., McPhee, M., and Miller, H.R.P. 2002. Nematode-induced mucosal mast cell hyperplasia is dependent on coexpression of TGF- β 1 and Integrin β 6 in the intestinal epithelium. *The Journal of Immunology*, 107, 12-17.

[28] Soboslay, P.T., Hamm, D.M., Pfäfflin, F., Fendt, J., Banla, M., and Schulz-Key, H. 2006. Cytokine and chemokine responses in patients co-infected with *Entamoeba histolytica/dispar*, *Necator americanus* and *Mansonella perstans* and changes after anti-parasite treatment. *Microbes and Infection*, 8(1), 238-247.

[29] Estrada-Reyes, Z., Lopez-Arellano, M.E., Torres-Acosta, F., Lopez-Reyes, A., Lagunas-Martinez, A., Mendoza-de-Gives, P., Gonzalez-Garduno, R., Olazaran-Jenkins, S., Reyes-Guerrero, D., and Ramirez-Vargas, G. 2017. Cytokine and antioxidant gene profiles from peripheral blood mononuclear cells of Pelibuey lambs after *Haemonchus contortus* infection. *Parasite Immunology*, 39(6), 11-27.

تقييم مستويات بعض المعايير المناعية في الفئران المصابة بطفيلي *Enterobius vermicularis* و المعالجة بجسيمات الذهب النانوية ومستخلص النيم والبيندازول

سيناء ناجي محسن¹, أشرف جمال محمود² اكتفاء عبد الحميد محمد³

1,2,3 قسم علوم الحياة, كلية التربية للبنات, جامعة تكريت, تكريت, العراق

الملخص

المقدمة والهدف: إن دودة الأمعاء *Enterobius vermicularis* والمعروفة أيضاً باسم الدودة الدبوسية، هي أكثر أنواع العدوى الطفيلية شيوعاً في العالم، وتكون معظم الحالات بين الأطفال. هدفت الدراسة الحالية إلى بيان مستويات بعض المعايير المناعية في الفئران المصابة بالدودة الدبوسية *Enterobius vermicularis* وتأثير العلاج بتركيزين من جزيئات الذهب (1.5% و 2%) ومستخلص النيم ومستخلص النيم مع جزيئات الذهب وعلاج البيندازول على تلك المعايير. **طرائق العمل:** تم جمع 640 عينة براز من المرضى الذين يراجعون مستشفى تكريت والمختبرات الخاصة في مدينة تكريت، للفترة من كانون الثاني إلى تشرين الأول 2024. تم تقسيم الفئران إلى خمس مجموعات شملت: مجموعة السيطرة السالبة: غير المصابة، ومجموعة السيطرة الموجبة: المصابة بدون علاج، ومجموعة السيطرة الموجبة: المصابة والمعاملة بالعلاج، ومجموعة الفئران المحصنة (المحقونة بـ 1 مل من لقاح البيض المضعف) المصابة والمعاملة بالعلاج ومجموعة التحكم السلبية (مجموعة العلاج فقط).

النتائج: أظهرت التراكيز المختلفة من جزيئات الذهب النانوية والمركب النانوي المحمل ومستخلص النيم والعلاج الطبي تأثيراً على مستويات المعايير المناعية في المجموعات المعالجة مقارنة بالمجموعة المصابة بدون علاج. في الفئران المصابة دون علاج، وُجدت زيادة ملحوظة في تركيزات عامل النمو المحول بيتا بمتوسط قيمته 371.8 بيكوغرام/مل، والإنترلوكين-8 بمتوسط قيمته 288.8 بيكوغرام/مل، عند مستوى معنوي ($P \leq 0.01$)، بينما وُجد انخفاض ملحوظ في تركيزات عامل نخر الورم ألفا بمتوسط قيمته 18.3 بيكوغرام/مل، والإنترلوكين-12 بمتوسط قيمته 9.65 بيكوغرام/مل، عند مستوى معنوي ($P \leq 0.01$). في الوقت نفسه، كانت مستويات الهيستامين بين المجموعات ضمن النطاق الطبيعي عند مستوى معنوي ($P \leq 0.05$). لم تُلاحظ أي تغييرات ملحوظة في تلك المعايير للمجموعات المعالجة الأخرى، والتي بقيت ضمن القيم الطبيعية.

الاستنتاج: ظهرت إصابة فئران المختبر بدودة الأمعاء الدبوسية *Enterobius vermicularis* ارتفاعاً ملحوظاً في تركيز كل من عامل النمو المحول بيتا والإنترلوكين-8 عند مستوى معنوي ($P \leq 0.01$)، بينما لوحظ انخفاض ملحوظ في تركيز كل من عامل نخر الورم ألفا والإنترلوكين-12 عند مستوى معنوي ($P \leq 0.01$). في الوقت نفسه، كانت مستويات الهيستامين ضمن المعدل الطبيعي بين المجموعات عند مستوى معنوي ($P \leq 0.05$).

الكلمات المفتاحية: مستخلص النيم ، الدودة الدبوسية ، جزيئات الذهب النانوية ، مناعة ، معايير مناعية