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Potential role of biosynthesized silver nanoparticles from *Aaronsohnia factorovskyi* on *Hymenolepis nana* in BALB/c mice

[*Potencial função das nanopartículas de prata biossintetizadas de Aaronsohnia factorovskyi sobre Hymenolepis nana em camundongos BALB/c*]

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ABSTRACT

Hymenolepiasis is the most common intestinal tapeworm infection in humans caused by an intestinal cestode, *Hymenolepis nana.* Praziquantel (PZQ) is the most effective drug among other compounds, however, many cases of drug resistance have been reported. Recent research projects have been focused on finding novel therapeutic agents from medicinal plants. In the present study, *Aaronsohnia factorovskyi* was used against hymenolepiasis in the forms of plant extract (AF) and biosynthesized nanoparticles (AF-NPs) in comparison to PZQ. The results showed that 100 mg/kg AF and 0.5 mg/kg AF-NPs were the most effective doses at suppressing the fecal egg output by 98.39% and 100%, respectively. After the 10th day of treatment, it was not feasible to detect the presence of *H. nana* eggs in the fecal sample's examination in the AF-NPs group. Upon treatment with AF-NPs, there were more improvements in the structure of the intestinal tissue than the effect of AF alone and in comparison, to PZQ. Collectively, results showed that *A. factoryviski* can be used as an anti-hymenolepiasis treatment with minimum side effects and less cost. Also, it was found that NPs are the most effective way, as it offers a faster recovery rate in comparison to natural plant extract.

Keywords: hymenolepiasis, drugs, medicinal plants

RESUMO

A himenolepíase é a infecção intestinal por tênia mais comum em humanos, causada por um cestódeo intestinal, o Hymenolepis nana. O Praziquantel (PZQ) é o medicamento mais eficaz entre outros compostos; entretanto, muitos casos de resistência a medicamentos foram relatados. Projetos de pesquisa recentes têm se concentrado em encontrar novos agentes terapêuticos a partir de plantas medicinais. No presente estudo, a Aaronsohnia factorovskyi *foi usada contra a himenolepíase nas formas de extrato vegetal (AF) e nanopartículas biossintetizadas (AF-NPs) em comparação com o PZQ. Os resultados mostraram que 100 mg/kg de AF e 0,5 mg/kg de AF-NPs foram as doses mais eficazes na supressão da produção fecal de ovos em 98,39% e 100%, respectivamente. Após o 10º dia de tratamento, não foi possível detectar a presença de ovos de H. nana no exame da amostra fecal no grupo AF-NPs. Após o tratamento com AF-NPs, as melhorias na estrutura do tecido intestinal foram maiores do que o efeito do AF sozinho e em comparação com o PZQ. Coletivamente, os resultados mostraram que o A. factoryviski pode ser usado como um tratamento anti-himenopiase com efeitos colaterais mínimos e menor custo. Além disso, verificou-se que as NPs são a forma mais eficaz, pois oferecem uma taxa de recuperação mais rápida em comparação com o extrato natural da planta.*

Palavras-chave: himenolepíase, medicamentos, plantas medicinais

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INTRODUCTION

Hymenolepis nana is a parasite known as the dwarf tapeworm, it is distributed worldwide, specifically in warm climates. It is known to infect both human beings and rodents (Shirvan *et al*., 2016). The infection occurs directly from the ingestion of contaminated food and water with *H. nana* eggs (Al-Olayan *et al*., 2020). Infections might cause a variety of symptoms such as irritability, abdominal pain, loss of appetite, diarrhea and even dizziness (Lin *et al*., 2014).

Drugs are available for the treatment of the infection of the intestinal tapeworm including Praziquantel (PZQ) (Shirvan *et al*., 2016). Due to the decreased effectiveness of antiparasitic drugs and antibiotics based on the reports that showed a high drug resistance in different microbes, current studies in the field of medicinal microbiology and parasitology have been focusing to find better natural alternatives (Al-Otibi *et al*., 2021). Many studies have shown the antiparasitic and antimicrobial activities of different plants and herbs. Of those plants, are the members of *Asteraceae* family which contains over 23,000 species that include classes which have proven to have high efficiency effects as antimicrobial, anti-parasitic, anti-inflammatory, and anti-cancer as they are crucial agents which are rich in antioxidants (Okafor *et al*., 2013). *Aronsohnia factorovskyi* is a member of the *Asteraceae* family that contains different medicinal plants, such as *Helianthus annuus* (sunflower), and *Matricaria chamomilla*, this family of plants is known for its rich content of coumarins, flavonoids, and sesquiterpenes which are of excellent therapeutic importance (Kuete *et al*., 2011) and known as an herbal treatment.

Currently, new trends such as nanotechnological approaches have been innovative antiparasitic agents (Gujjari *et al*., 2022). Silver nanoparticles (AgNPs) have proven its strong potential as an antifungal, anti-inflammatory activities as well as antibacterial effects (Kumar *et al*., 2014). The method of green synthesis of AgNPs with different biological material has proven an advantage over other methods as it is simple, safe, stable, and cost effective (Zimmermann, 2012). The extracts of plants have been used as synthesis mediators of metal nanoparticles and metal ions, as *Ziziphus spina christi*, *Eucalyptus camaldulensis*, *Calligonum comosum*, and the marigold flower (Maki and Yanagisawa, 1987).

Therefore, the aim of the present study is to investigate effect of *Aaronsohnia factorovskyi* as an extract and as NPs on *H. nana* infection in BALB/c mice compared to Praziquantel.

MATERIALS AND METHODS

Plant collection and extract preparation: The plant was obtained from the Department of Botany and Microbiology, King Saud University, Riyadh. Preparation of *Aaronsohnia factorovskyi* (AF) was conducted through the following steps, adding 0.25g of the plant (including flowers and green leaves) followed by 25mL dist. H_2O was in a flask and it was heated at 90ºC, then the mixture was filtered, lyophilized, and stored at -20ºC until used (Al-Otibi *et al*., 2021).

Analysis of the plant extract: The concentrations of the phenolic and flavonoid contents were evaluated using Folin–Ciocalteu technique and the aluminum chloride colorimetric method as described by Dkhil *et al*. (2022) and determined as mg gallic acid/gram dry weight and mg quercetin/gram dry weight, respectively.

Preparation and characterization of the bio-synthesized silver nanoparticles from the A. factorovskyi extract (AF-NPs): 0.0084 gm of $AgNO_3$ was mixed with 50 mL of dist. H_2O produced a colorless solution which was later mixed with 0.25 gm plant extract. The mixture was mixed and heated for 45 min on the hotplate. The color, later changed to reddish brown which is an indication of the formation of AgNPs (Makhloufi *et al*., 2015). Transmission electron microscopy (TEM) was used to characterize the shape of AF-NPs, using a JEOL JEM-1011 (JEOL Ltd., Tokyo, Japan) high-resolution TEM at an accelerating voltage of 80 kV.

Parasite collection: *Hymenolepis nana* was used as a model cestode murine parasite. For the propagation of the parasite, five laboratory mice (*Mus musculus*) were inoculated with 200 *H. nana* eggs/mouse by oral gavage. Feces were collected at 15th-day post-infection (p.i.), and eggs were separated by the floatation technique (Steinmann *et al*., 2012). Part of these eggs was washed in a phosphate buffer solution (Sigma

Aldrich, Taufkirchen, Germany) and used for *in vivo* study.

Experimental animals: A total of 45 male BALB/c mice, 9-12 weeks old, approximately weighing 20-25 gm are obtained from the animal house of Saudi Food and Drug Authority (SFDA), Riyadh, Saudi Arabia. Mice received care in the animal house in Zoology Department, College of Science, King Saud University; under controlled conditions of temperature (24±2°C), light (12 hr light/dark cycle), and relative humidity 40-70%. They received a standard diet and water.

Experimental design: Mice were divided into nine groups (5 mice per group), as follows: ***Group*** *1*: Non-infected-non-treated (negative control). ***Group*** *2*: Infected-non-treated (positive control). ***Group*** *3*: Infected and treated group with 25 mg/kg of Praziquantel (reference drug). ***Group*** *4*: Infected and treated group with 50 mg/kg of AF. ***Group*** *5*: Infected and treated group with 100 mg/kg of AF. ***Group*** *6*: Infected and treated group with 200 mg/kg of AF. ***Group*** *7*: Infected and treated group with 1 mg/kg of AF-AgNPs. ***Group 8***: Infected and treated group with 0.5 mg/kg of AF-AgNPs. ***Group*** *9*: Infected and treated group with 0.25 mg/kg of AF-AgNPs. All groups except group 1 were orally inoculated with 200 *H. nana* eggs/mouse. After 60 min, group (3) was orally treated with 25 mg/kg of Praziquantel, groups (4-6) were orally treated with three doses of AF (50, 100, and 200 mg/kg), and groups (7-9) were orally treated with three doses of AF-NPs (1, 0.5, and 0.25 mg/kg), respectively. Treatment was daily for 15 days. On day 15th-day p.i., animals were slaughtered and then the intestines were collected and rapidly excised from each animal. Parts of the intestine were trimmed and fixed in 10% formalin for histopathological study.

Determination of H. nana eggs output: Fresh fecal pellets were collected after 5, 10, and 15th days p.i. from the mice of the infected untreated and treated groups and the egg viability and output per gram of feces were calculated by the McMaster's counting technique, according to Esch and Petersen (2013).

Histopathological examination: Pieces of fixed intestine were dehydrated in ascending series of ethyl alcohol and then embedded in paraffin wax. Sections of 5 µm thickness were prepared and stained with hematoxylin-eosin (H&E) according to the protocol of Adam and Caihak (1964). Slides were examined and photographed under an Olympus B×61 microscope (Tokyo, Japan).

Statistical analysis: Data were analyzed with one-way analysis of variance (ANOVA) using a statistical package program (SPSS version 17.0). All values were expressed as mean ± standard deviation (SD).

RESULTS

Total concentration of phenolics and flavonoids in the investigated plant extract was found to be 40.37 ± 1.41 mg gallic acid/gm dry weight and 70.52 ± 1.03 mg quercetin/gm dry weight, respectively (Figure 1).

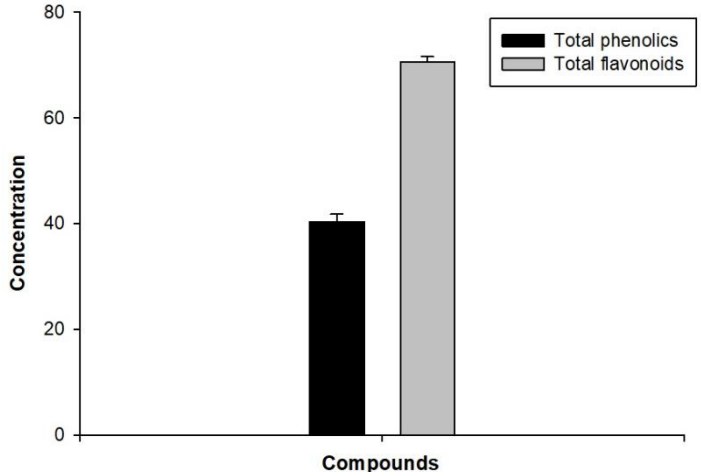

Figure 1. Concentration of phenolics (mg) and flavonoids (mg) in *A. factorovskyi* extract.

Synthesized AF-NPs are spherical morphology with smooth surface (Figure 2). The image also depicts that there are no residues related to the AF remain in the prepared product, which again confirms that the prepared nanostructure material is highly pure with good morphology.

Experimental cestode infection in mice with *H. nana* in both infected and infected-treated groups was established as revealed egg output in fecal pellets with a maximum level at the highest level on the 15th-day p.i. in the infected group. It was thus quite evident that the 100 mg/kg of AF and 0.5 mg/kg of AF-NPs were the most effective doses at suppressing the fecal egg output by 98.39% and 100%, respectively, therefore both were used for subsequent investigations (Figure 3).

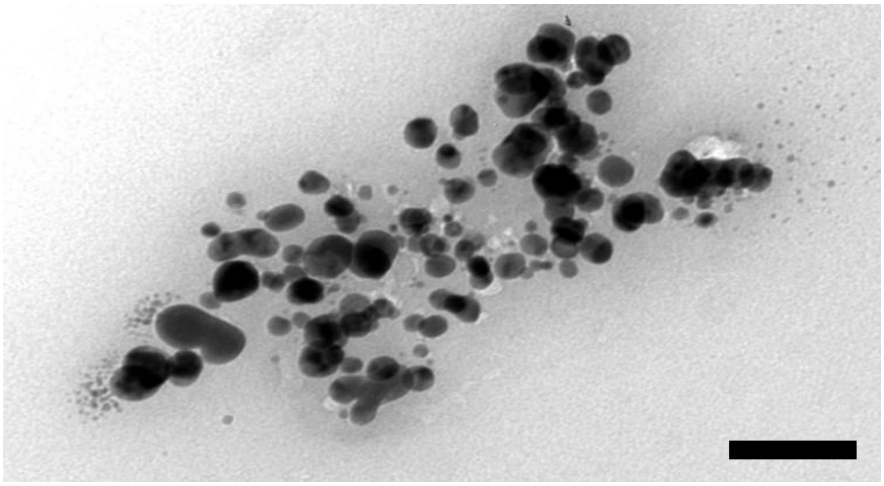

Figure 2. Characterization of AF-NPs by TEM. Scale =100 nm.

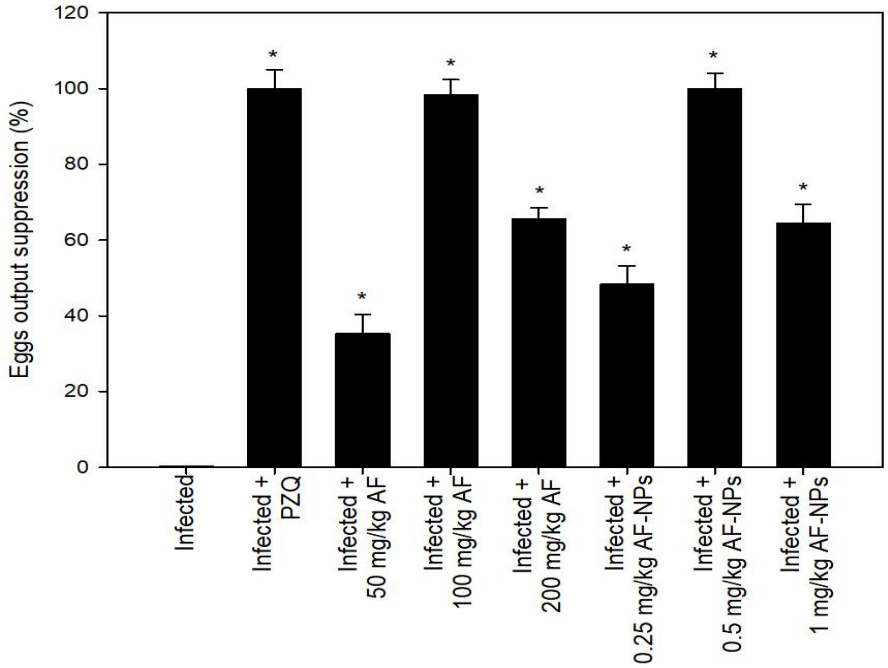

Figure 3. Suppression of *H. nana* eggs in infected and infected-treated mice. Significance at $p \leq 0.05$ against infected group (*).

On the 5th day p.i., the number of eggs in PZQ group (0.00±0.00) has significantly decreased $p \leq 0.05$ in comparison to the infected group (226.66±30.33). Moreover, AF-NPs group also showed a significant decrease in the number of *H. nana* eggs (90.33±1.57). Furthermore, AF-group showed a significant difference in the number of eggs (114.00±16.09) (Figure 4 A).

On the 10th day p.i., the number of eggs in PZQ group remains to be 0.00±0.00 at $p \leq 0.05$ in comparison to infected group (470.00±10.00) which has increased during the 10th day as the life cycle progresses. Moreover, AF-NPs group also showed a significant decrease in the number of eggs (1.66±2.88). Furthermore, AF-group has showed a significant difference in the number of eggs (96.66±1.52) (Figure 4 B).

On the 15th day p.i., the AF-NPs group showed a highly significant difference of the number of *H. nana* eggs in comparison to the infected group (0.00±0.00), also, in PZQ group remains to be 0.00±0.00 at $p \leq 0.05$ in comparison to infected group (704.66±5.50) which showed an increased number of eggs during the 15th day is the life cycle progresses. Moreover, AF group showed a significant decrease in the number of eggs as well (11.33±2.08) (Figure 4 C).

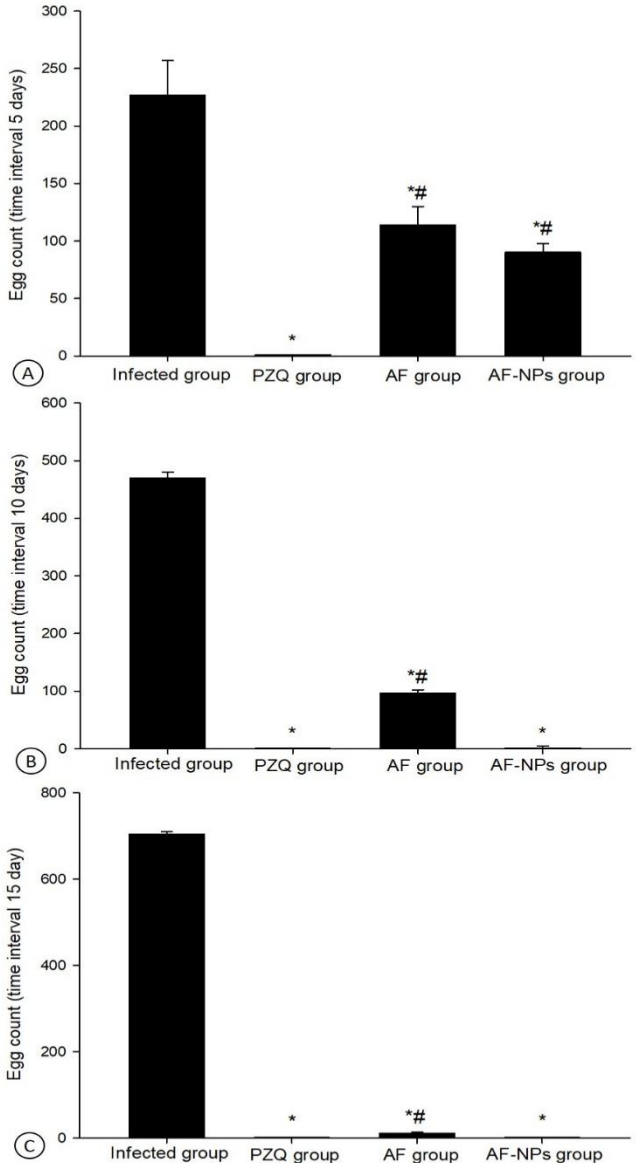

Figure 4. Egg count after different time intervals of 5 days (A), 10 days (B), and 15 days (C) after infection. *significance ($p \leq 0.05$) between the infected group and the PZQ group. #significance ($p \leq 0.05$) between the PZQ group and both AF and AF-NPs groups.

Microscopic examinations showed that the small intestine of the control group showed normal intestinal structure with long villi covered by the columnar epithelia (Figure 5 A). Severe pathological alterations were observed in the intestinal tissue of the infected mice group manifested by a change in the shape of columnar epithelia that changed to small cuboidal cells with hyperplasia besides wide degeneration of the lamina propria (Figure 5 B), on the other hand, peptic ulcers were seen accompanied with the splitting of the muscularis layer (Figure 5 C). Microscopical investigation of intestinal tissue of post-treated mice with PZQ to infection revealed the improvement of the intestinal tissue except for the presence of ulcers (Figure 5 D). Whereas the intestinal tissue of mice post-treated with AF or AF-NPs displayed the development of healthy columnar epithelia and no peptic ulcers were observed (Figure 5 E, F).

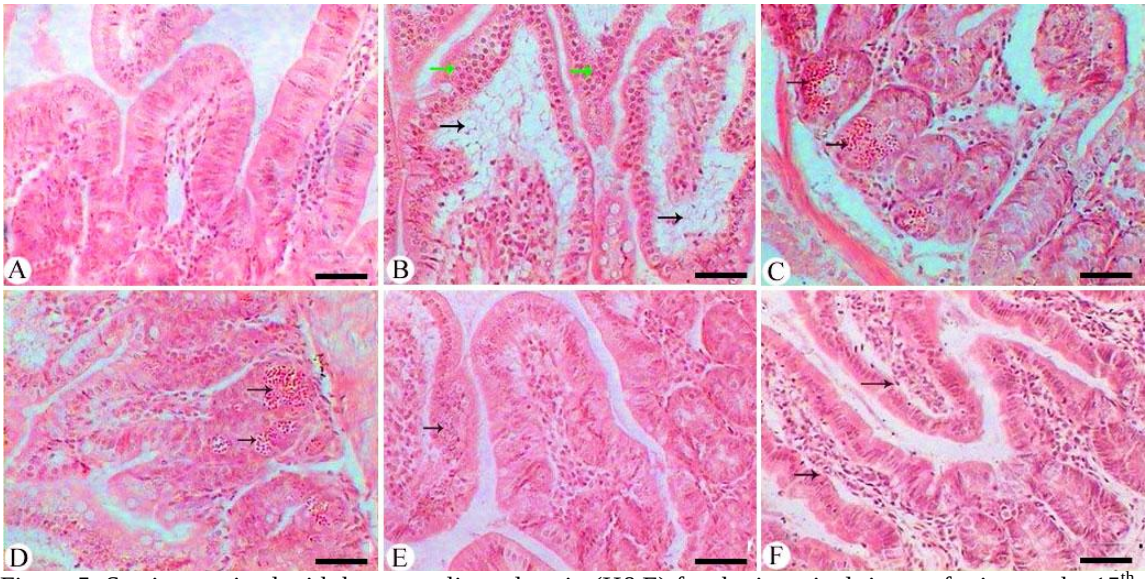

Figure 5. Sections stained with hematoxylin and eosin (H&E) for the intestinal tissue of mice on the 15th day p.i. (A) control small intestine showing normal villi. (B) untreated infected small intestine revealing degeneration of lamina propria (black arrows), hyperplasia (green arrows), (C) infected small intestine revealing peptic ulcer (black arrows). (D) infected small intestine treated with PZQ showing peptic ulcer. (E) infected small intestine treated with AF displaying less hyperplasia (black arrow). (F) infected small intestine treated with AF-NPs showing healthy intestinal tissue (black arrows). Scale Bar = 25 µm

DISCUSSION

The dwarf tapeworm, *Hymenolepis nana*, is a common parasite of mice, hamsters, and rats. This parasite species of *Hymenolepis* is of great importance due to its ability to transmit to humans. Once established in a host, it does not require an intermediate host to complete its life cycle (Al-Megrin, 2010). Chemotherapy is known to cure and control hymenolepiasis. Three compounds are currently used: albendazole, niclosamide, and praziquantel. All these drugs are approved and recommended by the world health organization's (WHO) list of essential drugs. Praziquantel is the drug of choice for the treatment of *H. nana*, however, the repeated regiments are required after ten to fifteen days to control the infection from spreading (Doenhoff *et al*., 2008). Due to the drug resistance, there is a need for a prompt solution and intensive research in finding natural treatments for the parasitic infections with minimum side effects. Therefore, this study investigates the potential anti-hymenolepiasis effect of biosynthesized AgNPs utilizing *A. factorovskyi* in comparison to PZQ.

In this study, egg output showed a constantly high level among infected group, meanwhile, the AF-NPs and PZQ groups had no *H. nana* eggs appearance on the 15th day, on the other hand, AF group showed a decrease in the number of eggs, which indicated the effectiveness of AF-NPs over the extract alone, this agreed with Sayyah and Mandgary (2003). High levels of

polyphenolics found in AgNPs and the plant extract (Al-Otibi *et al*., 2021) may be responsible for the anti-cestodal properties. El Shenawy *et al*. (2008) found a positive relationship between egg output and worm burden, where the reduction of ova count is directly correlated with reduction of worms. In addition, this study used PZQ, it was found to be highly effective with a 100% rate of recovery, and it was slightly higher than that induced by AF but extremely close to the effect of AF-NPs, effects were statistically significant as compared to the infected group. This agreed with Campos *et al*. (1984) in terms of the AF and PZQ results.

In the present study, the intestinal tissue showed abnormal architecture after the infection with *H. nana*, which agreed with the previous report by Mohammed and Sulaiman (2014) and Al-Olayan *et al*. (2020) reported severe damage for the intestinal tissue upon infection. Moreover, treatment with AF and AF-NPs against *H. nana* has eliminated the infection while protecting the jejunum from parasite-induced injury, whereas the intestinal tissue of post-treated mice with PZQ revealed an improvement in the tissue except for the presence of ulcers this presents similar findings to Chiamah *et al*. (2019). This is probably because the aqueous extract of *A. factorovskyi* has a great influence on the healing of ulcers by increasing the proliferation of the epithelial cell and the blood vessel formation and accelerating the inflammatory process.

CONCLUSION

The present study provides new insights for the uses of medical herbs in the treatment of *H. nana* infection. *A. factorovskyi* showed a great effect in the treatment of *H. nana* infection, specifically when synthesized with the silver nanoparticles. Further investigation should be focused on the active ingredient in *A. factorovskyi* to be used in the future treatments.

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