http://dx.doi.org/10.1590/1678-4162-13127



*Arq. Bras. Med. Vet. Zootec.*, v.76, n.1, p.67-76, 2024

# Therapeutic effectiveness of *Ferula asafetida* against *Hymenolepis nana*

[*Eficácia terapêutica da Ferula asafetida contra Hymenolepis nana*]

E. *Al-Olayan*, N. *Al-Kahtani*, F. *Al-Arifi*, R. *Abdel-Gaber**

Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

## ABSTRACT

*Hymenolepis nana* is a common intestinal tapeworm that affects humans. Drugs, including praziquantel (PZQ), are essential for managing this infection. Natural products are now considered as an alternative agent to control hymenolepiasis. Three doses of the herb *Ferula asafetida* (FAH) (100-150-200 mg/ml) were used to assess the appropriate dose and right time to eliminate *H. nana*. It was found that 150 mg/ml gives no movement in 5 min and is an appropriate dose affecting H. nana. This study showed that FAH completely controls mature worms. Treatment with FAH induced a significant reduction in worm burden and complete healing after 14 days relative to a single dose PZQ drug. Moreover, histological studies for the infected-treated mice with FAH demonstrated improvement in the intestinal tissue and less accumulation of inflammation relative to those treated with PZQ. In addition, the cestodal infection significantly upregulated the inflammatory cytokines. This increase in mRNA expression of TNF-$\alpha$, iNOS, and IL-2 was 6.80, 5.65, and 8.95-fold, respectively, which significantly downregulated upon treatment. Collectively, *F. asafetida* is a promising medicinal plant with anti-cestodal and anti-inflammatory activities and could be used for the treatment of hymenolepiasis.

Keywords: *Ferula asafetida*, *Hymenolepis nana*, histology, inflammation

## RESUMO

A *Hymenolepis nana é uma tênia intestinal comum que afeta os seres humanos. Os medicamentos, inclusive o praziquantel (PZQ), são essenciais para o controle dessa infecção. Atualmente, os produtos naturais são considerados um agente alternativo para o controle da himenolepíase. Três doses da erva Ferula asafetida (FAH) (100-150-200 mg/ml) foram usadas para avaliar a dose adequada e o momento certo para eliminar a H. nana. Verificou-se que 150 mg/ml não produz nenhum movimento em 5 minutos e é uma dose adequada para afetar a H. nana. Este estudo mostrou que o FAH controla completamente os vermes maduros. O tratamento com FAH induziu uma redução significativa na carga de vermes e a cura completa após 14 dias em relação a uma dose única do medicamento PZQ. Além disso, os estudos histológicos dos camundongos infectados tratados com FAH demonstraram melhora no tecido intestinal e menor acúmulo de inflamação em relação aos tratados com PZQ. Além disso, a infecção cestodal aumentou significativamente as citocinas inflamatórias. Esse aumento na expressão de mRNA de TNF-$\alpha$, iNOS e IL-2 foi de 6,80, 5,65 e 8,95 vezes, respectivamente, que diminuiu significativamente com o tratamento. Coletivamente, a F. asafetida é uma planta medicinal promissora com atividades anticestodal e anti-inflamatória e pode ser usada para o tratamento da himenolepíase.*

*Palavras-chave:* Ferula asafetida, Hymenolepis nana, *histologia, inflamação*

## INTRODUCTION

Intestinal parasites invade approximately three billion people globally, contributing to an augmented risk of developmental deficiencies, and even deaths (Fauziah *et al*., 2022). Parasitic infection is a frequent cause of morbidity in tropical countries along with mortality (Eyayu *et al*., 2021). These infections in developing countries are primarily elevated due to inadequate sanitary conditions, the usage of contaminated drinking water, and poor personal hygiene (Hussain *et al*., 1997).

*Corresponding author: rabdelgaber.c@ksu.edu.sa
Submitted: August 21, 2023. Accepted: October 5, 2023.

Hymenolepiasis is a parasitic disease triggered by intestinal Cestoda helminths (Waugh *et al*., 2006), and *Hymenolepis nana* is the only human tapeworm in which an intermediate host is optional (Schmidt and Roberts, 2010), which flourished in temperate and warm climates and is still a public health problem, especially in the child populations of rural areas (Malheiros *et al*., 2014; Ul Haq *et al*., 2015; Cabada *et al*., 2016). A lot of our knowledge of the efficacy of specific anti-helminthic against *H. nana* comes from rodent infection studies. Few reports document the outcomes of treatments used for human infections. While many drugs are effective against intestinal helminths, the fact remains that about one-third of the world's population still lacks regular access to vital drugs, with the number in several developing countries rising to over 50% (Temjenmongla and Kumar, 2005).

Several herbal medicines have been used to treat intestinal helminth infections since ancient times, based on the traditional beliefs of different cultures (Al Akeel *et al*., 2018). A significant portion of the population in rural regions often considers herbal medicines conveniently accessible and inexpensive (Tandon *et al*., 2011; Mala *et al*., 2018; Abdelmaksoud *et al*., 2020). Several studies have been made to validate the anthelmintic effects of medicinal plants (Behnke *et al*., 2008). For example, Abdel-Ghaffar *et al*. (2011) recorded that the extracts produced from coconut, onion, garlic, fig, date tree, chicory, ananas, and cistrose have important effects on cestodes (*Hymenolepis diminuta*, *Hymenolepis microstoma*, and *Taenia taeniaeformis*) and trematodes (*Fasciola hepatica* and *Echinostoma caproni*). Similarly, it was also observed that leaf extracts of *Adhatoda vasica* and *Clerodendrum colebrookianum* are effective against *H. diminuta* infections in rats. Recently, Deori and Yadav (2016) have documented the antihelmintic effects of the stem bark extract of *Oroxylum indicum*, a traditional anthelmintic plant of India, on the larval and mature worms of *H. diminuta*, a zoonotic tapeworm.

In the available published literature on *Ferula asafetida*, reports elucidating its anti-*Hymenolepis nana* activity are scarce. Accordingly, this study focused on assessing the efficacy of *F. asafetida* intervention on the adult and larval cysticercoid stages of *H. nana*.

## MATERIALS AND METHODS

A total of 118 Swiss Albino mice, 9-12 weeks old, approximately 20-25 g, were purchased from the Laboratory of Animal Breeding Council (King Saud University of Medical Science). They were housed under regulated temperature (24±2°C), lighting (12 hr light/dark cycle), and 40-70% relative humidity conditions. They were given a standard diet and water *ad libitum*. All animals were handled as suggested by the Ethics Committee at King Saud University, Riyadh, Saudi Arabia.

The *F. asafetida* herb (FAH) was purchased from a local herb store in Riyadh (Saudi Arabia). A total of 100 g of the herb will be crushed into powder with a pestle and mortar and then extracted by maceration with 1000mL of 70% methanol (MeOH). The mixture was removed continuously and stirred in the dark at 4°C for 24 hr. Then it was centrifuged at 5000 rpm for 15 min. The supernatant was collected, filtrated, and concentrated in a rotary evaporator under reduced pressure at 50ºC. The collected extract was freeze-dried and kept at -80°C until use.

Three doses of FAH (100-150-200 mg/mL) were prepared then placed in the group containing 10 worms of *H. nana* placed in 50 mL of solution and were checked to determine the appropriate dose and the correct time to eliminate the worms through movement monitored by anatomical microscope.

Feces were obtained from naturally infected mice (collected from the Animal House at Department of Zoology, Faculty of Science, College of Science (Saudi Arabia)), then mixed with dist. $H_2O$, centrifuged for 10 min at 5000 rpm, decanted supernatant, examined the precipitate to ensure that it included eggs, and then used for mice infection. The McMaster technique was used to count *H. nana* eggs. About 100 eggs per mouse were taken via a feeding tube of the Gavage Needle, having a volume for mice equal to 0.1 mL/10g.

A total of 70 mice were divided into seven groups each comprising ten mice, as follows; ***Group A*****:** Treatment with FAH (200 mg/kg at $4^{th}$-day post-infection (p.i.)), to investigate the effect on *H. nana* cysticercoids. ***Group B*****:** Treatment with FAH (150 mg/kg at $4^{th}$-day p.i.), to investigate the

effect on *H. nana* cysticercoids. ***Group C*:** Treatment with FAH (150 mg/kg at 10$^{th}$-day p.i.), to investigate the herbal action on the parasite that has achieved maturity but has not yet begun oviposition. ***Group D*:** Treatment with FAH (150 mg/kg at 14$^{th}$-day p.i.), to investigate the herbal action on fully matured worms laying eggs. ***Group E*:** Treatment with FAH of concentration dose 100 mg/kg at 4$^{th}$-day p.i., to investigate the effect on *H. nana* cysticercoids. ***Group F*:** Non-treated, to study natural parasite growth for comparison with those affected by the herb. ***Group G*:** Treatment with PZQ of concentration dose 25 mg/kg once p.i., to investigate drug action on the parasite that has achieved maturity but has not yet begun oviposition.

A total of 48 mice were divided into four; the ***first group*** (n=12) served as a normal non-infected control group, the ***second group*** (n=12) was an infected non-treated group, the ***third group*** (n=12) was infected and treated group with FAH (150 mg/kg at 10 days p.i.), and the ***fourth group*** (n=12) was an infected and treated group with a single oral dose of 25 mg/kg PZQ. The control group should be checked under the test of specific-pathogen-free (SPF) to ensure that they are guaranteed free of any pathogens. Three mice from each group were euthanized subsequently along the 1$^{st}$, 7$^{th}$, 14$^{th}$, and 20$^{th}$ days p.i. for sample collection.

Blood samples were collected from mice through the cardiac puncture into sterile vacuum tubes with and without anticoagulant (EDTA) to establish erythrocytic count (RBCs), hemoglobin concentration (Hb), and differential leucocytic count (WBCs) according to Feldman *et al*. (2000), during the 1$^{st}$, 7$^{th}$, 14$^{th}$ days, using Hematology Analyzer Device.

Immediately after scarification, samples of the small intestine were isolated from each group and fixed for 24 hr in 10% neutral buffered formalin, and paraffin blocks were produced and processed routinely for light microscopy. Slices of 5 µm were obtained from the prepared blocks and stained with hematoxylin and eosin (H&E). To determine the pathological changes in the tissues, the preparations obtained were visualized using a Nikon microscope.

Total RNA was isolated from intestinal tissue using the RNeasy Plus Mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. cDNA was synthesized using 500 ng of total RNA in a reverse transcription (RT) reaction by RevertAid H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Ontario, Canada) following the manufacturer's procedure. Real-Time PCR was performed in ViiA7 RT-PCR System (Applied Biosystems, Foster City, CA) using Power SYBR Green PCR Master Mix 2× (Life Technologies, Carlsbad, CA) at a final volume of 10 µL. The reaction mixture comprised 2 µM primers and 100 ng of template cDNA. Sequences of primers used for iNOS (5'-TTC CTC AGG CTT GGG TCT T-3' and 5'-GGG GGA ACA CAG TAA TGG C-3', IL-2 (5'-ACT TCA CCA TGG AAC CCG T-3' and 5'-GAG ACT GCC CAT TCT CGA C-3'), TNF-α (5'-GAA CTCA GCG AGG ACA CCA A-3' and 5'-CTT GGT GGT TTG CTA CGA C-3'), and GAPDH (5'-CAT CTT CTT GTG CAG TGC C-3' and 5'-ATG GTG ATG GGT TTC CCG T-3'). The PCR conditions were 95°C for 10 min, followed by 40 cycles for 15 s at 95°C and 1 min at 60°C. PCRs were performed as previously described by Dkhil *et al*. (2013). The Ct method ($2^{-\Delta\Delta CT}$) described by Livak and Schmittgen (2001) was used to evaluate the differences in the expression of genes and the reference gene was glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

All values were expressed as mean and standard deviation (SD). Statistical significance between groups ($p$-value $\leq 0.05$) was compared using one-way analysis of variance (ANOVA) using the statistical package program SPSS ver. 22 (Chicago, IL, USA).

## RESULTS

Treatment of *H. nana* was provided with different concentrations of FAH (100, 150, and 200 mg/mL), the appropriate dose affected on worms being 150 mg/mL giving no movement in 5 min compared to 200 mg/mL with no movement in 15 min. 100 mg/mL, which provided no movement in 25 min was the longest-time effect (Table 1).

Table 1. The appropriate dose for treatment of infected mice with *H. nana* by FAH

| Time/min | Worm movement in different doses of FAH | | |
|---|---|---|---|
| | 100mg/mL | 150mg/mL | 200mg/mL |
| **5** | 10 | 0 | 8 |
| **10** | 9 | 0 | 3 |
| **15** | 5 | 0 | 0 |
| **20** | 2 | 0 | 0 |
| **25** | 0 | 0 | 0 |

In comparison to the infected group, the counting of cysticercoids in infected-treated mice revealed an average reduction of 35%. On 4$^{th}$-day p.i., cysticercoids were not observed in mice of the infected-treated group with FAH (150 mg/kg). Nevertheless, the FAH action was not enough to prevent that from mice treated with FAH (100 mg/kg) and a few later in those treated with FAH (200 mg/kg). The fecal examination started to become positive; and at autopsy, performed on the 20$^{th}$ day, intact adult worms were found although in smaller numbers than in the infected group (Table 2). Moreover, FAH (150 mg/kg), at 10$^{th}$ and 14$^{th}$ days p.i., proved to be efficacious against mature parasites, those that had not yet initiated oviposition, and those that were already laying eggs. In fact, after treatment, it was not feasible to detect the presence of adult worms either by finding eggs in fecal examination or by observing parasites at autopsy on the 20$^{th}$ day (Table 2).

Table 2. Examination of fecal matter for *H. nana* eggs

| Days after infection | Infected | FAH (100 mg/kg) | FAH (150 mg/kg) | FAH (150 mg/kg) | FAH (150 mg/kg) | FAH (200 mg/kg) | PZQ (25 mg/kg) |
|---|---|---|---|---|---|---|---|
| | | 4$^{th}$ day p.i. | 4$^{th}$ day p.i. | 10$^{th}$ day p.i. | 14$^{th}$ day p.i. | 4$^{th}$ day p.i. | 10$^{th}$ day p.i. |
| 8$^{th}$ | - | - | - | - | - | - | - |
| 9$^{th}$ | - | - | - | - | - | - | - |
| 10$^{th}$ | - | - | - | - | - | - | - |
| 11$^{th}$ | - | - | - | - | - | - | - |
| 12$^{th}$ | + | - | - | - | - | - | - |
| 13$^{th}$ | + | - | - | - | - | - | - |
| 14$^{th}$ | + | + | - | - | - | - | - |
| 15$^{th}$ | + | + | - | - | - | - | - |
| 16$^{th}$ | + | + | - | - | - | + | - |
| 17$^{th}$ | + | + | - | - | - | + | - |
| 18$^{th}$ | + | + | - | - | - | + | - |
| 19$^{th}$ | + | + | - | - | - | + | - |
| 20$^{th}$ | 80 | 21 | None | None | None | 14 | None |
| Autopsy | 100% | 26% | 0% | 0% | 0% | 18% | 0% |

+= presence of *H. nana* eggs in the feces, - = negative fecal examination

A significant difference in total worm burden on comparing PZQ and FAH-treated infected groups to an infected group. PZQ showed a significant effect on total worm burden on 1$^{st}$ day after treatment (0±0). FAH-treated infected mice showed a statistically significant reduction in worm count that started on the 7$^{th}$ day after treatment (4±1.73). Also, compared to the infected group, FAH worm burden changes significantly after the 14$^{th}$ day (0±0) (Table 3). While the number of adults who developed in the intestinal lumen on the 20$^{th}$ day after infection showed a significant difference in those treated with PZQ and FAH (2±0.9 and 0±0, respectively), compared to the infected group.

Table 3. Number of adult worms recovered from the intestines of experimental groups

| Time of scarification | Worms count | | | |
|---|---|---|---|---|
| | Control group | Infected group | Infected-FAH group | Infected-PZQ group |
| 1$^{st}$ day | 0±0 | 30±1 [a] | 14.33±0.57 [a] | 0±0 [b] |
| 7$^{th}$ day | 0±0 | 30±1 [a] | 4±1.73 [a] | 0±0 [b] |
| 14$^{th}$ day | 0±0 | 30±1 [a] | 0±0 [b] | 0±0 b |
| 20$^{th}$ day | 0±0 | 30±1 [a] | 0±0 [b] | 2±0.9 [b] |

[a] $p \leq 0.05$, significant against control group, [b] $p \leq 0.05$, significant against infected group

There are different changes in the hematological parameters, especially on the 14th day p.i. The effect of FAH and PZQ on infected mice with *H. nana* through RBCs showed a significant increase in count (8.15±0.16 and 7.96±0.51, respectively), compared to the infected group was 5.36±0.22 (Table 4). Moreover, The Hb level after treatment with FAH and PZQ showed a significant increase in level (15.60±0.46 and 15.08±0.45, respectively), compared to the infected group was 9.15±0.19 (Table 4). The effect of FAH and PZQ through WBCs showed a significant decrease in count (8.63±0.83 and 9.22±0.59, respectively), compared to the infected group was 22.98±0.77 (Table 4).

Table 4. Hematological parameters in different experimental groups

| Traits / Treatment | | Red blood cells (RBC) | White blood cells (WBC) | Hemoglobin (Hb) |
|---|---|---|---|---|
| Control group | 1st day | 8.10±0.26 | 7.27±0.37 | 14.05±1.06 |
| | 7th day | 8.10±0.42 | 7.02±0.86 | 15.18±0.92 |
| | 14th day | 8.11±0.13 | 6.22±1.41 | 14.35±0.41 |
| Infected group | 1st day | 7.59±0.40[a] | 8.73±2.58[a] | 13.90±0.89[a] |
| | 7th day | 6.55±0.43[a] | 8.88±2.75[a] | 12.40±0.40[a] |
| | 14th day | 5.36±0.22[a] | 22.98±0.77[a] | 9.15±0.19[a] |
| Infected-FAH group | 1st day | 6.99±0.27[ab] | 12.18±4.71[ab] | 13.48±0.66[ab] |
| | 7th day | 7.48±0.12[ab] | 9.60±0.54[ab] | 14.20±0.0[ab] |
| | 14th day | 8.15±0.16[ab] | 8.63±0.83[ab] | 15.60±0.46[ab] |
| Infected-PZQ group | 1st day | 6.28±0.42[ab] | 13.93±3.81[ab] | 12.05±1.34[ab] |
| | 7th day | 7.29±0.04[ab] | 10.20±0.79[ab] | 14.15±0.05[ab] |
| | 14th day | 7.96±0.51[ab] | 9.22±0.59[ab] | 15.08±0.45[ab] |

[a] $p \leq 0.05$, significant against control group, [b] $p \leq 0.05$, significant against infected group

Normal architecture was observed in the control non-infected group (Figure 1A, B). However, the intestinal tissue of the infected group was demonstrated with thick and ulcerative villi with a large accumulation of inflammatory cells along the villi (Figure 1C, D). After treatment with PZQ, improvement in the injured intestinal tissue was observed with the inflammatory cells accumulated in high quantities (Figure 1E, F). Improvement in the injured intestinal tissue was observed after the FAH treatment with less quantity of the inflammatory cells accumulated (Figure 1G, H).

qRT-PCR was used to detect changes in the levels of mRNA for inflammatory cytokines in the mice intestine (Figure 2). The *H. nana* infection-induced upregulation in the mRNA expression of the TNF-α gene. This increase in mRNA expression of this gene was about 6.80 when compared to the non-infected control group (Figure 2). Moreover, the *H. nana* infection-induced upregulation in the mRNA expression of the iNOS gene. This increase in the mRNA expression of this gene was 5.65-fold when compared to non-infected control group (Figure 2). The *H. nana* infection-induced upregulation in the mRNA expression of the IL-2 gene. This increase in mRNA expression of this gene was 8.95-fold when compared to the non-infected control group (Figure 2). Treatment with FAH was associated with a significant downregulation for the expression of these genes more than the reference drug (PZQ).

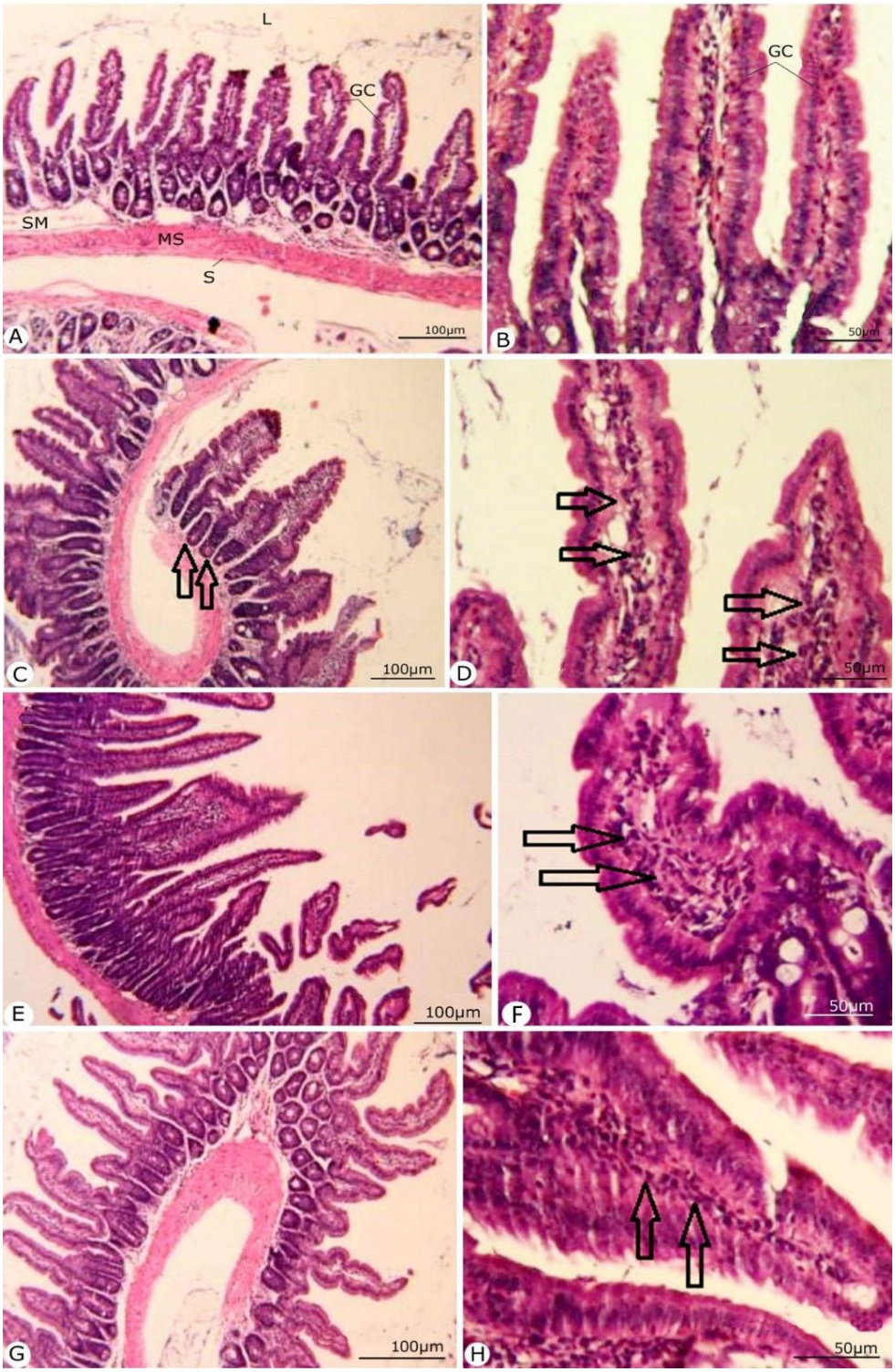


Figure 1. Histological examination of the intestine in all experimental groups: **A, B** Control group (high magnification in B). Note: S, Serosa; SM, Submucosa; GC, Goblet cells; ME, Muscularis external; L, Lumen. **C, D** Infected group showing thick and ulcerative villi (arrows) with inflammatory cells (arrows)**. E, F** Infected-treated group with PZQ, with high accumulation of inflammatory cells (arrows). **G, H** Infected-treated group with FAH, with less accumulation of inflammatory cells (arrows).

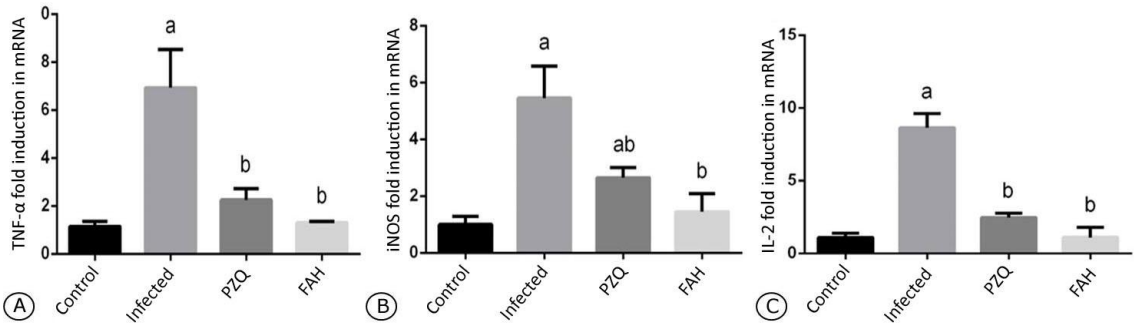


Figure 2. Effect of FAH on the mRNA gene expressions in the intestinal samples from experimental groups. (**A)** TNF-α. (**B)** iNOS. (**C)** IL-2. The expression values obtained by RT-PCR analysis were normalized to the reference gene GAPDH mRNA level and are shown as fold induction (in log 2 scale) relative to the mRNA level in the control. [a] Significance changes concerning the control group, [b] significance changes concerning the infected group

## DISCUSSION

*Hymenolepis nana* is a ubiquitous parasite, found throughout many developing countries; it's the only cestode capable of completing its cycle without an intermediate host (Schmidt and Roberts, 2010). Autoinfection occurs when gravid proglottids release eggs inside the gut, hatch in the small intestine, and liberate the oncospheres embryo which penetrates the lamina of the intestinal villi (Ortega, 2006). Chemotherapy is important for controlling hymenolepiasis, three compounds are currently in use, Niclosamide, Albendazole, and PZQ, and all these drugs are recommended by WHO's list of essential drugs (WHO, 2015). The most effective drug for the treatment of *H. nana* is PZQ. However, repeated regimens after the $10^{th}$ to $15^{th}$ days are required to control the spreading of infections, especially in institutional and familial settings (Bannerman *et al*., 2006). In addition, the deficient understanding of its mechanism of action till now delays efforts to combat its resistance (Doenhoff *et al*., 2008).

Today most of the research used the plant as an anti-parasitic which not only targeted parasites but also has organ protective properties in parasite-infected target hosts. In addition, FAH showed a significant suppressive effect on anti-convulsant (Sayyah and Mandgary, 2003), anti-mycobacterial (Appendino *et al*., 2004), antioxidant (Kartal *et al*., 2007), anti-diabetic (Abu-Zaiton, 2010), anti-viral (Amalraji and Gopi, 2016). This study used treatment with FAH in mice infected with *H. nana* and compared it with PZQ, to assess their efficacy of action. The obtained results showed a significant effect on the parasite using the herb concentration of 150 mg/kg which considers the shortest time effect compared with 100mg/kg and 200 mg/kg. This result disagreed with a previous report by Mali and Wadekar (2008) and Gundamaraju (2013). Also, this study revealed that FAH showed high efficacy against adult stages, starting from the $7^{th}$ day after treatment, and proved to have a statistically significant effect as compared to the infected group, these results agreed with previous reports of Mohammed and Sulaiman (2014), and Shady *et al*. (2014) against hymenolepiasis.

Shenawy *et al*. (2008) found a positive relationship between egg output and worm burden, where the reduction of worm numbers is correlated with the reduction in ova count. On the other side, our study used the PZQ, given at a dose of 25 mg/kg. It showed highly effective against adult worms with a 100% cure. In this case, the reduction in worm burden by PZQ was slightly higher than that induced by plant extract, so the worms disappeared by the $1^{st}$ day after treatment. These effects were statistically significant as compared to the infected group, but some adult worms developed in the intestinal lumen $20^{th}$ days p.i., this result agreed with Campos *et al*. (1984).

Lymphocytes are responsible for the host's immune response. The present increase in lymphocyte count, Lymphocytosis, reflects the host's immune response to overcome parasitic stress after phagocytic neutrophils failed in checking the invading parasites, this is following

the findings of Parvathi and Karemungikar (2011) who found an increase in immunity to *H. nana* infection by transfer from spleen cells. This study showed that infected mice have anemia and inflammation due to Hb reduction, also increased WBCs. On the other side, the treatment with FAH had been showing improvement in Hb rate, a significant increase in RBC, and a decrease in WBCs, when compared with a treated-PZQ group which is consistent with Singh *et al*. (2014). Our result about variations in blood parameters of the infected host indicates various defense mechanisms adopted by the host to combat *H. nana* infection. The values of all parameters in treated batches of host blood are nearer to uninfected control host blood value, which proved the efficacy of FAH and PZQ as a single oral dose regimen in restoring normal hematological profile in an infected host.

In the present study, on the $10^{th}$ and $14^{th}$ days post-treatment with FAH, histopathological examinations showed an improvement in the intestinal tissue, however, inflammatory cells accumulated in less quantity, compared with PZQ-treatment. This is agreed with the previous report by Mohammed and Sulaiman (2014). This is probably because the aqueous extract of *F. asafetida's* has a great influence on the healing of diabetic ulcers by increasing epithelial cell proliferation and blood vessel formation and accelerating the inflammatory process. The dose of 150 mg/kg of this extract for 14 days is safe and showed no side effects, this result agreed with data obtained by Goudah *et al*. (2015). FAH treatment showed good efficacy in reducing the severity of murine hymenolepiasis and can be cured within 7 days of continued treatment.

Cytokine production stimulated mesenteric lymph node cells after egg infection of mice with *H. nana* and showed that cytokine production varies during parasite development, this agreed with Conchedda *et al*. (1997). TNF-α is a pleiotropic cytokine playing an important role in the regulation of immune response. Proteins with pro- and anti-apoptotic functions play an important role in the regulation of programmed cell death (Ryazantseva *et al*., 2010). Nitric oxide (NO) produced by iNOS is an important host defense molecule. iNOS is expressed in many cell types in response to a diverse range of inflammatory cytokines, including IL-1β, IL-2, IFN-γ, TNF-α, and bacterial metabolites such as LPS (Connelly *et al*., 2001). The Th1 subtype is involved in protective immunity against *H. nana*, secreted IL-2 and IFN-γ after stimulation with egg antigens (Conchedda *et al*., 1997). FAH was more effective than PZQ due to its ability to down-regulate the expression of various pro-inflammatory cytokines including TNF-α, IL-2, and iNOS, most likely through the inactivation of the transcription factor NF-kB, which agreed with the previous report by Madpouly *et al*. (2011).

## CONCLUSION

Findings of this study indicated that *F. asafetida* has an active role in treating infection of *H. nana* due to its potential anti-cestodal and anti-inflammatory activities. *F. asafetida* was also more effective than PZQ and improved the immune system. Further studies are recommended to investigate the potential effective component of *F. asafetida* and produced pharmaceutically as a drug available for treatment purposes.

## ACKNOWLEDGMENTS

This study was supported by Researchers Supporting Project (RSP2023R111), King Saud University, Riyadh, Saudi Arabia.

## REFERENCES

ABDEL-GHAFFAR, F.; SEMMLER, M.; AL-RASHEID, K.A. *et al.* The effects of different plant extracts on intestinal cestodes and on trematodes. *Parasitol. Res.*, v.108, p.979-84, 2011.

ABDELMAKSOUD, H.F.; EL-ASHKAR, A.M.; ELGOHARY, S.A. *et al.* Potential therapeutic and prophylactic effects of *Asafoetida* in murine cryptosporidiosis. *J. Parasit. Dis.*, v.44, p.646-653, 2020.

ABU-ZAITON, A.S. Anti-diabetic activity of *Ferula asafetida* extract in normal and alloxan-induced diabetic rats. *Pak. J. Biol. Sci.*, v.13, p.97-100, 2010.

AL AKEEL, M.M.; AL GHAMDI, W.M.; AL HABIB, S. *et al.* Herbal medicines: saudi population knowledge, attitude, and practice at a glance. *J. Family Med. Prim. Care*, v.7, p.865-875, 2018.

AMALRAJI, A., GOPI, S. Biological activities and medicinal properties of *Asafoetida*: a review. *J. Tradit. Complement. Med.*, v.7, p.347-359, 2016.

APPENDINO, G.; MERCALLI, E.; FUZZATI, N. *et al.* Antimycobacterial coumarins from the sardinian giant fennel (*Ferula communis). J. Nat. Prod.*, v.67, p.2108-2110, 2004.

BANNERMAN, T.L.; PEACOCK, S.J.; MURRAY, P.R. *et al.* Staphylococcus, Micrococcus, and other catalase-positive cocci. *Man. Clin. Microbiol.*, v.1, p.390-411, 2006.

BEHNKE, J.M.; BUTTLE, D.J.; STEPEK, G. *et al.* Developing novel anthelmintics from plant cysteine proteinases. *Parasit. Vectors*, v.1, p.29, 2008.

CABADA, M.M.; MORALES, M.L.; LOPEZ, M. *et al. Hymenolepis nana* impact among children in the Highlands of Cusco, Peru: an emerging neglected parasite infection. *Am. J. Trop. Med. Hyg.*, v.95, p.1031-1036, 2016.

CAMPOS, R.; BRESSAN, M.C.R.V.; EVANGELISTA, M.G.B.F. Activity of praziquantel against *Hymenolepis nana*, at different development stages, in experimentally infected mice. *Rev. Inst. Med. Trop. São Paulo*, v.26, p.334-340, 1984.

CONCHEDDA, M.; BORTOLETTI, G.; GABRIELE, F. *et al.* Immune response to the cestode *Hymenolepis nana*: cytokine production during infection with eggs or cysts. *Inter. J. Parasitol.*, v.27, p.321-327, 1997.

CONNELLY, L.; PALACIOS-CALLENDER, M.; AMEIXA, C. *et al.* Biphasic regulation of NF-κB activity underlies the pro-and anti-inflammatory actions of nitric oxide. *J. Immunol.*, v.166, p.3873-3881, 2001.

DEORI, K.; YADAV, A.K. Anthelmintic effects of *Oroxylum indicum* stem bark extract on juvenile and adult stages of *Hymenolepis diminuta* (Cestoda), an *in vitro* and *in vivo* study. *Parasitol. Res*., v.115, p.1275-1285, 2016.

DKHIL, M.A.; DELIC, D.; AL-QURAISHY, S. Goblet cells and mucin related gene expression in mice infected with *Eimeria papillata*. *Sci. World J.*, v.2013, art.439865, 6p., 2013.

DOENHOFF, M.J.; CIOLI, D.; UTZINGER, J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr. Opin. Infect. Dis.*, v.21, p.659-667, 2008.

EYAYU, T.; KIROS, T.; WORKINEH, L. *et al.* Prevalence of intestinal parasitic infections and associated factors among patients attending at Sanja Primary Hospital, Northwest Ethiopia: An institutional-based cross-sectional study. *PLoS One*, v.16, p.e0247075, 2021.

FAUZIAH, N.; AVIANI, J.K.; AGRIANFANNY, Y.N. *et al.* Intestinal parasitic infection and nutritional status in children under five years old: a systematic review. *Trop. Med. Infect. Dis.*, v.7, p.371, 2022.

FELDMAN, B.F., ZINKL, J.G., JAIN, N.C. Schalm's veterinary haematology. 5th ed. Philadelphia, PA: Lippincott Williams and Wilkins, A Wolters Kluwer Company, 2000.

GOUDAH, A.; ABDO-EL-SOOUD, K.; YOUSEF, M.A. Acute and subchronic toxicity assessment model of *Ferula asafoetida* gum in rodents. *Vet. World*, v.8, p.584-589, 2015.

GUNDAMARAJU, R. Evaluation of anti-helminthic activity of *Ferula asafetida* "Hing-A natural Indian spice" aqueous extract. *Asian Pac. J. Trop. Dis.*, v.3, p.189-191, 2013.

HUSSAIN, S.M.; RAZA, M.I.; NAEEM, S. Prevalence of intestinal parasites in northern areas of Pakistan (Baltistan division-Skardu). *Biomedica*, v.5, p.60-63, 1997.

KARTAL, N.; SOKMEN, M.; TEPE, B. *et al.* Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. *Food Chem*., v.100, p.584-589, 2007.

LIVAK, K.J.; SCHMITTGEN, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, v.25, p.402-408, 2001.

MADPOULY, H.M.; SAIF, M.A.; HUSSEIN, A.S. Curcuma longa for protecting chicks against Newcastle disease virus infection and immunosuppressive effect of Marek's disease viral vaccine. *Inter. J. Virol.*, v.7, p.176-183, 2011.

MALA, K.N.; THOMAS, J.; SYAM, D.S. *et al.* Safety and efficacy of *Ferula asafetida* in functional dyspepsia: a randomized, double-blinded, placebo-controlled study. *Evid. Based Compl. Altern. Med.*, v.2018, art.4813601, 2018.

MALHEIROS, A.F.; MATHEWS, P.D.; LEMOS, L.M.S. *et al.* Prevalence of *Hymenolepis nana* in indigenous Tapirapé Ethnic group from the Brazilian Amazon. *Am. J. Biomed. Res*., v.2, p.16-18, 2014.

MALI, R.G.; WADEKAR, R.R. *In vitro* anthelmintic activity of *Baliospermum montanum* Muell. Arg roots. *Indian J. Pharm. Sci.*, v.70, p.131-133, 2008.

MOHAMMED, S.T.; SULAIMAN, N.M. Anthelmintic and hematological changes of natural plant *Carica papaya* seed extract against gastrointestinal nematode *Hymenolepis nana*. *J. Biol. Agric. Healthcare*, v.4, p.8-13, 2014.

ORTEGA, Y.R. Toxoplasmosis. In: ORTEGA, Y. (Ed.). *Foodborne parasites*. New York: Springer, 2006. p.109-134.

PARVATHI, J., KAREMUNGIKAR, A. Leucocyte variation, an insight of host defenses during hymenolepiasis and restoration with praziquantel. *Indian J. Pharm. Sci*. v.73(1), p.76–79, 2011.

RYAZANTSEVA, N.V.; NOVITSKII, V.V.; ZHUKOVA, O.B. *et al.* Role of NF-kB, p53, and p21 in the regulation of TNF-α mediated apoptosis of lymphocytes. *Bull. Exp. Biol. Med*., v.149, p.50-53, 2010.

SAYYAH, M.; MANDGARY, A. Anticonvulsant effect of *Ferula gummosa* root extract against experimental seizures. Iran. *Biomed. J.*, v.7, p.139-143, 2003.

SCHMIDT, G.D.; ROBERTS, L.S. Tapeworms. In: *Foundations of parasitology*. 8th ed. New York: McGraw-Hill, 2010. p.341-367.

SHADY, O.M.A.; BASYONI, M.M.; MAHDY, O.A. *et al.* The effect of praziquantel and *Carica papaya* seeds on *Hymenolepis nana* infection in mice using scanning electron microscope. *Parasitol. Res*., v.113, p.2827-2836, 2014.

SHENAWY, E.L.; NAHLA, S.; SOLIMAN, M.F. *et al.* The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. *Rev. Inst. Med. Trop. São Paulo*, v.50, p.29-36, 2008.

SINGH, G.; URHEKAR, A.D.; MAHESHWARI, U. *et al.* Effects of malarial parasitic infections on human blood cells. *Int. J. Curr. Microbiol. Appl. Sci.*, v.3, p.622-632, 2014.

TANDON, V.; YADAV, A.K.; ROY, B. *et al.* Emerging trends in zoology In: SRIVASTAVA UC, KUMAR S. (Eds.). *Phytochemicals as Cure of worm infections in traditional medicine systems*. New Delhi*:* Narendra Publishing House, 2011. p.351-378.

TEMJENMONGLA, T.; KUMAR, Y.A. Anticestodal efficacy of folklore medicinal plants of Naga tribes in North-east India. *Afr. J. Trad. CAM*, v.2, p.129-133, 2005.

UL HAQ, K.A.; GUL, N.A.; HAMMAD, H.M. *et al.* Prevalence of *Giardia intestinalis* and Hymenolepis nana in Afghan refugee population of Mianwali district, Pakistan. *Afr. Health Sci.*, v.15, p.394-400, 2015.

WAUGH, C.A.; LINDO, J.F.; FORONDA, P. *et al.* Population distribution and zoonotic potential of gastrointestinal helminths of wild rats *Rattus rattus* and *R. norvegicus* from Jamaica. *J. Parasitol*., v.92, p.1014-1018, 2006.

WHO model list of essential medicines: 19.ed. (updated). Rome: WHO, 2015.