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Article

Effect of non-aqueous extracts of *Artemisia ludoviciana mexicana* and *Chenopodium vulvaria* during experimental murine infection with *Hymenolepis diminuta*

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Abstract:

The study assessed the effect of non-aqueous extracts of *Artemisia ludoviciana mexicana* and *Chenopodium vulvaria* on an experimental infection with *Hymenolepis diminuta* in mice. Twenty (20) mice were infected with 10 cysticercoids each, which were obtained from *Tenebrio molitor* fed with feces from infected rats. Egg excretion was monitored for 30 d. Subsequently, the mice were divided into four groups (n= 5): control with DMSO, nitazoxanide (50 mg/kg), *Artemisia* (375 mg/kg), and *Chenopodium* (375 mg/kg). Egg shedding began on day 12 postinfection, with a peak between d 19 and 21. After 24 h of treatment (d 30), excretion decreased by 50 %, and at 72 h, by 70 % in the nitazoxanide and *Chenopodium* groups. At 33 d, the recovery of adult worms was 50 % in the groups with extracts and 25 % in the nitazoxanide group, with damage to the integument and uterine branches. The extracts increased the roughness of the integument without affecting the scolex. The extracts reduced the parasite load, but more studies are required to determine their cestocidal effect or if they only favor the expulsion of the parasite. In addition, it is necessary to evaluate their impact on gastroenteric nematodes. The absence of adverse reactions suggests their potential use in the control of cestodes.

Keywords: Cestode, Anthelmintic, Herbalism, Non-aqueous extract.

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Introduction

H. diminuta is a gastroenteric cestode commonly distributed in rats and mice; humans are an accidental host[(1)]. The adult worm settles in the small intestine and has a disarmed scolex, and gravid proglottids are 20 to 60 mm long by 4 mm wide. The round-shaped eggs measure between 60 and 79 µm in diameter and do not have polar filaments. The larval form (cysticercoid) settles in the hemocoel of coprophagous insects, such as fleas (*Nosopsyllus* and *Xenopsylla*) and cereal beetles (*Tribolium* and *Tenebrio*); however, cockroaches, lepidopterans, myriapods, and coprophilous beetles can also act as intermediate hosts[(2)]. Parasitosis is usually asymptomatic; nevertheless, a massive infection can cause catarrhal enteritis. Zoonotic transmission can be prevented by protecting food and water for human consumption to prevent rodent access. Diagnosis is made by coproparasitological identification of eggs[(3)]. The feasibility and viability of maintaining and propagating the life

cycle of *H. diminuta* in the laboratory makes it an experimental model with a number of immunological, molecular, and biochemical aspects, among which are the search for alternative treatments to drugs.

In recent years, it has been reported that several of the helminths that impact animals with livestock importance have developed resistance to antiparasitic pharmacological treatment; therefore, several strategies have been implemented to prevent this resistance, ranging from the replacement of the production animal with a breed resistant to parasites to the application of drugs in different rotation and combination schemes[4]. An alternative strategy to control parasitosis is the use of medicinal herbs.

The aromatic herbs *Chenopodium* spp. (skunk epazote, paico, or Mexican tea) and *Artemisia* spp. (estafiate) are used in traditional Mexican medicine; the former has been used as an anthelmintic, antispasmodic, and antirheumatic, whereas the latter, in addition to being employed as a diuretic, is used as an analgesic and gastrointestinal anti-inflammatory[5]. The primary constituent of *Chenopodium* oil is the bicyclic monoterpene ascaridole[6], which has sedative, antifungal, and analgesic properties with an antiparasitic effect in experimental infections to reduce the parasite load of *Cryptosporidium parvum* and *Eimeria* spp. in chickens and *H. diminuta* in mice[7]. *Artemisia* essential oils have components with antimicrobial, antioxidant, pesticide, nematicide[8], and ovicidal activity in mice naturally infected with *Hymenolepis nana*[9].

Non-aqueous extracts have been used in the manufacture of cosmetics, nutraceuticals, paints, lubricants, and biodiesel. Vegetable oils, as alternative solvents, are valued for their physicochemical properties, high global availability, biodegradability, low cost, and low toxicity for humans and animals; they are even effective in extracting chlorophyll and antioxidant compounds from avocado leaves for the food industry[10]. Therefore, this study aimed to examine the effect of non-aqueous extracts of *Artemisia ludoviciana mexicana* and *Chenopodium vulvaria* on experimental murine infection with *H. diminuta*.

Material and methods

Hymenolepis diminuta

To propagate the life cycle of *H. diminuta*, young female rats 6 to 8 wk old weighing approximately 180 to 200 g were experimentally infected. Each rat was given 20 cysticercoids through a gastric tube. The cysticercoids were obtained from the hemothorax

of imagoes of *Tenebrio molitor*. One hundred (100) adult beetles were used because, unlike larvae, adults actively seek food with moisture, thus favoring parasitosis. To obtain the cysticercoids, adult beetles with reddish-brown exoskeletons, recently released from the chrysalis and deprived of food for 12 h, were fed with freshly expelled feces from rats infected with *H. diminuta*. Ten days later, the insects were sacrificed and the cysticercoids of the hemothorax were recovered; they were counted and administered using the intragastric tube[11].

Dynamics of expelled eggs

A total of 20 14-mo-old female mice, *Mus musculus* strain Balb/c, were individually infected gastrically with 10 cysticercoids. To determine oviposition kinetics, the mice's feces were analyzed for 30 d using a formalin-ether sedimentation concentration coproparasitological technique[12]. Daily, during the early hours of the day, five fecal samples were collected from each mouse and hydrated in physiological saline solution before being processed. To construct the kinetics, it was arbitrarily decided to count the total number of eggs found in three fields at 10x using a brightfield microscope (CME; Leica Microsystems; Wetzlar, Germany) and then the daily average of all samples was calculated.

Non-aqueous extracts of *Artemisia ludoviciana* and *Chenopodium vulvaria*

Artemisia and *Chenopodium* extracts were made from the stems and leaves of the dehydrated plant in the flowering stage. The plants were acquired commercially (Las Plantas Medicinales de América; Mexico City, Mexico). The plant matter was pulverized with an Oster® stainless steel blender with a toggle switch (Oster®, model BLST4125, Acuña, Coahuila, Mexico) until particles of 0.2 to 0.7 mm in diameter were obtained. To prepare the non-aqueous extract of each herb, the pulverized biological material was homogenized in corn oil (Maceite, Promotora de Productos y Mercados Mexicanos S.A. de C.V.; Jalisco, Mexico), because it is biodegradable, easily accessible, low-cost, and non-toxic. The extraction was performed using a Thermomix® Kitchen Robot (Thermomix®, model TM31, Vorwerk & Co.; Wuppertal, Germany), programming the following parameters: 60 ºC, homogenizing at 1,100 rpm for 45 min in a ratio of 2 g of dry matter to 5 g of oil, equivalent to 0.4 g/ml. The oil was then filtered using sterile gauze with a vacuum pump to form the non-aqueous extract. Each extract was stored at room temperature in a hermetically sealed amber bottle for 15 d until use[10].

Treatment and dosage

On d 30 postinfection, mice infected with *H. diminuta* were divided into four groups of five individuals each and treated for 3 consecutive days (d 30, 31, and 32 postinfection), determining the number of eggs excreted per animal daily by means of a coproparasitoscopic sedimentation examination as described in the Dynamics of expelled eggs section. Group 1 was administered with 1 % dimethyl sulfoxide (DMSO), group 2 with 50 mg/kg of nitazoxanide (Cryptofin, Laboratorio Aranda, S.A. de C.V., Mexico City, MX), group 3 with the non-aqueous extract of *Artemisia*, and group 4 with the extract of *Chenopodium*. Groups 3 and 4 received a dose of 375 mg/kg body weight, according to the dose of highest effectiveness previously reported[7,13]. Animal welfare was conducted in accordance with Mexican regulations for the care and use of laboratory animals[14]. At 24 h after the end of treatment administration (d 33 postinfection), adult worms were recovered from each mouse, and the parasite load per host was determined.

Evaluation of adult helminths

The recovered helminths were washed with physiological saline solution and fixed for 3 h with 10 % formaldehyde, then placed in a container with 70 % ethanol, where they were kept until their evaluation. The specimens were stained according to the procedure described by Beltrán-Fabián-de-Estrada *et al*[15], with some modifications. Briefly, each helminth was stained with 1.6 % carminic acid for 15 min, and the excess dye was removed with three washes with 70 % ethanol. Helminths were dehydrated in a "train" of 80, 90, 96 and 100 % ethanol, incubating for 10 min at each step. Finally, the helminths were transported in clove essence for 24 h. The evaluation was carried out in brightfield microscopy to identify morphological alterations in the scolex and in the gravid proglottids of each recovered cestode. In particular, alterations in uterine branches and integuments were sought in proglottids, which was also evaluated by scanning electron microscopy according to a previously described procedure[16]. The data obtained were recorded qualitatively.

Statistical analysis

All results were expressed as mean values and standard deviation. The analysis of the results between samples was performed with a two-factor ANOVA test; statistical differences were

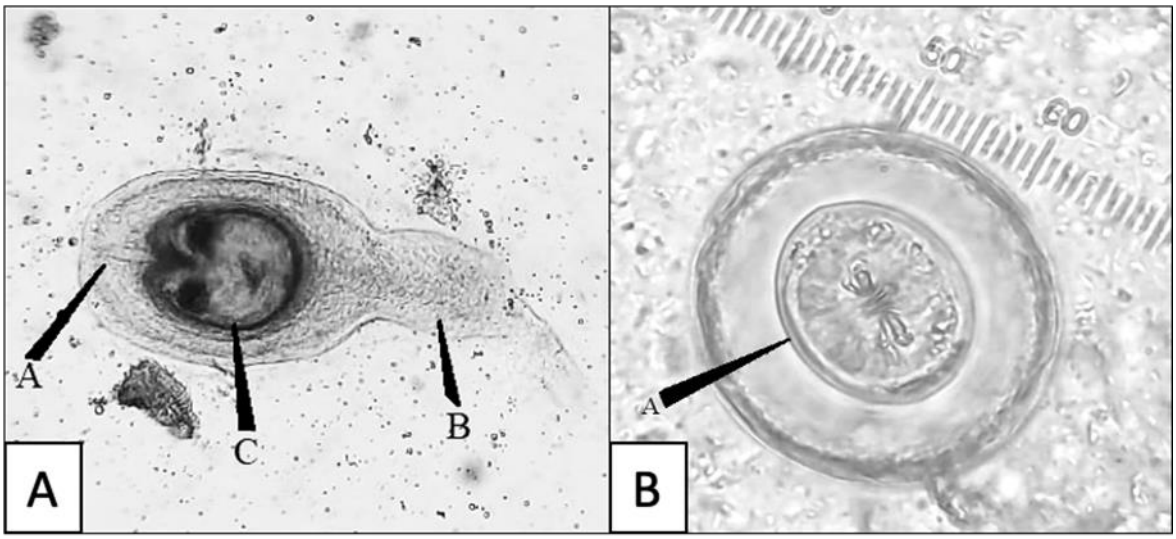
determined at 95 % confidence with the Tukey contrast test (Prism, GraphPad Software, Boston, USA).

Results and discussion

Experimental infection of *Tenebrio molitor*

Ten days after infection, the beetles were decapitated and a 60 % infection frequency was found. The cysticercoids had an average size of 80 to 100 μm in length. Other authors previously reported an experimental infection frequency of 39 to 41.7 % for *Tribolium confusum* beetles[11]; however, as in this study, the dose per beetle was not quantified. The cysticercoids were recovered by manually macerating the hemothorax in saline solution (Figure 1A).

Figure 1: Cysticercoid (A) at 10x and egg (B) at 40x of *Hymenolepis diminuta*



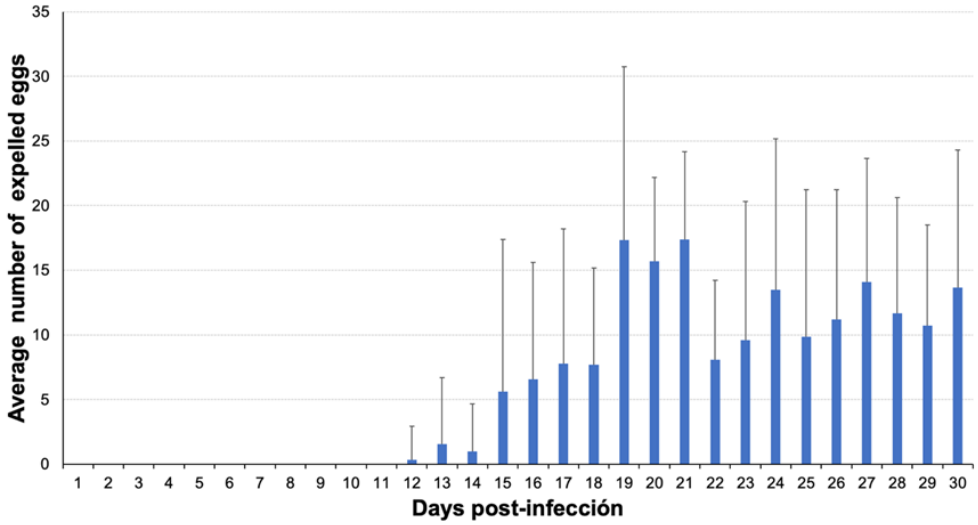
Panel A shows the pore (A), the tail (B), and the protoscolex (C). Panel B shows the membrane of the hexacanth embryo (A).

Dynamics of expelled eggs

To monitor the establishment of *H. diminuta*, the fecal samples of each of the experimentally infected mice were analyzed daily for 30 d, prior to the administration of any treatment. The

eggs of the cestode were detected from d 12 postinfection and remained present until the end of the experiment. The eggs measured between 30 and 38 µm in diameter (Figure 1B). Other authors have previously reported that the maximum length reached by the helminth is at 9 d postinfection, followed by a decrease in size from d 11 due to the detachment of gravid proglottids[17], which is consistent with the data recorded in this study since egg detection began on d 12 postinfection. Regarding the dynamics of expelled eggs, the data contrast with a 21-d dynamic in experimentally infected rats, where the authors recorded a gradual increase in the number of eggs excreted, starting with 1,106 and reaching 1,722 eggs per gram of feces[18]. The kinetics reported here are made up of 30-d data, in which a behavior with increases is observed every 8 d. Figure 2 shows that the largest number of eggs detected was found between days 19 and 21 postinfection.

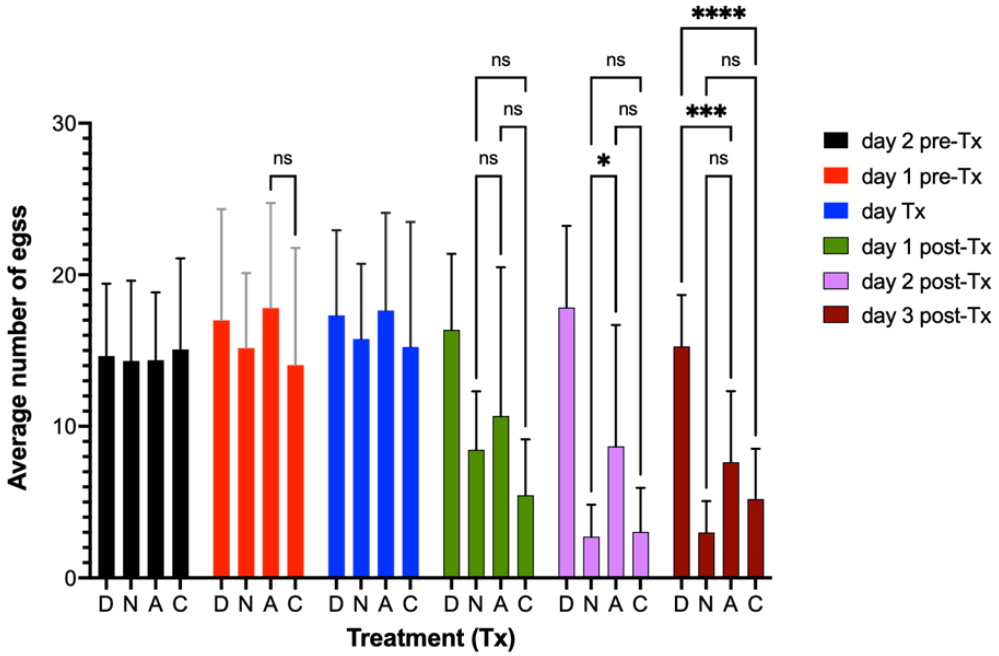
Figure 2: Dynamics of *Hymenolepis diminuta* eggs expelled by experimentally infected mice



Effect of non-aqueous extracts on egg excretion

Overall, a 50 % reduction in egg excretion was observed at 24 h post-treatment; nevertheless, between 48 and 72 h, there was a 75 % reduction in the nitazoxanide and *Chenopodium* groups, whereas the group treated with *Artemisia* maintained the 50 % reduction; the analysis of variance of two factors showed statistically significant differences between the groups, so Figure 3 shows the results of the non-significant (ns) and significant comparisons, $P<0.05$ (*), $P<0.001$ (***), and $P<0.001$ (****) of the Tukey contrast test.

Figure 3: Excretion of *Hymenolepis diminuta* eggs post-treatment



Results obtained with DMSO (D), nitazoxanide (N), *Artemisia ludoviciana* (A), and *Chenopodium vulvaria* (C). Non-significant (ns) and significant comparisons, $P<0.05$ (*), $P<0.001$ (***), and $P<0.001$ (****).

The results with the non-polar extract of *Artemisia* are similar to those previously published by other authors, where it was observed that in mice experimentally infected with *Hymenolepis nana*, the aqueous extract of *A. absinthium* induces, in a dose-dependent manner, the reduction of egg excretion by 61.8 % (400 mg/kg) and 98.8 % (800 mg/kg)[19], whereas the ethanolic extract of *A. abrotanum* induces a 75 % reduction (150 mg/kg)[9]. Nevertheless, the results of this study contrast with those obtained with naturally infected sheep, where there was a 20 % reduction in nematode egg excretion by using an aqueous extract of *A. campestris* (1:1.5; 50 ml/animal; 21 d)[20]. To explain this controversy, at least four factors must be considered: the geographical region of origin of the plant, the anatomical region of the plant from which the extract is prepared, the nature of the extract (aqueous or alcoholic), and the type of parasite under study[19]. The increase in antiparasitic resistance in addition to the residual risk of drugs has led to consider that some plants used in traditional medicine could be an alternative to this problem and the first thing that has been sought is to study the plants that have been used in some cultures as an antiparasitic method[9]. Although many plants have been studied, only some have been properly characterized, especially considering that the growing conditions are not necessarily homogeneous worldwide and not necessarily all plant species, even of the same genus, have the same metabolism and therefore the active ingredients may differ in variety and quantity[20]; they can even vary in the same plant, depending on the anatomical region from which the extract is prepared, and evidently the extraction method, aqueous or ethanolic, will also influence the metabolites obtained.

Once the heterogeneity of the extracts has been considered, it must be borne in mind that the parasites are also heterogeneous and, although the word helminth considers cestodes and nematodes, the former carry out all the nutritive and gaseous exchanges through their integument and its damage will cause irreversible losses in the metabolism. In the case of nematodes[20], feeding is carried out by ingestion and their body is surrounded by a very resistant cuticle of scleroprotein composition, which protects the worm from its environment, so the effect of plant extracts is associated with intake.

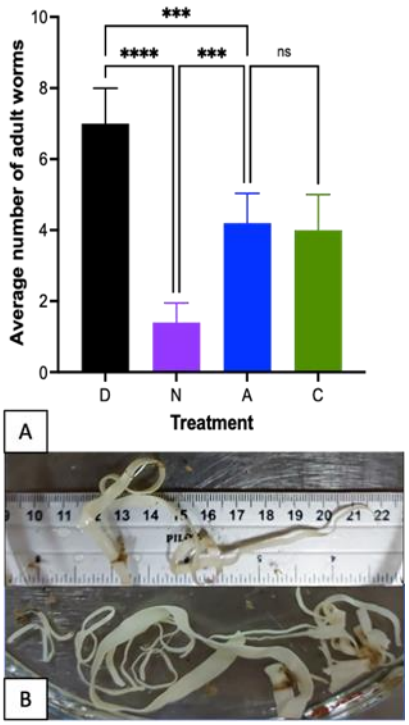
Regarding the non-aqueous extract of *Chenopodium*, the results differ from those reported for conventional oil extracts of *C. graveolens* (100 mg/kg) and *C. ambrosioides* (400 mg/kg), which showed no reduction in the excretion of *H. diminuta* eggs after 5 d of treatment[7]. Nonetheless, methanolic and aqueous extracts of *C. album* induced a gradual reduction (from 86 to 63.5 %) in the excretion of *Haemonchus contortus* eggs in naturally infected animals treated for 15 d at a dose of 2 mg/kg body weight[21]. Although the viability of the excreted eggs was not proven in the work reported here, it has been suggested that extracts of *C. ambrosioides* solubilized with Tween 80 have an ovicidal effect against *H. diminuta*[7] and nematicidal effect against *H. contortus*[6].

Effect of extracts on adult helminth numbers

During the course of infection or treatment, no mice with adverse clinical effects, with pasty or diarrheal feces, were observed, nor was the expulsion of worms observed. The recovered helminths measured between 27 and 35 cm in length (Figure 4B), the size expected in experimentally infected mice[17]. In the groups that receive *Artemisia* and *Chenopodium*, the number of adults decreased by 50 % ($P<0.005$), whereas in the nitazoxanide group, it decreased by up to 75 % ($P<0.001$) compared to the group treated with DMSO. Similar results to those of this work have reported that mice experimentally infected with *H. nana* and treated with an aqueous extract of *A. absinthium* obtained a 56.5 % reduction with 400 mg/kg and a 98.5 % reduction with 800 mg/kg[19]; in contrast, hydroalcoholic extracts of *A. dracunculus* and *A. absinthium* showed *in vitro* dose-dependent (50 to 250 mg/ml) cestocidal effects against protoscolices of *Echinococcus granulosus*[22]. In this way, *Artemisia* extract, regardless of the species, can be an alternative strategy for the control of gastrointestinal cestodes; however, the dose at which it should be administered and the characterization of the metabolites that constitute the *Artemisia* extract are still being studied, which is important because the aqueous extracts of *A. herba-alba* and *C. ambrosioides*, the first, administered orally in pigeons, had no effect on the number of *Raillietina* spp cestodes[23], and the second had no effect on the number of cestodes recovered from naturally infected rodents[7]. This could lead to the proposal of a new strategy for the control of cestode infections through the

combination of antiparasitic drugs used in a traditional way and *Artemisia* extracts, which would also have the advantage of reducing the development of antiparasitic resistance and environmental pollution.

Figure 4: Adult cestodes recovered post-treatment

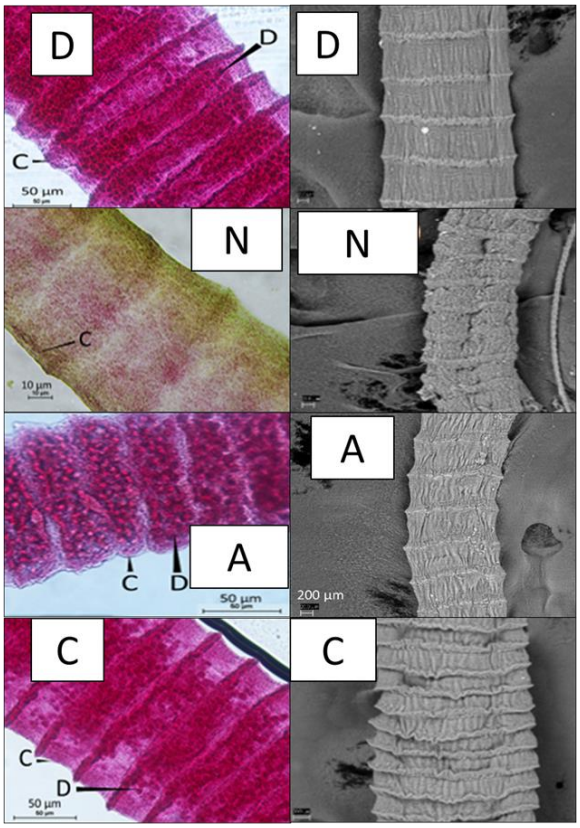


Results obtained with DMSO (D), nitazoxanide (N), *Artemisia ludoviciana* (A), and *Chenopodium vulvaria* (C). In panel A, non-significant (ns) and significant comparisons at $P<0.001$ (***) and $P<0.001$ (****). In Panel B, there is a cestode recovered from a mouse treated with DMSO-1%.

Effect on adult helminth integument

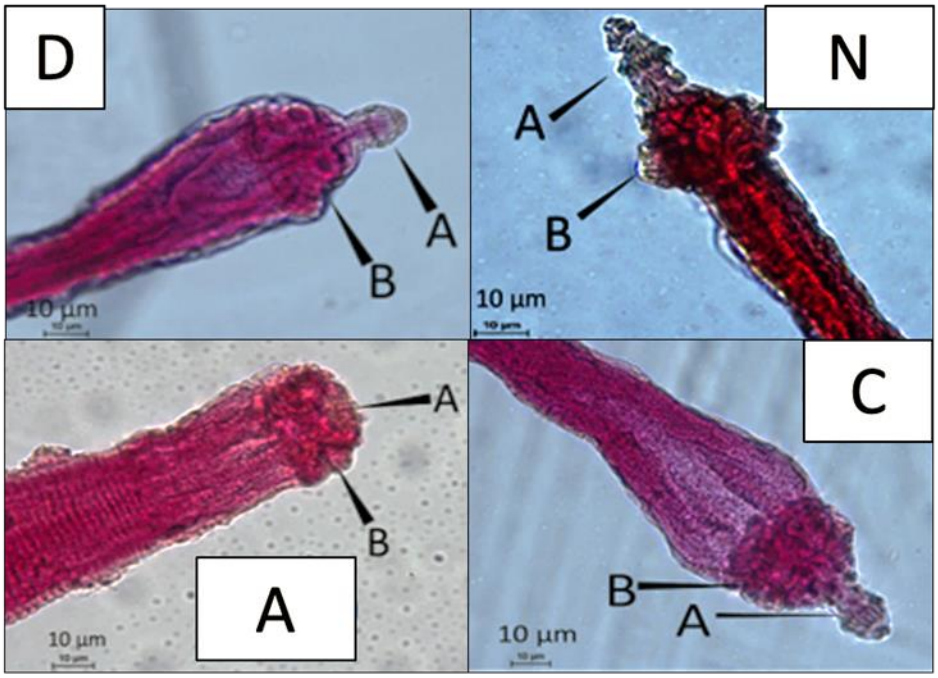
Gravid proglottids treated with 1 % DMSO showed a uniform stain that allowed the internal ramifications of uterus with eggs to be observed (Figure 5), whereas scanning electron microscopy showed an integument with uniform striations (Figure 6). Analysis of proglottids treated with nitazoxanide and then stained with carminic acid showed that proglottids retain stain homogeneously, i.e., there are regions with stain clusters and unstained regions, suggesting damage to internal structures. On the other hand, with clove oil, used as a means of lightening tissues, sections of the integument that took on a greenish brown coloration were observed, suggesting damage.

Figure 5: Gravid proglottids of *Hymenolepis diminuta* post-treatment



The proglottids were stained with carminic acid (left panels) and subjected to scanning electron microscopy (right panels). The figure shows the results obtained with DMSO (D), nitazoxanide (N), *Artemisia ludoviciana* (A), and *Chenopodium vulvaria* (C), genital atrium (C) and eggs in the uterine branches (D).

Figure 6: Scolex recovered 72 hours post-treatment



Results obtained with DMSO (D), nitazoxanide (N), *Artemisia ludoviciana* (A), and *Chenopodium vulvaria* (C). Each panel shows the rostellum (A) and suckers (B).

In summary, nitazoxanide caused deformations and abnormal grooves in the proglottid integument, as well as damage to uteruses without eggs. The analysis of the proglottids in scanning electron microscopy showed deformation of the integument associated with fissures and the generation of vesicles that give a velvety appearance without a striation pattern. It has been previously described that lesions in the integument of *Taenia crassiceps* cysticercoids caused by nitazoxanide treatment consist of separation of the integument from the body of the parasite and the rupture of the vesicle; similarly, scanning electron microscopy shows disintegration of the integument[24].

In the proglottids treated with *Artemisia*, the staining with carminic acid was observed to be uniform; the uterine branches were intact. The integument presented depressions. Scanning electron microscopy showed an increase in integument roughness, areas of fissures, depressions in some portions of the proglottids, small regions with vesicles, and degraded tissue. In 2017, by transmission electron microscopy, Beshay[19] demonstrated that the cestocidal effect (1 mg/ml during 7 h of exposure) of *A. absinthium* against adult *H. nana* is associated with morphological changes of the integument due to the accumulation of lipids and, internally, the proglottid showed destruction of the nephridial canal and intrauterine eggs, as well as apoptosis and autophagy processes, which could be attributed to the alteration of nutrient absorption due to integumental damage[19]. There are reports of the induction of

expulsion of nematodes with macroscopically observable damage when the host is treated with *Artemisia*[25].

In proglottids treated with *Chenopodium graveolens*, it was observed that carminic acid staining showed unstained areas inside the proglottids, but no damage to the uterine branches or morphological changes in the eggs was observed. With electron microscopy, it was observed that the main change was depressions in the integument; nonetheless, when observing the eggs after staining with carminic acid, they did not show changes in the morphology of the eggs. It has previously been documented that in a dose-dependent manner, *Artemisia* extract is capable of inducing apoptosis in gravid proglottids of *H. nana*[19] and thus induce damage in eggs; however, if the damage to the integument is superficial, as in the data reported here, there would be no damage to the eggs. On the other hand, other authors have described that extracts of *C. ambrosioides* and *C. album* have a paralyzing effect on the annelids *Pheretima posthuma*[26] and *Eisenia foetida*[27].

At the scolex level, treatment with nitazoxanide showed damage to the suckers and rostellum, as has been reported by other authors using the nitazoxanide-albendazole combination (40 mg/kg) in the murine cysticercosis model. The combination resulted in deformations of the suckers and the rostellum[28]. The extracts of *Chenopodium* and *Artemisia* showed no damage to the rostellum, but the staining with carminic acid showed slight deformation of the suckers. It has been formerly reported by other authors that the extract of *Senna alexandrina* produces roughness in the proglottids of *H. diminuta* and deformation in the suckers of the parasite[16].

Conclusions and implications

This study determined the effect of non-aqueous extracts of *Artemisia ludoviciana mexicana* and *Chenopodium vulvaria* during experimental murine infection with *H. diminuta*. The extracts reduced the parasite load by 50 % during the first 24 h of administration; nevertheless, with *Chenopodium* extract, the parasite load is reduced by up to 70 % in the following 72 h. Additional studies should be done to determine doses, active ingredients and, where appropriate, combination with drugs to establish the cestocidal effect. It is also necessary to verify the effect on other genera of gastroenteric cestodes, flukes, and nematodes, including the larval stages. The absence of adverse reactions makes it possible to use the extracts as an alternative strategy for the control of gastroenteric cestode infections.

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Conflict of interest

The authors of this paper declare that there is no type of conflict of interest, nor any economic, personal, political, financial, or academic interest that may influence their judgment.

Literature cited:

1. Panti-May JA, Servían A, Ferrari W, Zonta ML, Hernández-Mena DI, Hernández-Betancourt SF, *et al*. Morphological and molecular identification of hymenolepidid cestodes in children and synanthropic rodents from rural Mexico. Parasitol Int 2020; 75:102042-102048. DOI: 10.1016/j.parint.2019.102042.

2. Barriga OO. CIE-10 B71.0 Himenolepiasis. En: Organización Panamericana de la Salud editor. Zoonosis y enfermedades transmisibles comunes al hombre y a los animales, Volumen III Parasitosis. 3a ed. Washington, DC, USA: Organización Mundial de la Salud; 2003:211-216.

3. Rajeev S, ShuHua L, Derek MM. *Hymenolepis diminuta*. Trends Parasitol 2022;38(11):991-992. doi:10.1016/j.pt.2022.07.002.

4. Mpofu TJ, Nephawe KA, Mtileni B. Prevalence and resistance to gastrointestinal parasites in goats: A review. Vet World 2022;15(10):2442-2452. doi: 10.14202/vetworld.2022.2442-2452.

5. Assadpour E, Can KA, Fasamanesh M, Mahdavi SA, Shariat-Alavi M, Feng J. Jafari SM. *et al*. Application of essential oils as natural biopesticides; recent advances. Crit Rev Food Sci Nutr 2023;64(19):6477-6497. doi:10.1080/10408398.2023.2170317.

6. Kasali FM, Tusiimire J, Kadima JN, Agaba AG. Ethnomedical uses, chemical constituents, and evidence-based pharmacological properties of *Chenopodium ambrosioides* L.: extensive overview. Futur J Pharm Sci 2021;7(153):1-3. doi:10.1186/s43094-021-00306-3.

7. Khalifa MM, Mahrous LN, El-Gayed SH. *Chenopodium ambrosioides* oil extract as broad spectrum Parasiticides. Egyp Vet Med Soc Parasitol J 2017;13(1):25-37. doi: 10.21608/evmspj.2017.37458.

8. Abidi A, Sebai E, Dhibi M, Alimi D, Rekik M, B'chir F, *et al*. Chemical analyses and anthelmintic effects of *Artemisia campestris* essential oil. Vet Parasitol 2018;263:59-65. doi: 10.1016/j.vetpar.2018.10.003.

9. Amirmohammadi M, Khajoenia S, Bahmani M, Rafieian-Kopaei M, Eftekhari Z, Qorbani M. *In vivo* evaluation of antiparasitic effects of *Artemisia abrotanum* and *Salvia officinalis* extracts on *Syphacia obvelata*, *Aspiculoris tetrapetra* and *Hymenolepis nana* parasites. Asian Pac J Trop Dis 2014;4(1):S250-S254. doi: 10.1016/S2222-1808(14)60449-7.

10. Plazola-Jacinto C, Pérez-Pérez V, Pereyra-Castro S, Alamilla-Beltrán L, Ortiz-Moreno A. Microencapsulation of biocompounds from avocado leaves oily extracts. Rev Mex Ing Quím 2019;18(3);1261-1276. doi: 10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Plazola.

11. Yezerski A, Luyten Y, Dubiel T. Comparison of the effects of multiple variables on the levels of infection of the rat tapeworm, *Hymenolepis diminuta*, in its intermediate host, the confused flour beetle *Tribolium confusum*. J Helminthol 2020;94(e116):1-9. doi: 10.1017/S0022149X19001123.

12. de-la-Rosa-Arana JL, Tapia-Romero R. Frequency of helminth eggs in faeces of puppies living in urban or rural environments of Mexico City. Iran J Parasitol 2018;13(4):632-636.

13. Alvarez-Mercado JM, Ibarra-Velarde F, Alonso-Díaz MÁ, Vera-Montenegro Y, Avila-Acevedo JG, García-Bores AM. *In vitro* antihelmintic effect of fifteen tropical plant extracts on excysted flukes of *Fasciola hepatica*. BMC Vet Res 2015;11(1):45-51. doi: 10.1186/s12917-015-0362-4.

14. NOM-062-ZOO-1999. Norma Oficial Mexicana. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación: Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. México DF. 1999.

15. Beltrán-Fabián-de-Estrada M, Tello-Casanova R, Náquira-Velarde C. Métodos de coloración para helmintos, coloración carmín clorhídrico. En: Instituto Nacional de Salud (Lima) editor. Manual de procedimientos de laboratorio para el diagnóstico de los parásitos intestinales del hombre. 1a ed. Lima. Perú: Ministerio de Salud de Perú; 2003: 34-36.

16. Kundu S, Roy S, Nandi S, Ukil B, Lyndem LM. *Senna alexandrina* Mill. induced ultrastructural changes on *Hymenolepis diminuta*. J Parasit Dis 2017; 41(1):47-154. doi: 10.1007/s12639-016-0768-3.

17. Hopkins CA, Subramanian G, Stallard H. The development of *Hymenolepis diminuta* in primary and secondary infections in mice. Parasitology 1972;64(3):401-412. doi: 10.1017/S0031182000045479.

18. Sapaat A, Satrija F, Mahsol HH, Ahmad AH. Anthelmintic activity of papaya seeds on *Hymenolepis diminuta* infections in rats. Trop Biomed 2012;29(4):508-512.

19. Beshay EVN. Therapeutic efficacy of *Artemisia absinthium* against *Hymenolepis nana*: *in vitro* and *in vivo* studies in comparison with the anthelmintic praziquantel. J Helminthol 2018;92(3):298-308. doi: 10.1017/S0022149X17000529.

20. Castagna F, Piras C, Palma E, Musolino V, Lupia C, Bosco A, Rinaldi L, *et al*. Green veterinary pharmacology applied to parasite control: Evaluation of *Punica granatum*, *Artemisia campestris*, *Salix caprea* aqueous macerates against gastrointestinal nematodes of sheep. Vet Sci 2021;8(10):237-251. doi: 10.3390/vetsci8100237.

21. Lone BA, Chishti MZ, Bhat FA, Tak H, Bandh SA, Khan A. Evaluation of anthelmintic antimicrobial and antioxidant activity of *Chenopodium album*. Trop Anim Health Prod 2017;49:1597-1605. doi:10.1007/s11250-017-1364-y.

22. Norouzi R, Abedi-Maleki R, Siyadatpanah A, Fiad A, El-Zowalaty ME. *In vitro* scolicidal effect of *Calendula officinalis*, *Artemisia dracunculus*, *Artemisia absinthium*, and *Ferula assafoetida* extracts against hydatid cyst protoscolices. Ann Parasitol 2022;68(3):543-55. doi: 10.17420/ap6803.461.

23. Saad KA, Alsanousi SA, Alkailani HA. Evaluation of the effects of *Artemisia herba-alba* on intestinal parasite infections in domestic pigeons (*Columba livia*) in Albeida City, Libya. SOJUOB 2021;34(2)188-192. doi:10.37376/sjuob.v34i2.3229.

24. de Lima, NF, de Andrade P G, Valencia DGR, Villegas EOL, Mellado MDRE, Ambrosio JR, Vinaud MC. Alterations in *Taenia crassiceps* cysticerci cytoskeleton induced by nitazoxanide and flubendazole. Acta Trop 2021;221:106027-106037. doi: 10.1016/j.actatropica.2021.106027.

25. Dağ, ŞRO, Erez MS, Kozan E, Özkan AMG, Çankaya İİT. *In vitro* anthelmintic activity of five different *Artemisia* L species growing in Türkiye. Pakistan Vet J 2023;43(4):1-7. doi:10.29261/pakvetj/2023.087.

26. Chakraborty M, Pradhan R. Phytochemical analysis and potential anthelmintic activity of *Chenopodium ambrosioides* leaf extract. Int J Pharm Sci 2023;1(11):1-36. doi: 10.5281/zenodo.10203953.

27. Choudhary N, Khatik GL, Choudhary S, Singh G, Suttee A. *In vitro* anthelmintic activity of *Chenopodium album* and in-silico prediction of mechanistic role on *Eisenia foetida*. Heliyon 2021;7(1):1-8. doi:10.1016/j.heliyon.2021.e05917.

28. Sampaio GA, Zago LV, de Lima NF, Picanço G de A, Lino JR de S, Vinaud MC. Histopathologic evaluation of experimental murine neurocysticercosis after treatment with albendazole/nitazoxanide combination. Parasitology 2020;147(7):822-827. doi: 10.1017/s0031182020000505.