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Abstract

Strongyloidiasis is a parasitosis that represents a public health problem, in tropical regions. The present study aimed to investigate the anthelmintic effects of several extracts of Argemone mexicana, as well as its main component berberine (Ber) against the third-stage larvae (L3) of Strongyloides venezuelensis in-vitro experiments. Also, the anti-hemolytic activity of the extract, fractions and Ber were tested in human erythrocytes. A dose-response anthelminthic bioassay demonstrated Ber as the most effective component, followed by methanolic subfraction (Fr3) and finally the crude extract of A. mexicana (Am) showing LC50 response values of 1.80, 101 and 92 µg/mL, respectively. Also, Am, Fr3 and Ber did not produce significant hemolysis against human erythrocytes ($p \leq 0.05$). Am and Fr3 showed cytoprotective capacity at the membrane level ($p \leq 0.05$). Furthermore, Ber was found to have an antioxidant activity of 168.18 µg / mL. According to the results, the Fr3 of A. mexicana, and particularly Ber, exhibited potent in-vitro effects against L3 of S. venezuelensis, without cytotoxic activity against human erythrocytes and presented good antioxidant capacity. In conclusion the extracts of A. mexicana and the main component have activity against S. venezuelensis, nevertheless, further studies are required to elucidate the activity in animal models and the mechanism of action.

Keywords	Argemone mexicana; berberine; natural products; drug discovery; strongyloidiasis.

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Highlights

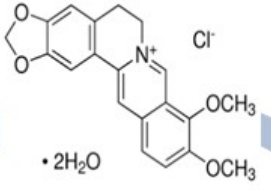
Anthelmintic, cytotoxic and cytoprotective activity of *A. mexicana*.

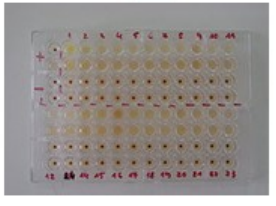
Development of a new alternative to conventional anthelmintics.

The capacity of the berberine alkaloid is reported, which has a response against the viability of *S. venezuelensis* (L3), being able to compare this activity with the reference drug.

To evaluate the safety of the extract, subfractions, and berberine, an *in-vitro* cytotoxicity test was performed.

Main component: Berberine





Cytotoxic activity

Argemone mexicana

***In – vitro* activity against L3**

Antioxidant activity

Berberine: A nematocidal alkaloid from *Argemone mexicana* against *Strongyloides venezuelensis*

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ABSTRACT

Strongyloidiasis is a parasitosis that represents a public health problem, in tropical regions. The present study aimed to investigate the anthelmintic effects of several extracts of *Argemone mexicana*, as well as its main component berberine (Ber) against the third-stage larvae (L3) of *Strongyloides venezuelensis in-vitro* experiments. Also, the anti-hemolytic activity of the extract, fractions and Ber were tested in human erythrocytes. A dose-response anthelminthic bioassay demonstrated Ber as the most effective component, followed by methanolic subfraction (Fr3) and finally the crude extract of *A. mexicana* (Am) showing LC_{50} response values of 1.80, 101 and 92 µg/mL, respectively. Also, Am, Fr3 and Ber did not produce significant hemolysis against human erythrocytes ($p \leq 0.05$). Am and Fr3 showed cytoprotective capacity at the membrane level ($p \leq 0.05$). Furthermore, Ber was found to have an antioxidant activity of 168.18 µg / mL. According to the results, the Fr3 of *A. mexicana*, and particularly Ber, exhibited potent *in-vitro* effects against L3 of *S. venezuelensis*, without cytotoxic activity against human erythrocytes and presented good antioxidant capacity. In conclusion the extracts of *A. mexicana* and the main component have activity against *S. venezuelensis*, nevertheless, further studies are required to elucidate the activity in animal models and the mechanism of action.

Keywords

Argemone mexicana; berberine; natural products; drug discovery; strongyloidiasis

1. Introduction

Parasitosis caused by helminths is a major public health problem worldwide in developing countries. These diseases are closely linked to poverty and poor hygienic-sanitary conditions, which is why parasitic diseases are more frequent in developing countries. Especially in geographical areas where ecological conditions favor the persistence of parasites besides, it is estimated that approximately more than two billion individuals in the world are home to at least one kind of geo-helminth (FAO/WHO, 2014). Concerning strongyloidiasis, the World Health Organization (WHO) mentions that between 30 - 100 million people are infected in the world; but there is a possibility that these data may be underestimated due to the number of undiagnosed individuals (Bisoffi et al., 2013).

Strongyloidiasis is a helminthiasis caused by *Strongyloides stercoralis* that causes rash and pulmonary symptoms (cough and wheezing), eosinophilia and abdominal pain with diarrhea, this disease is endemic in the poorest tropical and subtropical regions of the world (Siddiqui and Berk, 2001). The clinical strongyloidiasis ranges from asymptomatic to life threatening disseminated hyperinfection depending upon the immune status of the patient. Disseminated hyperinfection involves a massive spread of the parasite in immunosuppression with high mortality (Montes et al., 2010; Schär et al., 2013).

Infective third-stage larvae (L3) penetrate the skin and migrate to the lungs, reaching small intestine where became mature. Parthenogenetic females lay eggs that hatch into rhabditiform larvae (L1), which are eliminated in faeces, some L1 remain and molt into L3 establishing reinfection cycles (Ruano et al., 2005). So far, there are no vaccines for strongyloidiasis, however, some care can help in its prevention such as basic sanitation, adequate disposal of excreta and use of drinking water, hygienic hand washing measures, food disinfection and use of footwear adequate in areas where there is a possibility that the

74 soil is infected (Wang et al., 2013). *S. venezuelensis* is a laboratory analog of the human
75 parasite, *S. stercoralis* (Sato and Toma, 1990).

76 The treatment is performed with ivermectin (IV) or albendazole. The treatment of
77 strongyloidiasis is complex because the complete eradication of these helminths. The low
78 parasite load and irregular larval production hampers to identify a true cure only based on
79 examination of fecal matter. The most recommended treatment of strongyloidiasis is to use
80 200 mg/kg/day of IV by mouth for two days. This treatment must be repeated after 14 days
81 (Gotuzzo et al., 2016). Immunocompromised patients should receive three cycles of
82 treatment with intervals of 14 days between cycles (Corti, 2016). Since the introduction of
83 Iv for the treatment of strongyloidiasis, it was considered the drug of choice for this and
84 have been used against human and veterinary parasites (von Samson-Himmelstjerna, 2012).
85 IV resistant-nematode strains have also been reported in grazing livestock and the issue of
86 decreased susceptibility in human nematodes or the possibility of resistance is also
87 increasing (Parasitol and Article, 2013).

88 Natural products are an important source of metabolites with multiple diverse
89 biological properties that can be used as active ingredients for the treatment of diseases
90 since the therapeutic use of plants considered medicinal is the basis for the discovery and
91 development of new active ingredients (Patel et al., 2011). In Latin America, plants are an
92 abundant and accessible resource, and traditionally used since pre-Hispanic times, among
93 these plants is *Argemone mexicana* which is an endemic plant of Mexico but is widely
94 distributed in many tropical and subtropical countries (Bhattacharjee et al., 2010). Different
95 parts of this plant have been used for the treatment of different diseases, and several
96 investigations have demonstrated the effect of this plant against fungi, and parasites for
97 example *Plasmodium falciparum* and *Plasmodium berghei* (Brahmachari et al., 2013). The

presence of various types of chemical constituents such as alkaloids has been reported from this plant, with berberine (Ber) (Figure 1) being the most abundant component (Chang et al., 2003). Ber has been used as traditional medicine and dietary supplement. This alkaloid has activity against fungal, yeast, parasite, viral and bacterial infections (Swayze et al., 2007; Verma and Sharma, 2018). As a natural product, its effects against cancer and diabetes are studied (Raju et al., 2019).

In this study, the activity of methanolic extracts of the aerial part of *A. mexicana*, as well as their fractions and berberine were evaluated against L3 filariform larvae of *S. venezuelensis*. The treatment that showed good activity against *S. venezuelensis in-vitro* were selected to evaluate their hemolytic, cytoprotective and antioxidant activity.

2. Materials and Methods

2.1. Ethical statement

Animal procedures complied with Spanish (RD 53/2013) and European Union (European Directive 2010/63/CE) guidelines regarding animal experimentation for the protection and humane use of laboratory animals. The University of Salamanca's accredited Animal Experimentation Facilities (Registration number: PAE/SA/001) were used for such procedures. The University of Salamanca's Ethics Committee also approved the procedures used in this study (CBE 335). All efforts were made to minimize the animal suffering. The study with human erythrocytes was carried out under the approval of the ethics committee of the Universidad Autónoma de Nuevo León (UANL), College of Medicine (Registration Number HI11002) and under the consent of healthy donors, following the provisions of the Official Mexican Technical Standard NOM-253-SSA1-2012.

2.2. Chemicals used

2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), Benzylpenicillin streptomycin sulfate, Berberine (Ber), Dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ethylenediaminetetraacetic acid (EDTA), and Quercetin were purchased from Sigma-Aldrich (Merck Chemical Co. USA). All other chemicals and solvents were analytical grade.

2.3. Plant material, preparation phytochemical analysis of methanolic extracts

The aerial part of *A. mexicana*, leaves and stems, were collected between January and March 2017 in the city of San Nicolás de Los Garza [25°46'24.38" N & 100°20'59.28"W] in the state of Nuevo León, Mexico. The taxonomic identification of the plant was carried out in the Botany Laboratory of the Facultad de Ciencias Biológicas at the Universidad Autónoma de Nuevo León with Registration number 029127. The aerial part extracts of *A. mexicana*, were obtained as follows the plant was dried at room temperature and macerated with methanol (MeOH). For this, 100 g stems and leaves of *A. mexicana* were ground and successive extractions with absolute MeOH were performed in a flask, using an orbital shaker LAB-LINE 3508, (LAB-LINE Instruments, Inc. USA) with stirring at 100 rpm, at room temperature and solvent replacement every 24 h (3 x 250 mL of absolute MeOH). The extract obtained was filtered with Whatman No. 1 (Merck® KGaA, Germany) filter paper, concentrated under reduced pressure at 45 ºC with a rotary evaporator Laborota 4010 (Heidolph Instruments GmbH & CO KG, Germany) and the residual solvent was evaporated at room temperature. Finally, the extract obtained was calculated the percentage of yield, cataloged as Am and stored in dark at 4 °C until later use. For the fractionation process of the raw extract Am, serial partitions were made from the extract using solvents of different polarity, first, the extract was dissolved in 100 mL of hexane by magnetic stirring for 20 min at 25 °C, then was filtered through Whatman No. 1 paper (Fr1), the residue was extracted in

100 mL of $CHCl_3$ (Fr2), filtered through Whatman No. 1 paper again, and the residual solid was extracted with 50 mL of absolute MeOH and was filtered again (Fr3), finally, the solid obtained was solubilized in 20 mL of distilled water (Fr4), it was frozen at -20 °C and lyophilized at -40 °C and 0.22 mBar in a Free Zone 2.5 Liter Benchtop Freeze Dryer, (LABCONCO, USA). The procedure described was applied to the extract of the aerial part of *A. mexicana*, the yield for the extraction, was calculated with the following formula Yield = (Final weight / Initial weight) X 100.

To determine the functional groups present in the methanolic extract and its active fractions, conventional phytochemical tests were carried out and the following metabolites were detected qualitatively (Singh et al., 2012): potassium permanganate (unsaturations), 2,4-dinitrophenylhydrazine (carbonyl group), Baljet (coumarins and sesquiterpene lactones), Bornträger (quinones), Liebermann-Burchard (triterpenes and sterols), sodium bicarbonate (carboxyl group), ferric chloride (phenols and tannins), saponin test, Shinoda (flavonoids), anthrone (carbohydrates) and Dragendorff (alkaloids).

2.4. Strongyloides venezuelensis strain and its maintenance of the life cycle

The filariform infectious larvae (L3) of *S. venezuelensis*, were donated by the Department of Parasitology of the University of Minas Gerais, Belo Horizonte, Brazil, and their life cycle was maintained by serial passes on 4-week-old male Wistar rats with a weight of 150 - 200 g (Charles River Laboratories, Barcelona, Spain). The animals were kept in the bioterium of the University of Salamanca (registration number PAE / SA / 001) and kept in standard polycarbonate and wire cages with food and water *ad-libitum*, with controlled light and dark periods of 12 h temperature from 23 to 25 °C. The rats were infected subcutaneously with 12,000 third-stage larvae (L3) in 500 µL phosphate-buffered saline (PBS pH 7.4) using a 25-gauge needle syringe. Feces of infected rats (5-18 days post-

infection) were cultured in 250 mL polyethylene containers with vermiculite, mixed with distilled water for 4 - 7 days, with 90% relative humidity at 28 ºC, subsequently, the L3 were collected using the Baermann method, the viability of the L3 larvae was verified using an optical microscope before the tests. The larvae were washed 3 times with distilled water containing 100 IU/mL of benzylpenicillin and 0.1 mg/mL of streptomycin sulfate (Martins et al., 2000). Subsequently, they were kept in distilled water containing 0.25% sodium hypochlorite for 10 minutes and then the larval suspension was carried out for the corresponding tests.

2.5. In-vitro activity against S. venezuelensis third-stage larvae

A batch of 8,000 L3 were rinsed twice with PBS and 150 larvae per well were distributed in 96-well flat bottom culture plates. Extract, fractions, Ber and IV, were solubilized in DMSO (5%) and appropriate dilutions were made to perform assays. Larvae were incubated at 28 °C for 1 hour to allow adaptation, and treated with the methanol extract, fractions and Ber at 1, 10, 50, 100, 250 and 500 µg/mL, and then incubated during 96 h at 28 °C (Legarda-Ceballos et al., 2016a). Mortality was assessed as the lack of any movement detected during 2 min of observation under the microscope with the 4× objective and the 10× eyepiece (magnification 40×), at 0, 24, 48, 72 and 96 h after treatment (Legarda-Ceballos et al., 2016b). As controls, *S. venezuelensis* larvae were incubated in the presence of distilled water (Ctrl -) or treated with ivermectin 10 µM (Ctrl +) (Legarda-Ceballos et al., 2016b).

2.7. Cytotoxicity by hemolysis test

The evaluation of cytotoxicity was determined by hemolysis of a suspension of human blood erythrocytes from healthy donors, for which human blood was obtained from healthy donors and allowed to stand at room temperature for 25 min. After removing the serum with a transfer pipette and adding EDTA (1.5 mg/mL blood) to wash out the red blood cells, they

were carefully mixed and separated by centrifugation at 1,000 rpm (5 min / 37°C). The cell pack obtained was washed and centrifuged four times in phosphate buffer (PBS - 10 mM) pH 7.4 with supernatant removal. The erythrocytes obtained were then used to prepare a red cell suspension for the tests at 5% v/v in PBS. For the evaluation of the cytotoxicity of Am, Fr.3, and Ber on the human erythrocytes, the previously prepared red cell suspension was incubated with different concentrations of the extract (100, 200, 400, 600, 800 and 1,000 µg/mL) in 2 mL Eppendorf (Eppendorf® AG, Germany) microcentrifuge tubes, for 30 min at 37°C protected from light, these were labeled as treatments (Tr). As a negative control, a solution of erythrocytes without treatment (C-) was used, the positive control consisted of erythrocytes without treatment with sterile distilled water to produce osmotic hemolysis (C+) (Kumar et al., 2011). Once the incubation time has elapsed, all treatments are centrifuged at 13,000 rpm for 3 minutes at 4 °C. 200 µL of supernatant is taken and placed in a transparent plastic microplate (Costar® Corning Incorporated, New York, USA) of 96 flat-bottomed wells. The degree of hemolysis was determined by spectrophotometric readings at 540 nm, the wavelength of maximum absorption of the hemoglobin released in the supernatant (Lakshmi et al., 2014), on a microplate reader EPOCH Microplate Spectrophotometer (BioTek Instruments, Inc., USA). The readings were recorded as the absorbance (Abs) obtained by each treatment (Abs Tr) and finally, the percentage of hemolysis was calculated using the formula: % Hemolysis = [(Abs Tr - Abs C-) / (Abs C+ - Abs C-)] × 100.

2.8. Cytoprotection bioassay of treatments by the AAPH method

For this assay, a suspension of erythrocytes was obtained in the manner described in the hemolysis assay. The suspension was adjusted to 10% v / v with a phosphate buffer pH = 7.4 to be used the same day. Hemolysis was Induced adding 150 mM AAPH reagent prepared in the same phosphate buffer. To evaluate the cytoprotective effect of Am, Fr.3, and Ber, the

previously obtained red blood cell suspension were incubated in 2 mL Eppendorf microcentrifuge tubes with different concentrations of the extract (100, 200, 400, 600, 800 and 1,000 µg / mL) plus AAPH at 200 rpm (5 h / 37 °C) in a rotation incubator THZ-100 (Luzeren Co. China) protected from light, these were classified as treatments (Tr). As a negative control of hemolysis (C-), the phosphate buffer with the suspension of erythrocytes without including AAPH was used and as a positive control, the erythrocyte solution with AAPH (C +) was used (Karimi et al., 2011). After the incubation time had elapsed, all treatments were centrifuged at 13,000 rpm (3 min / 4 °C). 200 µL of supernatant was taken and placed in a flat bottom, transparent 96 well plastic microplate. The degree of hemolysis was determined by spectrophotometric readings at 540 nm, in a microplate reader. The readings were recorded as the absorbance (Abs) obtained by each treatment (Abs Tr) and finally, the percentage of cytoprotection was calculated using the formula: % Cytoprotection = [1 - (AbsTr - Abs C-) / (AbsC+ - AbsC-)] × 100.

2.9. Antioxidant Activity by the DPPH free radical scavenging method

The antioxidant activity, the method of reduction of the DPPH radical was used (Ledy et al., 2012). The treatments were evaluated at concentrations of 20 to 2,500 µg/mL. The DPPH was prepared to 125 μM in methanol, 100 μL of each sample was taken, and 100 μL of DPPH was added; the samples were allowed to stand for 30 min protected from light (Rodríguez-Magaña et al., 2019). The absorbance at 517 nm was measured using a spectrophotometer Spectronic Genesys 20 (Thermo Fisher Scientific, USA). As a positive control, a solution of quercetin was used and as negative control (C-) MeOH; the reduction percentage was calculated using the formula: % Reduction = [(Abs C- - Abs Tr) / (Abs C-)] × 100

2.9. Statistical analysis Data

242 The results were expressed as the mean and standard error of the mean (SEM).
243 Significant differences between groups were found using the one-way ANOVA test, followed
244 by the post hoc Tukey´s honest significance test (HSD) was performed to determine any
245 statistical differences between treated and untreated controls. Also, the Probit test was used to
246 determine the mean lethal concentration (LC_{50}) and half maximal effective concentration
247 (EC_{50}) of the radical scavenging activity. Statistical analyses were performed with SIMFIT
248 Statistical Package 7.4.1 (Manchester University, UK) and SPSS software (IBM – SPSS Ver.
249 24). All statistical analyses were considered significant at the $p < 0.05$ level.
250
251 **3. Results**
252 *3.1. Phytochemical analysis*
253 The percentages of maceration extraction yield for Am and Fr3, together with the
254 results of the basic phytochemical study, indicate the presence of unsaturations, quinones,
255 triterpenes - sterols, phenols, saponins, flavonoids, carbohydrates and alkaloids for both. In
256 the case of phenols, these were found abundantly in Am and the alkaloids abundantly in the
257 active fraction. The extraction yield of Am was 10.55% and for Fr.3 it was 1.72%.
258 *3.2. In-vitro activity against L3*
259 To investigate the *in vitro* anthelmintic effects of *A. mexicana* as well as its main
260 component, Ber against L3 filariform larvae of *S. venezuelensis* we performed in a
261 microassay technique. All the tested treatments were active against L3. All of the various
262 concentrations of the *A. mexicana* methanolic extract, fractions and berberine revealed
263 significant larvicidal effects against L3 of *S. venezuelensis* ($p < 0.05$) in a dose-dependent
264 manner as compared with the control groups (Table 2). When L3 was exposed in a range of

concentration to Fr3 and Ber the mortality rate was 100% after 24 h of incubation at the concentration of 100, 250 and 500 µg/mL, (data not shown).

3.1. In-vitro activity of A mexicana extract, fractions and Berberine against L3

This study was carried out to assess the in vitro anthelmintic effects of *A. mexicana* extract, fractions as well as its main component Ber against L3 filariform larvae of *S. venezuelensis* in comparison with reference drug ivermectin (IV). The *A. mexicana* methanolic extract, fractions and Ber revealed significant larvicidal effects against L3 of *S. venezuelensis* in a dose-dependent manner as compared with the control groups ($p < 0.05$. Table 1). The pure molecule Ber yielded the best nematocidal activity with LC_{50} of 1.8 ± 0.0 µg/mL only 1.5 less activity than those of IV at 72 hours post-treatment. Moreover, the best fraction of *A. mexicana* Fr3, showed LC_{50} of 29.3 ± 3.3 µg/mL. The other extract are actives but in high concentrations.

3.3. Cytotoxic activity by hemolysis test

The cytotoxicity of Am, Fr3, and Ber was evaluated by the human erythrocyte hemolysis test. It was observed that none of the extracts showed to be significantly cytotoxic, since, at the highest concentration tested (1 mg/mL), it was found that Am has hemolysis of 2.8%, and that caused by the Fr3 was of 2.3%. For the lowest concentrations, 100 µg/mL hemolysis presented in erythrocytes was 0.3 and 0.1% respectively. The negative control did not show detectable hemolysis and the positive control consisting of distilled water showed 100% hemolysis (data not shown). Ber was found to be hemolytic at concentrations greater than 200 µg/mL (Table 2).

3.4. Cytoprotection bioassay

The Am, Fr3 and Ber treatments were evaluated for cryoprotection by the AAPH (2,2′-Azobis (2-methylpropionamidine) dihydrochloride) method using human erythrocytes. The

289 less cytoprotector treatment was Ber, presenting a maximum percentage of protection of 3.5
290 % at the concentration of 200 µg/mL. The most effective treatment was Am with a
291 percentage of protection greater than 99% from concentrations of 400 µg/mL or more. And
292 Fr3 was less efficient since its maximum percentage of cytoprotection was given at 100
293 µg/mL after that the cytoprotection stopped (Table 3).

294 *3.5. Antioxidant bioassay*

295 The results of the antioxidant activity of The Am, Fr3 and Ber treatments determined
296 by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test showed that the amount of sample
297 necessary to decrease the concentration of DPPH by 50%, EC_{50}, for Am was 312.5 ± 10.5
298 µg / mL and for Ber (168.2 ± 12.1 µg / mL), however, the quercetin standard was very low
299 (2.1 ± 0.1 µg / mL), while Fr3 presented the highest value (629.5 ± 21.0 µg / mL).

300

301 **4. Discussion**

302 Chemical compounds derived from plants have been used since the origin of human
303 beings to counteract a number of diseases, recent investigations on plant extracts and plant-
304 derived compounds due to having fewer side effects, and high availability have been shown
305 a successful approach to treat a wide range of diseases (Adebayo and Krettli, 2011). As an
306 alternative, several medicinal plant extracts had been investigated to develop a new drug for
307 human parasites (Vargas et al., 2011). Some of the phytochemical compounds such as
308 flavonoid, phenolics, terpenoid and alkaloids that could be extracted from natural products
309 were suggested to have an effect against different pathogens (Rosmalena et al., 2019).
310 Mexican poppy, *Argemone Mexicana*, grows in Asia and America, the different parts of
311 this plant have been used widely as in ethno-medicine for the treatment and prevention of
312 various diseases including infectious diseases (Rajvaidhya and Pradesh, 2012; Vazquez-

313 Flota, 2013). Mexican plants such as *A. mexicana* had been investigated as a potential
314 antiparasitic and showed promising results (Singh et al., 2017). In this study, the aerial part
315 of the extract of *A. mexicana* was evaluated, as well as its subfractions recovered using
316 solvents of increasing polarity to determine their *in-vitro* nematocidal, and anticytotoxic
317 properties. Moreover, the main component, berberine was also assessed.

318 Previous studies have also been carried out on chemical composition of the *A.*
319 *mexicana* that showed the most important constituents of this plant are isoquinoline
320 alkaloids such as Ber, up to now, various studies have been demonstrated antibacterial,
321 antifungal, antiviral and antiparasitic effects of *A. mexicana* and its main constituent
322 berberine (Elizondo-Luevano et al., 2020). Our results agree with what is expected since
323 the alkaloids are the compounds that stand out for their presence, the yields were greater
324 than 10%, although it should be taken into account that the extraction yield and chemical
325 composition of the plant may differ according to the parts used of the plant, as well as the
326 harvest season and the geographical area (Brahmachari et al., 2013). The natural
327 isoquinoline alkaloid berberine has been employed in Chinese and Mexican Medicine for
328 hundreds of years with a wide range of pharmacological and biochemical effects (Tillhon et
329 al., 2012). Isoquinoline alkaloids as berberine are a group of molecules, which have shown
330 a wide variety of biological functions and have also been assayed against several
331 microorganisms, protozoa and nematodes (Journal, 2014; Rajvaidhya and Pradesh, 2012).
332 Ber is present in a wide variety of medicinal plants such as *Berberis aristata*, *Berberis*
333 *vulgaris*, *Coptis chinensis*, and *A. mexicana*.

334 The lethal effective concentrations (LC_{50}) more interesting were found in fraction Fr3
335 and Ber wih high larvicidal activity, in contrast with of *A. mexicana* extract and Fr1, Fr2
336 and Fr4 fractions. Also, Ber offers additional advantages as an antiparasitic drug and, could

make this drug of interest for the treatment of strongyloidiasis patients coinfected with *E. hystolitica*, *Leishmania* spp. or *Plasmodium falciparum* parasites (Raghav et al., 2017; Wright et al., 2000). In other studies high antiparasitic potential of *A. mexicana* against *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis, Echinococcus granulosus* and some *Leishmania* spp. have been proven (Mahmoudvand et al., 2014; Tillhon et al., 2012; Wright et al., 2000). A previous study showed that a component of the methanolic extract of *A. mexicana* showed the highest amount of Ber compound, and the methanolic subfraction extract showed the highest effect against different protozoa and Dengue virus and with low toxicity (Nonaka et al., 2018; Rosmalena et al., 2019). There are no previous reports in the literature concerning the use of *A. mexicana* or Ber for the treatment of strongyloidiasis. There is also evidence that Ber is active against filarial infections caused by *Brugia malayi*, *Wuchereria bancrofti*, *Onchocerca volvulus* (Rana and Misra-Bhattacharya, 2013), and against protozoa such as *T. vaginalis* and *E. histolytica* (Elizondo-Luevano et al., 2020; Elizondo-Luévano et al., 2018).

Although its activity is not as effective as IV, currently the primary drug for the treatment of strongyloidiasis that is why it is important to find alternatives for the treatment of this disease. These situations make the identification of alternative chemotherapies a high priority issue, our results indicate that Ber significantly reduces worm viability, recovery and kills L3 *in-vitro*. The activity of berberine opens the possibility to the combination of drugs that promote synergistic action or minimize the possibility of resistance of certain strains to drugs (Panic et al., 2014).

In this study extract, fractions and pure molecule did not present significant toxicity according to the criteria of Karimi et al. (Karimi et al., 2011), in which it is mentioned that hemolysis could be related to the mechanical stress of agitation and manipulation.

Relatively low concentrations are used in hemolysis studies, (Lakshmi et al., 2014). In the present research where we tested concentrations of 100 to 1000 μg/mL, none of our extracts can be considered to be significantly ($p < 0.05$) haemolytic (Vinjamuri et al., 2015). Regarding the cytoprotection of human erythrocytes, Am had an important protective activity greater than 99%; these results, with significant concentration-dependent inhibition, is attributed to the high levels of total phenols and its antioxidant effects (García-Becerra et al., 2016). On the other hand, Chiang et al. 2013, mentions that the protective effect depends on the dose and incubation time, in addition, a correlation of the protective effect with the powerful antioxidant capacity (Chiang et al., 2013). It is important to emphasize that the AAPH method is useful for the study of the damage induced by free radicals (Pieroni et al., 2011). Since the decomposition of this compound gives rise to molecular nitrogen and alkyl radicals that react with molecular oxygen to give rise to peroxyl radicals which are responsible for predominantly attacking the erythrocyte membranes and at a certain stage during oxidation, these radicals permeate the cells causing hemolysis (Chisté et al., 2014); Shiva Shankar Reddy et al., 2007). The cytoprotective effect of Am seems to be related to its antioxidant capacity since polyphenols seem to be responsible for the resistance of erythrocytes to oxidative stress, they can result in their fluidity hindering the diffusion of free radicals, thus decreasing the kinetics of free radical reactions (Chaudhuri et al., 2007). Due to the fact that the extracts were not toxic to human erythrocytes, it is suggested that the extracts with relevant activity can be considered for purposes for evaluation in a murine model.

5. Conclusions

A. mexicana methanolic extracts of aerial parts and its main compound, Ber, in particular, demonstrated strongyloidicidal activity against the third stage larvae (L3) of *S. venezuelensis* in low concentration *in-vitro* model. In addition to the crude extracts in concentrations of up to 1 mg/mL of *A. mexicana* did not turn out to be hemolytic against human erythrocytes. However, the *in-vivo* efficacy of *A. mexicana* and Ber requires to be evaluated using an animal model of L3 infection. Also, action mechanisms should be studied.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceived and designed the experiments: J.L.A., P.F.S. and A.M. Performed the experiments: J.H.E.L. and O.G.G. Analyzed the data/materials/analysis tools: R.C.R., B.V. and J.L.A. Writing—original draft preparation, J.H.E.L. and R.C.R. Writing—review and editing: J.L.A. and A.C.M. Funding acquisition/project administration: B.V. and P.F.S. Supervision: A.M., J.L.A. and A.C.M. All authors have read and agreed to the published version of the manuscript.

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595 **Table 1.** Lethal concentration (μg/mL) that causes the death of 50% (LC_{50}) at a 95%
596 confidence interval of L3 treated with the extract (Am), fractions (Fr1, Fr2, Fr3, Fr4) and
597 Berberine (Ber) compared with the effects of the reference drug ivermectin (IV).

Treatment	Incubation time (h)			
	24	**48**	**72**	**96**
Am	186.5 ± 3.6	136.9 ± 7.6	101.0 ± 5.5	92.1 ± 4.7
Fr1	ND	127.7 ± 3.8	77.2 ± 2.5	25.4 ± 3.2
Fr2	ND	194.1 ± 0.8	60.9 ± 3.8	44.4 ± 2.1
Fr3	58.9 ± 3.8	31.4 ± 2.4* $P < 0.05$	29.3 ± 3.3* $P < 0.05$	19.5 ± 2.1* $P < 0.001$
Fr4	ND	ND	224.7 ± 0.3	154.5 ± 1.4
Ber	6.6 ± 0.5* $P < 0.001$	3.2 ± 0.6* $P < 0.001$	1.8 ± 0.0* $P < 0.001$	1.6 ± 0.1* $P < 0.001$
IV	11.5 ± 0.8* $P < 0.001$	4.9 ± 0.3* $P < 0.001$	1.2 ± 0.0* $P < 0.001$	0.9 ± 0.0* $P < 0.001$
ANOVA	$F_{(6,21)} = 5.9$, $P < 0.001$	$F_{(6,21)} = 33$, $P = 0.08$	$F_{(6,21)} = 6.9$, $P = 0.05$	$F_{(6,21)} = 97.8$, $P = 0.001$

598 ANOVA and post-hoc Tukey's honest significance (HSD) test was used. *Significant
599 activity against L3 Larvae compared with to non-treated L3. ND: Activities lower than 50%
600 in all the range tested. Results are depicted as Mean ± SD.
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Table 2. Evaluation of the cytotoxicity of the *A. mexicana* extract (Am), and Fr3 extract and berberine (Ber) by the hemolysis test.

	Treatments			
Concentration (µg/mL)	**Control**	**Am**	**Fr3**	**Ber**
100	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	2.8 ± 0.4
200	0.3 ± 0.0	0.8 ± 0.1	0.4 ± 0.1	11.8 ± 1.4* $P < 0.001$
400	0.3 ± 0.0	1.2 ± 0.5	0.8 ± 0.0	23.8 ± 3.2* $P < 0.001$
600	0.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.3	55.9 ± 4.1* $P < 0.001$
800	0.7 ± 0.2	2.1 ± 0.4	2.1 ± 0.2	72.9 ± 2.3* $P < 0.001$
1000	0.7 ± 0.1	2.8 ± 0.4	2.3 ± 0.3	90.2 ± 9.3* $P < 0.001$
ANOVA	$F_{(2,15)} = 7.5$, $P = 0.25$	$F_{(2,15)} = 7.5$, $P = 1.00$	$F_{(2,15)} = 7.5$, $P = 0.99$	$F_{(2,15)} = 99.0$, $P < 0.001$

ANOVA and post-hoc Tukey's honest significance (HSD) test was used. *Significant differences in comparison with the control group. The results are given in % of hemolysis in duplicate and a minimum of three repetitions in different times. The values are shown as the mean ± SD.

633 **Table 3.** Evaluation of the cytoprotection of the *A. mexicana* extract (Am), and Fr3 extract
634 and berberine (Ber) by the AAPH method.

	Treatments			
Concentration(µg/mL)	**AAPH**	**Am**	**Fr3**	**Ber**
100	0.0 ± 0.0	23.6 ± 3.5* $P < 0.001$	29.8 ± 2.4* $P < 0.001$	3.5 ± 0.0
200	0.0 ± 0.0	77.0 ± 2.3* $P < 0.001$	4.8 ± 0.3* $P < 0.05$	2.6 ± 0.1
400	0.0 ± 0.0	99.1 ± 1.3* $P < 0.001$	4.2 ± 0.2* $P < 0.05$	1.6 ± 0.0
600	0.0 ± 0.0	99.3 ± 0.2* $P < 0.001$	2.9 ± 0.3	0.9 ± 0.1
800	0.0 ± 0.0	99.3 ± 0.1* $P < 0.001$	1.6 ± 0.0	0.8 ± 0.1
1000	0.0 ± 0.0	99.7 ± 3.1* $P < 0.001$	0.6 ± 0.1	0.1 ± 0.1
ANOVA	$F_{(3,68)} = 0.59$, $P = 1.00$	$F_{(3,68)} = 29.0$, $P = 0.00$	$F_{(3,68)} = 3.28$, $P = 0.50$	$F_{(3,68)} = 0.58$, $P = 0.99$

635 ANOVA and post-hoc Tukey's honest significance (HSD) test was used. *Significant
636 differences in comparison with the control group (AAPH). The results are given in
637 percentage of cytoprotection (%) in duplicate and a minimum of three repetitions in
638 different times. The values are shown as the mean ± SD.
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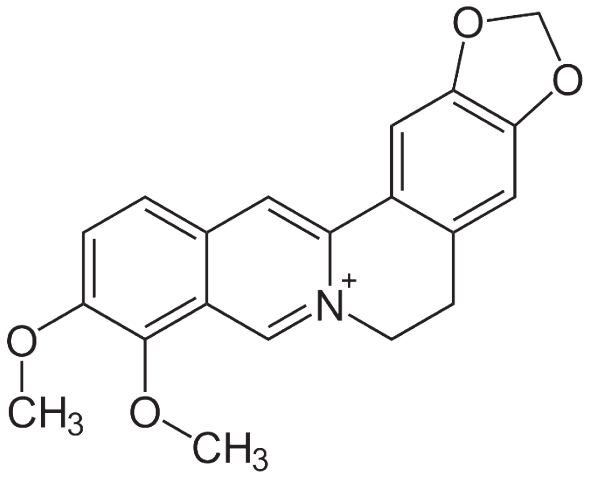

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655 **Figure 1.** The chemical structure of the alkaloid berberine (Average mass: 336.361).

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Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare no conflict of interest.

Author contributions

Joel Horacio ELIZONDO-LUEVANO (J.H.E.L.): Creation and writing the initial draft and performing the experiments.

Rocío CASTRO-RÍOS (R.C.R.): Evidence collection and Tools.

Julio LÓPEZ-ABAN (J.L.A.): Ideas; formulation and conceptualization of overarching research goals and aims.

Oscar GORGOJO-GALINDO (O.G.G.): Analyzed the data, materials and analysis tools.

Pedro FERNÁNDEZ-SOTO: Funding acquisition and project administration.

Ana Belén VICENTE-SANTIAGO (B.V.): Provision of study materials, reagents, materials and instrumentation.

Antonio MURO-ÁLVAREZ (A.M.): Conducting the research and supervision.

Abelardo CHÁVEZ-MONTES (A.C.M.): Writing – review, editing and correspondence.