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journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2015.06.011>Oral administration of Sauce llorón extract to growing lambs to control gastrointestinal nematodes and *Moniezia* spp.J. Cedillo<sup>1</sup>, A.E. Kholif<sup>2</sup>, A.Z.M. Salem<sup>3\*</sup>, M.M.Y. Elghandour<sup>3</sup>, J.F. Vázquez<sup>3</sup>, M.U. Alonso<sup>3</sup>, A. Barbabosa<sup>3</sup>, J.C.V. Chagoyán<sup>3</sup>, A.G. Reyna<sup>4</sup><sup>1</sup>University Center of UAEM-Temascaltepec, Autonomous University of the Mexico State, Mexico<sup>2</sup>Dairy Science Department, National Research Centre, Giza, Egypt<sup>3</sup>Faculty of Veterinary Medicine and Animal Science, Autonomous University of the Mexico State, Mexico<sup>4</sup>Autonomous University of the Tamaulipas, Faculty of Engineering and Science, Tamaulipas, Mexico

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## ABSTRACT

**Objective:** To explore anthelmintic effects of oral administration of aqueous extract of Sauce llorón (*Salix babylonica*; SB) against gastrointestinal nematodes and *Moniezia* spp.**Methods:** Sixteen Pelibuey male lambs of 3–4 months of age and (23.7 ± 3.3) kg body weight were used in a completely randomized design to be fed a total mixed ration (Control; SB0), or Control plus SB extract using 20 (SB20), 40 (SB40) and 60 (SB60) mL/lamb/day for 45 days. Lambs had a natural gastrointestinal nematodes and *Moniezia* spp. infection and had never been treated with chemical anthelmintic drugs. Individual faecal samples were collected for ova counting using McMaster procedure after 0, 7, 14, 21, 30 and 45 days post extract administration.**Results:** No extract dose × day interactions for both gastrointestinal nematodes and *Moniezia* spp. egg count were found. Administration of SB extract had a higher effect (quadratic effect,  $P = 0.0064$ ) at dose of 20 mL SB/lamb/day for gastrointestinal nematode eggs during the first 21 days; however, the dose of SB40 tended (linear effect,  $P = 0.0897$ ) to be more effective than the others for *Moniezia* spp. egg during the first 7 days. Sampling day had a linear ( $P = 0.0436$ ) effect on *Moniezia* spp. egg count.**Conclusions:** The aqueous extract of SB could be more effective against nematodes at 20 and at 40 mL/lamb/day for *Moniezia* spp. The use of the SB extract could represent a promising alternative to synthetic anthelmintics for the treatment of gastrointestinal nematodes and *Moniezia* spp. in small ruminants from organic and conventional production systems.

## 1. Introduction

Nematode parasitic diseases have been classified as a major health and welfare problems in small ruminants. It is a major cause of sheep and goat mortality in tropical Mexico[1] and other tropical countries[2,3] where climatic factors favor the development of parasitic infection[4]. This action leads to

serious economic loss for small holder farmers[5], delay in achieving target animal weights, the increase in feed requirements, reduced quality of carcass, and predisposition to other diseases[6]. It would be expected that reductions in the level of parasitism would be followed by improvement on the performance of parasitized hosts[7]. The husbandry system in which livestock are raised could affect the exposure to nematode parasites. In situations where Mexican farmers are almost entirely dependent on grazing, exposure to nematode larvae is continuous throughout the year[8].

A lot of money are annually used to combat helminth parasites in livestock[2]. To date, the repeated use of chemical anthelmintic drugs is a usual method for gastrointestinal parasitism control. However, chemical anthelmintic drugs have

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several disadvantages, including lack of availability in some areas, inconsistent quality in some countries, prohibitive cost, as well as environmental contamination[9]. Furthermore, regular and misuse of chemical anthelmintics have resulted in nematode resistance[10], along with the risk of contamination of animal products; a problem which is most serious in sheep and goats in the tropics and developing countries. Routine use of chemical anthelmintics has also reduced the development of natural immunity against helminthes[11]. This has compiled to search for alternatives on helminth control methods[3,12,13].

One of the alternative methods is the use of ethnoveterinary medicine (*i.e.*, phytotherapy) using traditional herbs with anthelmintics activity[13–15]. It is a new, safe, convenient and environmentally friendly product with reduced potential for the development of nematode resistance[6]. However, ethnoveterinary knowledge and plant-based anthelmintics were the mainstays of anthelmintic treatment, and are still widely used in many traditional societies[16]. The potential benefits of ethnoveterinary livestock anthelmintics are clear, as the latter societies often depend on livestock, and live in areas where synthetic anthelmintics are unavailable, unaffordable, and/or of poor quality. However, the demonstration of ovicidal, larvicidal and adulticidal activities of traditional medicinal plants extracts and determination of therapeutic doses remain in the preliminary stages.

Mejía-Hernández *et al.*[15] tested the anthelmintic effects of *Salix babylonica* L. (*S. babylonica* L.) (*i.e.*, SB) and *Leucaena leucocephala* Lam. water extracts at level of 30 mL/lamb/day for a 63 d trial in growing lambs and concluded that both extracts could be promising alternatives to conventional chemical anthelmintics for the control of gastrointestinal parasites in small ruminants. Therefore, this work aimed to evaluate the efficacy of Sauce llorón (*S. babylonica* L.) aqueous extract against gastrointestinal nematodes and *Moniezia* spp. in growing Pelibuey lambs of tropical regions in Mexico.

## 2. Materials and methods

### 2.1. Lamb's management, treatments and feeding

Sixteen Pelibuey male lambs with 3–4 months of age and (23.7 ± 3.3) kg live body weight, after weaning, were used in a completely randomized design to study the anthelmintic effects of oral administration of aqueous extract of *S. babylonica* (*i.e.*, SB) against gastrointestinal nematode and *Moniezia* spp. Selected lambs had a natural gastrointestinal nematodes and *Moniezia* spp. infection and had never been treated with any chemical anthelmintic drugs or traditional herbs with anthelmintic activity. Lambs were individually housed in pens of 1.24 m × 0.82 m. After 2 weeks of adaptation for consuming a total mixed ration (TMR) composed of [g/kg dry matter (DM) basis] alfalfa hay 150, sorghum grain 530, soybean meal 220, molasses 20, fish meal 35, salt 20 mineral and vitamin premix, 25 (containing per kg of mineral premix: 19.60 g/kg calcium, 22.10 g/kg sulfur, 4 mg/kg cobalt, 15.93 mg/kg iodine, 15.49 mg/kg selenium; 860.73 mg/kg copper, 224.07 mg/kg zinc). The TMR had the following nutritional composition (g/kg DM): organic matter 912.4, crude protein 173.6, neutral detergent fiber 131.0, acid detergent fiber 80.3 and hemicelluloses 51.0. The SB extract content for secondary metabolites was determined according to Cowan[17].

The TMR used was the same that was previously fed to lambs of the experiment done at the same farm by Salem *et al.*[18]. Lambs were fed the same TMR with the addition of 0, 20, 40 and 60 mL SB extract/lamb/day for Control (SB0), SB20, SB40 and SB60, respectively. Extract was orally administered daily at 7:00 h before morning feeding to each lamb for 45 days of the experimental period. Lambs were fed at 7:00, 13:00 and 17:00 h with a TMR that was formulated to meet all of their nutrient requirements[19]. Feed and water intake was recorded daily during the experimental period.

### 2.2. Parasitological test

The egg count was performed using the same methods described before in Mejía-Hernández *et al.*[15]. Briefly, faecal samples from each lamb, within each experimental group, were collected rectally before morning feeding. Ova were counted using McMaster procedure[20]. The egg count was performed after 0 (pre-extract administration), 7, 14, 21, 30 and 45 days post extract administration. Faecal samples were evaluated for the presence of worm eggs by a salt flotation technique[21], where the eggs were counted by the McMaster method. Faecal pellets were collected and weighed, and 60 mL of saturated salt solution added per gram of faeces. The pellets were broken up using a mechanical stirrer, and then strained in a sieve with an aperture of 250 µm. Ten milliliters of the strained solution was used for determination of faecal egg counts using a 2 chamber McMaster slide with a limit of detection of 200 eggs/g faeces. Identification of nematodes and *Moniezia* spp. eggs in the faeces were achieved according to the standard methods of MAFF[21]. Faeces contents of individual animal's samples were brought up to 500 mL saturated salt solution. Five aliquots of faecal content (one gram of fresh faeces) from each lamb were used to identify the worm egg species of nematodes and *Moniezia* spp. in the sub-sample by counting using a stereoscope (40×). Faecal cultures were prepared for each experimental period as 5 replicates of pooled samples from each lamb as described by Terrill *et al.*[22] to allow counting and identification of parasite nematode larvae to species. Mean egg counts of nematodes and *Moniezia* spp. from each lamb, within each experimental treatment, were used for statistical comparisons among experimental groups.

### 2.3. Proximate analysis of TMR and secondary metabolites assay

Samples of diet were analyzed for DM, ash, crude protein according to the Association of Official Analytical Chemists[23]. Neutral detergent fiber and acid detergent fiber contents were analyzed using the ANKOM F-57 filter bags in an Ankom<sup>200</sup> Fiber Analyzer unit (Ankom Technology, Macedon, NY USA) according to Van Soest *et al.*[24]. The neutral detergent fiber was assayed using α-amylase (Sigma A-3403 Sigma-Aldrich<sup>®</sup> Co., Louis MO, USA) but with sodium sulfite in the neutral detergent fiber and expressed without residual ash. Hemicellulose content was calculated from the difference between neutral detergent fiber and acid detergent fiber.

*S. babylonica* extract was weekly prepared (5 L) as previously described in Salem *et al.*[25]. Briefly, fresh leaves were

randomly collected during summer season from several young and mature trees of SB, chopped into 2–3 cm lengths and immediately extracted at 1 g leaf/8 mL of water. Plant materials were soaked and incubated in water in the laboratory temperature of 25–30 °C for 72 h in closed jars of 5 L. After incubation, jars were heated at 39 °C for 1 h, and then immediately filtered, and the filtrates were collected and stored at 4 °C for further use.

Secondary metabolites of SB extract were determined in triplicate according to the method described in Salem *et al.*[26]. Briefly, 10 mL of extract was fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolic by drying and quantifying the phenolic layer in the funnel. After phenolic separation, 20 mL of n-butanol was added to fractionate the saponins. The remaining solution in the funnel was considered to be the aqueous fraction that has the other secondary compounds, such as lectins, polypeptides and starch[17].

#### 2.4. Statistical analysis

The experimental design was a completely randomized design with repeated measures through time, where lambs were the experimental units. Data were analyzed using the MIXED procedure of Statistical Analysis System[27] for repeated measures[28]. The structure of the variance-covariance error matrix employed was unstructured, based on Bayesian criteria observed with several alternative structures. Terms in the model were extract dose (*i.e.*, SB0, SB20, SB40 and SB60), day of sampling (*i.e.*, 0, 7, 14, 21, 30 and 45 days) and diet × day, with lamb (lambs 1 to 4 within each treatment) included as random effects. The result repeated term was day of sampling, with lamb within diet. Results reported in tables and in text are least square means of fixed effects with their corresponding standard errors. Tests of simple effects were used to partition (slice) interaction effects by diet in order to test effects of period separately for each diet[27]. The statistical model used for the analysis was:

$$y_{ijk} = \mu + E_i + a(E)_{j(i)} + p_k + (Ep)_{ik} + \varepsilon_{ijk}$$

where:  $y_{ijk}$  is the value measured at day of sampling  $k$  on the  $j$ th lamb assigned to the  $i$ th dose (extract),  $\mu$  is the overall mean effect,  $E_i$  is the  $i$ th fixed dose (extract) effect,  $a(E)_{j(i)}$  is the random effect of the  $j$ th lamb within the  $i$ th extract dose,  $p_k$  is the fixed  $k$ th day of sampling (time) effect when the measurement was taken,  $(Ep)_{ik}$  is the fixed interaction effect between extract dose (E) and day of sampling (p),  $\varepsilon_{ijk}$  is the random error associated with the  $j$ th lamb assigned to the  $i$ th extract dose at sampling day  $k$ .

Turkey's test was used for multiple comparisons among mean values for each run and linear and quadratic effects were calculated at  $P < 0.05$ .

### 3. Results

#### 3.1. Secondary metabolites

Analyzing the secondary metabolites of SB extract showed its content (g/kg) from secondary metabolites as: 12.80 of total phenolic, 4.80 of saponins, and 72 of aqueous fraction of lectins, polypeptides and starch.

#### 3.2. Gastrointestinal nematodes and *Moniezia* spp. egg count

No extract dose × day interactions were observed ( $P > 0.05$ ) for both gastrointestinal nematodes and *Moniezia* spp. egg count. The response varied between both the gastrointestinal nematode and *Moniezia* spp. egg counts among different extract doses. Oral administration of SB extract was more effective (quadratic effect,  $P = 0.0064$ ) at 20 mL/lamb/day dose for gastrointestinal nematode eggs during the first 21 days, where the dose of 40 mL/lamb/day tended to be more effective (linear effect,  $P = 0.0897$ ) than the other doses for *Moniezia* spp. egg count during the first 7 days (Table 1).

Independent of day effect, the dose of 20 mL/lamb/day followed by the dose of 60 mL/lamb/day was the most effective ( $P = 0.0360$ ) than the other ones to reduce the number of gastrointestinal nematodes. However, both the moderate and highest doses of SB extract (*i.e.*, 40 and 60 mL/lamb/day) were more effective ( $P = 0.0430$ ) on *Moniezia* spp. egg count (Figure 1).

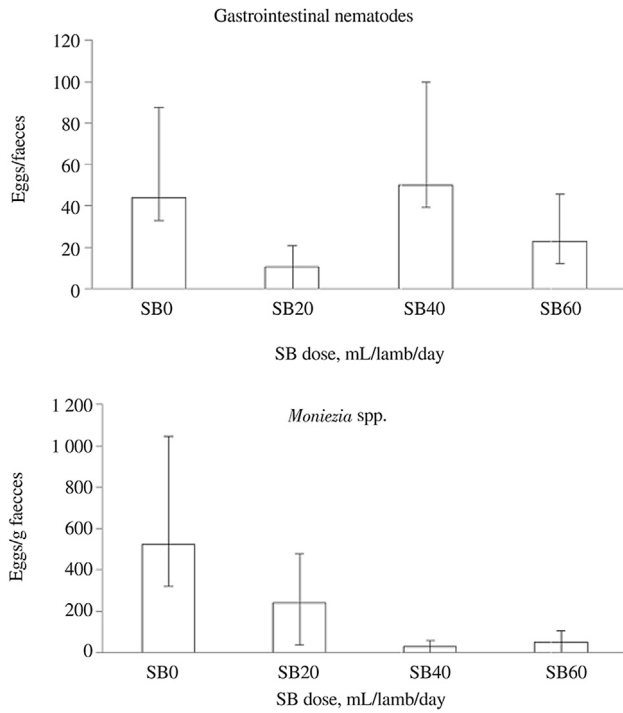
In general, sampling day had no effect ( $P > 0.05$ ) on gastrointestinal nematode egg count (Figure 2). However, a linear significant ( $P = 0.0436$ ) effect on *Moniezia* spp. egg

**Table 1**

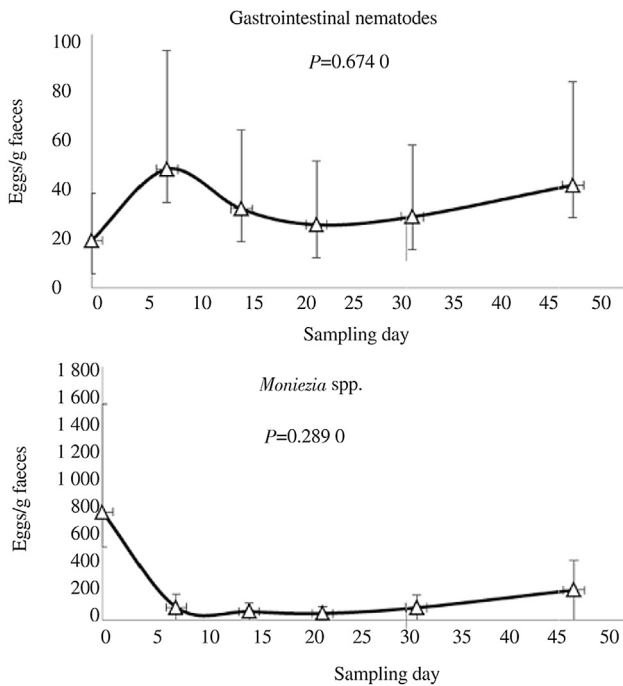
Effect of different doses of *S. babylonica* L. (SB) on the mean egg count of gastrointestinal nematodes and *Moniezia* spp. per gram of feces in growing lambs after 0 (pre-treatment), 7, 14, 21, 30 and 43 days of the experiment (n = 4 lambs).

Extract doses (mL/lamb/day)	Sampling day	Gastrointestinal nematodes <sup>a</sup>	<i>Moniezia</i> spp.
SB0	0	0.0	43.8
	7	75.0	62.5
	14	62.5	37.5
	21	75.0	50.0
	30	37.5	87.5
SB20	45	12.5	462.5
	0	0.0	587.5
	7	0.0	262.5
	14	0.0	137.5
	21	0.0	87.5
SB40	30	12.5	162.5
	45	50.0	200.0
	0	25.0	0.0
	7	87.5	0.0
	14	50.0	12.5
SB60	21	12.5	50.0
	30	25.0	25.0
	45	100.0	87.5
	0	50.0	50.0
	7	25.0	37.5
SEM	14	12.5	50.0
	21	12.5	0.0
	30	37.5	75.0
	45	0.0	100.0
			10.61
Extract dose (E)			
Linear		0.6781	0.0897
		0.0064	0.8838
Quadratic			
Day (D)			
Linear		0.7347	0.0436
		0.5575	0.2562
Quadratic			
E × D		0.2138	0.6231

<sup>a</sup> Including: *Haemonchus contortus*, *Cooperia* spp., *Chabertia* spp., *Trichostrongylus* spp., *Trichostrongylus* spp., *Ostertagia* spp. and *Nematodirus* spp.



**Figure 1.** Bar diagram of anthelmintic activity of *S. babylonica* L. (SB) extract dose-dependent (0, 20, 40 and 60 mL/lamb/day) on the egg count of gastrointestinal nematodes and *Moniezia* spp. per gram of faeces in growing lambs.  $n = 4$ .



**Figure 2.** Bar diagram of anthelmintic activity of *S. babylonica* L. (SB) extract at 0 (pre-treatment), 7, 14, 21, 30 and 45 days post extract administration on the egg count of gastrointestinal nematodes and *Moniezia* spp. per gram of faeces in growing lambs.  $n = 4$ .

count was noted (Table 1). Independent of the dose effect, the effects of sampling days were insignificant ( $P > 0.05$ ) on both gastrointestinal nematodes and *Moniezia* spp. egg counts means (Figure 2).

#### 4. Discussion

This work was carried out in order to find a phytotherapeutic for the control of gastrointestinal nematodes and *Moniezia* spp. in lambs. Prevention of parasitism should be a major goal for producers who lack the access to a constant use of parasiticides or need to keep their herds under a certified organic program[29]. If the consumption of some types of plants or their extracts can be combined with high performance and anthelmintic effects, they should be considered as an alternative to synthetic anthelmintics and ideal candidates in ruminant production systems[6].

The results of the current study indicated that the aqueous extract of SB reduced the faecal egg counts of both gastrointestinal nematode and *Moniezia* spp. This activity was more visible (100% elimination) at the dose rate of 20 mL/lamb/day after 21 days of treatment and 40 mL/lamb/day after 7 days of treatment for gastrointestinal nematode and *Moniezia* spp., respectively. The activity was dose and time dependent in some cases. The nematicidal activity observed could be due to the secondary metabolite present in plants[3,13,15]. Salem *et al.*[30] identified about 59 chemical constituents from SB alcohol extract from which some of them may have anthelmintic effects in lambs[31]. SB Extract contains saponins, alkaloids, tannins, other polyphenols[32], non-protein amino acids, lignins and glycosides; which are all secondary metabolites with anti-parasitic effects[33]. Many hypotheses were put forward to explain the anti-parasitic action of these compounds. Plant secondary metabolites in SB aqueous extract can bind to a specific building block, beta tubulin, and prevent its incorporation into micro-tubules. These processes are essential for worm energy metabolism resulting in a paralysis of worm tissues making them unable to feed, thereby leading to death[34]. Moreover, phenolics could cause larvae starvation and death through binding to the cuticle of larvae, which is rich in glycoprotein, and therefore reduce nutrient availability[35]. Tannins have also an ability to interact with proteins of the cuticle, oral cavity, esophagus, cloaca and vulva of nematodes; changing their chemical and physical properties or faecal egg proteins of larvae[36]. Another indirect mechanism is that condensed tannins can bind to dietary proteins and protect them from rumen degradation. This process can cause an increased protein and amino acid flow and absorption by the small intestine that may result in improved host immune response against worms [36]. Méndez-Ortíz *et al.*[37] reported a 58.8% reduction in *Haemonchus contortus* eggs when *Havardia albicans*, a tannin rich fodder, was fed to sheep. It is also likely that alkaloids in the plant extract could also have contributed to the paralysis and consequent worm death. Alkaloid salts are competitive antagonists at muscarinic acetylcholine receptor sites. The direct result is a prevented binding of acetylcholine. Moreover, this process can lead to excitation of cells and neurological dysfunction[38]. All previous processes could reduce larval growth and development, or inhibit egg hatching, consequently resulting in larval death[36]. Mejía-Hernández *et al.*[15] stated that oral administration of *Leucaena leucocephala* and *S. babylonica* extracts reduced egg and worm counts in lamb feces by 54% and 47%, respectively versus control lambs which were fed no extracts.

However, the effects of secondary metabolites, and in particular the condensed tannin consumption, on the performance of parasitized herbivores are not always straightforward.

Some of the active compounds may also have anti-nutritional effects, such as reducing feed intake and performance. So, it is very important to validate the anti-parasitic effects of plant products, in relation to their potential anti-nutritional and other side effects. More efforts for the isolation, development, and validation of effects of these herbal remedies will have to be undertaken before their wider acceptance<sup>[6]</sup>.

Results indicated that the aqueous extract of SB could be a promising alternative to conventional anthelmintics. The most effective doses against nematodes of naturally infected lambs, under the current experiment conditions, were, 20 mL SB/lamb/day and 40 mL SB/lamb/day for *Moniezia* spp. Further experiments incorporating polyvalence assay for other uses, purification, reproducibility, dosage, application regime, toxicity and identification of active compounds and toxicological investigation are necessary. In addition, the development of a commercial product, with economic evaluation based on SB aqueous extract should be performed in order to define if it could be used in large scale sheep production systems of tropical regions.

### Conflict of interest statement

We declare that we have no conflict of interest.

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