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Activity of the *Hura crepitans* (Linn.) seeds as gastrointestinal antiparasitic in fattening lambs

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The seed latex of *Hura crepitans* (Linn.) has been used for managing various diseases in both humans and animals, including as an intestinal antiparasitic against nematodes and coccidia in sheep. This research aimed to evaluate the antiparasitic efficacy of *H. crepitans* seeds, determine the effective dose for this effect, and assess any potential toxic effects on fattening lambs and their impact on blood parameters. Six ewe lambs were administered different doses of *H. crepitans* seeds (ranging from 0.16 to 6 g/kg b. w.). The antiparasitic efficacy was assessed by measuring the presence of gastrointestinal parasites, oocysts per gram of feces (opg), and eggs per gram of feces (epg). No significant changes were observed in feed intake or body weight. Vital signs, blood parameters, and most biochemical variables evaluated (AP, GGT, and TP) remained stable, with no increases in AST, ALT, or LDH even at the highest doses (4 and 6 g/kg b. W.). The absence of significant adverse effects or alterations in the measured blood variables suggests that *Hura crepitans* seeds can be administered to lambs as a gastrointestinal antiparasitic at doses up to 6 g/kg b. w. without causing side effects.

Keywords: Blood, *Hura crepitans,* Natural antiparasitic, Phytotoxicity, Sheep, Vital signs

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In veterinary medicine, the search for alternatives to control certain parasites should be considered a priority due to the increasing resistance to commercial treatments. The use of bioactive plants is one such alternative[1], but to generate credibility in the use of plants in conventional medicine, empirical arguments must be transformed into evidence-based arguments[1].

The ingestion of toxic plants, after pesticides, is the second most common cause of poisoning. In animals, in most cases these intoxications are accidental and frequently occur in unfavorable situations, such as when food is scarce and especially pasture, and particularly after periods of drought[2]. In this sense, we can point out several members of the Euphorbiaceae family, among which is *Hura crepitans*, which grows in tropical climates, surviving in desert, hot and dry conditions. *H. crepitans* (Linnaeus, 1753), also called sandbox tree or possum wood, is an evergreen tree, native to the tropical regions of America and widely distributed throughout the world. In the dry season, before the rains, this tree releases the seeds that, despite representing an important food source, can also be toxic[3].

For some researchers, the poisoning of livestock by plants that contain certain phytochemicals, as occurs with *H. crepitans*, is unusual since the plants are not palatable, besides the toxic effects of the plants may depend on multiple conditions, such as the amount consumed, individual susceptibility, growth phase and part of the plant ingested[1,2,4]. In this regard, it can be mentioned that according to farmers, some ruminants (and especially cows) are capable of consuming the leaves of this tree without showing signs of intoxication. On the other hand, it has been described that macaws habitually consume this seed without suffering alterations in their health, although we must mention that these birds consume a certain type of clay, possibly to counteract the toxic effects of the seed.

Phytochemical evaluation has shown that *H. crepitans* contains multiple substances (tannins, saponins, flavonoids, coumarins, glycosides and

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triterpenoids). The presence of these phytochemicals confirms the multiple applications of *H. crepitans* in traditional medicine[5], being used as purgative, emetic, antimicrobial, anti-inflammatory and hepatoprotective agents. It has also been used in the treatment of mucous diarrhea in dogs and humans[5-7]. The seed latex of *H. crepitans* has been used for the management of skin diseases in humans as an intestinal antiparasitic, and has even been used with success in sheep, as an intestinal antiparasitic against *Haemonchus contortus* and *Strongyloides venezuelensis*[8].

The toxic effects of these plants arise from their interference with various organs in animals, including the cardiovascular, nervous, gastrointestinal, liver, skin, hematopoietic, respiratory, reproductive, and urinary systems[9]. Blood tests can be useful in identifying damaged tissues, as phytotoxins can induce specific types of damage[9]. For example, liver damage caused by pyrrolizidine alkaloids results in increased serum activity of specific enzymes, which differs from those elevated due to muscle damage induced by white snakeroot[10]. Other injuries, such as kidney disease, lead to elevated concentrations of specific metabolites, such as urea and creatinine[11]. Thus, changes in certain blood parameters are helpful in identifying damaged tissues and supporting a specific diagnosis. Therefore, the aim of this research is to confirm the antiparasitic effect of *H. crepitans* seed, to identify the dose that provides this action without inducing toxic effects in fattening lambs, and to assess its impact on some blood parameters to support its use in further investigations.

Materials and Methods

Animal care and use

This research adhered to the approved standards for the use and welfare of animals, specifically the Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio (Technical specifications for the care and use of laboratory animals, livestock farms, farms, production, reproduction and breeding centers)[12], which outlines technical specifications for the production, care, and use of laboratory animals. These guidelines were published in the Diario Oficial de la Federación (2001). The experimental protocol was approved by the Ethics Committee of Autonomous University of Nayarit, Mexico (OBA-UAMVZ-03-2022).

Study location

The research was carried out at the Sheep and Goat Production Unit of the Academic Unit of Veterinary Medicine and Animal Husbandry (UAMVZ), part of the Autonomous University of Nayarit UAN), located in Compostela, Nayarit, México (21°14'14"N, 104°54'03" W, at 840 meters (a.s.l.).

Handling and feeding

Six clinically healthy female Pelibuey/Katahdin sheep in the fattening phase, with an average body weight (b.w.) of 25.33±1.8 kg, a body condition score of 3.5/5, and 4 months of age were used. The lambs were individually housed in elevated cages (163 X 100 cm) with raised-slatted floors, each equipped with individual water troughs and feeders. They were fed a diet formulated according to National Research Council recommendations[13]. The composition of the diet is provided in Supplementary Table S1. Food and water were available ad libitum, and the feed offered and rejected, was weighed daily. Lambs were weighed daily and acclimatized to the pens and diet for eight days prior to the experiment.

Obtaining and handling of *Hura crepitans* seeds

Whole fruits of *H. crepitans*, identifiable by their brown cover when ripe, were collected in April. The seeds were extracted by breaking the shells with a hammer, then quartered and ground using a Thomas-Wiley mill (model 4, Arthur H. Thomas Company, Philadelphia, PA, USA). Finally, the ground seeds were dried in a forced-air oven at 65°C for 48 h.

Hura crepitans seed dosage

According to the opinion of the local people, one *H. crepitans* seed (approximately 5 g) is sufficient to produce effects in humans. This informed the initial dosage range for the lambs, which varied from 0.16 to 6 g/kg b.w. Doses were randomly assigned to the six lambs following the recommendations of Kramer and Font[14], aiming to minimize the number of animals used in biomedical research (Table 1). The seeds were administered orally once, marking day 0 of the experiment, immediately after the first sampling.

Animal sampling

Vital signs were recorded first, followed by blood and fecal sample collection. Rejected feed from the previous day was then documented, and the lambs were subsequently fed with the base diet at 8:00 a.m. Sampling occurred at 0, 24-, 48-, 72-, 96-, and 120 h post-administration.

Table 1 — Body weight of ewe lambs and total dose of *Hura crepitans* (single dose) administered to each animal

Lamb number	Body weight (kg)	Doses (g/kg b.w.)	Total doses (g, once)	Clinical signs	Length of clinical signs
1	21.5	0.16	3.44	None	-
2	22.8	0.5	11.4	None	-
3	24.0	1.0	24.0	None	-
4	23.0	2.0	46.0	Diarrhea	> 5 h
5	25.0	4.0	100.0	Diarrhea	> 5 h
6	26.0	6.0	156.0	Diarrhea	> 5 h

$\bar{X}$: average value in the 5 days.

Blood samples (3 mL) were collected via jugular venipuncture using Vacutainer® tubes (Becton Dickinson, NJ, USA) containing EDTA. Blood counts were performed, and the remaining sample was centrifuged at 3000 rpm for 10 min (VE-4000 VELABTM). Plasma was stored in Eppendorf tubes at -20°C until analysis.

Fecal samples (approx. 20 g) were collected from the rectum, stored in screw-cap plastic cups, refrigerated, and immediately transported to the UAMVZ-UAN Parasitology Laboratory for analysis. Feces were classified according to Burke *et al*.[15].

Vital signs and measurements

Heart rate (HR), respiratory rate (RR), body temperature (BT), body weight (b.w.), feed intake (FI), and stool consistency (SC) were assessed.

Blood analysis

Hematology

Included red cell counts [erythrocytes, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV)] and white cell counts (leukocytes, lymphocytes, monocytes, eosinophils, basophils, neutrophils, segmented neutrophils, band neutrophils, and platelets) using a Coulter AcT-5diff Hematology Analyzer (Beckman Coulter International S.A., Nyon, Switzerland).

Biochemistry

Parameters were analyzed using a spectrophotometer (Miura 200, ISE S.r.l brand, Levate, Beramo, Italy) and Diasys reagents (DiaSys Diagnostic Systems GmbH, Holzheim, Germany), with the following wavelengths (λ) for measurement: protein profile [total proteins (PT, λ = 540 nm), albumin (ALB, λ = 628 nm), globulins (GLOB), and albumin/globulin ratio (ALB/GLOB)], enzyme profile [lactate dehydrogenase (LDH, λ = 340 nm), aspartate aminotransferase (AST, λ = 340 nm), alanine aminotransferase (ALT, λ = 340 nm), gamma-glutamyl transferase (GGT¸ λ = 405 nm), and alkaline phosphatase (AP, λ = 405 nm)], and other blood variables [urea (λ = 540 nm), creatinine (λ = 510 nm), and glucose (λ = 505 nm)].

Coprological analysis

Gastrointestinal parasites were identified using the Willis flotation technique. The McMaster technique[16] was used to quantify oocysts and eggs per gram of feces (opg and epg, respectively). Each sample was analyzed in triplicate to estimate mean values.

Data analysis

Data were recorded in an Excel file. Due to the limited sample size, the average value for the five days post-seed administration was calculated.

Results

Productive performance and vital signs

The animals that received the highest doses of seeds (2, 4 and 6 g/kg b.w.) stopped consuming food within 5 h after administration. After this time, they continued with their usual diet, with no difference in food consumption (Supplementary Table S2). There were no changes in body weight, body temperature, heart rate or respiratory rate. Although lambs 2, 3 and 4 initially had a decreased heart rate, compared to the rest of the animals, the same stabilized in the following days (Supplementary Table S3).

Blood analysis

Leukocyte and platelet counts, as well as MCV and MCHC, did not show variations, while erythrocytes, hemoglobin, and hematocrit, as well as total and segmented neutrophils, were slightly decreased. Only lymphocytes were slightly increased. The presence of monocytes, eosinophils, basophils, or band neutrophils was not determined (Supplementary Table S4).

The protein profile (TP, albumin, globulins and Alb/Glob ratio) did not change with the administration of *H. crepitans* (Supplementary Table S5). While urea showed a slight increase with all doses tested, creatinine did not show significant changes. Glycemia decreased slightly throughout the experience, although in the two lambs with the highest doses it showed an increase on the first post-treatment day, later showing the same trend in all the ewe lambs (Supplementary Table S5). There was no difference between AF and GGT activity, while AST, ALT and LDH seem to have decreased with the highest doses of seeds (4 and 6 mg/kg b.w., Supplementary Table S6).

Coprological analysis

The greatest elimination of eggs/oocysts of nematodes and coccidia occurred with the highest doses (4 and 6 g/kg b.w.; Fig. 1 and Fig. 3), while the elimination of cestodes was only observed with the highest dose (6 mg/kg p.v., Fig. 2). Likewise, we were able to verify that the greatest elimination of eggs/oocysts occurred on the day after the administration of the seeds (Fig. 1-3).

Discussion

Many plants have been used in ethnoveterinary medicine to control parasites in small ruminants, and many of them with promising results[14,15,17,18].

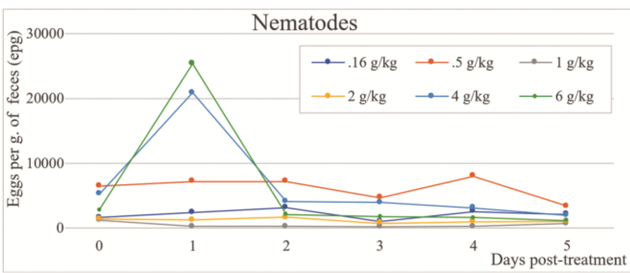

Fig. 1 — Number of nematode eggs eliminated per day, depending on dose of *H. crepitans* seeds

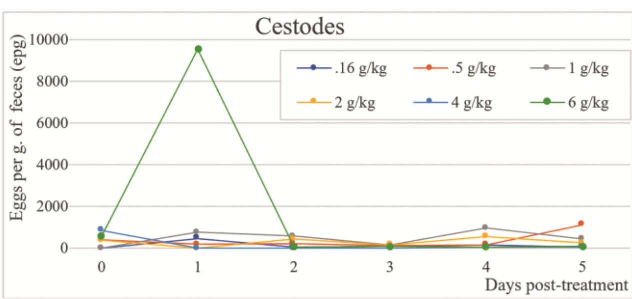

Fig. 2 — Number of cestode eggs eliminated per day, depending on dose of *H. crepitans* seeds

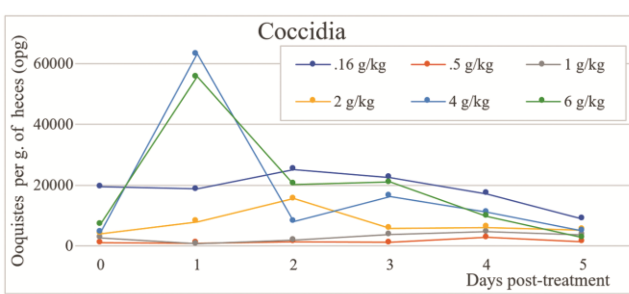

Fig. 3 — Number of coccidia oocysts eliminated per day, depending on dose of *H. crepitans* seeds

The inclusion of traditional methods for parasite control based on bioactive plants and related secondary metabolites aimed at improving livestock health are gaining increasing importance. This is due, on the one hand, to the growing resistance to commercial treatments[1], and on the other hand, because these practices can be particularly useful among low-resource livestock breeders or those in remote areas who cannot afford or access conventional medical options[15,19,20].

Even products like onion (*Allium cepa*) and garlic (*Allium sativum*) have been used as important ingredients in traditional ethno-veterinary medicine among popular communities for a long time[19,20]. In the first case, onions have been used in the treatment of gastrointestinal problems (indigestion and bloat), as insect repellents, antiparasitic, and even as antiseptic[19]. Garlic, on the other hand, has been used in veterinary medicine to cure cough, cold, fever, urinary problems like oliguria and anuria, bloat, foot-and-mouth disease, pleuropneumonia, otalgia, dog bites and snake bites, trembling, respiratory system disorders like pleuritis and pneumonia, yoke gall, bone fractures, flatulence, wounds, and gangrene. Allicin and other sulfur compounds have been cited as providing garlic with antibiotic, antibacterial, and antifungal properties[20].

However, the ingestion of some plants or their derivatives can cause adverse effects in different animal species, including sheep. Therefore, the main objective of the present research was to validate the beliefs of traditional farmers, who claimed that *H. crepitans* seeds could be used as antiparasitic agents, while also verifying that they did not trigger adverse effects in lambs. The latex of *H. crepitans* (sandboxtree or possumwood, but also called ceiba blanca, ceiba lechosa, tronador, achohó, javillo, tronador, habillo, salvadera, molino, arceira, catauá, arbre du diable, posentri, among other names) is considered toxic and highly irritating to skin and mucous membranes. In humans, ingestion of *H. crepitans* seeds produces a burning sensation in the mouth, with vomiting, diarrhea, dyspnea and headache[21].

Plants contain many phytochemicals, which is why in recent years they have been used for various purposes, ranging from mosquito repellents, larvicidal agents, and insect growth regulators[22] to even combating ectoparasites like ticks in buffaloes and bovids[23].

Although we have not carried out the characterization of the phytochemical components of the *H. crepitans* seed in this research, some references have shown the presence of multiple substances depending on the part of the tree sampled. Thus, toxalbumins, hurine and crepitin, are distributed in all the organs of the plant[7], the stem, bark and leaves of *H. crepitans* contain tannins, saponins, flavonoids, coumarins, glycosides and triterpenoids, although dafnane, diterpenes, acid daphnetoxin and huratoxin have also been isolated[24-27], along with apocynin and methylpentadecanoate[6]. Other phytochemicals such as alkaloids, steroids, crepitin, glucosamine or lectinhave also been isolated[5-7,25]. The presence of phospholipids (phosphatidylcholine and phosphatidylethanolamine) has also been noted as being highly abundant in the seed oil[25], and *H. crepitans* seeds also contain phytic acid (phytate) and hydrogen cyanide[27].

Productive performance

The analysis of *H. crepitans* seeds revealed a high content of crude fat (36.7±1.1%), crude fiber (0.50±0.00%), and crude protein (36.62±0.88%) in the seeds of *H. crepitans*, demonstrating that the seed is potentially nutritional[27]. Investigating the nutritional properties of the seed oil showed that it has a high amount of fat (37.5%) and protein (27.31%), and it is rich in β-sitosterol, campesterol, stigmasterol, and vitamin A. It also has a high percentage of bioactive lipids (linoleic and oleic acids, total unsaturated fatty acids of 80.31%), and a high content of phospholipids, suggesting that this underutilized plant could have numerous applications in animal feed[25]. Furthermore, twenty amino acids were found in the seed flour, with significant amounts of arginine, glutamic acid, and valine, while methionine was the limiting amino acid[28]. In line with this, some studies have been conducted to explore the use of *H. crepitans* seeds as animal feed, aiming to understand their nutritional characteristics. Escalera *et al*.[29] found good degradability of the seed in the first three hours of incubation and determined the presence of multiple essential amino acids, with glutamic acid, arginine, and aspartic acid being the most abundant.

In our case, we observed that with the highest doses of seed (2, 4 and 6 g/kg b.w.), the lambs stopped consuming food within 5 h post-administration, although they quickly recovered their usual diet. In our opinion, this is due to the fact that the lambs were not affected in their feeding, and as a consequence, they did not show important changes in body weight, even with any of the doses tested.

On the other hand, it is considered that appetite may have decreased due to secondary compounds (phenols, terpenes, oxalates) that reduce palatability and can even cause harmful effects to those who consume them. This may be an adaptive strategy to avoid being consumed, as many of these compounds have an unpleasant odor and taste for animals[30]. In this study, we observed that at low doses, the seed was not perceptible to the animals' taste, which opens the door to future research aimed at evaluating the palatability of the seed and exploring possible mixing alternatives to be offered to animals at concentrations higher than 2 g/kg b.w.

Ahumada *et al*.[31] found that in quinoa grains, the saponins contained in the outer tissues are responsible for the bitter taste. In our study, whole seeds were used, including both the seed and the husk, and it is highly likely that the husk contains a large amount of saponins, imparting a bitter taste, which may have reduced the lambs' appetite for a maximum period of 5 h.

The reviewed literature also describes anti-nutrients in *H. crepitans* seeds, such as phytic acid (phytate), flavonoids, alkaloids, hydrogen cyanide, oxalate, and tannins[27,28]. Among these, tannins are present in high concentrations, while phytate was found in the lowest concentration of all the analyzed anti-nutrients[27]. These compounds may be responsible for the initial hyporexia and adverse effects at high doses[27]. It is important to note that, according to Ofoedum *et al*.[28], anti-nutrients can be reduced or removed through certain processing techniques, such as fermentation and wet cooking (or wet thermal treatment)[27].

Clinical signs

The vital signs measured, both prior to the ingestion of the seeds and throughout the investigation, are within the ranges considered physiological[32], so we can affirm that the animals were in good state of health. Checking and sampling an animal produces stress in it and as a consequence causes an alteration of the cardiac and respiratory rate, although the physiological state (estrus, pregnancy, parturition, etc.) in which the animal is found also influences[32].

Nor have we verified changes in the consistency of feces. Although many of the poisonous plants induce diarrhea, and in humans the ingestion of this seed also produces it, which did not last long, with normalization of appetite and fecal consistency[33].

In vitro studies on the antileishmanial activity of *H. crepitans* latex concluded that the toxicity of hydroalcoholic extracts and phytocomponents (tannins, flavonoids, coumarins, saponins, and alkaloids) is considerable[34]. However, the negative reputation of the *H. crepitans* tree should be reassessed, and further studies are needed to determine its true therapeutic value[34]. Recent research has demonstrated its beneficial effects on liver diseases[7] and in the treatment of colorectal cancer[24]. It has been found that, in rats with liver injury, it exhibits antifibrotic, anti-inflammatory, and antioxidant effects[5]. Additionally, some plant compounds (huratoxin and epoxihuratoxin) have been shown to have significant and selective inhibitory effects against certain colorectal cancer cell lines[24].

Blood parameters

The hematological analysis showed that most of the values were within the reference interval, contrary to what was indicated by Chong Dubé *et al.*[35] in goats intoxicated by Mulato grass (*Brachiaria ruziziensis, Brachiaria brizantha*), where they found an animal with anemia and several with slight hemoconcentration. The total leukocyte count also showed values within the reference range in most of the animals, and only in some cases did they present slight leukocytosis and generalized neutrophilia, differing from what was described in goats intoxicated with grass Mulatto[35].

Likewise, the biochemical variables measured in the ewe lambs treated with *H. crepitans* seeds presented values within the interval described for this species[11,36,37], and totally differ from a clinical case described where 73% of the sheep died intoxicated by *Astragalus pehuenches* plants. In this case, serum protein and albumin values increased significantly, while blood glucose and urobilinogen remained normal[38].

Neither uremia nor plasma creatinine changed significantly in this research, ruling out any type of injury and especially kidney disease. It is important to note that serum creatinine levels in lambs raised under tropical conditions are lower[37] than reported by Kaneko *et al.*[11], and that creatinine values may be slightly altered due to the age of the animals. According to Gregory *et al.*[39] young animals, up to 12 months of age, have lower serum values than adults, and tend to increase as animals age[37]. Although serum creatinine levels are little affected by nutrition[37], both feeding and handling can influence blood urea levels in ruminants, as higher protein intake causes an increase in uremia and mainly due to ammonia production in the rumen[37].

Glycaemia tends to decrease in all lambs throughout the experience, although with the two highest doses it showed an increase on the first post-treatment day, perhaps as a consequence of the stress and discomfort produced by the administered seeds. In the following days, glycaemia returned to the initial values and its evolution was similar in all the ewe lambs.

The non-increase in the activity of the enzymes ALT, AST, GGT, AP, show that there have been no significant organic injuries[11,37], ruling out liver or muscle injuries. It is important to keep in mind that the reference values found in the literature come from lambs of different breeds, raised in different environmental and nutritional conditions, which reduces the precision of the comparison[37].

There is limited data on the influence of *H. crepitans* seed ingestion on biochemical parameters. We have found only one reference[26] in which different amounts of *H. crepitans* seed oil (5%, 10%, and 15%) were administered to male rats to investigate functional and proinflammatory responses over eight weeks. Functional responses and the expression of proinflammatory cytokines and their receptors were evaluated. Biomarkers from different organs were statistically similar to those of the control rats (fed peanut oil), except for uric acid and creatinine levels, which were significantly lower in the groups fed *H. crepitans* oil, while urea levels were elevated in all groups fed this oil. Additionally, in the experimental groups, there was a significant reduction in the expression of proinflammatory cytokines (TNF-α, IL-1α, IL-1β, and IL-6) and their receptors (IL-1R and IL-6R) compared to the control group. Therefore, Ugbaja *et al.*[26] highlight the renoprotective, cardioprotective, and anti-inflammatory potential of the oil present in these seeds.

Performance parasite infection

Gastrointestinal parasites affect both humans and animals. In both, but especially in veterinary medicine, the search for alternatives to control gastrointestinal parasitosis is a priority, due to the increase in resistance to commercial antiparasitics[1].

In our trial, the greatest elimination of eggs and oocysts was observed with the highest doses of seeds, as well as the day following seed administration. It has been shown that the seeds of *H. crepitans* are capable of causing a significant elimination of the oocyst load, possibly due to the chemical components of the seeds, including latex, since it has been shown that lambs treated with *Calotropis procera* latex expelled large numbers of coccidia oocysts, possibly due to the latex's purging effect[33]. Perhaps the significant reduction in oocysts can also be attributed to condensed tannins, since the *H. crepitans* tree contains these compounds, although in low concentrations[40]. The effect of condensed tannins in the control of coccidia has also been demonstrated in several investigations[15].

Condensed tannins also seem to be responsible for the anthelmintic activity, since several studies have shown the effect in sheep fed forage containing condensed tannins, or in the bark of *Ziziphus nummularia* and in the fruit of *Acacia nilotica*[41]. While in the first case they are more potent inhibitors of egg hatching and larval development than of larval motility, in the second they were more potent in inhibiting larval development compared to hatching from eggs. The anthelmintic effects of tannins can be attributed to their ability to alter the nutrition of the larvae, causing their death, as well as by affecting vital processes such as feeding, reproduction of the parasite or by altering the integrity of the cuticle of the parasites[42-44].

As previously indicated, the inclusion of traditional methods for controlling ecto- and endoparasites based on plants to improve livestock health is becoming an important intervention to enhance livestock productivity in resource-limited areas[23]. Thus, due to its economic importance, there is a need to seek alternatives to the indiscriminate use of chemical compounds, among which the application of biocidal extracts from the *H. crepitans* plant should be noted.

Conclusion

The no finding of alterations in the clinical signs and in the laboratory variables due to the experimental ingestion of *Hura crepitans* seeds allows us to conclude that this seed can be administered in lambs as a gastrointestinal antiparasitic, up to doses of 6 mg/kg b.w., without causing side effects.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(09)(2024)853-861_SupplData.pdf

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

F. E-V: conceptualization, investigation, writing-original draft writing-review and editing; J.L. L-O: investigation, software, writing-review and editing; R.G-L: data curation, methodology, project administration; C.A. C-G: conceptualization, methodology; S. M-G: supervision, validation, visualization; H.J. L-C: data curation, formal analysis, project administration; J.R. G-M: conceptualization, methodology, writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The authors declare that the handling of lambs used in the current research complied all times with the Mexican and international regulations for the use and welfare of animals (Humanitarian care of animals during the animal mobilization (NOM-051-ZOO-1995); Technical specifications for the care and use of laboratory animals (NOM-062-ZOO-1999); Directive regulating the use of animals for scientific purposes in the European Union (European Union, 2010). The research protocol was approved by the Dirección de la Unidad Académica de Medicina Veterinaria y Zootecnia, with the number OBA-UAMVZ-03-2022.

Data Availability

Data will be made available on request.

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