Journal of Helminthology

cambridge.org/jhl

Research Paper

Cite this article: Dixit AK, Das G, Dixit P, Sharma RL (2019). Efficacy of herbal extracts and closantel against fenbendazole-resistant *Haemonchus contortus*. *Journal of Helminthology* **93**, 529–532. https://doi.org/10.1017/S0022149X18000627

Received: 24 January 2018
Accepted: 30 June 2018
First published online: 24 July 2018

Key words:
anthelmintics; faecal egg count reduction test; fenbendazole resistance; goats; *Haemonchus contortus*; herbal extracts

Author for correspondence:
A.K. Dixit, E-mail: alokdixit7@yahoo.com

Efficacy of herbal extracts and closantel against fenbendazole-resistant *Haemonchus contortus*

A.K. Dixit[1], G. Das[1], P. Dixit[2] and R.L. Sharma[3]

[1]Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur 482001, Madhya Pradesh, India; [2]Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur 482001, Madhya Pradesh, India and [3]843-44, Ranisati Nagar, P.O. Shyam Nagar, Ajmer Road, Jaipur-302019, Rajasthan, India

Abstract

This study assessed the efficacy of closantel vis-à-vis herbal extracts with known anti-parasitic properties, against fenbendazole-resistant nematodes in goats maintained under a semi-intensive system of management at the University goat farm, Jabalpur. Fifty goats were randomly assigned to five groups, each comprising 10 animals, irrespective of their breed, age and sex. Each animal in Group I, II and III was orally administered with aqueous leaf extracts of neem (*Azadirachta indica*) at 1 g/kg body weight, sitaphal (*Annona squamosa*) at 1.5 g/kg body weight and tobacco (*Nicotiana tabacum*) at 1 g/kg body weight, respectively, whereas Group IV was an untreated control group. Each animal in Group V was orally treated with closantel at 10 mg/kg body weight. During the course of the study, all animals were maintained under an identical semi-intensive system of management. Compared to the untreated control group (Group IV), there was no conspicuous reduction in post-treatment (day 10) faecal egg counts (FEC) in animals administered with the herbal extracts (Groups I, II and III), which is suggestive of poor anti-parasitic activity. However, using the faecal egg count reduction test (FECRT), the overall efficacy of closantel was recorded as 95.64%. This supports the rotational use of closantel as a preferred choice over the benzimidazole group of anthelmintics and/or herbal extracts to meet the acute challenge of *in situ* development of drug-resistant gastrointestinal nematodes, especially *Haemonchus contortus*.

Introduction

In developing countries such as India, small ruminants make an important contribution to human livelihood. Thirty-seven percent of the world's sheep population (1.2 billion) and 56% of the world's goat population (1 billion) are bred and reared in Asia (FAO, 2015). Parasitic gastroenteritis accounts for heavy production losses in the small ruminant industry (Dhar *et al.*, 1982). Amongst helminthic infections of small ruminants, infection with *Haemonchus contortus* has been a major threat (Swarnkar *et al.*, 2008; Santos *et al.*, 2012). The ubiquitous prevalence of *H. contortus* makes it a predominant helminth in both tropical and temperate climates. In India alone, the annual cost of anthelmintic treatment against *H. contortus* has been estimated to be USD103 million (McLeod, 2004). For decades, the application of broad spectrum anthelmintics has been the primary strategy for the control of *Haemonchus* infection. However, resistance to these anthelmintics continues to be documented in *Haemonchus* populations around the world, including in India (Singh *et al.*, 2002). In earlier studies at the University goat farm in Jabalpur, levamisole and fenbendazole resistance was detected in the strongyles (Das *et al.*, 2015). Further, the resistant nematodes were identified as *H. contortus* by coproculture and molecular methods, and the mechanism of fenbendazole resistance was linked to a single nucleotide polymorphism at position 200 of the β-tubulin isotype I gene (Dixit *et al.*, 2017).

Rotational use of anthelmintics with different pharmacokinetics and/or herbal medicine is a possible alternative treatment to counter the heavy losses that are associated with gastroenteritis caused by nematodes resistant to the benzimidazole group of anthelmintics. Herbal products offer the advantage of sustainable supply and are ecologically acceptable. Evaluation of the anti-parasitic effect of plants with respect to *H. contortus* has been previously reported (Ferreira *et al.*, 2013). However, studies on the efficacy of botanicals against known drug-resistant gastrointestinal nematodes are scarce. The present study was therefore planned to determine the efficacy of a conventional dewormer (closantel) and some unexploited herbal extracts possessing anti-parasitic activity in goats infected with fenbendazole-resistant *H. contortus*.

Materials and methods

The herbal leaves

The plant leaves were identified and authenticated by the botanist in the Department of Botany, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India.

Annona squamosa (sitaphal)

This is commonly known as sugar apple, custard apple or sitaphal, and is cultivated as an edible fruit throughout India.

Azadirachta indica (neem)

The tree is synonymously known as *Melia azadirachta* L and is commonly referred to as the Neem tree. It is a hardy tree growing to 15–20 m in height and is usually found throughout the tropics and subtropics.

Nicotiana tabacum (tobacco)

Nicotiana tabacum leaves are well-known to contain nicotine as a main alkaloid, which is probably responsible for the anti-nematicidal activity of the plant extract.

The animals

Fifty goats with faecal egg counts of >600 eggs, from whom anthelmintic medication was withheld for 8–12 weeks prior to the trial, were randomly selected and assigned to five groups of 10 animals for the experiment, irrespective of age, sex and breed. Each animal was weighed, and anthelmintics were administered on the basis of individual body weight.

Aqueous herbal extract

Leaves of *A. indica*, *A. squamosa* and *N. tabacum* were harvested directly and/or purchased from the local market. The leaves were dried under shade, powdered using a commercial stainless steel electrical blender and weighed. Powdered leaves of *A. indica* (1025 g), *A. squamosa* (950 g) and *N. tabacum* (550 g) were soaked in 6.8 l, 6.75 l and 3 l, respectively, of distilled water at room temperature (30°C). The suspension was shaken vigorously every 24 hours for 10 days. The aqueous suspension was filtered using muslin cloth. The filtrate was concentrated using a rotary vacuum evaporator until it acquired a pasty consistency, and was poured into Petri dishes. The paste was further dried at 45°C using a hot air oven, and was then stored at 4°C until use. The percentage yield of extract was calculated as follows:

$$\%\,\text{yield of extract} = \left[\text{Weight of extract (g)}/\text{Weight of dried leaves (g)}\right] \times 100$$

The yield of aqueous leaf extract from *A. indica*, *A. squamosa* and *N. tabacum* was 21.96%, 34.21% and 68.19%, respectively. The extracts were administered to the animals by adding 10–15 g jaggery for easy and complete administration.

Medication schedule

Group I animals were given aqueous leaf extract of *A. indica* at 1 g/kg body weight (Chandrawathani *et al.*, 2013a), Group II animals received a single oral dose of aqueous leaf extract of *A. squamosa* at 1.5 g/kg body weight (Githiori *et al.*, 2004) and Group III animals were given aqueous leaf extract of *N. tabacum* at 1 g/kg body weight (Hamad *et al.*, 2013). Group IV animals were treated as a control group. Group V animals were given closantel at 10 mg/kg body weight orally.

Faecal egg counts

Faecal samples were collected from each goat on day zero (pre-treatment) and again on days 10 and 14 post treatment. Samples were collected rectally and placed in plastic bags bearing the animal's identification number, and parasitological analysis was undertaken in the laboratory. One gramme of faeces from each goat was mixed in 14 ml of saturated sodium chloride solution and the eggs were counted in one chamber of a McMaster's egg counting slide. The total egg count was multiplied by 100 (Zajac and Conboy, 2012).

Faecal egg count reduction test (FECRT)

The percentage reduction in faecal egg count was calculated as per Coles *et al.* (1992) using the following formula:

$$R = 100(1 - Xt/Xc)$$

where R is the percentage reduction in faecal egg count, Xt is the mean FEC of the treatment group and Xc is the mean FEC of the control group.

Faecal culture

Thirty grammes of faeces from the infected animals were mixed in a Petri dish, which was placed in an incubator at 27°C for 10 days to obtain third-stage larvae. One hundred larvae were obtained from faecal cultures and identified to genus level based on morphological characteristics such as the shape of the anterior portion and tail, as well as caudal and sheath length (Van Wyk *et al.*, 2004).

Results

The mean FEC on day 10 in Groups I, II and III was 2020, 3800 and 2380, respectively, and 2570 in the untreated control group (Group IV). Comparison of FEC revealed no reductions on day 10 post infection in animals treated with herbal extracts. The mean FEC of the control group (Group IV) and closantel-treated individuals (Group V) on day 14 post infection was 2750 and 120, respectively. FEC reduction and lower 95% confidence interval for closantel were 95.64 and 87, respectively (Table 1). *Haemonchus contortus* (81%), *Strongyloides* spp. (8%), *Oesophagostomum* spp. (6%), *Trichostrongylus* spp. (4%), and other larvae (1%) were identified in the pre-treatment faecal cultures. Post-treatment coproculture in the closantel-treated group revealed *Strongyloides* spp. (54%), *Oesophagostomum* spp. (38%), *Trichostrongylus* spp. (6%) and *H. contortus* (2%). Evidently, the *H. contortus* strain of goats in Central India was found to be susceptible to closantel.

Discussion

Resistance in *H. contortus* in Central India to fenbendazole was documented recently using allele-specific polymerase chain reaction (PCR). The frequency of resistant allele (r) was quite high

Table 1. Mean faecal egg count (FEC) and FEC reduction (FECR, %) in goats treated with three botanical aqueous extracts and closantel.

Parameter	*Azadirachta indica* aqueous leaf extract, Group I	*Annona squamosa* aqueous leaf extract, Group II	*Nicotiana tabacum* aqueous leaf extract, Group III	Control, Group IV	Closantel, Group V
No. of goats	10	10	10	10	10
Mean FEC on day 0	1700 ± 323	1910 ± 562	2190 ± 493	1690 ± 292	1440 ± 161
Mean FEC on day 10 post treatment	2020 ± 312	3800 ±672	2380 ± 181	2570 ± 472	–
Mean FEC on day 14 post treatment	–	–	–	2750 ± 575	120 ± 59
FECR, %	21.40	−51	7.39	–	95.64
95% upper confidence interval	–	–	–	–	99
95% lower confidence interval	–	–	–	–	87
Resistance status	Resistant	Resistant	Resistant	–	Susceptible

(74%) incidental to widespread occurrence of resistance to benzimidazoles (Dixit *et al.*, 2017). Consequently, closantel is currently used widely to control the parasite in small ruminants. Clearly, closantel has been a drug of choice, being an anthelmintic in the salicylanilide drug class. Salicylanilides become highly bound to plasma proteins, and therefore they specifically target parasites that ingest blood, such as *H. contortus*. Salicylanilides uncouple oxidative phosphorylation, decrease the availability of adenosine triphosphate and nicotinamide adenine dinucleotide in the mitochondria and thus decrease the energy available to the parasites. Closantel also disrupts mechanisms that maintain pH homeostasis in the parasite (Lanusse *et al.*, 2009). However, periodically, reports have suggested developing resistance to closantel as well (Chandrawathani *et al.*, 2013b; Premaalatha *et al.*, 2014). According to Coles *et al.* (1992), if FEC reduction is <95% and the lower confidence limit <90%, only then is it considered to be resistant. If only one of these two criteria is fulfilled, then resistance is suspected. In our study the FEC reduction was above 95% and the lower confidence limit was below 90%. Therefore, as per the guidelines of the World Association for the Advancement of Veterinary Parasitology, it should be considered to be a case of suspected resistance. Closantel is an anthelmintic with persistent effect only against haematophagous parasites such as *H. contortus*. After treatment with closantel, *Haemonchus* larvae were reduced from 81% to 2%. Therefore, when the percentage FEC reduction was calculated only for *H. contortus* using RESO software, it was found to be susceptible to closantel, which is similar to observations reported by Westers *et al.* (2016).

In the present study, following administration of a single dose of crude aqueous extract of *N. tabacum*, a 7% reduction in FEC was found on day 10 post treatment, whereas Hamad *et al.* (2013) reported 87.5% and 88.6% reductions in sheep on day 14 post treatment using crude aqueous methanolic extract of *N. tabacum* at 2 and 4 g/kg body weight, respectively, against benzimidazole-resistant *H. contortus*. In our study, the comparatively smaller reduction in FEC may be due to the difference in solvents used (water vs 70% methanol), host species (goat vs sheep), dosage (1 g vs 2 g) and assessment days post treatment (10 vs 14). *Nicotiana tabacum* leaves contain nicotine, which may cause spastic paralysis of worms via action on nicotinic receptors. Levamisole is also known to stimulate nicotinic receptors for exhibiting anthelmintic activity. In our study, the nematodes were both levamisole- and fenbendazole-resistant, whereas Hamad *et al.* (2013) studied benzimidazole-resistant *H. contortus* only. This may also explain the non-effectiveness of *N. tabacum* extract as the gastrointestinal nematodes were also levamisole-resistant.

Iqbal *et al.* (2006) studied the effect of *N. tabacum* aqueous and methanolic extract on *H. contortus* in sheep and found that the aqueous extract was comparatively less effective. In addition, the efficacy of aqueous extract was dose dependent and increased efficacy was evident when 3 g/kg body weight was used instead of 1 or 2 g/kg body weight. Interestingly, the anthelmintic effect was rapid. It was observed at 5 days post treatment but was not evident at 10 days post treatment after a single dose of *N. tabacum* extract.

The efficacy of *A. indica* administered to small ruminants as fresh leaves (Chandrawathani *et al.*, 2006; Chagas and Vieira, 2007) and crude leaf powder (Akhtar and Riffat, 1984; Dongre *et al.*, 2015) was variable. In an experiment, a significant reduction in the number of worms was recorded among sheep that received 3 g/kg of fresh neem leaves for 6 weeks, on necropsy, compared to the control group, although this was not reflected in a reduction in the FEC (Chandrawathani *et al.*, 2006). Chagas and Vieira (2007) reported another experiment that revealed no anthelmintic effect of neem at a dosage of 30 g of dried leaves per goat/day given for 5 days. In the present study, compared to the control group, only a 21% reduction in FEC was observed at day 10 post treatment in the group treated with *A. indica*. Similar to our findings, Worku *et al.* (2009) reported that aqueous leaf extract containing water-soluble proteins from neem was not an effective anthelmintic in goats. Nawaz *et al.* (2014) reported 89% efficacy of aqueous extract of *A. indica* leaves against *H. contortus* in sheep 12 days post treatment. This is the only study whose findings are dissimilar to our own.

Investigations carried out with different species of the genus *Annona* have shown that aqueous leaf extracts of *Annona senegalensis* (Ndjonka *et al.*, 2011) and *A. muricata* (Ferreira *et al.*, 2013), and methanol and ethyl acetate leaf extracts of *A. squamosa* (Kamaraj and Rahuman, 2011) demonstrate *in vitro* anthelmintic activity against different nematodes, including *H. contortus*. The anthelmintic activities of *Annona* extracts have been attributed to the annonaceous acetogenins, a class of natural compounds extracted from leaves and seeds. Ferreira *et al.* (2013) indicated the presence of phenolic compounds in the aqueous leaf extract of *A. muricata*. In our study, aqueous leaf extract of *A. squamosa* has shown no effect on fenbendazole-resistant *H. contortus* when used *in vivo* in goats. However, compounds or substances that are effective *in vitro* do not necessarily work equally well *in vivo*. In addition, acetogenins may not be extracted effectively from a plant using water as a solvent. Vieira *et al.* (1999) administered *A. squamosa* at 1 g/kg for four consecutive days and found a 51.90% reduction in the adult *Oesophagostomum columbianum* population, although it was ineffective in eliminating *H. contortus*, *Trichostrongylus colubriformis* and *Strongyloides papillosus* in goats. Githiori *et al.* (2004) evaluated *in vivo* anthelmintic efficacy of fresh leaves of seven plants, including *A. squamosa* and *A. indica*, and found no significant difference in FEC 2–3 weeks post treatment in lambs. These findings are consistent with the observations of the anti-parasitic efficacy of herbal extracts reported here.

In conclusion, the rotational use of closantel is the preferred choice over the benzimidazole group of anthelmintics and/or herbal extracts to combat the problem of *in situ* development of drug-resistant gastrointestinal nematodes, especially *H. contortus*, and to ensure increased productivity in small ruminants in Central India.

Acknowledgements. The authors are grateful to the Dean of the College of Veterinary Science and Animal Husbandry NDVSU, Jabalpur, for providing the facilities to enable this study to be undertaken.

Financial support. The authors thank ICAR, New Delhi, for financial assistance under the All India Network Programme on Gastrointestinal Parasitism.

Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals.

References

Akhtar MS and Riffat S (1984) Efficacy of *Melia azedarach* linn. (Bakain) and morantel against naturally acquired gastro-intestinal nematodes in goats. *Pakistan Veterinary Journal* **4**, 176–179.

Chagas ACS and Vieira LS (2007) Açao de *Azadirachta indica* (Neem) em nematódeos gastrintestinais de caprinos. *Brazilian Journal of Veterinary Research and Animal Science* **44**, 49–55.

Chandrawathani P *et al.* (2006) Daily feeding of fresh neem leaves (*Azadirachta indica*) for worm control in sheep. *Journal of Tropical Biomedicine* **23**, 23–30.

Chandrawathani P *et al.* (2013a) Testing neem products on goats in Infoternak, Perak: a preliminary trial for neem capsules, neem juice, neem extract and neem decoction for worm control. *Malaysian Journal of Veterinary Research* **4**, 33–39.

Chandrawathani P *et al.* (2013b) Severe anthelmintic resistance in two free grazing small holder goat farms in Malaysia. *Journal of Veterinary Science & Technology* **4**, 137.

Coles GC *et al.* (1992) World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* **44**, 35–44.

Das G *et al.* (2015) Levamisole and fenbendazole resistance in gastrointestinal nematodes in goats at Jabalpur, Madhya Pradesh. *Journal of Veterinary Parasitology* **29**, 98–102.

Dhar DN, Sharma RL and Bansal GC (1982) Gastrointestinal nematodes in sheep in Kashmir. *Veterinary Parasitology* **11**, 271–277.

Dixit AK *et al.* (2017) An assessment of benzimidazole resistance against caprine nematodes in Central India. *Tropical Animal Health & Production* **49**, 1471–1478.

Dongre S *et al.* (2015) Anthelmintic efficacy of *Azadirachta indica* (neem) against strongyles in goats. *Indian Journal of Veterinary Science & Biotechnology* **10**, 18–21.

FAO (2015) *The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture*, Scherf BD and Pilling D (eds). FAO Commission on Genetic Resources for Food and Agriculture Assessments, Rome (available at http://www.fao.org/3/a-i4787e/index.html).

Ferreira LE *et al.* (2013) *In vitro* anthelmintic activity of aqueous leaf extract of *Annona muricata* l. (annonaceae) against *Haemonchus contortus* from sheep. *Experimental Parasitology* **134**, 327–332.

Githiori JB *et al.* (2004) Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. *Parasitology* **129**, 245–253.

Hamad KK *et al.* (2013) Antinematicidal activity of *Nicotiana tabacum* L. leaf extracts to control benzimidazole-resistant *Haemonchus contortus* in sheep. *Pakistan Veterinary Journal* **33**, 85–90.

Iqbal Z *et al.* (2006) *In vitro* and *in vivo* anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. *Phytotherapy Research* **20**, 46–48.

Kamaraj C and Rahuman AA (2011) Efficacy of anthelmintic properties of medicinal plant extracts against *Haemonchus contortus*. *Research in Veterinary Science* **91**, 400–404.

Lanusse CE, Guillermo LV and Alvarez LI (2009) Anticestodal and antitrematodal drugs. In Riviere JE and Papich MG (eds), *Veterinary Pharmacology & Therapeutics*, 9th edn. Ames, IA: Wiley-Blackwell, pp. 1104–1106.

McLeod RS (2004) Economic impact of worm infections in small ruminants in South East Asia, India and Australia. In Sani RA, Gray GD and Baker RL (eds), *Worm Control of Small Ruminants in Tropical Asia*. ACIAR Monograph 113. Canberra: Australian Centre for International Agricultural Research, pp. 23–33.

Nawaz M *et al.* (2014) *In vitro* and *in vivo* anthelmintic activity of leaves of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* against *Haemonchus contortus*. *Global Veterinaria* **13**, 996–1001.

Ndjonka D *et al.* (2011) *In vitro* activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. *Journal of Helminthology* **85**, 304–312.

Premaalatha B *et al.* (2014) Anthelmintic resistance in small ruminant farms: an ongoing challenge for perak farmers to control helminths. *Malaysian Journal of Veterinary Research* **5**, 31–38.

Santos MC, Silva BF and Amarante AFT (2012) Environmental factors influencing the transmission of *Haemonchus contortus*. *Veterinary Parasitology* **188**, 277–284.

Singh D, Swarnkar CP and Khan FA (2002) Anthelmintic resistance in gastrointestinal nematodes of livestock in India. *Journal of Veterinary Parasitology* **16**, 115–130.

Swarnkar CP *et al.* (2008) *Epidemiology and Management of Gastrointestinal Parasites of Sheep in Rajasthan*, 1st edn. Avikanagar: Central Sheep and Wool Research Institute, pp. 38–57.

Van Wyk JA, Cabaret J and Michael LM (2004) Morphological identification of nematode larvae of small ruminants and cattle simplified. *Veterinary Parasitology* **119**, 277–306.

Vieira LS *et al.* (1999) Evaluation of anthelmintic efficacy of plants available in Ceará State, north-east Brazil, for the control of goat gastrointestinal nematodes. *Revista de Medicina Veterinaria* **150**, 447–452.

Westers T *et al.* (2016) Efficacy of closantel against ivermectin- and fenbendazole-resistant *Haemonchus* sp. in sheep in Ontario, Canada. *Veterinary Parasitology* **228**, 30–41.

Worku M, Franco R and Miller JH (2009) Evaluation of the activity of plant extracts in Boer goats. *American Journal of Animal & Veterinary Sciences* **4**, 72–79.

Zajac AM and Conboy GA (2012) *Veterinary Clinical Parasitology*, 8th edn. Chichester: UK, pp. 3–170.