

Research Paper

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Comparison of *in vivo* anthelmintic efficacy of *Artemisia absinthium* extract and albendazole in Awassi breed sheep with natural gastrointestinal nematode infection

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Abstract

This study was conducted to determine the *in vivo* anthelmintic efficacy and clinical safety of crude ethanolic extract (CEE) obtained from *Artemisia absinthium* in naturally infected Awassi breed sheep with gastrointestinal nematodes (GIN). A total of 18 non-pregnant sheep were divided into 3 groups of 6 animals each: CEE (1.0 g/kg), albendazole (5 mg/kg), and negative control (untreated). Changes in egg counts (EPG) were quantitatively monitored in faecal samples collected before treatment (day 0) and after treatment (days 7 and 15). The research findings showed that *A. absinthium* CEE significantly reduced the parasite load over time, with the highest efficacy achieved on day 15 ($p < 0.001$), demonstrating statistically significant efficacy in suppressing the fecundity of GIN agents. According to the faecal egg count reduction test (FECRT), therapeutic success was observed in 76.77% of the CEE group and 100% of the albendazole group. The performance demonstrated by CEE confirmed the potential of plant secondary metabolites to reduce dependence on synthetic drugs, and the absence of any adverse effects during the trial period demonstrated the plant's high biosafety profile. Consequently, *A. absinthium* offers a powerful, economical, and residue-free biotherapeutic alternative to synthetic anthelmintics in sustainable and organic livestock models, countering global anthelmintic resistance. To the best of our knowledge, this study represents the first investigation in Türkiye evaluating the anthelmintic efficacy of *A. absinthium* CEE in Awassi breed sheep naturally infected with GIN, providing the first regional efficacy data from Şanlıurfa.

Introduction

Small ruminant farming plays a strategic role in rural economies by providing protein supply and income sources in pastoral regions where limiting factors such as drought and resource scarcity prevail (Pulina et al 2017). However, gastrointestinal nematode (GIN) infections are an endemic health problem in pasture-based sheep and goat farming worldwide (Mpofu et al 2022). These parasitic infections suppress the immune system in host animals, leading to serious health problems and irreparable production losses (Paul et al 2020).

Although the most common *Trichostrongylidae* members infecting sheep and goats are reported to be *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Teladorsagia circumcincta* (Julienne et al 2021), *H. contortus* and *T. circumcincta* are prominent in the clinical picture in terms of pathogenicity (Mpofu et al 2020). GIN infections are characterised by severe diarrhoea, anaemia, liveweight loss, growth retardation, and high mortality rates in small ruminants (Paul et al 2020).

The population distribution of parasites varies across geographic regions depending on variations in local ecological conditions (Paul et al 2020; Sharma and Ganguly 2016). Particularly in the tropical climate zone, grazing activities under semi-intensive management systems (Chandrawathani et al 1999) and high humidity levels that enhance the environmental survival of parasites are key factors increasing infection prevalence (Chandrawathani et al 1999; Paul et al 2020).

Control of GIN infections has traditionally relied on the periodic use of broad-spectrum anthelmintic drugs (Coles et al 1992, 2006). Modern parasite control strategies involve the regular administration of major drug classes such as benzimidazoles (e.g., albendazole), imidazothiazoles (e.g., levamisole), macrocyclic lactones (e.g., ivermectin), and pyrimidines (e.g., pyrantel) to minimise the density of infective larvae in pastures (Coles et al 2006; Kotze et al 2020; Mickiewicz et al 2021; Sonibare et al 2016). Although these chemotherapeutic agents have historically demonstrated high efficacy in improving productivity (Sonibare et al 2016), the global spread

of multiple anthelmintic resistance has become a serious concern today (Charlier et al 2014). Although anthelmintic resistance development has become apparent in genera such as *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Teladorsagia* spp., and *Trichostrongylus* spp. (Baudinette et al 2022), the species with the highest prevalence of resistance is *H. contortus*. This situation places the aforementioned parasite in the position of being the most economically destructive gastrointestinal nematode in small ruminant husbandry (Fleming et al 2006).

The mechanisms underlying anthelmintic resistance involve multifaceted processes such as the restriction of drug uptake in parasites, changes in metabolic pathways, and genetic modifications that reduce target-site sensitivity (Shalaby 2013). This resistance observed in GIN populations, and the associated risk of chemical residues in animal products, toxicity concerns, and increased treatment costs, severely limit the effectiveness of chemotherapeutics (Gasbarre et al 2001; Jabbar et al 2007). In particular, the phenomenon of 'multiple resistance' developed against most broad-spectrum drug classes has necessitated the development of sustainable, effective, and highly biosafe alternatives for parasite control (Kaplan 2004; Varady et al 2011). In this context, ethnoveterinary practices and plant secondary metabolites represent a rich source for modern pharmacology. Numerous studies in the literature have investigated the potential nematocidal activities of various plant extracts against *Trichostrongylidae* parasites (Githiori et al 2006; Hussain et al 2011; Vatta et al 2011). In traditional societies, leaf or seed extracts from plants such as garlic, mint, walnut, and wormwood have been used safely for many years in the treatment of gastrointestinal parasitism (Guarrera 1999).

Artemisia absinthium, commonly known worldwide as 'wormwood' or 'absinthe', is an aromatic plant that is also widespread in the flora of Turkey (Quinlan et al 2002). Preferred in traditional medicine systems for its antispasmodic, antiseptic, tonic, and anthelmintic properties (Baytop 1984; Kaul 1997), this plant has also been reported to possess antimicrobial and antifungal activities (Juteau et al 2003; Tariq et al 2009). The phytochemical profile of *A. absinthium* is rich in thujone (α and β) found in the volatile oil structure, tannins, and sesquiterpene lactones. Previous studies have reported that these bioactive compounds exhibit potent anthelmintic effects on nematodes (Seaman 1982; Tariq et al 2009).

This study was designed to evaluate the *in vivo* anthelmintic efficacy of *A. absinthium* extracts against gastrointestinal nematodes in sheep, compared to albendazole, a reference drug.

Materials and methods

Animal material and care conditions

In this study, 18 non-pregnant Awassi breed sheep aged 2–3 years were used and housed at the Dicle University Faculty of Veterinary Medicine Education, Research, and Application Farm. The animals were kept under observation for 14 days prior to the experiment to allow them to adapt to the environmental conditions. During the study period, the animals' water, hay, and concentrate feed requirements were met without restriction (*ad libitum*) within the limits of the ration calculations.

Parasitological examination and grouping

Prior to the study, the status of the animals regarding GIN infection was confirmed using standard parasitological procedures (Soulsby 1982) and the Fülleborn flotation method. Sheep found to be naturally infected were divided into 3 groups, each

consisting of 6 animals, to be homogeneous in terms of age, sex, and faecal egg count (FEC). The average EPG values on day 7 of the adaptation period were used as the basis for balancing the groups. The McMaster method and faecal culture techniques were applied to diagnose the parasite genera and species and to determine egg density (Ministry of Agriculture, Fisheries and Food (MAFF) 1986).

Procurement and preparation of plant material

The *Artemisia absinthium* plant used in the study was procured from a commercially certified supplier, and the plant species was also verified by the pharmacologist involved in the study. The plant material was dried at room temperature in a well-ventilated environment away from direct sunlight to preserve its phytochemical content. Following drying, the plant parts were pulverised using a mechanical grinder to produce a homogeneous powder. The resulting pure plant powder was stored under appropriate conditions until extraction stages.

Preparation of crude ethanolic extract (CEE)

The crude ethanolic extract (CEE) of *A. absinthium* was prepared by modifying the method described by Tariq et al (2009). Two hundred grams of pulverised plant powder was macerated in 1000 mL of 95% ethanol at room temperature for 16 hours. The resulting mixture was filtered through a non-absorbent cotton wool filter to remove coarse particles, and this process was repeated three times to increase extraction efficiency. The solvent (ethanol) in the collected filtrate was removed under low pressure in a rotary evaporator at 50 °C. The resulting concentrated extract was transferred to airtight sterile glass bottles and stored at +4 °C until *in vivo* applications.

Trial plan and treatment protocols

To prevent cross-contamination between groups during the study, the sheep groups used in the study were housed in physically isolated pens. All groups were fed a standard basal ration, and the treatment and control protocols applied are outlined below:

Group I (CEE): *A. absinthium* CEE was administered orally in a single dose of 1 g/kg (live weight) by mixing it into the morning ration on the first day of the study (Tariq et al 2009).

Group II (albendazole – positive control): Animals in the positive control group received a single oral dose of 5 mg/kg (live weight) of commercial albendazole.

Group III (negative control): Animals were fed only the standard ration without any anthelmintic treatment or herbal supplement.

All groups were clinically and parasitologically monitored for 15 days after treatment to evaluate efficacy.

Laboratory procedure and faecal egg count (FEC)

To determine changes in GIN egg counts, faecal samples were collected in the morning directly from the rectum of each animal in the treatment and control groups on day –7 (before treatment), on the day treatment began (day 0), and on days 7 and 15 post-treatment (PT). The samples were analysed using a modified McMaster technique integrated with the Visser filter system (Van Schalkwyk et al. 1995). The method was applied as follows: 4 g of faecal sample was placed in a three-stage nested sieve system

with pore sizes of 110 µm (top), 70 µm (middle), and 25 µm (bottom), respectively. The faecal material was washed and filtered under medium-pressure water flow, ensuring that the nematode eggs were retained in the bottom (25 µm) sieve. After filtration, the suspension remaining on the bottom sieve was transferred to a calibrated container. To create a flotation solution, sufficient sucrose was added to the suspension to achieve saturation, and the total volume was adjusted to 60 mL with distilled water. Samples taken from the prepared homogeneous mixture were transferred to a three-compartment McMaster slide and examined under a microscope. The detection limit (sensitivity) of this method was calculated as 25 EPG (eggs per gram of faeces).

Calculation of treatment efficacy (FECR)

The anthelmintic efficacies of *A. absinthium* CEE used in the trial and albendazole, selected as the positive control, were calculated based on the percentage reduction in faecal egg count (FECR). The calculation was performed using the Henderson–Tilton formula in accordance with VICH guidelines, which accounts for both pre- and post-treatment egg counts in both treated and control groups, as follows:

$$FECR(\%) = \left(1 - \frac{T_2 \times C_1}{T_1 \times C_2} \right) \times 100$$

where FECR: Faecal egg count reduction;

T1: Mean EPG of faeces in the treated groups (*Artemisia absinthium* CEE or albendazole) prior to treatment (day 0);

T2: Mean EPG of faeces in the treated groups after treatment (day 7 or 15);

C1: Mean EPG of faeces in the untreated negative control group prior to treatment (day 0); and

C2: Mean EPG of faeces in the untreated negative control group after treatment (day 7 or 15).

When interpreting the results, the standard efficacy threshold values determined by Coles et al (1992) were taken into account for the positive control group (albendazole). Conversely, for the *A. absinthium* CEE group, the reduction rate was evaluated as 'anthelmintic potential' data, reflecting the plant extract's capacity to mitigate the parasite load rather than establishing commercial resistance profiles.

Statistical analysis

Statistical analysis of the data was performed using R (v4.3.1) software (R Core Team 2023) and the rstatix (Kassambara 2023b), tidyverse (Wickham et al 2019), and ggpubr (Kassambara 2023a) packages. Descriptive statistics are expressed as mean ±

standard error (SE). A two-way repeated-measures ANOVA was applied to determine differences between groups (untreated, albendazole, and CEE) and time points (−7, 0, 7, and 15 days), as well as group-time interactions. Following the assessment of assumptions, group differences by day were analysed using a Bonferroni-corrected post hoc pairwise *t* test for main effects found to be significant. A *p*-value of <0.05 was considered statistically significant in all analyses. Treatment efficacy was calculated as the percentage reduction in EPG (number of eggs per gram of faeces) values on days 0, 7, and 15 (VICH 2021).

Results

In naturally infected sheep with GIN, the anthelmintic efficacy of the CEE of *A. absinthium* and albendazole, selected as the positive control, was prospectively and comparatively evaluated. When faecal egg counts on days 7 and 15 post-treatment (PT) were analysed for the treatment groups (CEE and albendazole), a statistically significant reduction was observed in all experimental groups compared to the negative control group (*p* < 0.005; Table 1). In the evaluation of anthelmintic efficacy, the reduction rates in faecal egg counts (FECR%) on days 7 and 15 post-treatment were calculated using the Henderson–Tilton formula in accordance with VICH guidelines. According to the findings obtained, 100% anthelmintic efficacy was observed on both days 7 and 15 in the albendazole group. In the CEE group, an increase in anthelmintic efficacy over time was determined; the FECR, which was 66.79% on day 7, reached 76.77% on day 15. In the group treated with albendazole, which was evaluated as a positive control, no GIN eggs were detected in faecal samples from treatment day 7 onwards (complete cure); in the group treated with CEE, examinations using the McMaster method showed that the parasite load was significantly suppressed, but EPG persisted at different levels. The high efficacy of the CEE against GIN infections in sheep (*p* < 0.001) was associated with the higher solubility capacity of the plant's anthelmintic active components in organic solvents. As a result of clinical observations, no local irritation, systemic toxicity, or adverse effects related to the extracts administered to the animals in the experimental groups were detected during the study period and the follow-up period after treatment. All animals included in the experiment remained clinically stable throughout the study protocol; clinically significant improvements were observed in stool consistency, appetite, and overall condition (body condition score) at the end of the treatment process, particularly in the CEE and albendazole groups. These findings support the potential of *A. absinthium* CEE as a reliable and effective herbal alternative for controlling GIN infections.

Table 1. Comparison of average fecal egg counts (EPG) and percentage reduction (FECR %) in sheep treated with *Artemisia absinthium* CEE with positive (albendazole) and negative control groups

Group	Dose (mg/kg bw)	Mean ± S.E. of eggs per gram of faeces pre- and post-treatment				P-value ^a
		Pre-treatment		Post-treatment		
		Day -7	Day 0	Day 7	Day 15	
A. absinthium CEE	1.0 g/kg bw	1187.5 ± 38.13	1129.17 ± 25.54	375 ± 8.01(66.79)	262.5 ± 7.21(76.77)	P<0.001
Albendazole (Positive Control)	5 mg/kg bw	1166.67 ± 40.14	1116.67 ± 33.33	0.0 ± 0.00(100.0)	0.0 ± 0.00(100.0)	P<0.0001
Untreated (Negative Control)	–	1158.33 ± 45.49	1166.67 ± 42.16	1166.68 ± 33.54	1167.53 ± 40.82	P>0.05

Figures in parenthesis indicate mean egg count percent reduction (FECR %); SE, standard error of mean; bw, body weight. a The values represent the rows.

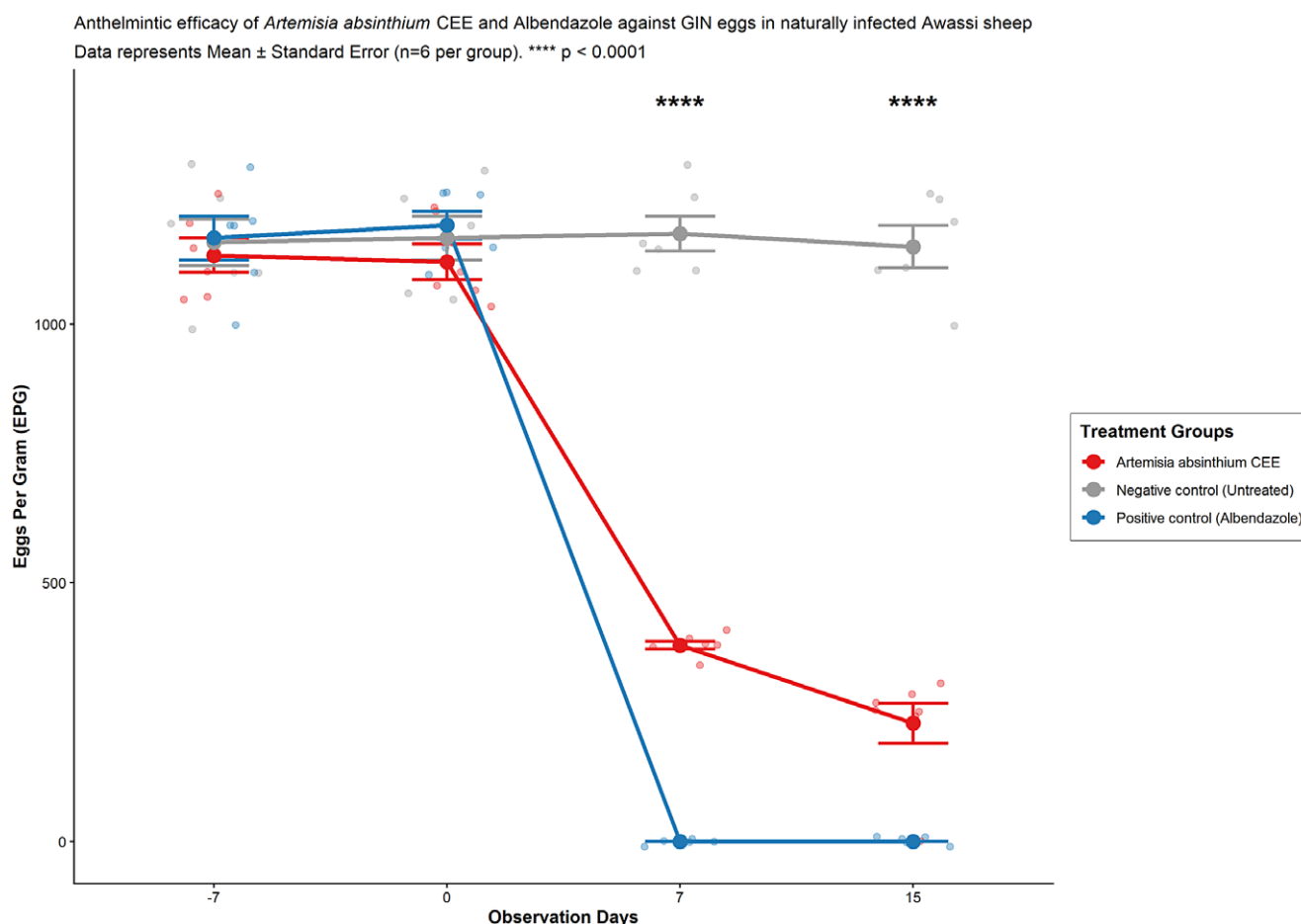


Figure 1. Anthelmintic efficacy of *A. absinthium* CEE and Albendazole against gastrointestinal nematode (GIN) eggs in naturally infected Awassi sheep. Data points represent Mean $\pm$ Standard Error (n=6 per group). **** indicates significant difference from the untreated control group ($p < 0.0001$).

The EPG counts and treatment efficacy obtained during the study are presented in detail in Figure 1. At the start of the experiment (days -7 and 0), a homogeneous distribution was observed among the groups, and no statistically significant difference was detected ($p > 0.05$). On day 7 post-treatment, while the albendazole group completely halted GIN egg shedding (100%), the CEE group reduced the EPG value from 1129.17 ± 25.54 on day 0 to 375.00 ± 8.01 , demonstrating a 66.79% decrease by day 7 (Figure 1). By the final day of the experiment (day 15), the decline in GIN egg count in the CEE group continued, reaching an EPG value of 262.50 ± 7.21 , achieving a total treatment efficacy of 76.77%. Statistical analyses (repeated-measures ANOVA) indicated that both the group and time interactions were highly significant ($p < 0.001$). In the negative control group (untreated), no significant decrease in egg count was recorded throughout the process; on the contrary, a slight increase of 1.42% was observed. No statistically significant difference was detected between the EPG values determined on different days of the study in the untreated group ($p > 0.05$).

Discussion

GIN infections are endemic in sheep and cause serious economic losses in the livestock sector (Doğanay 1993; Güralp 1981). The resistance developed against synthetic anthelmintics commonly used against these parasites (Bremner et al 1983; Kieran 1994) has necessitated the development of alternative control strategies. In their review

examining plants used for GIN control in ruminants, Githiori et al (2006) listed 32 different plant species, 13 of which are directly effective against the *Trichostrongylidae* family; however, they reported that in most cases, the efficacy of these plants was lower than that of their chemical counterparts. In this context, research on tannin-containing plants and phytochemical extracts with prominent anthelmintic properties has gained importance (Ketziş et al 2006).

This study was conducted to evaluate the *in vivo* anthelmintic efficacy and clinical safety of the CEE of *A. absinthium* in naturally infected sheep. The data obtained demonstrated that the CEE of *A. absinthium* produced a significant time- and dose-dependent inhibitory effect on the fecundity of nematodes belonging to the *Trichostrongylidae* family. The reduction rate in the faecal egg count (EPG) detected on day 15 post-treatment was recorded as 76.77% in the CEE group. Compared to the 100% cure rate provided by albendazole, the positive-control group, the performance demonstrated by CEE highlights the potential for plant-based alternatives in parasite control strategies.

In the literature, it has been reported that lambs fed diets containing *A. absinthium* had significantly lower parasite egg loads (Seaman 1982); this effect is thought to result from sesquiterpene lactones in the plant suppressing egg formation. Furthermore, it has been reported that *A. absinthium* extracts inhibit acetylcholinesterase activity, negatively affecting the motility and viability of nematodes (Korayem et al 1993).

The efficacy levels obtained in our study show a high degree of similarity with the findings reported by Tariq et al (2009). The aforementioned study reported that CEE at a dose of 1.0 g/kg resulted in an 82.85% reduction in eggs (FECR), and our result of 76.77% at a dose of 1.0 g/kg close agreement with these data. In both studies, the anthelmintic effect of CEE can be attributed to the higher concentration of active components in ethanol and the faster absorption of these components through the parasite cuticle (transcuticular absorption) (Tariq et al 2009). Furthermore, the fact that 90.46% efficacy was achieved at a dose of 2.0 g/kg in the same study demonstrates the wide therapeutic window of *A. absinthium* and its potential for increased efficacy with increasing doses.

Comparisons with other *Artemisia* species more clearly demonstrate the superiority of *A. absinthium*. For example, in a study by Iqbal et al (2004), the crude aqueous extract (CAE) form of *Artemisia brevifolia* was only able to achieve a 67.2% reduction in GIN infections in sheep at a high dose of 3 g/kg using the FECR test. In our study, the CEE form of *A. absinthium* achieved 76.77% anthelmintic efficacy even at a dose of 1 g/kg. Although none of the doses tested in the *A. brevifolia* study were comparable to synthetic drugs (levamisole, 99.2%), the fact that the CEE group in our study approached a statistically similar level of efficacy to albendazole suggests that the *A. absinthium* plant has a higher affinity for GIN species in terms of phytochemistry.

However, in Peru, Flores-Prado et al (2025) reported that, despite the *in vitro* anthelmintic success of *A. absinthium*, it did not show a significant effect under *in vivo* conditions (at a dose of 600 mg/kg) in a study conducted on Creole goats. This failure is thought to stem from dosages below the 1000 mg/kg used in our study or from the resistance profile developed by parasite strains in the relevant region. Furthermore, the ecological conditions of the region where the plant was collected and differences in extraction methods may also have played a role in these contrasting results.

In the present study, the 100% efficacy observed in the albendazole group serves as a critical historical indicator that GIN populations in the region had not yet developed significant resistance to benzimidazole-class drugs during the study period. Given the increasing global trend of anthelmintic resistance, these findings provide an indispensable baseline dataset for monitoring the development of resistance in Awassi sheep. In a region like Şanlıurfa, which is the heart of small ruminant breeding, the fact that this is the first *in vivo* study in this field (particularly on the Awassi breed) indicates that the data are 'foundational' rather than 'historical'. The data obtained in this study provide a critical baseline dataset for understanding long-term trends in parasite load and drug resistance in Awassi sheep in the Şanlıurfa region. The lack of fundamental change in livestock practices in the region since the study was conducted and the continued dependence on synthetic anthelmintics ensure that the 100% albendazole efficacy (positive control) and the potential of plant extracts identified at that time remain a reference point for current discussions on resistance development.

Conclusion

Based on the data obtained in this study, it was determined that CEE of *Artemisia absinthium* exhibits high anthelmintic activity against GIN infections in sheep under *in vivo* conditions at a dose of 1.0 g/kg. In particular, the 76.77% reduction in egg production in faeces (EPG) achieved by CEE at a dose of 1.0 g/kg proves that the

plant could be a strategic biotherapeutic alternative to synthetic anthelmintics. The findings of this study suggest that *A. absinthium* extracts can be used not only as a therapeutic intervention but also as a protective strategy. The inclusion of *A. absinthium* in integrated parasite management systems not only reduces reliance on synthetic anthelmintics but also offers the dual benefit of continuously suppressing the environmental larval population. These fundamental findings remain highly relevant for contemporary and sustainable livestock farming models, particularly organic farming, where residue-free interventions are prioritised. Particularly in small ruminant husbandry, adding low amounts of *A. absinthium* to rations (concentrated feeds) during periods of intense parasite pressure is predicted to be a sustainable supportive method for controlling GIN infections and maintaining herd health. Such an application could both reduce the frequency of synthetic drug use and minimise pasture infestation by continuously suppressing parasite egg shedding. However, to validate the true potential of *A. absinthium* in parasite control programmes, further research is needed to determine the variable dose-response kinetics against different parasite stages (larvicidal effect) and specific nematode species. In this context, the standardisation of the plant's active components is critical for the development of residue-free and sustainable new preparations in veterinary pharmacology.

In conclusion, our findings confirm that *A. absinthium* CEE is a reliable and highly effective biotherapeutic option for controlling GIN infections in sheep. Standing out as a promising alternative in integrated parasite management, this plant-based approach offers a dual benefit: it significantly reduces the chemical burden on animal products while providing a cost-effective solution for farmers. Most importantly, it represents a sustainable frontier in the fight against anthelmintic resistance, ensuring long-term health and productivity in livestock systems. To the best of our knowledge, this study represents the first investigation in Türkiye evaluating the anthelmintic efficacy of *A. absinthium* CEE in Awassi breed sheep naturally infected with GIN, providing the first regional efficacy data from Şanlıurfa.

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Competing 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.

Ethical approval. This study was conducted with permission and approval granted by Harran University's Local Animal Experiments Ethics Committee under decision number 11/01. All experimental procedures were conducted in accordance with animal welfare laws and international ethical standards regarding the use of animals in experiments.

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