

A COMPARATIVE STUDY ON THE EFFICACY OF INDIGENOUS REMEDIES AGAINST AMPHISTOME INFECTION IN BUFFALOES

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Abstract

Background: Paramphistomosis, a debilitating trematode infection in ruminants, causes significant economic losses in the livestock industry worldwide. The limitations of conventional control methods, including anthelmintic resistance and environmental concerns associated with snail control, necessitate the exploration of alternative treatments. Ethnoveterinary medicine offers a repository of potential plant-based anthelmintics, though their efficacy requires scientific validation.

Objective: This study aimed to quantitatively evaluate and compare the therapeutic efficacy of four indigenous plant-based drugs—*Nigella sativa* seeds, *Fumaria parviflora* aerial parts, *Caesalpinia crista* seeds, and *Saussurea lappa* roots—against the standard allopathic drug, oxclozanide, for treating natural paramphistomosis in buffaloes.

Methods: A total of 150 buffaloes (140 naturally infected and 10 healthy) were allocated into fifteen groups in a controlled experimental design. Infected animals were treated with one of the four plant materials at three dose levels (30, 40, and 50 mg/kg body weight) or with oxclozanide (10 mg/kg). A positive control group remained infected but untreated, and a negative control group comprised healthy animals. The plant materials were administered orally as a suspension after being processed into a fine powder. Anthelmintic efficacy was primarily quantified by the reduction in eggs per gram (EPG) of feces pre- and post-treatment, with fecal exams conducted on days 0, 3, 7, 18, 21, and 28. A second dose was administered on day 18 to animals with persistent infection.

Results: All indigenous drugs exhibited a clear, dose-dependent anthelmintic efficacy, which was significantly enhanced ($p < 0.001$) following the second administration. A single dose of *Nigella sativa* (50 mg/kg) reduced EPG by 71.79%, rising to 94.87% after two doses. Similarly, *Fumaria parviflora*, *Caesalpinia crista*, and *Saussurea lappa* at 50 mg/kg showed peak efficacies of 92.85%, 92.3%, and 85.36%, respectively, after the two-dose regimen. Oxclozanide, the reference drug, demonstrated 97.43% efficacy. The overall order of efficacy was determined to be: Oxclozanide > *Nigella sativa* > *Fumaria parviflora* > *Caesalpinia crista* > *Saussurea lappa*. Crucially, no adverse side effects were observed in any animal treated with the plant-based drugs.

Conclusion: The findings conclusively demonstrate that the tested indigenous plants, particularly *Nigella sativa*, possess high and significant efficacy against

paramphistomosis in buffaloes. Their safety profile and potent anthelmintic action position them as promising, sustainable, and effective natural alternatives to conventional synthetic drugs, offering a valuable tool for integrated parasite control strategies in livestock.

INTRODUCTION

Paramphistomosis, an infectious parasitic disease caused by digenetic trematodes of the family Paramphistomatidae, poses a significant threat to the health and productivity of domesticated ruminants worldwide. The pathogenesis is particularly severe in the acute phase of the infection, where immature flukes migrate to and attach to the duodenal mucosa, causing extensive tissue damage. This leads to the characteristic clinical signs of profuse, often fatal, diarrhea, profound weakness, severe dehydration, and a dramatic reduction in milk yield. Additional symptoms such as submaxillary edema and anorexia are common, frequently culminating in high mortality rates, especially in young and immunocompromised animals. The cumulative effect of mortality, stunted growth, reduced feed conversion efficiency, and decreased milk and meat production inflicts substantial economic losses upon the global livestock industry, making it a disease of critical concern for farmers and veterinarians alike (Liu et al., 2024; Maqbool et al., 2013).

The control of paramphistomosis traditionally relies on a two-pronged approach: managing the population of the intermediate snail host and the strategic use of anthelmintic drugs in infected animals (Khan & Maqbool, 2012; Masood ul Haq et al., 2013). However, both strategies present considerable challenges. In regions like Pakistan, the incidence of the disease is witnessing an alarming annual increase, exacerbated by climatic conditions favorable to the intermediate hosts (Khan et al., 2006; Khan et al., 2008). Snail control is notoriously difficult and economically burdensome, as it requires extensive environmental management, including improved drainage of pastures, fencing of water bodies, and the application of molluscicides such as copper compounds. These chemical interventions are not only costly but also lack specificity, posing a significant ecological threat to non-target poikilothermic animals and disrupting local aquatic ecosystems (Khan et al., 2008; Tanveer & Khan, 1989).

While anthelmintic treatment remains a cornerstone of disease management in enzootic areas, its success is often limited. Conventional allopathic drugs, such as oxcyclozanide, are not always fully effective against all parasitic stages and their continuous, often prophylactic, use has led to the emergence of drug-resistant parasite strains, a growing problem in veterinary parasitology. Furthermore, many synthetic anthelmintics have mandatory withdrawal periods due to residual effects, adversely affecting milk and meat production during treatment and causing tangible economic losses for producers (Asif & Shaheen, 2022, Asif et al., 2025; Brander et al., 1991; Tellegen et al., 2018).

Given the limitations of conventional control methods, there is a pressing need to explore sustainable and safe alternatives. In this context, ethnoveterinary medicine offers a rich repository of knowledge, reporting the use of numerous medicinal plants with purported anthelmintic properties (Akhtar, 1988; Iqbal et al., 2005). Plants such as *Nigella sativa* (black seed), *Fumaria parviflora* (fine-leaved fumitory), *Caesalpinia crista* (fever nut), and *Saussurea lappa* (costus) have been traditionally employed in various communities to treat helminthic infestations in livestock (Akhtar, 1988; Maqbool et al., 2004; Masood ul Haq et al., 2013). These botanicals are thought to contain secondary metabolites like alkaloids, flavonoids, and essential oils that may possess potent antiparasitic activity. However, despite their widespread traditional use, their specific efficacy and optimal dosage against paramphistomosis in buffaloes, a major dairy and draught animal in Pakistan, have not been rigorously scientifically established through controlled trials. Therefore, the present study was designed to systematically evaluate and compare the therapeutic efficacy of these four indigenous plant-based drugs with a standard allopathic treatment for controlling paramphistomosis in naturally infected buffaloes.

MATERIALS AND METHODS

Animals Used

A total of 150 buffaloes of the *Nili Ravi* breed (140 naturally infected and 10 healthy), aged 4-10 years and of both sexes, were used. The animals were maintained under uniform feeding and management conditions. A detailed history was recorded for each animal.

Experimental Design

The infected animals were randomly divided into seven main groups (A, B, C, D, E, F, G). Groups A, B, C, and D were further subdivided into three subgroups each (A1, A2, A3; B1, B2, B3; C1, C2, C3; D1, D2, D3), with 10 animals per subgroup. Groups E, F, and G contained 10 animals each.

- **Group A (A1, A2, A3):** Treated with *Nigella sativa* at 30, 40, and 50 mg/kg body weight, orally.
- **Group B (B1, B2, B3):** Treated with *Fumaria parviflora* at 30, 40, and 50 mg/kg body weight, orally.
- **Group C (C1, C2, C3):** Treated with *Caesalpinia crista* at 30, 40, and 50 mg/kg body weight, orally.
- **Group D (D1, D2, D3):** Treated with *Saussurea lappa* at 30, 40, and 50 mg/kg body weight, orally.
- **Group E:** Treated control, given oxcyclozanide (Zanil) at 10 mg/kg body weight.
- **Group F:** Positive untreated control (infected, no treatment).
- **Group G:** Negative untreated control (healthy, no treatment).

Preparation and Administration of Herbal Drugs

The preparation of the indigenous plant materials was conducted with strict adherence to standard protocols to ensure consistency, stability, and accurate dosing. The seeds of *Nigella sativa* and *Caesalpinia crista*, the aerial parts of *Fumaria parviflora*, and the roots of *Saussurea lappa* were obtained from a reputable herbal supplier and authenticated by a botanist. The

plant parts were thoroughly washed with distilled water to remove any soil or debris and then shade-dried at room temperature for a period of two weeks to preserve their volatile active compounds. Once completely desiccated, the materials were pulverized into a fine, homogeneous powder using an electric grinder. This powder was then stored in opaque, airtight glass containers at 4°C to prevent photodegradation, moisture absorption, and thermal decomposition, thereby maintaining the potency of the phytochemicals throughout the experimental period.

For administration, a 2% (weight/volume) aqueous solution of gum tragacanth was prepared and used as a benign and effective suspending agent to ensure uniform dispersion of the hydrophobic plant powder. Immediately prior to treatment, the precise quantity of each powdered drug corresponding to the designated dose (30, 40, or 50 mg per kg of body mass) was weighed for each individual animal. The powder was then thoroughly mixed and suspended in 100 ml of the gum tragacanth solution by vigorous shaking. This suspension was administered orally to each buffalo using a dedicated drenching bottle, ensuring the entire dose was delivered. The control group receiving the allopathic drug was administered oxcyclozanide (Zanil) at 10 mg/kg body weight as per the manufacturer's instructions.

Parasitological Techniques

Parasitological monitoring was conducted to accurately diagnose the infection and quantitatively assess the anthelmintic efficacy of the treatments. Fresh fecal samples were collected directly from the rectum of each animal into clean, pre-labeled plastic bottles at each time point to avoid environmental contamination. A comprehensive diagnostic approach was employed to maximize the detection sensitivity of paramphistome eggs. Initially, a direct smear examination in saline was performed for a rapid qualitative assessment. This was followed by a formalin-ether sedimentation technique, which is highly effective for concentrating the large, heavy trematode eggs. To ensure no light infections were missed, a zinc sulphate flotation technique (specific gravity 1.18-1.20) was also utilized (Soulsby, 1986). Quantification of the parasitic load was performed using the standardized McMaster egg counting

technique (Kelly, 1974). This method allows for the determination of the number of eggs per gram (EPG) of feces, providing a reliable and reproducible metric for evaluating the intensity of infection and the subsequent reduction post-treatment. The identification of eggs and any adult flukes recovered during the study was based on established morphological characteristics, including size, shape, and opercular features, using standard parasitological keys (Foreyt, 2001; Yamaguti, 1975).

The fecal examination schedule was designed to monitor both the initial drug action and the sustained effect. Baseline (pre-treatment) EPG counts were established on day 0. Subsequent monitoring was conducted on days 3, 7, and 18 post-treatment to track the progression of the drug's efficacy. Animals that remained patent (positive for eggs) on day 18 were considered to have a residual infection and were administered a second, identical dose of their respective treatment. The fecal EPG for these animals was then re-evaluated on days 21 and 28 to assess the cumulative effect of the two-dose regimen.

Statistical Analysis

The data generated from the EPG counts were subjected to rigorous statistical analysis to ensure the validity and reliability of the findings. The primary analysis was conducted using a Linear Regression Model (Steel & Torrie, 1980) to evaluate the relationship between the dosage levels of each drug and the resulting reduction in EPG. The coefficient of determination (R^2) was calculated for each treatment group to quantify the proportion of the variance in efficacy that could be attributed to the dosage, providing a measure of the model's goodness-of-fit.

The therapeutic efficacy of each drug at different dose levels was calculated as a percentage reduction in EPG counts using the standard formula:

$$\text{Efficacy (\%)} = \frac{[\text{Pre-treatment EPG} - \text{Post-treatment EPG}]}{\text{Pre-treatment EPG}} \times 100$$

The mean percentage reductions for each treatment group were compared against the untreated control group (Group F) to account for any natural fluctuation in egg output. The statistical significance of the observed reductions, particularly after the second dose, was assessed, with a p-value of less than 0.05 ($p < 0.05$) considered statistically significant. This

comprehensive analytical approach allowed for a robust comparison of the relative efficacy among the different indigenous drugs and the standard allopathic control.

RESULTS

The efficacy of all drugs, based on the reduction in EPG counts, is summarized in Table I.

Efficacy of *Nigella sativa* Seeds

Nigella sativa at 30, 40, and 50 mg/kg showed efficacies of 65.85%, 68.29%, and 71.79%, respectively, after one dose. After the second dose, efficacy increased to 85.36%, 92.68%, and 94.87%. The R^2 values from linear regression were 0.9331, 0.9245, and 0.9545, indicating 93%, 92%, and 95% model fit, respectively.

Efficacy of *Fumaria parviflora* Aerial Parts

Fumaria parviflora at 30, 40, and 50 mg/kg showed efficacies of 46.34%, 53.65%, and 59.52% after one dose, which increased to 82.92%, 90.24%, and 92.85% after two doses. The R^2 values were 0.9019, 0.9794, and 0.9685, indicating a good fit (90%, 97%, and 96%, respectively). The second dose caused a significant reduction in EPG compared to the control ($p < .001$).

Efficacy of *Caesalpinia crista* Seeds

Caesalpinia crista at 30, 40, and 50 mg/kg was 47.61%, 52.63%, and 64.1% effective after one dose, and 80.95%, 86.84%, and 92.3% effective after two doses. The R^2 values were 0.9971, 0.9915, and 0.9714 (99%, 99%, and 97% fit). The second dose resulted in a significant reduction ($p < .001$).

Efficacy of *Saussurea lappa* Roots

Saussurea lappa at 30, 40, and 50 mg/kg demonstrated efficacies of 50%, 53.48%, and 56.09% after one dose, improving to 71.42%, 81.39%, and 85.36% after two doses. The R^2 values were 0.9702, 0.9598, and 0.9916 (97%, 95%, and 99% fit). The second dose caused a significant reduction ($p < .001$).

Efficacy of Oxyclozanide

Oxyclozanide at 10 mg/kg was 66.66% effective after one dose and 97.43% effective after two doses. The R^2 value was 0.9696 (96% fit).

Comparative Efficacy

The relative efficacy at optimal doses showed that all drugs had a significant anti-paramphistomic effect. The overall order of efficacy was: Oxyclozanide

> *Nigella sativa* > *Fumaria parviflora* > *Caesalpinia crista* > *Saussurea lappa*.

Table 1

Efficacy of Different Herbal and Allopathic Drugs against Paramphistomosis in Buffaloes.

Drug Used	Subgroups and Dose (mg/kg)	Efficacy (%) on 18th Day	Efficacy (%) on 28th Day
<i>Nigella sativa</i>	A1 (30)	65.85	85.36
	A2 (40)	68.29	92.68
	A3 (50)	71.79	94.87
<i>Fumaria parviflora</i>	B1 (30)	46.34	82.92
	B2 (40)	53.65	90.24
	B3 (50)	59.52	92.85
<i>Caesalpinia crista</i>	C1 (30)	47.61	80.95
	C2 (40)	52.63	86.84
	C3 (50)	64.10	92.30
<i>Saussurea lappa</i>	D1 (30)	50.00	71.42
	D2 (40)	53.48	81.39
	D3 (50)	56.09	85.36
<i>Oxyclozanide</i>	E (10)	66.66	97.43

Efficacy (%) was calculated based on the reduction in mean EPG counts compared to pre-treatment (Day 0) levels. The R² Value (Coefficient of Determination) from the Linear Regression Model indicates the proportion of the variance in efficacy that is predictable from the dose. The second dose of all drugs caused a statistically significant reduction in EPG compared to the untreated control group (p < .001).

At their optimal dose (50 mg/kg for herbal drugs and 10 mg/kg for oxyclozanide), all drugs exhibited a significant anti-paramphistomic effect. The overall order of efficacy, based on the maximum efficacy achieved after two doses, was:

Oxyclozanide (97.43%) > *Nigella sativa* (94.87%) > *Fumaria parviflora* (92.85%) > *Caesalpinia crista* (92.30%) > *Saussurea lappa* (85.36%).

DISCUSSION

The findings of this study provide compelling evidence that the selected indigenous plant-based therapeutics possess significant efficacy against paramphistomosis in buffaloes, with their anthelmintic action markedly potentiated by a two-dose regimen. This underscores the potential of phytotherapeutic interventions as a viable component of integrated parasite management strategies.

Among the herbal treatments investigated, *Nigella sativa* (black seed) emerged as the most potent, achieving a remarkable efficacy of 94.87% at the 50 mg/kg dose following the second administration. This result is in strong agreement with the work of Kailani et al. (1995) and Maqbool et al. (2004), who reported similar high efficacy against other trematodes like *Fasciola*. The pronounced anthelmintic activity of *N. sativa* can be attributed to its rich and complex phytochemical profile. The seeds are known to contain bioactive isoquinoline alkaloids such as



nigelline, metarbin, and melanthin, which are believed to interfere with parasite neuromuscular coordination. Furthermore, the presence of a high concentration of fixed oils (37.5%) and volatile oils (1.5%), including thymoquinone, may synergistically enhance its efficacy. These lipophilic compounds likely delay gastrointestinal transit, thereby prolonging the contact time between the active constituents and the parasites, leading to more effective paralysis and expulsion (Asif, 2024; Ghazanvi, 1988; Khan et al., 2025; Nath, 1983).

The aerial parts of *Fumaria parviflora* (fine-leaved fumitory) also demonstrated high efficacy, reaching 92.85% at the 50 mg/kg dose after two treatments. This robust activity is consistent with its documented phytochemistry; Akhtar (1988) identified that the plant contains approximately 4% isoquinoline alkaloids (e.g., fumaricine, parfumine) on a dry matter basis, which are probable active principles responsible for its antiparasitic properties. These alkaloids may exert their effects by causing spastic paralysis in the flukes. The correlation of our findings with those of Kailani et al. (1995) against fasciolosis suggests a broad-spectrum anti-trematodal activity for *F. parviflora*, likely effective against a range of digenean flukes through similar mechanisms of action (Khan et al., 2025; Usama et al., 2022).

Both *Caesalpinia crista* (fever nut) and *Saussurea lappa* (costus) produced substantial, dose-dependent reductions in fecal egg counts, validating their traditional use. The efficacy of *C. crista*, which reached 92.3%, is supported by its known content of bitter principles and cassane-type diterpenes. The results for *S. lappa* (85.36% efficacy), while lower than the other plants, still represent a significant anthelmintic effect. The findings for these two botanicals corroborate earlier research by Kailani et al. (1995) and Maqbool et al. (2004), and align with broader studies on plant-based anthelmintics (Asif & Sandhu, 2023; Diaz et al., 2006; Wang et al., 2023; Stafford et al., 2007), reinforcing the concept that diverse plant families have evolved secondary metabolites with potent antiparasitic properties.

As expected, the reference allopathic drug, oxclozanide, exhibited the highest efficacy at 97.43%, a result that is consistent with its well-established mechanism of uncoupling oxidative

phosphorylation in flukes, as documented in previous studies (Maqbool et al., 2004; Richards et al., 1990). Its performance serves as a robust benchmark, confirming the validity of our experimental model and the severity of the infections treated.

CONCLUSION

In summary, the results definitively establish the descending order of anthelmintic efficacy as: Oxclozanide > *Nigella sativa* > *Fumaria parviflora* > *Caesalpinia crista* > *Saussurea lappa*. Perhaps even more significant than their efficacy is the outstanding safety profile observed; no adverse physiological reactions or side effects were recorded in any animal treated with the plant-origin drugs throughout the study period. This stands in stark contrast to the potential residues and withdrawal periods associated with synthetic anthelmintics. Therefore, this research conclusively demonstrates that these indigenous plants, particularly *Nigella sativa*, are not only effective but also safe therapeutic agents. Their integration into control programs offers a sustainable, eco-friendly, and economically viable alternative for managing paramphistomosis in buffaloes, which could help mitigate drug resistance and reduce reliance on conventional chemicals, thereby contributing to more sustainable livestock production systems.

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