Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.245

RESEARCH ARTICLE

In vitro and In vivo Anthelmintic Activity of *Nicotiana tabacum* against *Haemonchus placei* in Cattle

Ali Haider[1], Kashif Hussain1, Mohammed M. Mares[2], Asghar Abbas[*1], Muhammad Mohsin[3], Atif Rehman[1], Ume Habiba Firdous[4], Muhammad Asif Raza[1]

[1]Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture (MNSUA), 66000, Multan, Pakistan; [2]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia; [3]Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong, China; [4]Department of Zoology, Wildlife and Fisheries, Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture (MNSUA), 66000, Multan, Pakistan
*Corresponding author: asghar.abbas@mnsuam.edu.pk; kashif.hussain@mnsuam.edu.pk

ARTICLE HISTORY (24-393)

Received: July 10, 2024
Revised: August 20, 2024
Accepted: August 22, 2024
Published online: September 20, 2024

Key words:
Haemonchus placei
Nicotiana tabacum
Crude Aqueous Extract
Anthelmintic Efficacy
Parasite Control

A B S T R A C T

In current study, anti-parasitic properties of *Nicotiana tabacum* leaves (*N. tabacum* L.) and their traditional use in ethno-veterinary therapy were investigated using both in-vitro and in-vivo approaches. Crude aqueous extracts (CAE) of *N. tabacum* L. were used to evaluate in-vitro antiparasitic effect of *Haemonchus (H.) placei* that had been directly retrieved from the abomasum of cows. The nematodes' death or paralysis within three hours subsequent to being exposed to different quantities 25, 50 and 100 mg/ml of CAE proved to be statistically significant ($P<0.05$) indicator. Cattle infected with gastrointestinal nematodes, specifically *H. placei*, were orally administered with crude aqueous extracts (CAE) and crude methanolic extracts (CME) at gradually higher dosage of 1.0 and 3.0 gm/kg, respectively to evaluate in-vivo anthelmintic efficiency. When administered with 3.0 gm/kg of the body weight, CME showed an ideal decrease of 75.8 and 79.03% parasitic infection but CAE showed 48.88 and 51.11% reduction after 5 and 10 days of treatment, respectively ($P<0.05$). The overall reduction in eggs per gram was 98.68% in positive controls treated with levamisole HCl. Being an initial investigation of its kind in Pakistan, the outcomes suggested that *N. tabacum* L. have potential to be used as anti-parasitic agent in ethno-veterinary medication.

To Cite This Article: Haider A, Hussain K, Mares MM, Abbas A, Mohsin M, Rehman A, Firdous UH, Raza1 MA, 2024. In vitro and in vivo anthelmintic activity of *Nicotiana tabacum* against *Haemonchus placei* in cattle. Pak Vet J, 44(3): 745-750. http://dx.doi.org/10.29261/pakvetj/2024.245

INTRODUCTION

The gastrointestinal tract of animals consists of a diversity of parasites responsible for both symptomatic and subclinical parasitism. Among these, helminth parasites insert negative effects on livestock wellbeing resulting significant economic losses for livestock sector. Pakistan is suffering from high level of parasitic infection having annual loss of almost 26.5 million rupees to livestock industry (Rashid *et al.*, 2019; Khan *et al.*, 2022). In Pakistan intestinal parasites affected growth of calves and impeded the improvement of livestock industry over time (Khan *et al.*, 2021; Khattak *et al.*, 2023).

Numerous deaths and financial losses in tropical and subtropical regions have resulted from the predominance of helminths which reduced the production potential of livestock development initiatives (Williams *et al.*, 2021). Ruminants affected by Helminthiasis may experience hematological and biochemical abnormalities (Alam *et al.*, 2020; Ul- Rahman *et al.*, 2021), weight loss, loss of appetite, hypo-proteinemia, reduced meat (27%) and wool production (40%), poor digestion, reduced reproduction and death of lambs (Raza *et al.*, 2007). Parasitic infection is primary threat resulting reduction in profitability of livestock business (Khan *et al.*, 2021).

Parasitic diseases frequently include intestinal nematodes, mainly *Haemonchus* parasite which have two species including *H. contortus* and *H. placei*. Among these, *H. placei* is an abomasal parasite of cattle, prevalent in tropical and subtropical areas of world. This nematode can be extremely pathogenic in summer rainfall areas resulting production losses as well as heavy mortality in cattle (Saminathan *et al.*, 2015; Fávero *et al.*, 2016).

In order to control infection of gastrointestinal nematodes, synthetic anthelmintic medications and chemicals are frequently utilized, however, parasites can acquire resistance to these drugs (Cotter *et al.,* 2015; Mughal *et al.,* 2023) and toxicity alarms of such chemicals (Ploeger and Everts, 2018) specify the main threat in improvement of chemo-therapeutically effective parasitic control program. These concerns highlight the need of exploring and inspecting anthelmintic effect of medicinal plants as a substitute for the common usage of synthetic drugs in clinics (Akkari *et al.,* 2014; Ahmed *et al.,* 2020; Romero-Jola *et al.,* 2023). Plants have historically been used as anthelmintics for both humans and animals, suggesting an alternative to synthetic chemicals (Rehman *et al.,* 2023; Hussain *et al.,* 2023; Hamad *et al.,* 2013; Lateef *et al.,* 2013; Aderibigbe *et al.,* 2022; Abbas *et al.,* 2023; Mughal *et al*., 2023; Pavičić *et al.,* 2023).

Nicotiana (N.) *tabacum* (tobacco), is well known plant containing 90% of alkaloidal content. Plants have diverse therapeutic, medicinal and insecticidal properties (Nouri *et al.,* 2016; Weber *et al.,* 2019; Al-Lahham *et al.,* 2020). Furthermore, this plant is used as anthelmintic, emetic, purgative anti- rheumatic and non-steroidal anti-inflammatory agent in human and livestock medicine (Nadkarni, 2007). Based on diverse medicinal properties of N. tabacum, a current study was designed to evaluate *in vitro* and *in vivo* anthelmintic potential of this plant against *H. placei* parasite.

MATERIALS AND METHODS

The present study was divided into two sets of experiments. The *in-vitro* study was conducted on adult *H. placei* specimens obtained from slaughtered dairy cattle at public abattoir named Punjab Agriculture and Meat Company (PAMCO) located in Lahore and the *in-vivo* study was based on adult cattle infected with *H. placei*, found in Lahore near River Ravi region.

Collection of *N. tabacum* leaves: The leaves of indigenous *N. tabacum* were collected from the local market in Lahore during February-March months. To obtain a consistent weight for subsequent processing, tobacco plant leaves were dried for 20 days in shade and further drying to attain constant weight was done in hot air oven at 55-60ºC.

Preparation of Aqueous extracts: Using an electric grinder, the leaves of *N. tabacum* were grinded into fine powder, which was then stored in plastic containers. For preparation the aqueous extracts, 10g of *N. tabacum* L. powder was mixed with 100ml of deionized water in 500 ml glass beaker. The solution was stirred on magnetic stirrer for 30 minutes at 6000rpm and left for 24 hours. First, fine cloth was utilized to filter extracts and subsequent filtration was performed using Whatman No. 1 filter paper. After that, the filtrate was concentrated by heating in water bath at 5℃ until it achieved a constant volume of 10 ml in round bottom flask. After the water evaporation, the concentrated solution was refrigerated at 4℃ until required for its anthelmintic properties (Amin *et al.,* 2009).

Preparation of methanolic extracts: The crushed plant parts (500 g) were thoroughly extracted using methanol at different temperatures ranging in between 60 to 80ºC in Soxhlet apparatus till whole extraction. A rotary vacuum evaporator was used to hold the CME and to remove the solvent. The dehydrated CME was stored at 4°C for future usage.

Stock solution preparation: Prior to anthelmintic screening, the stock solutions for the *in vitro* study were prepared by diluting the concentrated solution with standard quantity of distal water to obtain different concentrations i.e., 25, 50 and 100 mg/ml.

***In-vitro* anthelmintic activity:** Collection of the adult *H. placei* parasites was done directly from abomasum of slaughtered cattle. The contents were visually inspected and a curved needle was used to collect the adult parasites in normal saline (0.9% NaCl). The adult parasites were preserved in petri plates having normal saline and placed in incubator at 37ºC. Using morphological features, adult *H. placei* worms were identified as described by Lichtenfels *et al.* (1994). Five petri dishes were marked as Plate T1, T2, T3, T4 and T5 with 20 adult worms in each were preserved in PBS (Fig 1a). Aqueous extracts of *N. tabacum* leaves were administered to T1, T2, and T3 at 25, 50 and 100 mg/ml concentrations, respectively as described by Amin *et al.* (2009). T4 was given Levamisole HCl (0.55 mg/ml) as positive control and T5 treated as negative control containing no treatment. The petri dishes were placed in incubator for 3 hours at 37°C. After incubation, the effectiveness of *N. tabacum* aqueous extracts was determined by the number of dead *H. placei* worms (Fig 1b).

***In-vivo* anthelmintic activity:** For the *in-vivo* trials, adult cattle were selected from Lahore near River Ravi region. Prior to the research trials, fecal analysis was performed by using both qualitative and quantitative methods to confirm the presence of *H. placei* in the animals. Fecal samples were collected directly from rectum and examined salt flotation technique. A direct smear method was used to check the presence of nematode eggs. Using the conventional description of Foreyt (2013), nematode eggs found in the direct smear method and salt flotation technique were identified.

A total of 24 cattle were included in study trials after confirmation of *H. placei* infection. For treatment, six groups were created from selected (n=24) cows found positive for endo-parasitic infections, particularly for *H. placei* (Iqbal *et al.,* 2006a). The groups (T1 & T2) were treated with crude aqueous extract containing eggs per gram (EPG) standard maintained between 500 and 600. The T3 and T4 groups were maintained at 700–800 eggs per gram and treated with CME. Levamisole HCl-treated positive control group (T5) with 900–1000 eggs per gram. EPG >1000 was treated as negative control group (T6). T1 and T2 received CAE 1.0 g/kg, and 3.0 g/kg, while, T3 and T4 were treated with CME 1.0 g/kg and 3.0 g/kg dosages, separately. Positive control group was treated with single dose of Levamisole HCl (Nilverm 1.5% w/v Levamisole Hydrochloride from ICI Pakistan Limited) at dose rate 7.5 mg/kg, and negative control group received no treatment.

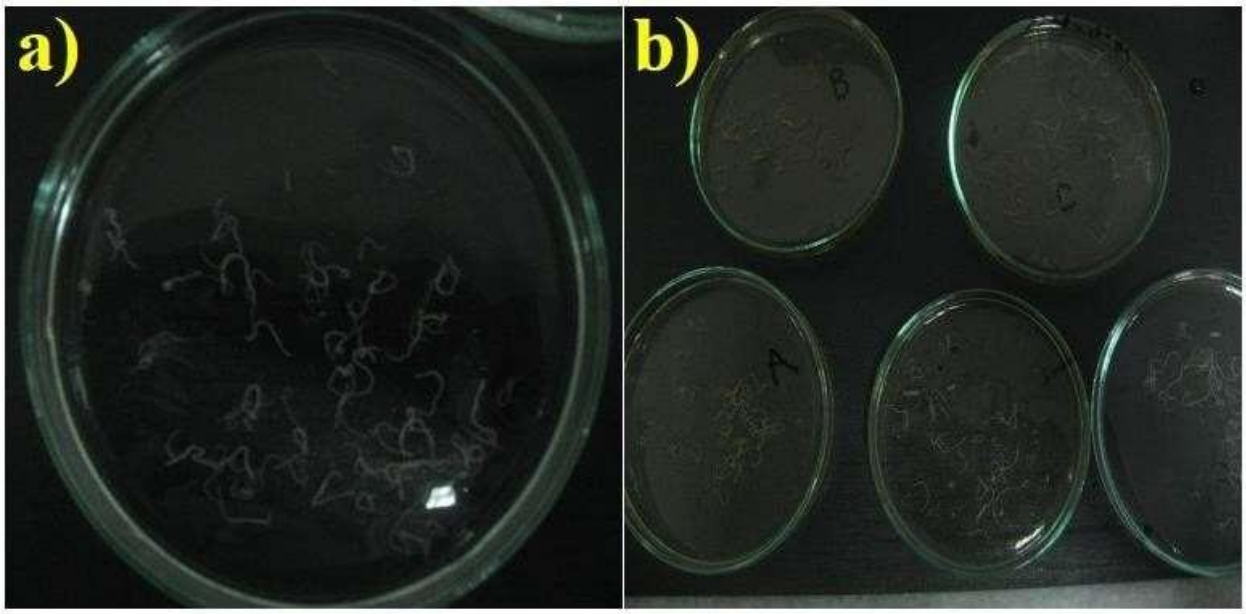

Fig. 1: Adult *H. placei* (a) *in-vitro* anthelmintic efficacy of *N. tabacum* (b) effectiveness of *N. tabacum* CAE.

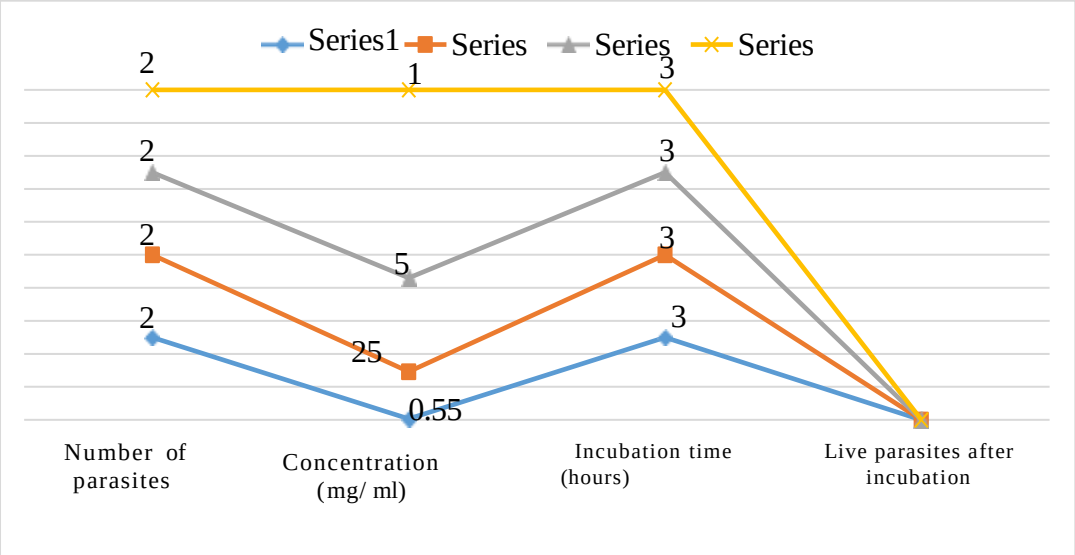

Fig. 2: Time dependent effect of various concentrations of *N. tabacum* CAE.

Fecal samples were collected from every animal in the morning and the salt flotation method was used to assess the existence of worm eggs beginning on day 0 pre-treatment, continuing days 5 and 10 post-treatment. The counting was done by McMaster technique as described by Zajac and Conboy (2012). Total number of eggs per gram of feces were calculated by multiplying number of eggs counted in both chambers by 50 (Zajac and Conboy, 2012).

RESULTS

***In-vitro* Trial:** The effectiveness of *N. tabacum* extracts at three different concentrations against adult *H. placei* parasites are shown in Fig. 1 and Table 1. Aqueous extracts against adult parasites demonstrated promising anthelmintic properties. Efficacy of aqueous extracts of *N. tabacum* (Tobacco) plant against adult *H. placei* parasites at different concentrations of 25, 50, and 100 mg/ml found same as 100 % during in-vitro study. After placing in incubator for three hours at 37ºC, all worms exposed to levamisole 0.55 mg/ml were found dead while, from the phosphate buffer solution (PBS), used as negative control, not a single dead worm was observed. These findings indicated the anthelmintic activity of *N. tabacum* CAE.

***In-vivo* Trial:** The effectiveness of *N. tabacum* CAE and CME as *in-vivo* anthelmintics was assessed in number of treatment groups. Microscopically examined *H. placei* positive cattle were divided into six (6) treatment groups T1, T2, T3, T4, Positive control and Negative control groups and each group contained four animals. Animal fecal samples were analyzed for the purpose of calculating eggs per gram (EPG) on days 0 pre-treatment, 5 and 10 day post-treatment as shown in Table 2 and Table 3. All treatment groups revealed a significant reduction ($P< 0.05$) in EPG at day 5 and 10 post-treatment. Maximum reduction was indicated by CAE at 3 gm/kg dose at day 5 and 10 post treatment as 48.88 % and 51.11 %. Reduction revealed by CME at dose rate of 3 gm/kg body weight was 75.8 % and 79.03 %, respectively at 5 and 10 days

post treatment as depicted in Table 3 and Table 4. Maximum reduction at 98.68 % in eggs per gram (EPG) was observed in positive control group that was treated with Levamisole HCl.

Table 1: In-vitro anthelmintic activity of CAE of *N. tabacum*.

Sr. No	Treatments	No. of live parasites used	Incubation time (hours)	Live parasites after incubation	Efficacy (%)
1	-ve ctrl	20	3	20	0
2	+ve ctrl	20	3	0	100
3	T 1	20	3	0	100
4	T 2	20	3	0	100
5	T 3	20	3	0	100

–ve ctrl = negative control = Phosphate buffer solution; +ve ctrl = positive control = 0.55 mg/ml levamisole HCl; T 1 = treatment 1 = 25 mg/ml; T 2 = treatment 2 = 50 mg/ml; T 3 = treatment 3 = 100 mg/ml

Table 2: In-vivo anthelmintic efficacy of *N. tabacum* against *H. placei*.

Sr. No	Treatments	EPG at 0 Day ($\bar{X}$)	EPG at 5th Day ($\bar{X}$)	EPG at 10th Day ($\bar{X}$)	P value
1	T 1	537.5	387.5	375	
2	T 2	562.5	287.5	275	
3	T 3	712.5	437.5	425	
4	T 4	775	187.5	162.5	
5	+ ve Ctrl	950	12.5	12.5	
6	-ve Ctrl	1037.5	1075	1087.5	P<0.05

T 1 = treatment 1 = Crude Aqueous Extract 1 gm/kg; T 2 = treatment 2 = Crude Aqueous Extract 3 gm/kg; T 3 = treatment 3 = Crude Methanolic Extract (CME) 1 gm/kg; T 4 = treatment 4 = Crude Methanolic Extract (CME) 3 gm/kg; +ve ctrl = positive control = Levamisole HCl 7.5 mg/kg; -ve ctrl = Negative control = No Treatment

Table 3: Effect of *N. tabacum* Extracts on EPG at 5th day Post Treatment.

Sr. No	Treatments	EPG at 0 Day ($\bar{X}$)	EPG at 5th Day ($\bar{X}$)	Efficacy % age
1	T 1	537.5	387.5	27.9
2	T 2	562.5	287.5	48.88
3	T 3	712.5	437.5	38.59
4	T 4	775	187.5	75.8
5	+ ve Ctrl	950	12.5	98.68

T 1 = treatment 1 = Crude Aqueous Extract 1 gm/kg; T 2 = treatment 2 = Crude Aqueous Extract 3 gm/kg; T 3 = treatment 3 = Crude Methanolic Extract (CME) 1 gm/kg; T 4 = treatment 4 = Crude Methanolic Extract (CME) 3 gm/kg; +ve ctrl = positive control = Levamisole HCl 7.5 mg/kg

Table 4: Effect of *N. tabacum* Extracts on EPG at 10^{th} Day Post Treatment.

Sr. No	Treatments	EPG at 0 Day ($\bar{X}$)	EPG at 10th Day ($\bar{X}$)	Efficacy % age
1	T 1	537.5	375	30.23
2	T 2	562.5	275	51.11
3	T 3	712.5	425	40.35
4	T 4	775	162.5	79.03
5	+ve ctrl	950	12.5	98.68

T 1 = treatment 1 = Crude Aqueous Extract 1 gm/kg; T 2 = treatment 2 = Crude Aqueous Extract 3 gm/kg; T 3 = treatment 3 = Crude Methanolic Extract (CME) 1 gm/kg; T 4 = treatment 4 = Crude Methanolic Extract (CME) 3 gm/kg; +ve ctrl = positive control = Levamisole HCl 7.5 mg/kg

DISCUSSION

The well-known herb *N. tabacum*, or tobacco, is utilized for its narcotic properties. The effectiveness of these plants and numerous *N. tabacum* alkaloids on various biological systems have been reported in different literatures. Traditionally many portions of this plant are used for anthelmintic, anti-inflammatory, and anti-rheumatic properties (Nouri *et al.*, 2016). The primary bioactive ingredient in *N. tabacum* is nicotine. The leaves of *Nicotiana rustica* and Tabacum have an average nicotine content of 2–6% of dry weight. It was discovered that nicotine was used to control an extensive range of insects, as aphid's thrips and whiteflies (González-Garduño *et al.*, 2013).

It has previously been documented that the traditional veterinary practices in pastoral Karamoja, Uganda, and the Sahiwal district of Punjab, Pakistan, used *N. tabacum* as a botanical anthelmintic (Hussain *et al.*, 2008; Gradé *et al.*, 2009). Usage of *N. tabacum* in ethno-veterinary practices for the management of parasitic diseases in livestock in Cholistan desert, (Pakistan) has also been reported (Farooq *et al.*, 2008). The effectiveness of *N. tabacum* aqueous extract as an in-vitro anthelmintic was assessed having concentrations 25, 50, and 100mg/ml and its was found that adult worms of *H. placei* of cattle were significantly and completely damaged by the tobacco plant. Iqbal *et al.* (2006b) studied *N. tabacum* leaves and their anthelmintic activity against GINs including *H. contortus* of sheep. Paralysis or death of worms after six hours of exposure demonstrated the in-vitro inhibitory effect. Raje and Jangde (2003), discovered that *N. tabacum* depicted in-vitro anthelmintic activity against goat *H. contortus*. Nematode muscles are known to feature excitatory neuromuscular junctions, where the neurotransmitter acetylcholine is paired with ganglion-type nicotinic receptors (Neal, 2020). Every ganglion stimulant has a tendency to stimulate these neuromuscular connections, paralyzing the worms spastically and ultimately causing them to die and remove from the host.

Tobacco leaf containing nicotine, a ganglion stimulant (Mughal *et al.*, 2023) show anthelmintic activity of plant extract. Levamisole, the standard drug employed in this investigation, also exhibited anthelmintic action via stimulating nicotinic receptors (Raje and Jangde, 2003).

Six medicinal plants were tested for their in-vitro ovicidal and wormicidal effects against *H. contortus* and *N. tabacum* demonstrated the strongest ovicidal effects. This study also reported a dose-dependent anthelmintic efficacy for crude extract of *N. tabacum*. *N. tabacum* was found to be just as effective as Levamisole HCl dosage 0.55 mg/ml, causing 100% mortality of *H. contortus* within three hours of treatment (Iqbal *et al.*, 2006b). *N. tabacum* also found effective against ectoparasitic infestation in goat. As in an experiment when the anti-louse properties of aqueous tobacco and ivermectin extracts were examined in highly parasitized West African dwarf goats, the leaves and stems of *N. tabacum* were found to be 100% effective against lice by the second day of treatment and it was maintained for approximately 56 days after the challenge (Fajimi *et al.*, 2003).

Sujon *et al.* (2008) reported similar types of effects of plant as in current study. In-vitro anthelmintic activity of *N. tabacum* was evaluated in goats. The proportional effectiveness of 10, 20, 50 and 100mg/ml concentrations was 60, 60, 70 and 80%, respectively. The results may vary because of differences in animal and parasite species as well as because different ethanolic and aqueous extracts were used in the previously reported investigation and the current study, respectively.

According to Iqbal *et al.* (2006b), the maximum anthelmintic activity demonstrated by *N. tabacum* extracts at 3 gm/kg dose rate was 49.4% and 73.6 % decrease in *H.*

contortus EPG on day 5 after therapy. In both cases the anthelmintic efficacy onset was rapid. The maximum reduction in *H. placei* EPG was observed at 5 days post treatment found 48.88 and 75.8% while, minimum decrease was observed at day 10 post treatment compared to day 5 as 51.11 and 79.03%. The variation in the results could be due to differences in animal and parasite species between the current investigation and previously reported study.

Recently, Aderibigbe *et al.* (2022) evaluated in-vitro anthelmintic effects of three plants including Vernonia amygdalina, *Garcinia kola* and *Leucaena leucocephala* against *Haemonchus placei* parasite. Selected plants were three well-known tropical plants used in African ethnomedicine and potential sources of alternative solution for controlling parasitic infections of livestock. Among three tested plants, chloroform extract of V. amygdalina leaf demonstrated excellent anthelmintic activity against adult *H. placei*. This study demonstrates that *N. tabacum* has potential and can be used in future as an anthelmintic phyto-medicine after proper formulation to control gastrointestinal nematodes infection in cattle.

Conclusions: The findings of the present study showed that CAE and CME of *N. tabacum* leaves demonstrated significant in-vitro and in-vivo anthelmintic activity for *H. placei* originated from cattle. Consequently, the ethno veterinary medical practices justify the application of *N. tabacum* as an anthelmintic, and this study provide Pakistan's traditional medicine with an evidence-based foundation.

Acknowledgements: We thank the Researcher Support Program (RSPD2024R/1084), at King Saud University, Saudi Arabia.

Authors contributions: AH and KH developed the idea, designed and executed the experiment. MMM and AA supervised the study. MM, AR and UHF Collected and analyzed the Data. MAR finally approved the manuscript for publication.

REFERENCES

Abbas A and Alkheraije KA, 2023. Immunomodulatory effects of *Carica papaya* extract against experimentally induced coccidiosis in broiler chickens. Pak Vet J 43: 628-632.

Aderibigbe SA, Opayemi, OS, Bolaji, SA, *et al.*, 2022. In vitro effect of three tropical plants on adult Haemonchus placei, an haematophagous nematode from cattle. Beni-Suef Univ J Basic Appl Sci 11: 72.1-10.

Ahmed AH, Ejo M, Feyera T, *et al.*, 2020. In vitro anthelmintic activity of crude extracts of Artemisia herba-alba and Punica granatum against Haemonchus contortus. J Parasitol Res 2020. 4950196.

Akkari H, Rtibi K, B'chir F, *et al.*, 2014. In vitro evidence that the pastoral Artemisia campestris species exerts an anthelmintic effect on *Haemonchus contortus* from sheep. Vet Res Commun 38:249–255.

Alam RTM, Hassanen EAA and El-Mandrawy SAM, 2020. Heamonchus contortus infection in Sheep and Goats: alterations in haematological, biochemical, immunological, trace element and oxidative stress markers. J Appl Anim Res 48:357–364.

Al-Lahham S, Sbieh R, Jaradat N, *et al.*, 2020. Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the roots of *Nicotiana tabacum* L. Eur J Integr Med 33:101039.

Amin MR, Mostofa M, Hoque ME, *et al.*, 2009. In vitro anthelmintic efficacy of some indigenous medicinal plants against gastrointestinal nematodes of cattle. J Bangladesh Agric Univ 7. 57–61.

Cotter JL, Van Burgel A and Besier RB, 2015. Anthelmintic resistance in nematodes of beef cattle in south-west Western Australia. Vet Parasitol 207:276–284.

Fajimi AK, Taiwo AA and Ajayi FT, 2003. Studies on the anti-louse activity of aqueous tobacco extract and ivermectin in heavily parasitized West African dwarf goats. Isr J Vet Med 58: 118-122.

Farooq Z, Iqbal Z, Mushtaq S, *et al.*, 2008. Ethnoveterinary practices for the treatment of parasitic diseases in livestock in Cholistan desert (Pakistan). J Ethnopharmacol 118:213– 219.

Fávero FC, Buzzulini C, Cruz BC, *et al.*, 2016. Experimental infection of calves with *Haemonchus placei* or *Haemonchus contortus*: Assessment of clinical, hematological and biochemical parameters and histopathological characteristics of abomasums. Exp Parasitol 170:125–134.

Foreyt WJ, 2013. Veterinary parasitology reference manual. John Wiley & Sons.

González-Garduño R, Mendoza-de Gives P and Torres-Hernández G, 2013. Variability in the fecal egg count and the parasitic burden of hair sheep after grazing in nematode infected paddocks. Pesqui Veterinária Bras 33:469–475.

Gradé JT, Tabuti JRS and Van Damme P, 2009. Ethnoveterinary knowledge in pastoral Karamoja, Uganda. J Ethnopharmacol 122:273–293.

Hamad KK, Iqbal Z, Muhammad G, *et al.*, 2013. Antinematicidal activity of Nicotiana tabacum High Educ Comm 35–40.

Hussain K, Abbas A, Alanazi HAH, *et al.*, 2023. Immunomodulatory effects of *Artemisia brevifolia* extract against experimentally induced coccidiosis in broiler chicken. Pak Vet J 43: 333-338

Hussain A, Khan MN, Iqbal Z, *et al.*, 2008. An account of the botanical anthelmintics used in traditional veterinary practices in Sahiwal district of Punjab, Pakistan. J Ethnopharmacol 119:185–190.

Iqbal Z, Sajid MS, Jabbar A, *et al.*, 2006a. Techniques in Parasitology. Islamabad, Pakistan.

Iqbal Z, Lateef M, Jabbar A, *et al.*, 2006b. In vitro and in vivo anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. Phyther Res 20:46–48.

Khan T, Khan W, Iqbal R, *et al.*, 2022. Prevalence of gastrointestinal parasitic infection in cows and buffaloes in Lower Dir, Khyber Pakhtunkhwa, Pakistan. Brazilian J Biol 83:e242677.

Khan T, Nasreen N, Shater AF, *et al.*, 2021. Risk factor analysis for the prevalence of gastrointestinal parasites found in large ruminants in Lower Dir Khyber Pakhtunkhwa Pakistan. Saudi J Biol Sci 28:7022–7026.

Khattak I, Akhtar A, Shams S, *et al.*, 2023. Diversity, prevalence and risk factors associated to gastrointestinal tract parasites in wild and domestic animals from Pakistan. Parasitol Int 97:102777.

Lateef M, Iqbal Z, Sajid MS, *et al.*, 2013. An account of botanical anthelmintics and methods used for their evaluation. Rev Vet Anim Sci 1:6–14.

Lichtenfels JR, Pilitt PA and Hoberg EP, 1994. New morphological characters for identifying individual specimens of Haemonchus spp.(Nematoda: Trichostrongyloidea) and a key to species in ruminants of North America. J Parasitol 107–119.

Mughal MAS, Abbas RZ, Khan MK, *et al.*, 2023. Traditional practices regarding the use of botanicals to treat Bovine Haemonchosis in Pakistan. In: Ethnobotany. CRC Press, 237–255.

Nadkarni AK, 2007. Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. Popular Prakashan.

Neal MJ, 2020. Medical pharmacology at a glance. John Wiley & Sons.

Nouri F, Nourollahi-Fard SR, Foroodi HR, *et al.*, 2016. In vitro anthelmintic effect of Tobacco (*Nicotiana tabacum*) extract on parasitic nematode, Marshallagia marshalli. J Parasit Dis 40:643–647.

Pavičić A, Zajíčková M, Šadibolová M, *et al.*, 2023. Anthelmintic activity of European fern extracts against *Haemonchus contortus*. Vet Res 54:59.

Ploeger HW and Everts RR, 2018. Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. Vet Parasitol 262:11–15.

Raje AA and Jangde CR, 2003. In vitro anthelmintic activity of decoction of *Nicotiana tabacum* against *Haemonchus contortus* of goats. Indian Vet J 80:364-365

Rashid M, Rashid MI, Akbar H, *et al.*, 2019. A systematic review on modelling approaches for economic losses studies caused by

parasites and their associated diseases in cattle. Parasitology 146: 129-141.

Raza MA, Iqbal Z, Jabbar A, *et al*., 2007. Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. J Helminthol 81:323–328.

Romero-Jola, NJ, Cubides-Cárdenas JA, Escobar, N, *et al*., 2023. Ovicidal effect on *Haemonchus contortus* of extract partitions shrubby plants of the tropical dry forest and potentially active compounds identification by UHPLC-Q/Orbitrap/MS/MS. Appl Sci 13: 7147.

Rehman TU, El-Mansi AA, Alhag SK, *et al*., 2023. Antiparasitic activity of methanolic and ethyl acetate extracts of *Azadirachta indica* against *Haemonchus contortus*. Pak Vet J 43: 199-203.

Saminathan M, Gopalakrishnan A, Latchumikanthan A, *et al*., 2015. Histopathological and parasitological study of blood-sucking *Haemonchus contortus* infection in sheep. Adv Anim Vet Sci 3:99-108.

Sujon MA, Mostofa M, Jahan MS, *et al*., 2008. Studies on medicinal plants against gastrointestinal nematodes of goats. Bangl J Vet Med 6: 179–183.

Ul-Rahman A, Shabbir MAB, Mehmood A, *et al*., 2021. Genotypic and Sub-genotypic Diversity of Avian Paramyxoviruses 2, 4 and 6. Pak Vet J: 156-159.

Weber SS, Kaminski KP, Perret JL, *et al*., 2019. Antiparasitic properties of leaf extracts derived from selected Nicotiana species and Nicotiana tabacum varieties. Food Chem Toxicol 132:110660.

Williams AR, Myhill LJ, Stolzenbach S, *et al*., 2021. Emerging interactions between diet, gastrointestinal helminth infection, and the gut microbiota in livestock. BMC Vet Res 17:1-9.

Zajac AM and Conboy GA, 2012. Veterinary clinical parasitology. A John Wiley and Sons. Inc, Publ.