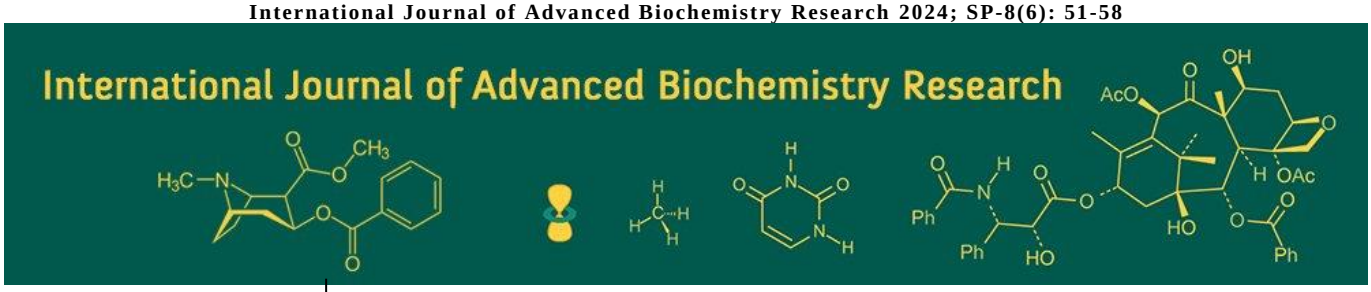

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(6): 51-58
www.biochemjournal.com
Received: 19-03-2024
Accepted: 22-05-2024

Dr. Ramakant
Ph.D. Scholar, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Tarun Kumar Sarkar
Professor, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Amit Kumar Verma
Professor & Head, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Prem Sagar Maurya
Associate Professor, Department of Veterinary Parasitology, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Vipul Thakur
Assistant Professor, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Arbind Singh
Assistant Professor, Department of Livestock Farm Complex, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Vikash Jaiswal
Assistant Professor & OIC, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Shriya Rawat
Veterinary Public Health & Epidemiology, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Pankaj Kumar Maurya
Ph.D. Scholar, Department of Veterinary Physiology & Biochemistry, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Dr. Sachin Gautam
Ph. D. Scholar, Department of Animal Nutrition, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Corresponding Author:
Dr. Ramakant
Ph.D. Scholar, Department of Veterinary Medicine, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

In vitro anthelmintic activity of aqueous extracts of seven medicinal plants against *Haemonchus contortus* in goats

Dr. Ramakant, Dr. Tarun Kumar Sarkar, Dr. Amit Kumar Verma, Dr. Prem Sagar Maurya, Dr. Vipul Thakur, Dr. Arbind Singh, Dr. Vikash Jaiswal, Dr. Shriya Rawat, Dr. Pankaj Kumar Maurya and Dr. Sachin Gautam

DOI: https://doi.org/10.33545/26174693.2024.v8.i6Sa.1254

Abstract
The aim of this study was to evaluate the *In vitro* anthelmintic efficacy of aqueous extracts from various plants against *Haemonchus contortus* in goats. The selected plants included of *Coriandrum sativum* seeds, *Allium sativum* bulb, *Carica papaya* seeds, *Zingiber officinale* rhizome, *Azadirachta indica* leaves, *and Swertia chirata* whole plant and *Prunus persica* leaves. For this purpose, an adult worm mortality assay was conducted using adult *Haemonchus contortus* worms collected from the abomasum of naturally infected goats. Adult mature parasites of *Haemonchus contortus* were collected from abomasum of naturally infected goats. Adequate amount of plant materials were collected. The collected plant materials were cleaned, dried under shade and then used to prepare aqueous extracts. In *In vitro* study, four concentrations of the aqueous extracts (50 mg/ml, 100 mg/ml, 200 mg/ml, and 400 mg/ml) were tested at regular time intervals, and viability of parasites was recorded over an 8 hours periods. Closantel (at the concentration of 1.25 mg/ml) and PBS were used as the positive and negative controls, respectively. The results showed that aqueous extracts of *Swertia chirata* whole plant of exhibited the highest adulticidal effects followed by *Coriandrum sativum* seeds, *Azadirachta indica* leaves, *Allium sativum* bulbs, *Carica papaya* seeds, *Zingiber officinale* rhizome *and lowest in Prunus persica* leaves against the *Haemonchus contortus* in goats. This indicates that these plant extracts have potential anthelmintic effects.

Keywords: *In vitro*, aqueous extracts, medicinal plants, *Haemonchus contortus* and goats

Introduction
Haemonchus contortus, commonly referred to as barber's pole worm, is a significant member of Trichostrongyloidea family and genus Haemonchus. This parasitic nematode stands out due to its considerable size, distinctive twisted shape, and the unique arrangement of its spicule in males (Flay *et al.*, 2022) [1].Among the gastrointestinal nematode parasites *Haemonchus contortus* poses the most significant pathogenic infecting to goats population worldwide (Shamim *et al.*, 2018) [2]. Infestations with this parasite can result in a range of detrimental effects including ascites, weight loss, anemia, and even mortality (Kelkele *et al*., 2012) [3]. Parasite control programs depend on the chemotherapeutic control, grazing and dietary management, biological control, vaccination, and ethno veterinary therapy (Sisay *et al*., 2012) [4]. However, synthetic anthelmintics pose challenges due to issues like resistance. *H. contortus* for instance, has shown resistance to the both broad and narrow spectrum anthelmintics (Singh *et al*., 2002) [5].
Herbal remedies may serve as valuable alternative treatments, particularly in cases of medication side effects and drug resistance (Ingle *et al*., 2017) [6]. Medicinal plants have been utilized for centuries to combat parasitism and various human and veterinary illness for centuries and they are still employed for these purposes in many regions worldwide (Kumsa and Hagos, 2020) [7]. Phytotherapeutic drugs, particularly plant anthelmintics, are deemed safe, non-toxic, biodegradable and do not leave residues in animal products.

Various extracts have exhibit potential for development as anthelmintic agents (Ndlela *et al.*, 2021) [8]. Several *In vitro* tests were utilized to screen plants for anthelmintic activity against various stages of parasites. In the present study, seven medicinal plants were selected to investigate *In vitro* anthelmintic activities of aqueous extracts. These extracts tested against *H. contortus* using the adult worm mortality (AWM) assay.

Materials and Methods

Ethical Approval

This study design was approved by Institutional Animal Ethics Committee (IAEC), Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110 (U.P.), India vide letter no. of IAEC/SVPUAT/2022/125 dated 03/12/2022.

Collection of plant materials

An adequate quantity of seeds of *Coriandrum sativum*, bulbs of *Allium sativum* and rhizomes of *Zingiber officinale* were purchased from the local market of Meerut. Additionally, ripe papaya (*Carica papaya*) fruits were also purchased from the same market for collecting the fresh seeds. Leaves of *Azadirachta indica* and *Prunus persica* tree were gathered from the SVPUAT campus in Meerut. The whole plant of *Swertia chirata* was acquired from authorized herbal medicine suppliers. After purchasing, the plant materials were brought to the laboratory for the further process. Plants materials were cleaned properly to remove the any impurities. The bulb of *Allium sativum* and rhizomes of *Zingiber officinale* were cut into small pieces to expedite the drying process. Subsequently, all the collected plant materials were to air dried under shade in well-ventilated conditions. Adequately dried the plant materials were grounded into powder using electric grinder machine. The resulting powdered materials were then stored in air tight properly labeled containers for further uses.

Preparation of extracts

Aqueous plant extracts were prepared by mixing 100 gram of the dry plant materials powder with 1000 ml distilled water. This mixture was then heated in a water bath at 100 °C for 30 minutes. The resulting solutions were filtered first through muslin cloth and then through Whatman No.1 filter paper. The obtained filtrate was concentrated using rotary evaporator and dried at 40 °C in hot air oven. The extracts were stored in a refrigerator at 4 °C until further use.

In vitro experiments

The *In vitro* anthelmintic activity was carried out following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) as outlined (Coles *et al.*, 1992) [9] with slight modifications to the procedures for parasites collection, preparation, and adult worm mortality assay.

Collection of parasite and preparation

Adult mature parasites of *Haemonchus contortus* were harvested from abomasum of naturally infected Barbari breeds of goats following their death and subsequent postmortem examination. The collection of *Haemonchus contortus* were to standard procedure (Rahman and Collins, 1990) [10]. The both ends of the collected abomasums were ligated after collections and they were then brought to the laboratory. The abomasum was rinsed thoroughly with running water then, opened along its greater curvature and its contents emptied into a 5 liter plastic bucket containing 2 and half liters of water. The parasites were separated by passing the contents through a sieve with a diameter of 100 micrometers and were subsequently collected using a wire loop. Adult mature *Haemonchus contortus* was identified and isolated from other parasites depend on their morphological characteristics utilizing the keys and description described by Taylor *et al.* (2007) [11]. The parasites were then, gathered, washed, and kept in phosphate buffered saline (PBS) until required for *In vitro* testing.

Adult Worm Mortality Assay (AWMA)

The experiment followed the protocol outlined in prior studies (Sharma *et al.*, 1971; Coles *et al.*, 1992) [12, 9].The test was carried out in 50 mm diameter glass petri dish. A total of 1080 adult *Haemonchus contortus* parasites were utilized. Four concentrations were examined for each plant extract. In each petri dish,10 actively moving parasites were introduced with varying concentration (50 mg/ ml, 100 mg/ml, 200 mg/ml and 400 mg/ml) of the aqueous extracts from seven plants materials, dissolved in distilled water or phosphate buffered saline. A negative control group was consisting of ten actively moving parasites in phosphate buffer saline in a total volume of 4 ml. In a positive control group closantel initially dissolved in DMSO (dimethyl sulfoxide) and then diluted in distilled water at the concentration of 1.25 mg/ml along with ten parasites. Each treatment concentrations were conducted in three replicates at room temperature (25–30ºC).

Worm motility of was observed, and motile worms were counted at a regular time intervals of 0, 2, 4, 6, and 8 hours of post treatment. Worms showing no motility were isolated and placed in lukewarm PBS for duration of 10 minutes. If there was a revival in motility during this time the worms were considered alive; otherwise, they were considered dead. Worm death was confirmed by the absence of motility for observed over a period of 5 to 6 seconds. After 8 hours post treatment, the plant extracts and closantel were washed away, and the parasites were suspended in PBS for duration of 30 minutes to allow for possible recovery of the motility of the parasite. Finally, the number of alive (motile) and dead (immotile) worms were counted under dissecting microscope. This was recorded for every concentration. Dead worms were simply identified by their straight, flat appearance with absence of movements observed at the head and tail regions of the body. The percent mortality of worms was calculated for each extract concentration using the formula: the mortality index was determined as the total number of dead worms divided by the total number of worms per petri dish.

Statistical Analysis

Collected raw data was stored in a Microsoft Excel database system used for data management. The collected data were analyzed using IBM SPSS version 20.

Results

The present study suggests that the aqueous extract derived from the seeds of *Coriandrum sativum*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizome of *Zingiber officinale*, leaves of *Azadirachta indica*, whole plant of

Swertia chirata and leaves *of Prunus persica* exhibit promising adulticidal effects on adult *Haemonchus contortus.* The highest adulticidal effects were observed with aqueous extracts from the whole plant of *Swertia chirata* followed by Seeds of *Coriandrum sativum*, leaves of *Azadirachta indica*, bulbs of *Allium sativum*, seeds of *Carica papaya*, rhizomes of *Zingiber officinale and lowest in* leaves of *Prunus persica* against the *Haemonchus contortus* in goats (Table 1).

The adulticidal efficacy of the extracts was assessed by measuring the percentage of adult parasites killed at the different time intervals and concentrations. Dose and time dependent anthelmintic activity responses were observed with across all concentrations of seven selected plant extracts. Complete mortality of worms was observed 8 hours post exposures to the 400 mg/ml concentration of aqueous extracts of *Swertia chirata*. In closantel treated group, all worms were found dead after 2 hours of post exposure. However, no worms were found dead in any of selected medicinal plants aqueous extracts groups after 2 hours of post exposure. In the negative control group, all worms remained alive during the first 8 hours of the test. Furthermore, none of the worms showed any signs of revival of motility after being placed in lukewarm PBS for 30 minutes

Table 1: Percentage of mortality of adult *Haemonchus contortus* at different concentrations of aqueous extract, positive control (closantel, 1.25 mg/ml) and negative control (PBS) for 8hours post treatments

Treatment	Time (hr)	Treatment Group					
		NC	PC	T_1	T_2	T_3	T_4
Coriandrum sativum	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	6.67	10.00
	4hr	0.00	100.00	16.67	23.33	50.00	56.67
	6hr	0.00	100.00	23.33	33.33	66.67	76.67
	8hr	0.00	100.00	26.67	43.33	76.67	90.00
	Mean ± SE	0.00±0.00	80.00±20.00	13.33±5.68	20.00±8.76	40.00±15.60	46.67±17.89
Allium sativum	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	3.33	3.33
	4hr	0.00	100.00	6.67	16.67	30.00	50.00
	6hr	0.00	100.00	13.33	26.67	50.00	53.33
	8hr	0.00	100.00	20.00	30.00	60.00	66.67
	Mean ± SE	0.00±0.00	80.00±20.00	8.00±3.89	14.67±6.38	28.67±12.05	34.67±13.77
Carica papaya	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	3.33	13.33	26.67	46.67
	6hr	0.00	100.00	10.00	20.00	36.67	43.33
	8hr	0.00	100.00	16.67	26.67	46.67	53.33
	Mean ± SE	0.00±0.00	80.00±20.00	6.00±3.23	12.00±5.33	22.00±9.52	28.67±11.81
Zingiber officinale	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	0.00	10.00	20.00	33.33
	6hr	0.00	100.00	6.67	16.67	26.67	33.33
	8hr	0.00	100.00	13.33	23.33	36.67	43.33
	Mean ± SE	0.00±0.00	80.00±20.00	4.00±2.67	10.00±4.59	16.67±7.30	22.00±9.17
Azadirachta indica	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	3.33	3.33
	4hr	0.00	100.00	10.00	20.00	40.00	46.67
	6hr	0.00	100.00	16.67	30.00	56.67	63.33
	8hr	0.00	100.00	23.33	33.33	66.67	76.67
	Mean ± SE	0.00±0.00	80.00±20.00	10.00±4.59	16.67±7.15	33.33±13.62	38.00±15.58
Swertia chirata	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	10.00	13.33
	4hr	0.00	100.00	20.00	33.33	53.33	63.33
	6hr	0.00	100.00	26.67	43.33	76.67	86.67
	8hr	0.00	100.00	36.67	53.33	86.67	100.00
	Mean ± SE	0.00±0.00	80.00±20.00	16.67±7.30	26.0±11.08	45.33±17.40	52.67±19.79
Prunus persica	0hr	0.00	0.00	0.00	0.00	0.00	0.00
	2hr	0.00	100.00	0.00	0.00	0.00	0.00
	4hr	0.00	100.00	0.00	6.67	16.67	26.67
	6hr	0.00	100.00	3.33	13.33	20.00	23.33
	8hr	0.00	100.00	10.00	20.00	30.00	36.67
	Mean ± SE	0.00±0.00	80.00±20.00	2.67±1.94	8.00±3.89	13.33±5.87	17.33±4.41

Note: NC, Negative control; PC, Positive control; T_1, 50 mg/ml dose; T_2, 100 mg/ml dose; T_3, 200 mg/ml dose; T_4, 400 mg/ml dose

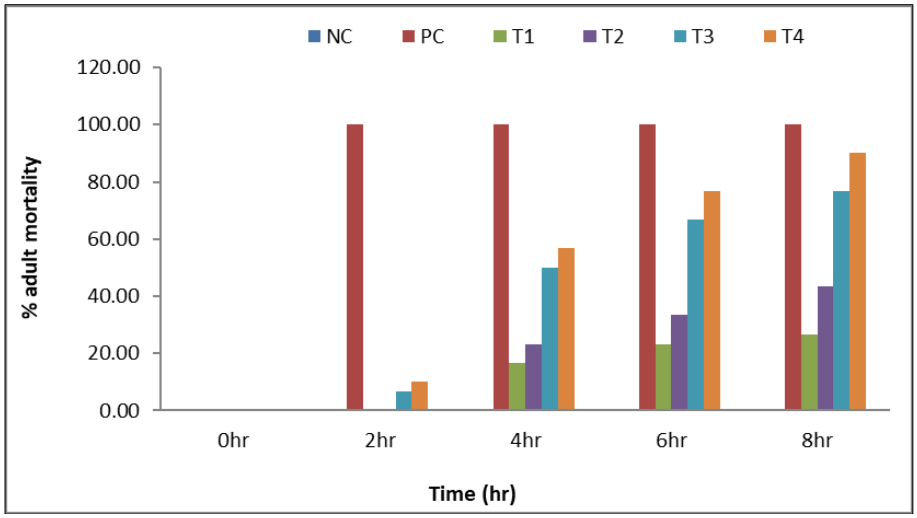

Fig 1: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1),100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of seeds of *Coriandrum sativum*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

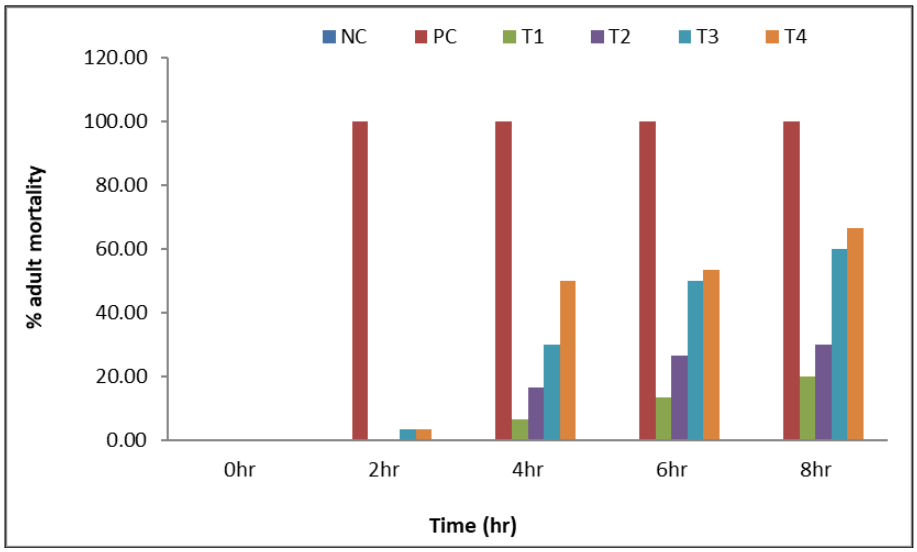

Fig 2: Percentage(%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1),100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of bulbs of *Allium sativum*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

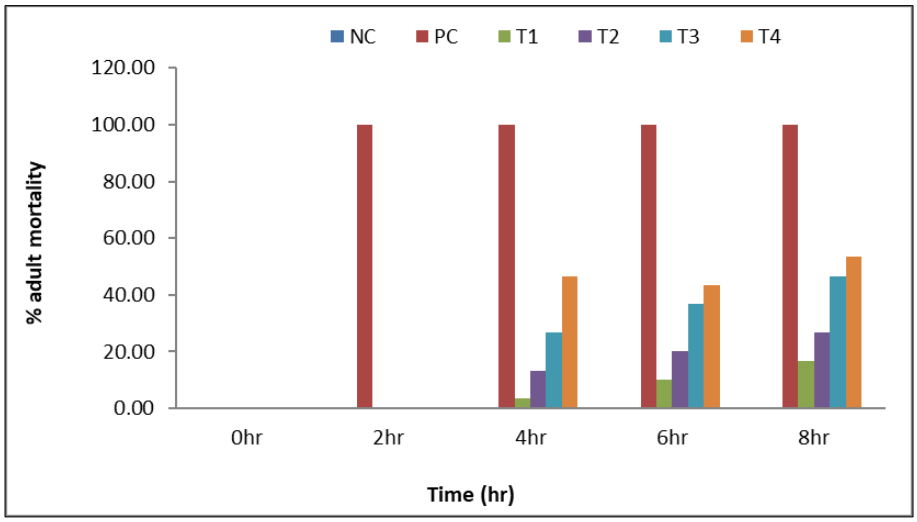

Fig 3: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1),100 mg/ml (T_2),200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of seeds of *Carica papaya*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

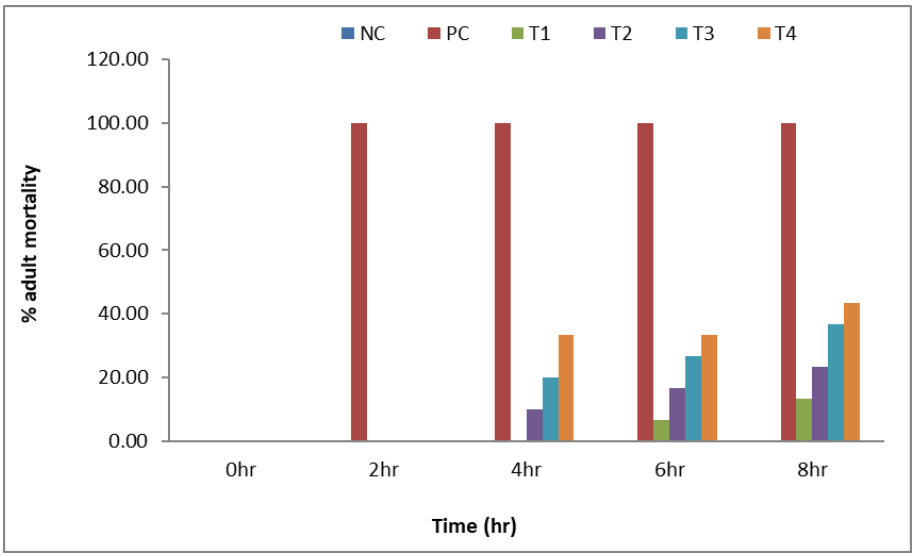

Fig 4: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1), 100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of rhizome of *Zingiber officinale*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

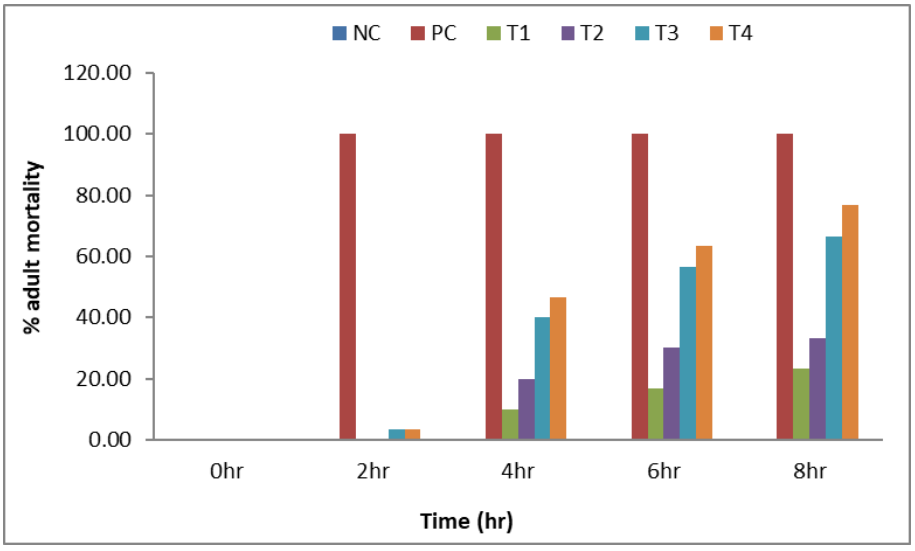

Fig 5: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1), 100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of leaves of *Azadirachta indica*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

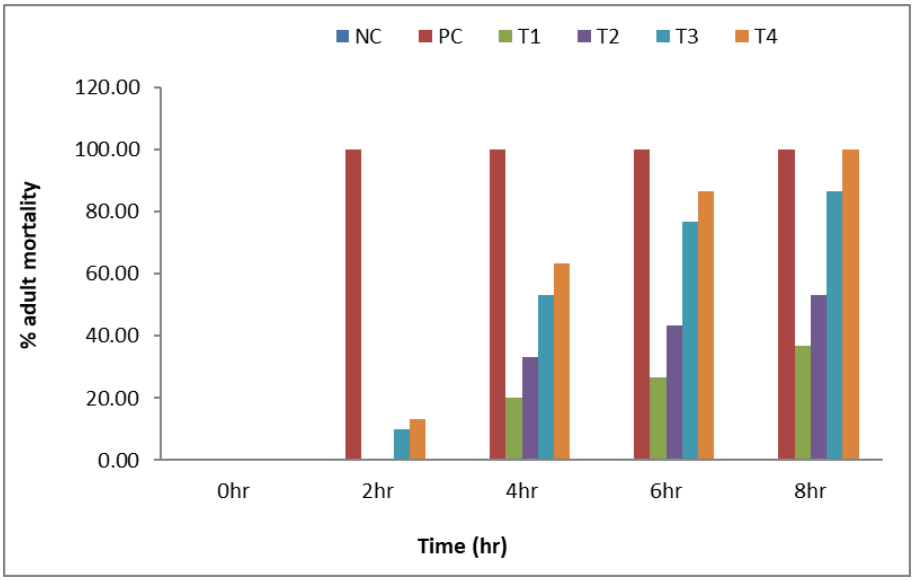

Fig 6: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1), 100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of whole plants of *Swertia chirata*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

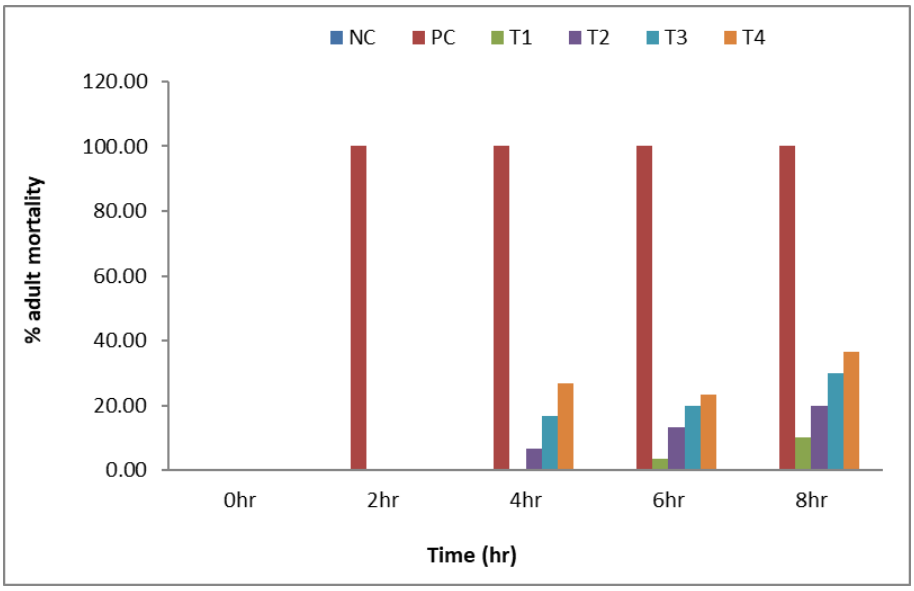

Fig 7: Percentage (%) mortality of adult *Haemonchus contortus* after 8 hours at concentration of 50 mg/ml (T_1), 100 mg/ml (T_2), 200 mg/ml (T_3) and 400 mg/ml (T_4) of aqueous extract of leaves of *Prunus persica*, closantel, 1.25 mg/ml (PC) and PBS, negative control (NC)

The aqueous extracts of *Coriandrum sativum* seeds at concentration of 50 mg/ml, 100 mg/ml 200 mg/ml and 400 mg/ml exhibited varying percentage of mortality in *Haemonchus contortus*. After 2 hours post exposure the mortality rates were 0%, 0%, 6.67% and 10% respectively. After 4 hours, the rates increased to 16.67%, 23.33%, 50%, and 56.67%. Subsequently, after 6 hours, the rates rose further to 23.33%, 33.33%, 66.67%, and 76.67%, and after 8 hours, they reached 26.67%, 43.33%, 76.67%, and 90% respectively (Figure 1).

The aqueous extracts of *Allium sativum* bulbs at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml exhibited varying percentage in mortality of *Haemonchus contortus.* After 2 hours post-exposure, the mortality rates were 0%, 0%, 3.33%, and 3.33%, respectively. After 4 hours post-exposure, the rates increased to 6.67%, 16.67%, 30%, and 50%. Subsequently, after 6 hours post-exposure, the rates further escalated to 13.33%, 26.67%, 50%, and 53.33%. Finally, after 8 hours of exposure, the mortality rates were 20.0%, 43.33%, 30%, and 66.67% respectively (Figure 2).

The aqueous extracts of *Carica papaya* seeds at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml exhibited varying percentage of mortality of *Haemonchus contortus.* After 2 hours post-exposure, the mortality rates were 0% in all the four concentrations. After 4 hours post-exposure, the rates increased to 3.33%, 13.33%, 26.67% and 46.67%. Subsequently, after 6 hours post-exposure, the rates further escalated to10%, 20%, 36.67% and 43.33%. Finally, after 8 hours of exposure, the mortality rates were16.67%, 26.67, 46.67% and 53.33% respectively (Figure 3)

The aqueous extracts of *Zingiber officinale* rhizome at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml showed 0% mortality of *Haemonchus contortus* after 2 hours of exposure. After 4 hours, the mortalities were 0%, 10%, 20%, and 33.33% respectively. This increased to 6.67%, 16.67%, 26.67%, and 33.33% after 6 hours, and further to 13.33%, 23.33%, 36.67%, and 43.33% after 8 hours of exposure (Figure 4).

Aqueous extracts of *Azadirachta indica* leaves at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml, showed varying percentages of mortality in *Haemonchus contortus.* After 2 hours of exposure, the mortality rates were 0%, 0%, 3.33%, and 3.33% respectively. After 4 hours, the rates increased to 10%, 20%, 40%, and 46.67% respectively. Subsequently, after 6 hours, the rates rose to 16.67%, 30%, 56.67%, and 63.33%, and after 8 hours, they further increased to 23.33%, 33.33%, 67.67%, and 76.67% respectively (Figure 5).

The aqueous extracts derived from whole plant of *Swertia chirata* at the concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml varying percentages of mortality in *Haemonchus contortus.* After 2 hours of exposure, the mortality rates were 0%, 0%, 10% and 13.33% respectively. After 4 hours, these percentages increased to 20%, 33.3%, 53.33%, and 63.33%, respectively. Subsequently, after 6 hours, the mortality rates rose to 26.67%, 43.33%, 76.67%, and 86.67%, and finally reached 36.67%, 53.33%, 86.67%, and 100% after 8 hours of exposure (Figure 6).

Aqueous extracts from Prunus *persica* leaves, at concentrations of 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml showed varying degrees of effectiveness against *Haemonchus contortus* over different exposure durations. After 2 hours post exposures all concentrated exhibited 0% mortality. However, after 4 hours post exposure the mortalities were 0%, 0%, 6.67%, 16.67% and 26.67% respectively. This trend continued with increased exposure time: at 6 hours, mortalities were 3.33%, 13.33%, 20% and 23.33%, at 8 hours they further increased to, 10%, 20, 30% and 36.67% respectively (Figure 7).

Discussion

The challenge of anthelmintic resistance, toxicity, and growing apprehension about drug residue in animal products has results into a renewal of interest in the use of plant based remedies (Zenebe *et al*., 2017) [13].*In vitro* assays employing the free living stages of parasitic nematodes provide a valuable method for assessing the anthelmintic activity of new plant compounds (Asase *et al*., 2005) [14].Furthermore, *Haemonchus contortus* has been proved as a excellent test worm for *In vitro* studies due to its prolonged survival in PBS and its widespread prevalence worldwide. This abomasal helminth has recently been utilized *In vitro* by

other workers (Zenebe *et al*., 2017) [13].Various extracts derived from plants exhibited capabilities for development as anthelmintic remedies (Ndlela *et al.*, 2021) [8].

In vitro findings demonstrated significant anthelmintic effect from *Coriandrum sativum* seeds, *Allium sativum* bulb, *Carica papaya* seeds, *Zingiber officinale* rhizome, *Azadirachta indica* leaves, *and Swertia chirata* whole plant and *Prunus persica* leaves on *H. contortus* showing a dose-dependent pattern and the effect was comparable to that of closantel. Moreover, the study indicated that mortality of adult *Haemonchus contortus* with escalating dosages and exposure duration following treatment with tested plant extracts. These results align with prior researchers by Avinash *et al*. (2017) [15], in which mortality effect of plant materials was depended on the dosage and exposure durations.

The aqueous extracts of various plant extracts exhibited differing percentage of mortality in *Haemonchus contortus* in the current study. This variance in mortality percentage among could be attributed to variations in the concentrations of secondary metabolites present in their extracts (Mumed *et al*., 2022) [16].

Eguale *et al*. (2007) [17] reported that aqueous extracts of *C. sativum* possess significant anthelmintic activity against nematode parasites, particularly *Haemonchus* spp. *In vitro.* Krstin *et al.* (2018) [18] reported that antiparasitic activity of *A. sativum* can be due to the sulfur containing compounds like ajoene and allicin. These compounds have potentially form disulfide bonds with free thiol groups, thereby inhibiting enzymes or other proteins crucial for parasites survival. Lee (1996) [19] observed that *ethanolic* extract of *A. sativum* hampers the motility of *H. contortus* by exerting a destructive and inhibitory effect on the enzyme acetyl cholinesterase. This enzyme plays a vital role in hydrolyzing acetylcholine a neurotransmitter involved in cholinergic synaptic transmission.

Benzyl isothiocyanate is main constituents found in papaya seeds has exhibits anthelmintic properties as reported by Rumiyati (2006) [20]. Yadav and Tangpu (2006) [21] conducted a study where they examined the efficacy of an aqueous extract derived from Carica papaya seeds against *Ascaris lumbricoides* and *Ascaridia galli*. Their findings demonstrated significant anthelmintic properties in the extract, suggesting its effectiveness in combating these parasitic infections.

Abdullahi *et al.* (2017) [22] reported several phytochemical constituents in *Zingiber officinale* that exhibit anthelmintic properties including flavonoids, tannins, saponins, phenols and terpenoids. Dubey *et al.* (2010) [23] observed that the aqueous extract of the rhizome of *Zingiber officinale* exhibited anthelmintic activity in Pheretima *posthuma.* They found that paralysis occurred within 32 to 34 minutes, 28 to 31 minutes, and 25 to 29 minutes, respectively, at concentrations of 25, 50, and 100 mg/ml. Subsequent death occurred at 86 minutes, 78 minutes, and 65 minutes, respectively, at the same concentrations.

Qiao, *et al.* (2013) [24] suggested that the anthelmintic properties of neem may be attributed to the presence of an active alkaloid, azadirachtin. This compound is thought to disrupt the central nervous system of parasites by inhibiting excitatory cholinergic transmission and partially blocking calcium channels, ultimately leading to the expulsion of parasites from the host body. Saktia *et al.* (2018) [25] found that an aqueous leaf infusion of *A. indica* at doses of 6% demonstrated *In vitro* anthelmintic activities against *H. contortus* by reducing egg hatch and adult worm motility. This suggests its potential as a bio-anthelmintic against *H. contortus.*

Khanal *et al.* (2014) [26] reported that *Swertia chirata* is also effective against intestinal worms. Iqbal *et al*. (2006) [27] reported that *In vitro* studies on anthelmintic properties of Swertia *chirata* have demonstrated that at a concentration of 25 mg/ml the crude aqueous (CAE) derived from S. *chirata* whole plant exhibited an anthelmintic effect against live *Haemonchus contortus.*

The leaves of *Prunus persica* possess anthelmintic was reported by Aziz and Rahman (2013) [28]. Kumar *et al.* (2014) [29] investigated the anthelmintic activity of crude powder, aqueous, diethyl ether and methanol extracts from *Prunus persica* leaves. They tested concentration of 0.25%, 0.5%, 1% and 2% against adult *Haemonchus contortus* finding that all extracts showed 100% efficacy after 24 hour. The variations observed among these studies could stem from difference in the solvents utilized for extraction. Research has demonstrated that organic solvent extracts exhibit higher biological activity compared to aqueous extracts (Chanda and Parekh, 2007) [30].

Due to the biotransformation of drugs potential interactions with food materials, and variations in absorption, the results obtained through *In vitro* methods may not accurately reflects *in vivo* activity. Therefore, it is essential to confirm these results in through *in vivo* evaluation (Zenebe *et al.*, 2017) [13].

Conclusions

The study findings suggest that plants could serve as promising alternative anthelmintic remedies for treating the haemonchosis. Essential steps toward establishing their therapeutic utility include identifying the active compounds in plant extracts, assessing their *in vivo* bioavailability, and studying their toxicity.

Conflicts of Interest: All authors declare no conflict of interest.

Acknowledgement

The authors are highly thankful to the Dean, College of Veterinary & Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut-250110, INDIA for providing the necessary facilities to carry out the research work.

References

1. Flay KJ, Hill FI, Muguiro DH. A review: *Haemonchus contortus* infection in pasture-based sheep production systems, with a focus on the pathogenesis of anaemia and changes in haematological parameters. Animals. 2022;12(10):1238.
2. Shamim A, Sajid MS, Khan MN, Imran M, Saqib M. Peptides isolation from crude somatic antigens of *Haemonchus contortus* through SDS-PAGE. Indian J Anim Res. 2018;52:914-916.
3. Kelkele FA, Tolossa YH, Kassa GM. Experimental infection of Ethiopian highland sheep by different infective doses of *Haemonchus contortus* (L3): haematological and parasitological parameters serum protein concentrations and clinical responses. Ethiop Vet J. 2012;16(1):41-57.

4. Sisay G, Nuraddis I, Belay A, Tadesse E. *In vitro* evaluation of anthelmintic activities of crude extracts of selected medicinal plants against *Haemonchus contortus* in Alemgena Wereda. Acta Parasitol Glob. 2012;3:20-7.
5. Singh D, Swarnkar CP, Khan FA. Anthelmintic resistance in gastrointestinal nematodes of livestock in India. Vet Parasitol. 2002;16:115-30.
6. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. J Pharmacogn Phytochem. 2017;6:32-36.
7. Kumsa B, Hagos Y. Antihelmintic medicinal plants used for animals in Ethiopia: A review. J Phytopharmacol. 2020;9(4):274-280.
8. Ndlela SZ, Mkwanazi MV, Chimonyo M. *In vitro* efficacy of plant extracts against gastrointestinal nematodes in goats. Trop Anim Health Prod. 2021;53:1-8.
9. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor M, Waller PJ. 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. Vet Parasitol. 1992;44(1-2):35-44.
10. Rahman A, Collins H. Changes in live weight gain, blood constituents and worm egg output in goats artificially infected with a sheep-derived strain of *Haemonchus contortus*. Br Vet J. 1990;146(6):543-550.
11. Taylor A, Coop L, Wall L. Veterinary Parasitology. 3rd ed. Blackwell Publishing; 2007. Vol. 33.
12. Sharma LD, Bhaga HS, Srivastava PS. *In vitro* anthelmintic screening of indigenous medicinal plants against *Haemonchus contortus* (Rudolphi, 1803) Cobbold, 1898 of sheep and goats. Indian J Anim Res. 1971;5:33-38.
13. Zenebe S, Feyera T, Assefa S. *In vitro* anthelmintic activity of crude extracts of aerial parts of Cissus quadrangularis L. and leaves of Schinus molle L. against *Haemonchus contortus*. Biomed Res Int; c2017. p. 1-6.
14. Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MS. Ethnobotanical study of some Ghanaian antimalarial plants. J Ethnopharmacol. 2005;99(2):273-9.
15. Avinash B, Santhi-Priya C, Kondaiah PM. *In vitro* evaluation of anthelmintic activity of Nicotiana tabacum extracts against *Haemonchus contortus*. Int J Sci Environ Technol. 2017;6(1):458-465.
16. Mumed HS, Nigussie DR, Musa KS, Demissi AA. *In vitro* anthelmintic activity and phytochemical screening of crude extracts of three medicinal plants against *Haemonchus contortus* in sheep at Haramaya Municipal Abattoir, Eastern Hararghe. J Parasitol Res. 2022:1-8.
17. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. *In vitro* and *in vivo* anthelmintic activity of crude extracts of Coriandrum sativum against *Haemonchus contortus*. J Ethnopharmacol. 2007;110:428-33.
18. Krstin S, Sobeh M, Braun M, Wink M. Anti-parasitic activity of Allium sativum and Allium cepa against Trypanosoma b. brucei and Leishmania tarentolae. Medicines. 2018;5(2):37.
19. Lee DL. Why do some nematode parasites of alimentary tract secrete acetylcholinesterase? Int J Parasitol. 1996;26(5):499-508.
20. Rumiyati S. Effect of protein fraction of Carica papaya L. leaves on the expression of p53 and Bcl-2 in breast cancers cells line. Maj Farm Indones. 2006;17(4):170-6.
21. Yadav AK, Tangpu V. *In vitro* anticestodal evaluation of some medicinal plants used by Naga traditional healers. Pharmacologyonline. 2006;3:90-95.
22. Abdullahi H, Karunakaran R, Sankar AU, Aye KM. Anti-inflammatory effects of Zingiber officinale on Sprague Dawley rats. Asian J Pharm Clin Res. 2017;10:353-355.
23. Dubey RD, Verma S, Rane D, Wani VK, Pandey AK, Paroha S. Comparative studies of anthelmintic activity of Zingiber officinale and Cassia tora. Int J Chem Pharm Sci. 2010;1(1):1-4.
24. Qiao J, Zou X, Lai D, Yan Y, Wang Q, Li W, Deng S, Xu H, Gu H. Azadirachtin blocks the calcium channel and modulates the cholinergic miniature synaptic current in the central nervous system of Drosophila. Pest Manag Sci. 2013;70:1041-1047.
25. Saktia AA, Kustantinah, Nurcahyoc RW. *In vitro* and *in vivo* anthelmintic activities of aqueous leaf infusion of Azadirachta indica against *Haemonchus contortus*. Trop Anim Sci J. 2018;41(3):185-190.
26. Khanal S, Shakya N, Nepal N, Pant D. Swertia chirata: The Himalayan herb. Int J Appl Sci Biotechnol. 2014;2(4):389-92.
27. Iqbal Z, Lateef M, Khan MN, Jabbar, Akhtar MS. Anthelmintic activity of Swertia chirata against gastrointestinal nematodes of sheep. Fitoterapia. 2006;77:463-5.
28. Aziz S, Rahman H. Biological activities of Prunus persica L. Batsch. J Med Plants Res. 2013;7(15):947-951.
29. Kumar RR, Vatsya SS, Yadav CL. *In vitro* anthelmintic activity of Prunus persica against *Haemonchus contortus*. Prog Res. 2014;9(Special):466-468.
30. Chanda SV, Parekh J. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 2007;31:53-58.