Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Screening of Anthelminthic Plants against *Haemonchus Contortus –In Vitro* Study

M. Mohanapriya*

Department of Zoology, Adhiyaman Arts and Science College for Women, Uthangarai, Krishnagiri District, Tamilnadu, India.

ABSTRACT

Livestock plays a major role in economic growth of India. In other hand, livestock also improved the economic status of the rural people. On global index, *Haemonchous contortus* is the gastrointestinal helminth, which causes haemonchosis an important disease leads the greatest economic loss in small ruminants (sheep and goats) husbandry. The disease results in retarded growth, weight loss, disorder fertility, less milk production and increased mortality rate. Many synthetic drugs used to controlling the infestation and to increase the livestock productivity in small ruminants include benzimidozoles (BZ), ivermectins, imidazothiazole.Since, synthetic anthelmintic drugs create resistance and side effects there are a need to search for a new alternative source to control the nematode. The plants always have been a rich source of phytochemicals and acts as storage house for secondary metabolites, hence used as alternative anthelmintic drug. Thus, this study focused to screen anthelmintic plants viz. *Acacia catechu*, *Cassia angustifolia*, *Eclipta prostrate*, *Piper longum* against the parasite, *Haemonchous contortus* using gross visual observation on the motility of the worm. The result of the motility of *Haemonchous contortus* in three concentrations (3%, 5% and 10%) of plant extracts for different periods of exposure was studied. *Piper longum* was found to highly effective in reducing the motility of the parasites followed by other plant extracts. Screening and proper evaluation of the medicinal plants could be substitute for chemotherapeutic anthelmintic drugs.

KEYWORDS: *Haemonchous*, Anthelmintic, Phytochemicals, Motility, *Piper longum.*

***Corresponding Author:**

Dr.M.Mohanapriya

Assistant Professor, Department of Zoology,

Adhiyaman Arts and Science College for Women,

Uthangarai, Krishnagiri District, Tamilnadu, India.

E Mail id: priyamohan729@gmail.com

Phone No: 9176555077

1. INTRODUCTION

The small ruminants have gained more attention in world-wide due to its great economic importance. In particular, sheep and goats play a major role in the socio economic status of rural people and value addition in the economy of country. The valuable contribution of the sheep and goats includes in terms of production of animal protein (milk and meat) drought power in the highlands, skins and fiber production and food security to the poor people in rural areas[1]. Sheep is considered as most important major sources of wool and meat production. But sheep are commonly affected mostly by helminthiasis, which leads to heavy productivity loss of livestock[2].

Gastro Intestinal (GI) parasitic infections are a world-wide problem for flock management breeders. Among them *Haemonchous contortus* commonly known as baber's pole worm is the predominant, blood sucking nematode, highly pathogenic that infects small ruminants[3]. The acute *H. contortus* infections are hemorrhagicanemia, hypoproteinemia, and oedema, diarrhoea results in fluid loss and dehydration leads to hypovolemic shock[4].

Epidemiological studies shows that infection rate of *H. contortus* were high in sheep when compared to goats. The infestation between the sexes was recorded high in female sheep 48.64% and 20.83% respectively in males. The histological examination of tissue samples of infected fourth stomach of sheep, the result indicated the presence of necrosis and thickening layer of muscle and hyperplasia, infiltration by polymorph inflammatory cells and fully fibrosis for some glandular cells[5].

Haemonchosis is mainly controlled by synthetic drugs[6]. Benzimidazoles, imidothiazoles, tertahydro pyrimidine and macrocycliclactones are the most common synthetic anthelmintic deworming drugs possess broad-spectrum activity and safety. Development of resistance against all anthelmintic drug classes has been reported by several researchers throughout the world and anthelmintic resistance is considered as most serious threat to the gastro intestinal nematodes of small ruminants[7]. Addition, to the development of high-level resistance of synthetic anthelmintic drugs, it's also results in toxicity and side effects in the animal[8]. However, resistance too many of these anthelmintic is now wide spread and therefore, there is a need to find alternative source to control the parasites effectively. There is an emergence need to develop novel and non – chemical approaches for the nematode control.

The medicinal plants and its extracts have promising anthelmintic activity and broadly containing a rich source of phytochemical constituents to combat helminthic diseases[9]. The use of crude medicinal plants assures great effect towards parasitic nematode without any side effects[10].

Acacia catechu willd belongs to family: Fabaceae and subfamily: Mimosoideae is widely distributed in different parts of India. It is commonly known as cutch tree or catechu tree. The bark contains bioactive compounds such as glycosides (poriferasterol, acylglucosides), tannins (gallic acid, phlobatannins), Sugars (D-galactose, d- rhamnose and l-arabinose)[11]. Catechu is bitter, acrid, thermogenic, digestive, appetizer, aphrodisiac, hepato – protective, haemostatic, vulnerary, anthelmintic, depurative and tonic[12].

Cassia angustifolia and it belongs to Leguminosae family. It is popularly known as Indian senna[13]. The plant is found in India and other tropical countries. Different parts of the plant such as leaves, seed and root are recorded for their medicinal value. The leaves contain more active pharmacological constituents responsible for hepatoprotective and anti-inflammatory activity. The whole plant is employed in the treatment of impetigo, ulcers, helmenthiasis and as a purgative, hepatoprotective, anti-inflammatory, antimicrobial and antioxidant activity[14,15].

Eclipta prostrata L. belongs to Asteraceae family, common name of the plant is false daisy. The herb is native to tropical and subtropical regions of the world[16]. The functional groups such as hydroxyl, alcohols, phenols, alkanes, alkenes, ether, ketone, amine, aromatic compounds, nitrile compounds, nitro compounds and phosphorus are present in the leaves would be responsible for various medical characteristics and properties[17]. The plant is well documented for its pharmacological activities such as anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective, antimicrobial, antinociceptive, analgesic, antiviral, immunomodulatory activity, etc[18,19].

Piper longum Linn.belong to family piperaceae[20]. It is commonly referred as long pepper. The active compound present in the plant is piperine. The alkaloid compound is mainly responsible for anthelmintic activity[21]. The roots of *Piper longum* possessing a high therapeutic value includes carminative, hepatoprotective, stomachic, abortificient, haematinic, diuretic, digestive and as a general tonic. It also cures inflammation of the liver, pains in the joint, lumbago, snakebite, scorpion-sting and night-blindness[22].

Hence, in present investigation the following plants such as *Acacia catechu*, *Cassia angustifolia*, *Eclipta prostrate*, *Piper longum* were selected and subjected to motility test to find which plant possess more anthelmintic activity.

2. MATERIALS AND METHODS

2.1 Collection of the parasites

The abomasum of infected sheep was collected from the slaughter house in Chennai and brought to the laboratory. Adult female *Haemonchus contortus* were isolated from the abomasal contents and washed thoroughly in physiological saline and used for subsequent studies.

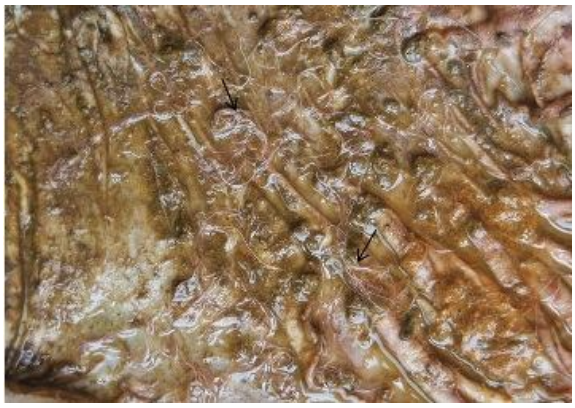

Figure: 1 Abomasum of infected sheep showing *H. contortus* indicated using arrows

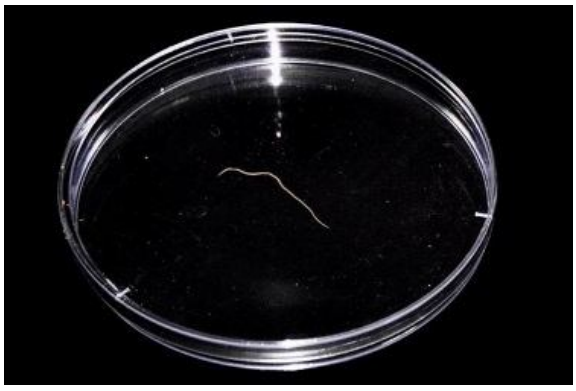

Figure: 2 *H. contortus*-entire worm

2.2 Preparation of plant extracts

The aqueous extract of bark of *Acacia Catechu* (*Ac*BaE), leaves of *Cassia angustifolia* (*Ca*LE) and *Eclipta prostrate* (*Ep*LE), root of *Piper longum* (*Pl*RE) were prepared. A stock solution of 20 % concentrations of each plant extract was prepared using standard procedure[23]. These stock solutions were then serially diluted to 3 %, 5 % and 10 % concentrations with the *in vitro* maintenance medium Hedon-fleig solution.

2.3 In vitro study

2.3.1 Gross visual observation on the motility of the parasites incubated in the plant extracts

Sixty millilitres of the extracts was individually distributed in airtight sterile plastic containers. The live and active parasites (n=10) were incubated in various concentrations of the extracts. The activity of the parasite was checked at various intervals (5, 15, 30 m, 1, 2, 4, 6 and 8 h) of incubation. Simultaneously, control was also maintained in the medium devoid of the plant extracts. Based on the motility of the parasites, the observations were categorized as very active (++++), moderately active (+++), slightly active (++), sluggish (+) and dead (-). Based on the motility of the parasite for 12 h of incubation, three different sub-lethal concentrations were selected. The parasites were incubated for 2, 4, and 8 h in the various sub-lethal concentrations of the plant extracts for further *in vitro* studies.

3. RESULTS AND DISCUSSION

The present investigation elucidated the *in vitro* effects of various plant extracts viz., *Ac*BaE, *Ca*LE, *Ep*LE and *Pl*RE on the viability and motility of *Haemonchus contortus*. Based on the observation on the motility and viability of treated parasites, one most potent anthelmintic plant was identified to treat against *H. contortus*.

3.1 Gross visual observation on the motility of the parasites incubated in various plant extracts

The results of the gross visual evaluation of the motility of *H. contortus* in three concentrations (3 %, 5 % and 10 %) of aforesaid plant extracts for different periods of exposure are given Table 1. *Pl*RE was found to be highly effective in reducing the motility of the parasites; whereas *Ac*BaE, *Ca*LE and *Ep*LE were moderately effective in reducing the viability and motility of *H. contortus.*

Table No. 1: "Chronological observation on the motility of *H. contortus* incubated in various concentrations of indigenous plants"

S.No	Indigenous Plants	Conc. %	5(m)	15(m)	30(m)	1h	2h	4h	6h	8h
1.	*Acacia catechu*	3	++++	++++	++++	++++	+++	++	+	+
		5	++++	++++	++++	+++	++	+	+	+
		10	+++	+++	+++	++	++	+	+	+
2.	*Cassia angustifolia*	3	++++	++++	++++	+++	++	++	+	+
		5	++++	+++	++	++	++	+	+	+
		10	+++	++	++	++	+	+	+	-
3.	*Eclipta prostrate*	3	++++	++++	+++	+++	+++	++	+	+
		5	++++	++++	+++	+++	++	+	+	+
		10	+++	+++	++	++	++	+	+	+
4.	*Piper longum*	3	++++	+++	++	+	+	+	-	-
		5	+++	++	+	+	+	-	-	-
		10	+++	++	+	+	+	-	-	-
	Untreated worm		++++	++++	++++	+++	+++	+++	+++	++

++++ Very active; +++ Active; ++ Moderately active; + sluggish; - Dead

The elucidation of anthelmintic activity *in vitro* assays is mostly relied on the visual observation on the motility and viability of drug-treated parasites. *Pl*RE significantly inhibited the motility of *H .contortus*, the inhibitory effect being concentration and time dependent. Several phytotherapeutic agents have been reported to inhibit the motility response of helmintic parasites. *In vitro* viz. adult motility test (AMT), egg hatch assay (EHA) and larval paralysis test (LPT) treatment with *Nicotiana tabacum* was conducted against gastrointestinal nematode, *Haemonchuscontortus* of goats[24].

In vitro assays such as gross visual motility, glucose uptake, glycogen and lactic acid contents and AChE activity were evaluated against *Fasciola gigantic* treated with plant extracts *Carica papaya* (papeeta), *mallotus philippinensis* (kamala) and *Azadirachta indica* (neem). It was that *M. philippinensis* and *A. indica* extracts possess inhibitory effects on the spontaneous muscular activity of *F. gigantica*[25]. The GI adult parasite most important characteristic feature is their motility, which helps in survive of the parasite by adhering in host's gut and also helps them in their feeding and reproduction process. Motility occurs by the contraction of body musculature, which is well developed in nematodes and considerable amount of energy is required for normal functioning of muscles. Thus, by arresting the motility is due to muscular paralysis, which brought by the deleterious effects on the structure and/or physiological functions of the parasite[26].The inhibitory

effect of the plant possessing anthelmintic property on the motility of the parasite is due to direct action on the morphology of the parasite includes cuticle and gut epithelium. The bioactive compound present in the anthelmintic plant which may also interfere with the biochemical and physiological functions of the parasite causes death of the parasite.

4. CONCLUSION

Based on the visual observation, *Piper longum* root extract significantly inhibited the motility of the parasites. Therefore, it is further subjected to structural analysis by using different sub-lethal concentrations to study the conspicuous morphological and structural damages in the parasite.

5. REFERENCES

1. Hafiz Muhammad Qasim. Haemonchosis in Small Ruminants: A Review. Scholar's Advances in Animal and Veterinary Research. 2015; 2(2): 76-89.
2. Jothi Karumari R, Vijayalakshmi K. Preliminary phytochemical analysis and anthelmintic activity of the aqueous extract of *Ocimum sanctum* (Linnaeus, 1767) leaves (Green and Black) against *Cotylophoron cotylophorum* (Fischoeder, 1901). International Journal of Pharma and Bio Sciences. 2014; 5 (2): 580-587.
3. Saeed El-Ashram, Ibrahim Al Nasr, Rashid mehmood, Min Hu, Li He, Xun Suo. *Haemonchus contortus* and Ovine host: A Retrospective Review. International Journal of Advanced Research. 2017; 5(3): 972-999.
4. Mani Saminathan, Arumugam Gopalakrishnan, Annamalai Latchumikanthan, Arockiasamy Arun Prince Milton, Manivasagam Aravind, Kuldeep Dhama and Rajendra Singh. Histopathological and parasitological study of blood-sucking *Haemonchuscontortus*infection in sheep. Advances in Animal and Veterinary Sciences. 2015; 3(2): 99-108.
5. Mohammad Hadi Mohammad AL-Hasnawy. Prevalence and pathogenicity of Haemonchosis in sheep and goats in Hilla city/Iraq. Journal of Babylon University/Pure and Applied Sciences. 2014; 7(22): 1989-1999.
6. Sathish Kumar S and Veerakumari L. Effect of ethanol extract of *Syzygium aromaticum* on fumarate reductase and succinate dehydrogenase of *Haemonchus contortus.* Journal of Global Trends in Pharmaceutical Sciences.2017; 8(4): 4648-4655.
7. Yitayew Demessie, Zewdu Seyoum, Kalkidan Getnet and Dagmawi Yitbarek.Anthelmintics resistance against gastrointestinal nematodes of sheep: A Review. World Journal of Agricultural Sciences. 2016; 12 (4): 245-253.

8. Manoj Dhanraj K and Veerakumari L. Effect of ethanol extract of *Areca catechu* on fumarate reductase and succinate dehydrogenase of *Cotylophoron cotylophorum.* International Journal of Research and Development in Pharmacy and Life Sciences.2016; 5(3): 2117-2123.
9. Rasika Kumarasingha, Sarah Preston, Tiong-Chia Yeo, Diana SL Lim, Chu-Lee Tu, Enzo A Palombo, Jillian M Shaw, Robin B Gasser and Peter R Boag. Anthelmintic activity of selected ethno-medicinal plant extracts on parasitic stages of *Haemonchus contortus.* Parasites & Vectors. 2016; 9: 187.
10. Bauri RK, Mary NishaTigga and Singray Saleeb Kullu. A Review on use of medicinal plants to control parasites. Indian Journal of Natural Products and Resources. 2015; 6(4): 268-277.
11. Monu, Milind Parle, Renu Kadian and Kalish Sharma. Phytopharmacology of *Acacia Catechu willd:* A Review. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(11): 1380-1389.
12. Mradu Gupta, Tuhin Kant Biswas, Shyamali Saha and Pratip Kumar Debnath. Therapeutic utilization of secretory products of some Indian medicial Plants – A Review. Indian Journal of Traditional Knowledge. 2006; 5(4): 569-575.
13. Pragati Khare, Kamal Kishore, Dinesh Kumar Sharma. A study on the standardization parameters of *Cassia angustifolia*. Asian Journal of Pharmaceuical and Clinical Research. 2017; 10(7):329-332.
14. Shoba Sundaramoorthy, Shylaja Gunasekaran, Sathiavelu Arunachalam, Mythili Sathiavelu. A Phytopharmacological Review on *Cassia* Species. Journal of Pharmaceutical Science and Research. 2016; 8(5): 260-264.
15. Shivjeet Singh, Sandeep Kumar Singh, Ashutosh Yadav. A Review on *Cassia* species: pharmacological, traditional and medicinal aspects in various Countries. American Journal of Phytomedicine and Clinical Therapeutics. 2013; 1(3): 291-312.
16. Somnath D Bhinge, Maheshwar G Hogade, Chetan Chavan, Mahesh Kumbhar, Vanita Chature. In Vitro Anthelmintic activity of herb extract of *Eclipta prostrate* L. against *Pheretima posthuma*. Asian Journal of Pharmaceutical and Clinical Research. 2010; 3(3): 229-230.
17. Kamble VM and Pawar SG. Characterization of *Eclipta Prostrata* (L.) L. leaves by FTIR Spectroscopy Method, CHNS and ICP-MS analysis techniques. International Research Journal of Biological Sciences. 2017; 6(2): 30-35.

18. Rownak Jahan, Abdullah Al-Nahain, Snehali Majumder and Mohammed Rahmatullah. Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae). International Scholarly Research Notices. 2014; 1-22.
19. Swati Mishra, Monalisa Jena, Mishra SS. Evaluation of anticonvulsant and muscle relaxant activities of *Eclipta alba* using animal models. Indo American Journal of Pharmaceutical Research. 2014; 4(3): 1397-1401.
20. Ravindra G Mali and Antia A Mehta. A Review on anthelmintic plants. Natural Product Radiance. 2008; 7(5): 446-475.
21. Bauri RK, Mary Nisha Tigga and Singray Saleeb Kullu. A Review on Use of Medicinal Plants to Control Parasites. Indian Journal of Natural Products and Resources. 2015; 6(4): 268-277.
22. Krutika Joshi, Kalpesh Panara, Nishteswar K and Suhas Chaudhary. Cultivation and pharmacological profiles of root of *Piper longum* Linn. An International Journal of Pharmaceutical Sciences. 2013; 4(1): 3617-3627.
23. Nama HS and Bhatnagar B. Laboratory evaluation of cercaricidal properties of certain plant extracts. Indian Journal of Parasitology. 1990; 14(1): 79-82.
24. Sushmita Sastya, Rajeev Ranajan Kumar and Stuti Vatsya. Evaluation of anthelmintic efficacy of *Nicotiana tabacum* against Gastrointestinal Nematodes of Goats. International Journal of Current Microbiology and Applied Sciences. 2017; 6(10): 780-789.
25. Kushwaha DS, Kumar D, Tripathi HC and Tandan SK. Effect of some indigenous medicinal plant extracts on *Fasicola giganticain in vitro.* Indian Journal of Animal Sciences.2004; 143-146.
26. Available from: URL:http://shodhganga.inflibnet.ac.in/bitstream/10603/173943/7/chapter%205.pdf