Available online at www.ijpsdronline.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(3): 259-262

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

In vitro Studies on the Effect of *Prosopis cineraria* On the Motility and Acetylcholinesterase of *Cotylophoron cotylophorum*

C. Manigandan, L. Veerakumari*

P.G. and Research Department of Zoology, Pachaiyappa's College, Chennai-30, Tamil Nadu, India

ABSTRACT

Paramphistomosis caused by the paramphistome *Cotylophoron cotylophorum* constitute a major group of disease in domestic ruminants. Chemical control of helminths coupled with improved management has important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening of medicinal plants for their anthelmintic activity. In the present investigation, the anthelmintic efficacy of bark of *Prosopis cineraria* was assessed based on its effect on the motility and on the enzyme acetylcholinesterase (AChE) of *Cotylophoron cotylophorum*. The flukes were exposed to five different concentrations (1, 2, 3, 4 and 5%) of ethanol extract of bark of *Prosopis cineraria* (*Pc*EE) for 2, 4 and 8 h. The motility response of the drug-treated parasites was quantitatively measured with the aid of Electronic Micromotility Meter (EMM). The electronic measurement of the motility of the drug-treated parasites clearly indicates the direct impact of the drugs on the motility of the parasite. Maximum level of inhibition of the motility response of the parasites was observed at 5% concentration after 8 h of exposure of *Pc*EE. AChE was assessed. *Pc*EE significantly inhibited the activity of AChE. As, AChE plays an important role in neurotransmission, inhibition of AChE results in muscular paralysis of parasites which loose its "biochemical hold-fast" and gets eliminated from the host. *Pc*EE possesses a remarkable anthelmintic activity against *C. cotylophorum*. It may serve as an alternative for anthelmintic chemotherapeutic agents and could be used in a safe and eco friendly manner. However, this study warrants further *in vivo* studies for practical utility.

Keywords: *Cotylophoron cotylophorum, Prosopis cineraria,* Acetylcholinesterase (AChE), Electronic Micromotility Meter (EMM).

INTRODUCTION

Paramphistomosis is one of the major pathogenic diseases in domestic animals and responsible for heavy economic loss in terms of reduced milk, meat and wool production. [1] *epiclitum, Paramphistomum cervi, Gastrothylax crumenifer, Gigantocotyle explanatum*, *Cotylophoron cotylophorum* and *Fischoederius elongatus* are the predominant species in ruminants. [2] species, *Cotylophoron cotylophorum* is more prevalent in Tamilnadu. [3]

Synthetic anthelmintics are used to combat helminth infection in livestock. However, synthetic drugs are usually associated with many limitations, such as non-availability of desired type, cost, drug resistance, environment pollution and presence of residues in milk, meat, wool and on pasture. Therefore, there is a need for developing cheaper, less toxic and eco-friendly novel anthelmintic drug. The use of medicinal plants

***Corresponding author: Dr. L. Veerakumari,**
Associate Professor and Head, P.G. and Research Department of Zoology, Pachaiyappa's College, Chennai-30, Tamil Nadu, India; **Tel.:** +91- 98404 48345;
E-mail: veerakumari_2002@yahoo.co.in
Received: 20 March, 2015; **Accepted:** 06 May, 2015

for the prevention and treatment of gastro-intestinal parasitism has its origin in ethnoveterinary medicine. [4] As, application of plant based anthelmintic drugs appears promising, an attempt has been made in the present study to elucidate the anthelmintic potential of bark of *Prosopis cineraria* against the paramphistome *C. cotylophorum*. Anthelmintic efficacy of *P. cineraria* has been assessed earlier using earthworm. [5] As the anatomy, physiology and mode of reproduction of earthworm which belongs to the phylum annelida and terrestrial in habitat entirely differ from endoparasitic helminths, use of earthworm as the animal model to elucidate anthelmintic efficacy of medicinal plants should be avoided. Hence, the anthelmintic efficacy of *P. cineraria* was investigated against *C. cotylophorum*, the endoparasite of domestic ruminants.

Prosopis cineraria belong to the family Fabaceae, commonly called vanni. *P. cineraria* is used as antihyperlipidemic, antioxidative, anthelmintic, antibacterial, antifungal, antiviral, anticancer, antipyretic and antidiabetic agent. [6-7] Phytochemical analysis of bark of *P. cineraria* by several investigators revealed the presence of flavonoids, tannins, alkaloids, diketones, phenolic substances, free amino acids, patulitrin, spicigerin, prosogerin, steroids namely campesterol, cholesterol, β-sitosterol, stigmasterol, alcohols namely octacosanol and triacontan-1-ol and alkanes hentriacontane, lipids, sugars and vitamins. [8-9] Tannins are known to produce anthelmintic activities and chemically tannins are polyphenolic compounds. [10]

Motility is an important parameter in assessing the anthelmintic efficacy of drugs. Electronic micromotility meter (EMM) provides quantitative measure of the motor activity of drug-treated parasites and could be used to assess motility of the parasites. [11] The enzyme AChE plays an essential role in neurotransmission. [12] The most common effect of the anthelmintic drugs given for worm infection is paralysis of the parasite musculature either by inhibiting neuromuscular transmission or enzymes involved in energy production. [13-15] AChE provides "biochemical hold-fast" to the parasite by causing local paralysis of the host gut and thus inhibiting the peristaltic movements of the host intestine required for parasite expulsion. [16] The drugs which affect neuromuscular activity and AChE are said to be associated with anthelmintic action. Hence, the present study was undertaken to evaluate the anthelmintic efficacy of bark of *P. cineraria* against *C. cotylophorum* based on its effect on the motility and on the enzyme acetylcholinesterase.

MATERIALS AND METHODS

In vitro maintenance of *Cotylophoron cotylophorum*

Cotylophoron cotylophorum (Fischoeder) [17] were collected from the rumen of infected sheep, slaughtered at Perambur abbatoir, Chennai. Adult live worms were washed thoroughly in physiological saline and maintained in Hedon-Fleig solution (pH-7.0) which is the best medium for the *in vitro* maintenance of *C. cotylophorum*. [3]

Collection and preparation of plant Extract

Bark of *Prosopis cineraria* were collected and shade dried. The dried bark was powdered and stored in closed bottles at room temperature in the dark until needed. Powdered bark of *P. cineraria* (2 kg) was soaked in ethanol. The extract was then filtered using Whatman No. 1 filter paper and concentrated by distillation using Rotary evaporator, (Evator). The concentrated extract was completely dried to remove the last traces of the solvent using Lyodol Freeze Dryer (DELVAC).

Different concentrations of ethanol extract of bark of *P. cineraria* (*Pc*EE) were prepared using Hedon-Fleig solution. Twenty five millilitre of Hedon-Fleig solution containing various concentrations (1, 3 and 5%) of the extracts were individually distributed to air tight containers. In each container five live flukes were introduced. The activities of the flukes were checked at various time intervals (5 min, 15 min, 30 min, 1, 2, 4, 6, 8, 12 and 24 h). Control was also maintained simultaneously in Hedon-Fleig solution without *Pc*EE. Based on the motility of the flukes, the observations were categorized as very active (++++), active (+++), moderately active (++), sluggish (+) and dead (-). The worms with no movement were regarded as dead. Based on the motility of the parasites for 24 h exposure to the plant extract, five different sub-lethal concentrations were selected for further *in vitro* studies.

Quantitative measure of the fluke's motility using Electronic micromotility meter

The motility of the flukes in sub-lethal concentrations of *Pc*EE was quantitatively assayed using Electronic micromotility meter (EMM). [11] The percentage of inhibition of motility of control and drug-treated flukes were calculated using the following formula:

$$\text{Percentage of inhibition of motility} = \frac{C-T}{C} \times 100$$

Where, C = Deviation of voltage signal caused by the control fluke

T =Deviation of the voltage signal caused by the fluke treated with plant extract

Acetylcholinesterase

Acetylcholinesterase (AChE, EC 3.1.1.7) activity was assayed using photometric method. [18] AChE in the sample hydrolyses acetylcholine, which is the substrate and forms thiocholine that will react rapidly and irreversibly with 5, 5 Di thio-bis 2-nitrobenzoic acid (DTNB). The increase in colour intensity was measured spectrophotometrically at 412 nm.

$$\text{Acetylcholine} \xrightarrow{\text{AChE}} \text{Thiocholine} + \text{Acetate thiocholine} + \text{Dinitrobenzoate} \longrightarrow \text{Yellow colour}$$

The flukes were incubated in 1, 2, 3, 4 and 5% concentration of *Pc*EE for 2, 4 and 8 h. The enzyme samples were prepared by homogenizing 100 mg of control and drug-treated flukes in 1 ml of 0.1M

phosphate buffer (pH 8.0). The homogenate was centrifuged at 1000 rpm for about 5 min. To 100µl of the supernatant, 1.3 ml of 0.1M phosphate buffer (pH 8.0) and 0.05 ml of 0.01M DTNB solution was added and transferred to quartz cuvette. The absorbance at 412 nm was set to zero in a UV visible double beam biospectrophotometer. 0.02 ml of 0.075M acetylthiocholine iodide was added to the reaction mixture in the cuvette and mixed well and the absorbance was noted for 5 min at 15 sec interval. The change in absorbance per minute was recorded. AChE activity was calculated using the formula

$$R = 5.74 \times 10^{-4} \times \frac{\Delta A}{\text{Protein content of the sample}}$$

Where, R- Rate of activity; Δ A- Increase in absorbance
The enzyme activity was expressed as number of moles of acetylthiocholine iodide hydrolyzed /min/mg protein.

Estimation of protein

Total protein content was estimated following the method of Lowry. [19] The carbamyl group of protein molecules reacts with copper and potassium of the reagent to give a blue coloured complex. This complex together with tyrosine and phenolic compounds present in the protein reduce the phosphomolybdate of the folin phenol reagent to intensify the colour of the reagents.

To 0.1 ml of the sample, 0.9 ml of 10% TCA (Trichloro acetic acid) was added to precipitate the protein. The sample was then centrifuged at 3000 rpm for 5 min and the supernatant was decanted. To this 5 ml of alkaline copper sulphate was added and mixed well and allowed to stand for 10 min. To this solution 0.5 ml of folin phenol reagent was added and mixed rapidly and allowed to stand for 30 min. 1N NaOH solution was used as blank and 100, 200 and 300 $mgml^{-1}$ of bovine serum albumin (BSA) were used as standard. The protein content in the sample was directly observed with the aid of UV-Visible double beam biospectrophotometer (ELICO), which is an autoanalyser. It automatically quantifies the protein content in the sample.

Statistical analyses

Statistical analyses were performed with the statistical program for the social sciences SPSS version 16.0. The significance of drug induced inhibition in the motility and AChE activity of the parasites was assessed using analysis of variance (ANOVA) for different concentrations of ethanol extract of *Prosopis cineraria* (*Pc*EE). The term significant had been used to indicate difference for which $P<0.005$.

RESULTS AND DISCUSSION

The present *in vitro* studies on the effect of *Pc*EE against *Cotylophoron cotylophorum* elucidated the anthelmintic potential of *Pc*EE. From the visual observation on the motility of the parasite, it was found that *Pc*EE was effective against *C. cotylophorum* causing 100% mortality at 5% concentration after 12 h exposure (Table.1). Based on the motility and survival of the parasite for 8h of exposure to *Pc*EE, five different sub lethal concentrations (1, 2, 3, 4 and 5%) were selected for further investigation.

The inhibition of the motility recorded with the aid of EMM showed that the percentage inhibition of motility is directly proportional to the concentration and period of incubation; Maximum inhibition (93.54%) of the motility was observed at 5% concentration after 8h of exposure (Fig. 1). The inhibitory effects *Pc*EE, of on the motility at different concentrations, for each period of incubation are significant ($P<0.005$).

Dose and time dependent inhibition in AChE activity was observed in drug-treated parasites. AChE activity was inhibited to 25.19, 59.09 and 67.12% at 1% concentration after 2h, 4h and 8h exposure to *Pc*EE respectively. The maximum level of inhibition was observed at 5% after 8h of exposure of *Pc*EE (Fig. 2). Inhibitory effects of the extracts among the different concentrations of *Pc*EE are significantly different for each period of incubation ($P<0.005$).

The survival status of any living organism depends on the motility. Several synthetic anthelmintic drugs were assessed for their efficacy based on their inhibitory effect on the motility of the parasites against helminth parasites. [20-23] The motility response of *Pc*EE-treated flukes recorded with the aid of EMM is highly supportive of the gross visual observation. The quantitative measurement of motility of drug-treated parasites clearly indicates the impact of the drugs on the motility of the parasite. Similar inhibitory effect of flukicidal drugs on the motility of *F. hepatica* and *C. cotylophorum* was reported by several investigators. [21-23]

The neuromuscular system of the parasites is particularly sensitive to the action of many anthelmintic drugs. [24-25] Acetylcholinesterase (AChE) is an enzyme broadly distributed in many species, including parasites. It occurs in multiple molecular forms that differ in their quaternary structure and mode of anchoring to the cell surface. It terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine. Initially, it was proposed that AChE was acting as a "biological hold-fast" by causing local paralysis of the host gut and thus inhibiting the peristaltic movements of the intestine required for parasite expulsion. [16]

Table 1: Gross visual observation on the motility of *C. cotylophorum* treated with *Pc*EE

Extract	Concentration (%)	5 min	15 min	30 min	1 h	2 h	4 h	6 h	8 h	12 h	24 h
Control		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
***Pc*EE**	**1**	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
	3	++++	++++	++++	++++	++++	+++	+++	++	++	-
	5	++++	++++	++++	++++	+++	+++	++	+	-	-

++++: Very active; **+++:** Moderately active; **++:** Slightly active; **+**: Sluggish; **-** : Dead; *Pc*EE: *Prosopis cineraria* ethanol extract

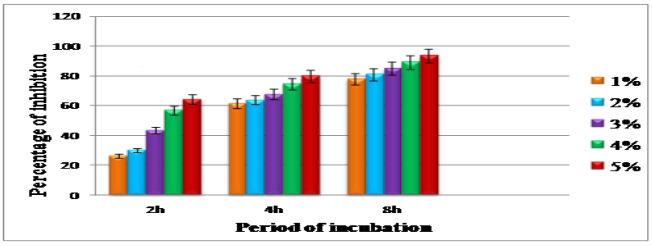

Fig. 1: Effect of *Pc*EE on the motility of *Cotylophoron cotylophorum*

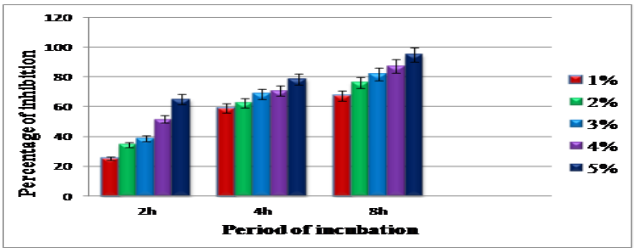

Fig. 2: Effect of *Pc*EE on the AChE activity of *Cotylophoron cotylophorum*

Later, it has been hypothesized that AChE may contribute to parasite survival by interfering with the process of mucus secretion from intestinal goblet cells. [26] It has also been proposed that AChE may be capable of regulating the immune and inflammatory response through messenger and membrane receptor modulation. [27] Therefore, it appears that if the host is to expel the parasite, inactivating the parasite AChE, it not only dislodge the parasite but also elevate the immune response of the host. Inhibition of AChE increases the concentration of endogenous acetylcholine, which results in the inhibition of motor activity of *C. cotylophorum*. This may lead to the expulsion of the parasite from the host. *Pc*EE possesses a remarkable anthelmintic activity against *C. cotylophorum*. It may serve as an alternative for anthelmintic chemotherapeutic agents to avoid their toxic side effects and development of resistance in a safe and eco friendly manner. However, this study warrants further *in vivo* studies for practical utility. In depth field trials of plant based anthelmintics along with best farm management practices can play a great role in parasite control strategies and in enhancing productivity of livestock farming.

REFERENCES

1. Hassan Syed Shabih, Juyal PD. Diagnosis of Paramphistomosis in Domestic Ruminants in Punjab (INDIA). Proceedings of the 11th Int Symp Vet Epidemiol Ecol. 2006.
2. Hassan SS, Kaur K, Joshi K, Juyal PD. Epidemiology of paramphistomosis in domestic ruminants in different districts of Punjab and other adjoining areas. J. Vet. Parasitol. 2005; 19(1): 43-46.
3. Veerakumari L. *In vitro* studies on the effect of some anthelmintics on *Cotylophoron cotylophorum*. (Fischoeder, 1901) (Digenea; Paramphistomidae). A structural and biochemical analysis. Ph.D, thesis University of Madras, Chennai. India, 1996.
4. Athanasiadou S, Githiori S, Kyriazakis I. Medicinal plants for helminth parasite control: facts and fiction. Animal. 2007; 1(9): 1392-1400.
5. Khatri A, Rathore A, Patil UK. Assessment of anthelmintic Activity of the *Prosopis cineraria* (Linn). Druce bark. Indian J Nat Prod Res. 2011; Vol. 2(4): 512-514.
6. Jabbar A, Raza MA, Iqbal Z, Khan MN. An inventory of the Ethnobotanicals used as Anthelmintics in the southern punjab (pakistan). J Ethnopharmacol. 2006; 108(1): 152-4.
7. Kumar A, Yadav SK, Singh S, Pandeya SN. Analgesic activity of ethanolic extract of roots of *prosopis cineraria* (L.) Druce. J Appl Pharmaceut Sci. 2011; 01(08): 158-160.
8. Robertson S, Narayanan N, Kapoor BR. Antitumor activity of *Prosopis cineraria* (L.) Druce against agrlich ascites carcinoma-induced mice. Nat Prod Res. 2011; 25(8): 857-862.
9. Velmurugan V, Arunachalam G, Ravichandran V. Anthelmintic potential of *Prosopis cineraria* (Linn.) druce stems barks. Asian J Plant Sci Res. 2011; 1(2): 88-91.
10. Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC. Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or Sulla (*Hedysarum coronarium)* which contains condensed tannins. J Agri Sci. 1995; 125: 281-289.
11. Veerakumari L. Micromotility meter: an instrument evaluates the efficacy of anthelmintic drugs. Fourteenth National Congress of Vet Parasitol. 2003; Pp. 87.
12. Lee DL. Why does some Nematode Parasites of Alimentary tract secrete Acetylcholinesterase? Int J Parasitol. 1996; 26: 499-508.
13. Van den Bossche HP. The Molecular Basis of Anthelmintic action. In: Biochemistry of Parasites and Host: Parasite Relationships van den Bossche H, (Ed.). Amsterdam, New York, 1976; 553-572.
14. Coles GC. The Biochemical mode of action of some modern anthelmintics. Pesti sci. 1977; 8: 536-543.
15. Manger BR. Anthelmintics veterinary applied pharmacology and therapeutics. (Brander GC, pugh Dm, Bywater RJ, Jenkins WL, eds) ELBS/ Bailliere, Tindall, 1991; 513-548.
16. Ogilvie M, Jones VE. *Nippostrongylus brasilienses*: a review of immunity and the host/parasite relationship in the rat. Exp Parasitol. 1971; 29: 138-177.
17. Fischoeder F. Die Paramphistomiden der Saiigethiere. Zool. Anx. 1901; 24: 365-375.
18. Ellman GL, Courtney KD, Andres VJr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; **7**: 88–95.
19. Lowry OH, Rosebrough NJ, Farr A, Randall RJ. Protein measurement with folinphenol reagent. J Biol Chem. 1951; 193: 265-275.
20. Fairweather I, Holmes SD, Threadgold, LT. *Fasciola hepatica:* Motility response to fasciolicides *in vitro*. Exp Parasitol. 1984; 57(3): 209-224.
21. Holmes SD, Fairweather I. *Fasciola hepatica*: The effects of neuropharmacological agents upon *in vitro* motility. Exp Parasitol. 1984; 58: 194-208.
22. Kumar D, Tripathi HC. Paralytic effect of fasciolicidal agents on rhythmic motility of *Fasciola gigantica*. Indian J Animal Sci. 1998; 68: 1126-1129.
23. Veerakumari L, Priya P. *In Vitro* effect of *Azadirachtin* on the motility and Acetylcholinesterase activity of *Cotylophoron cotylophorum* (Fishoeder, 1905). J Vet parasitol. 2006; 20: 1-5.
24. Barragry T. Anthelmintics - A review: Part II (1984). New Zealand. Vet J. 32: 191-199.
25. Sharma RK, Singh K, Saxena KK. Effect of parbendazole and piperazine adipate on the activity of some enzymes of *Ascardia galli* and *Heterakis gallinae*. Vet Parasitol. 1987; 24: 211-220.
26. Philipp M. Acetylcholinesterase secreted by intestinal nematodes: a reinterpretation of its putative role of "biochemical holdfast": Trans. Royal Soc. Trop Med Hygiene, 1984; 78: 138-139.
27. Rhoads ML. Secretory cholinesterases of nematodes: possible functions in the host-parasite relationships. Trop Vet. 1984; 2: 3-10.

Source of Support: Nil, Conflict of Interest: None declared.