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EFFECT OF ETHANOL EXTRACT OF *ALBIZIA LEBBECK* ON THE MOTILITY AND ACETYLCHOLINESTERASE ACTIVITY OF *COTYLOPHORON COTYLOPHORUM*

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ABSTRACT

Paramphistomosis is one of the major problems in the productivity of livestock throughout the world. Paramphistomosis is caused by digenetic trematode belongs to the family paramphistomidae. The effect of *Albizia lebbeck* on the motility and Acetylcholinesterase against a paramphistome *Cotylophoron cotylophorum* was study. The adult parasites were exposed to different concentrations of hexane, chloroform, ethyl acetate, ethanol and aqueous extract of *A. lebbeck* and the motility of the flukes were observed. As ethanol extract was very effective, further study was carried out with five different sub-lethal concentrations (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml) of ethanol extract of *A. lebbeck* (*Al*EE). The electronic measurement of the motility of the drug-treated parasites indicated the direct impact of the drugs on the motility of the parasites. AChE was assayed following the method of Ellman *et al.* (1961). Maximum inhibition in the motility (91.27%) and AChE activity (89.82%) was observed in 0.5 mg/ml concentration after 8h of exposure of *Al*EE. The principle physiological role of AChE is believed to be the termination of transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine and also involved with other non-enzymic functions like host-parasite interaction. Inhibition of AChE causes desensitization of the muscle receptor resulting in paralysis and the parasite lose its biochemical holdfast and get expelled from the host.

INTRODUCTION

Livestock is central to the livelihood of the rural poor in the country and contribute substantially to poverty alleviation by strengthening the socio-economic conditions of pastoralists (Gadahi*et al.*, 2009). Helminth parasites affect animal production by lowering their working efficiency, growth, bodyweight, milk yield and meat. Paramphistome is a digenetic trematode affecting various domestic ruminants Causing various digestive catarrhal enteritis which can lead to hemorrhagic death of the animal (Chandrasekharan *et al.*, 1982). Paramphistomosis caused by a number of closely related amphistome species of various genera often infecting concurrently, is one of the most common disease of livestock in the Indian sub-continent (Saifullah*et al.*,2001).

Synthetic drugs are used to treat ruminant parasites. The most commonly used anthelmintic drugs are niclosamide, oxyclozanide, triclabendazole, albendazole and praziquantel (Veerakumari and Munuswamy, 1999; Veerakumari and Munuswamy, 2000; Chaudhri, 2000; Sahoo, 2002; Galdhar and Roy, 2004). Natural plant items have been utilized for remedial purposes since the day of immemorial and their utilization is of a more noteworthy interest these days (Calixto, 2000). Several plants or plant derived formulations are used to cure helminth infection in man and animals (Satyavati, 1990). The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic efficacy (Veerakumari and Priya, 2006; Veerakumari *et al.,* 2012; Veerakumari, 2015). In the present study anthelmintic efficacy of *A. lebbeck* was investigated against the paramphistome *C. cotylophorum*.

Albizia lebbeck commonly called as Vaagai in Tamil belongs to the family Mimosaceae. It is unarmed deciduous woody tree, 12-21 meters in height, having pale bark which is widely used to treat various ailments (Vasanthi Padmanabhan, 2013). Phytochemical screening of *A. lebbeck* revealed the presence of steroids, terpenoids, saponins, alkaloids, flavonoids, anthraquinones, and phenolic compounds (Varshney,1976; Deshpande, and Shastri, 1977; Pal, 1995; Dixit, and Misra, 1997; Sanjay, 2003). *A. lebbeck* has biological activities such as antioxidant, anti-inflammatory, antipyretic, analgesic, estrogenic and anticonvulsant activity (Kasture *et al.*, 1996; Resmi *et al.*, 2006; Saha and Ahmed, 2009; Verma and Srivastav, 2011; Mohamed Farag, 2013). Motility is an important parameter in assessing the anthelmintic efficacy of drugs. Electronic micro motility meter (EMM) provides quantitative measure of the motor activity to assess motility of the parasites (Veerakumari, 2003). Inhibition of AChE secretion has given to be a decent parameter for the translation of *in vitro* anthelmintic action (Rapson *et al.,* 1986). The enzyme AChE plays as an essential role in neurotransmission (Lee, 1996). Any significant unsettling influences of its neuromuscular co-ordination are probably going to make the flukes to become detached, and this may eventually prompt it to elimination from the host. In the present study effect of ethanol extract of *A. lebbeck* on the motility and AChE of *C. cotylophorum* was investigated.

MATERIALS AND METHODS

In vitro maintenance of *Cotylophoron cotylophorum*

C. cotylophorum (Fig.1) was collected from the rumen of infected sheep, slaughtered at Perambur abattoir, Chennai. Adult live parasites were collected, washed completely in physiological saline and kept up in the Hedon-Fleig solution, which is the best mode for *in vitro* maintenance (Veerakumari, 1996). It is prepared by dissolving 0.3gm of potassium chloride, 7gm of sodium chloride, 1.5gm of sodium bicarbonate, 0.1gm of calcium chloride,0.5gm of disodium hydrogen phosphate, 1gm of glucose, 0.3gm of magnesium sulphate in 1000ml of distilled water.

Preparation of plant extracts

Albizia lebbeck (bark) (Fig.2) were collected from Lakshmi stores at Chennai, and it was authenticated by a botanist in the Department of Botany, Pachaiyappa's college, Chennai and vouchered specimens are deposited in the herbarium of Pachaiyappa's College, Chennai-30. The extraction of plant materials was finished after the strategy for Harborne (1998). The bark of *A. lebbeck* was coarsely powdered and splashed sequentially in hexane, chloroform, ethyl acetate acid derivation, and ethanol progressively. The aqueous extract was also prepared. Extracts were filtered using Whatman filter paper No.1 and concentrated by distillation using, rotary evaporator (EQUITRON). The concentrated extracts were totally dried to evacuate the last hints of the solvents utilizing Lyodel Freeze Dryer (DELVAC). The concentrates were diluted with Hedon-Fleig solution to obtain different concentrations (1, 3 and 5 mg/ml). Ten parasites were incubated in

25ml of every convergence of plant extract and the motility of the parasites was checked at different intervals viz. 5, 15, 30 min, 1, 2, 4, 6, 8, 12 and 24h. Simultaneously, control parasites were also maintained in the Hedon-Fleig solution without the plant extracts. The motility of the flukes was observed visually at a regular time interval. The motility response of the flukes was categorized as very active (++++), moderately active (+++), slightly active (++), sluggish (+), and dead (-). Supported the visual observations five different sub-lethal concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) of effective plant extracts were selected for further studies.

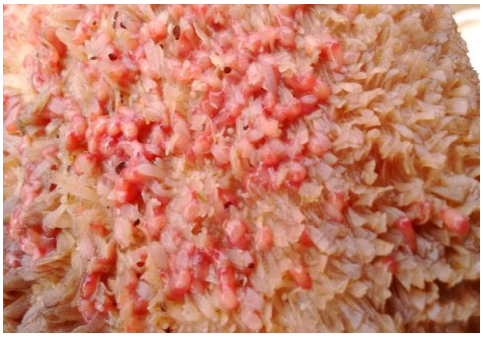

Fig. 1 *Cotylophoron cotylophorum*

Fig. 2 *Albizia lebbeck*

Estimation of Acetylcholinesterase

Acetylcholinesterase (AChE, EC 3.1.1.7) was assayed following photometric method as described by Ellman *et al*. (1961). AChE within the sample hydrolyses acetylcholine, which is the substrate and forms thiocholine that which will react rapidly and irreversibly with 5 thio-bis nitro benzoic acid. The rice in color intensity was measured spectrophotometrically at 412nm

The enzyme samples were prepared by homogenizing 500 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 100 µl of the supernatant, 1.3 ml of 0.1 M phosphate buffer (pH 8.0) and 0.05 ml of 0.01M 5- thio-bis-nitro benzoic acid (DTNB) solution was added and transferred to a quartz cuvette. The absorbance at 412 nm was set to zero in an exceedingly UV visible double beam bio-spectrophotometer. 0.02 ml of 0.075M acetyl thiocholine iodide was added to the reaction mixture in the cuvette and mixed well and the absorbance was noted for five min at 15 seconds interval. The rise in absorbance per minute was calculated. The protein content of the sample was estimated following the procedure of Lowry *et al*. (1951). The enzyme activity was expressed as the number of moles of acetyl thiocholine iodide hydrolyzed min/mg protein.

Statistical analyses

Statistical analyses were performed with the Statistical Program for the social sciences SPSS version 16.0. The importance of drug-induced inhibition within the motility and AChE activity of the parasites was assessed using analysis of variance (ANOVA) for different various concentrations of ethanol extract of *A. lebbeck* (*Al*EE).

RESULTS: The present study elucidated the anthelmintic potential of *A. lebbeck*. The motility of flukes (*C. cotylophorum*) incubated in different solvent extracts like hexane, chloroform, ethyl acetate, ethanol and aqueous extract of *A. lebbeck* was observed. The control flukes were highly flux and active throughout the experimental period,whereas the movement of the drug-treated flukes was severely affected. It is evident from Table 1 that ethanol extract of *A. lebbeck* (*Al*EE) is effective in reducing the motility of *C. cotylophorum* after 2h of exposure. Hence, *Al*EE was selected for subsequent *in vitro* studies. EMM is used to validate the results obtained in gross visual observation. *Al*EE effectively inhibited the motility of the parasite. The motility of the parasites was inhibited to 49.92, 76.32and

91.27% at 0.5 mg/ml concentration after 2, 4 and 8h of exposure (Table 2). Inhibition of motility of the parasites exposed to *Al*EE was linear with increase in concentration and period of exposure. The inhibition of motility of drug-treated flukes was significant (P<0.005) among different concentrations and different periods of incubation. Table 3 depicts the effect of *Al*EE on AChE activity of *C. cotylophorum*. The inhibitory effect was dose and time dependent; the maximum level of inhibition was observed in at 0.5mg/ml concentration after 8h of exposure.

DISCUSSION

The action of anthelmintics is reflected in their ability to reduce of motility of the parasites. EMM is an instrument designed with spectacular advancement and automation to detect the mortality and motility response of parasites (Veerakumari, 2003). Electronic measurement of the motility of the flukes indicates the direct impact of the drug on the flukes. Motility studies are directly correlated to the neuromuscular physiology of the trematodes (Kumar *et al.,* 1995). AChE is an important enzyme in helminths which is associated with the neuromuscular co-ordination (Bueding, 1952).

Sukhdeo *et al.*(1988) detected AChE activity within the cell bodies and the neuropile of the cerebral ganglia of adult *F. hepatica.* Mansour(2002) reported the presence of AChE in a number of helminth parasites. ACh and AChE inhibitors relaxed parasitic musculature, decreased motility and eventually induced paralysis in *Fasciola hepatica* (Sukhdeo *et al.,* 1986), *Dipylidium caninum* (Mansour, 2002) and the nematode, *Nippostrongylus brasiliensis* (Rapson *et al.,* 1986). AChE is believed to be the target for a wide array of anthelmintics, due to its crucial biological role in the termination of signal transmission at neuromuscular junctions and cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (Massoulie *et al.,* 1993). Inhibition of AChE causes desensitization of the muscle receptor leading to paralysis (Opperman and Chang, 1992). Kirby *et al.* (2000) reported that the inhibition of AChE leads to marked increase in acetylcholine levels that cause continuous and excessive stimulation of the nervous system. The present investigation disclosed the inhibitory effect of *Al*EE on AChE of*C. cotylophorum*. This is corroborated with the reports of earlier researchers on various plant extracts. Szwajgier and Borowiec (2012) asserted the inhibition of AChE by the ethanol extract of *Carum cervi.* Roy *et al*. (2012) reported that the AChE of *Fasciolosis buski* was inhibited when treated with *A. nigra* plant extract. Similarly, Manoj Dhanraj and Veerakumari (2014) and Manigandan and Veerakumari (2015) reported that the motility and AChE of *C. cotylophorum* was inhibited when treated with ethanol extracts of *Syzygium aromaticum* and *Prosopis cineraria*. More recent research indicated that one amongst the potential functions of nematode AChE is to change the host cytokine environment and depress the event of M2 macrophages which are deleterious to the survivalof the helminth parasites (Vaux *et al.,* 2016).

In the present investigation it has been observed that the percentage of inhibition of motility and AChE at higher concentration 0.5mg/ml after 8h of exposure were 91.27% and 89.82% respectively, indicating the direct correlation between the motility and AChE. Inhibition in AChE activity was significant (P<0.005) among different concentrations and different period of incubation.

The inhibition of AChE might be the numerous reasons for the drastic changes in the motility of the parasites. Consequent to the inactivation of neuromuscular co-ordination, active ingestion and movement of food through the gastrointestinal tract is arrested. The parasites thus enter a state of starvation and energy-deprivation. The energy deprived flukes unable to sustain themselves in place are expelled by the host.

Table 1: Chronological observations on the viability and motility of *C. cotylophorum* were exposed to various extracts of *Albizia lebbeck*

EXTRACTS	CONC. (%)	5min	15min	30min	1h	2h	4h	6h	8h	12h	24h
CONTROL		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
*Al*HE	1	++++	++++	++++	++++	++++	++++	+++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	+	-
	5	++++	++++	++++	++++	++++	+++	++	+	-	-
*Al*CE	1	++++	++++	++++	++++	++++	++++	+++	+++	++	-
	3	++++	++++	++++	++++	++++	++++	+++	++	-	-
	5	++++	++++	++++	++++	++++	+++	++	-	-	-
*Al*EaE	1	++++	++++	++++	++++	++++	+++	++	+	-	-
	3	++++	++++	++++	+++	++	++	+	-	-	-
	5	++++	++++	+++	++	++	+	-	-	-	-
*Al*EE	1	++++	++++	++++	+++	++	+	-	-	-	-
	3	++++	++++	+++	++	+	-	-	-	-	-
	5	++++	+++	++	+	-	-	-	-	-	-
AlAE	1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
	3	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++
	5	++++	++++	++++	++++	++++	++++	+++	+++	++	++

Very active (++++), moderately active (+++), slightly active (++), sluggish (+), and dead (-).

*Al*HE- Hexane extract of *Albizia lebbeck*, *Al*CE- Chloroform extract of *Albizia lebbeck*, *Al*EaE- Ethyl acetate extract of *Albizia lebbeck*, *Al*EE- Ethanol extract of *Albizia lebbeck* *Al*AE- Aqueous extract of *Albizia lebbeck*

Table 2- Quantitative analysis of motility of *C. cotylophorum* treated with *Al*EE using Electronic Micro motility Meter

Concentrations	% inhibition (mean ± SD of n = 5) at various periods of incubation**		
	2h	4h	8h
0.1 mg/ml	9.52±0.23	27.38±0.22	52.96±0.62
0.2 mg/ml	16.07±0.19	30.35±0.10	62.20±0.29
0.3 mg/ml	22.02±0.26	44.76±0.20	79.04±0.14
0.4 mg/ml	33.72±0.17	60.46±0.16	86.08±0.12
0.5 mg/ml	49.92±0.66	76.32±0.12	91.27±0.05

* Inhibitory effects of the extracts among the different concentrations of the respective plant are significantly different for each duration of incubation ($P < 0.05$) using Bonferroni test

** Inhibitory effects of the extracts among the different hours of incubation is significantly different for each concentration of the respective plants ($P < 0.01$) using

Bonferroni test

Table 3- Effect of *Al*EE on Acetylcholinesterase activity of *C. cotylophorum*

Concentrations	% inhibition (mean ± SD of n = 5) at various periods of incubation**		
	2h	4h	8h
0.1 mg/ml	25.48±0.83	34.48±0.96	63.69±0.23
0.2 mg/ml	31.24±0.93	44.46±0.99	78.12±0.66
0.3 mg/ml	40.67±0.10	56.62±0.74	81.13±0.16
0.4 mg/ml	48.65±0.07	60.94±0.10	86.12±0.15
0.5 mg/ml	53.50±0.19	75.97±0.43	89.82±0.10

* Inhibitory effects of the extracts among the different concentrations of the respective plant are significantly different for each duration of incubation ($P < 0.05$) using Bonferroni test

** Inhibitory effects of the extracts among the different hours of incubation is significantly different for each concentration of the respective plants ($P < 0.01$) using Bonferroni test

CONCLUSION:

In the present investigation, *Al*EE exhibited a remarkable anthelmintic potential against *C. cotylophoru*. *Al*EE significantly inhibited the motility and neurotransmitter enzyme AChE has myriad effects on helminth parasites. This study would target and fine-tune to use of herbal plant remedies with low cost, locally available to improve ruminant health and performance. It is concluded that *Albizia lebbeck* possess appreciable anthelmintic effects against a trematode parasite (*C. cotylophoru*), and could be used to treat paramphistomosis in livestock.

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