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Research Article

ANTHELMINTHIC ACTIVITY OF *CLITORIA TERNATEA* SEEDS AQUEOUS AND ALCOHOLIC EXTRACTS AGAINST *PARAMPHISTOMUM CERVI*

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ABSTRACT
The present study is an attempt to study the effect of *Clitoria ternatea* (aparajita) as an anthelminthic agent on *Paramphistomum cervi. Clitoria ternatea* plant is very useful in medico veterinary field and commonly called as aparajita. It is known for their traditional medicinal uses in many diseases like fever, arthritis, asthma and skin diseases. Different aqueous and alcoholic extract of aparajita shows efficacy, determine by the mortality and death time of *P. cervi*. The worm mortality, viability and tegumental alteration in *P. cervi* showed anthelminthic activity of *Clitoria ternatea* (aparajita) with used synthetic drug albendazole. The aparajita extracts were used for *in vitro* study at 25, 50 and 100 mg/ml concentration as tested extracts with albendazole in same concentrations. The synthetic drug albendazole was induced 72% and 60% mortality in 5 hours of incubation at 100 mg/ml and 50 mg/ml dose concentration. Whereas, aqueous *Clitoria ternatea* extracts induced lowest mortality 60% at 25mg/ml concentration in 4hrs and highest mortality 100% at 100 mg/ml in 5hrs were observed. Alcoholic *Clitoria ternatea* extract induced highest mortality 100% at 100mg/ml in 4 hour and lowest mortality in 60% at 25mg/ml in 3 hour exposure time was exhibited. The present study reveals that the *Clitoria ternatea* aqueous and alcoholic extracts showed paralysis and caused death of *Paramphistomum cervi* at lower concentrations, as compared to albendazole.

Keywords: *Albendazole, Antihelminthic, Clitoria Ternatea, Paramphistomum Cervi*

INTRODUCTION
Helminthiasis is a parasitic infection of livestock caused by nematode, cestode and trematode. Amphistome *Paramphistomum cervi* remains one of the most important helminth parasites of livestock in tropical and subtropical countries. Amphistome *P. cervi* is major health problem in ruminants thought out the world. This parasite is harmful for livestock and caused severe diseases called paramphistomiasis. Paramphistomiasis is due to the causes of *P. cervi* produce many illnesses in cattles health, also effect economic conditions of poor farmers. There are several synthetic anthelminthic drugs available at the present time to cure this parasite from livestock. Various synthetic anthelminthics drugs like albendazole, niclosamide and triclalbendazole were used for deworming in livestock. Frequently used of synthetic drugs in parasitic infection develop resistance power in helminths. Also, the world population still does not afford to pay for modern medicines particularly for poor cattle farmers (Bagavan *et al.*, 2009; Swarnakar *et al*., 2014a and 2014b & Swarnakar *et al*., 2015a and 2015b).
The traditional medicinal plants are easily available and known to provide a rich source of anthelminthic agents which is used to treat parasitic infection in developing countries. Many ethno medicinal plants have been used to eliminate the helminth parasites from animals and humans throughout the world which are more effective than synthetic drugs and eco-friendly and less costly as well for farmer (Dutt, 1980; Satyavati *et al.,* 1976; Satyavati, 1990; Tandon *et al.,* 1997 and Githioroi *et al.,* 2006).
Clitorea ternatea (Fabaceae) is a climbing herb known as aparajita (Ghani, 1998). Different parts of aparajita were used in ayurvedic medicines for deworming, skin diseases, arthritis, bronchitis and fever (Rai *et al.,* 2000 and 2001). Aparajita is anthelminthic plant and also used for cured to various diseases. In Aparajita, phytochemical reviews shows presence of tannins, alkaloids, resins, glycosides, flavonoids and phenols (Khadker *et al.,* 2008; Patil and Patil, 2011; Sing, 2015 and Lijon *et al.,* 2017).

None of the researcher worked on anthelminthic effect of aparajita that's why present research have been taken to study the effect of cumulative concentration of aqueous and alcoholic extracts of seeds of *Clitorea ternatea* on *Paramphistomum cervi* in buffalo.

MATERIALS AND METHODS

Plant Collection: Medicinal plant was selected *Clitoria ternatea* for their anthelminthic activity against the *Paramphistomum cervi* of buffalo. Fresh plant material seeds of *C. ternatea* were collected from the Kalka Mata, Sisarma herbal gardens and Jhadol villages of Udaipur (Rajasthan), washed with tap water and then distilled water (Figure 3 and 4).

Preparation of Plant Extracts

Plant material seeds of *C. ternatea* were collected then keep in dry in oven at 40 ℃ for 3-4 days for material become completely solid and dry. Dried plant material were homogenize to fine powder with the help of an electric blender and store in the dark at room temperature in close containers until required. Plant material was extracted by taking 20 g of each sample in 160 ml of water and organic solvent in 250 ml flask. Then, continuous shacking with an orbital shaker and an occasional stirring with a glass rode manually at 4 hours interval. After 72 hours the macerates of each plant part were filtered in separate flasks using a qualitative filter paper (Whatman No 4 filter paper). Then, centrifuged at x 10000 g for 15 min and supernatant were used for anthelminthic testing. The filtered supernatants were dried until constant dry weights of each extract were obtained. Dried plant extract were reconstituted in the respective solvent. Plant extracts were reconstituted in distilled water and organic plants extract were reconstituted in 10% DMSO. The extracts were stored in 15 ml black cap bottle, covered with aluminum foil for the prevention of plant extract directly from light. The residues were stored at 4 ºC for further used. Plant extracts were prepared at different concentration aqueous and alcoholic extracts of seeds of *Clitoria ternatea*.

Collection of Parasites: Live amphistome parasites *Paramphistomum cervi* were collected from the infected rumen of freshly slaughtered domestic ruminants; buffaloes *(Bubalus* bubalis) at the local zoo abattoir in Udaipur. After removing the parasites from the infected part of rumen, washed several times in tap water to remove debris and mucous, etc. and then it was transferred into 0.9% physiological saline (NaCl) for further investigations (Figure 1 and 2).

Experimental Design: The parasites were divided in four groups with five parasites in each group (five replicate in each group):

First Group (Control Parasites): Untreated *P. cervi* were used as control group.

Second Group: In Vitro Treatment with Synthetic Drug Albendazole: Second group of parasites were treated with synthetic drug Albendazole (Methyl 5-propylthio 2- benzimidazole carbonate) at different concentration.

Third Group: In Vitro Treatment with Clitoria Ternatea Aqueous Extracts: Third group of parasites were incubated in different concentrations (mg/ml) of the aqueous extracts with a volume of 10 ml in the petri dish for five hours and to evaluate the optimum cumulative concentrations of plant against *P. cervi.*

Fourth Group: In Vitro Treatment with Clitoria Ternatea Alcoholic Extracts: Fourth group incubated in concentration mg/ml of alcoholic extracts with a volume of 10 ml in the petri dish for five hours and to evaluate the optimum cumulative concentrations of plant against *P. cervi.*

.***Evaluation of Anthelmintic Activity of Albendazole and Aqueous and Alcoholic Extracts of Clitoria Ternatea:*** Ten ml of each concentration of the anthelmintic extracts and synthetic drug were applied to a group of 5 worms maintained in 10ml of selected medium and 2ml of the sterilizing solution. The experiment was performed in five replicates at the optimal temperature (37ºC) and pH 7.4. All experimented worms were examined after 1, 2, 3, 4, 5 hours and mortality was calculated for each experiment. Mortality was determined visually by activity and by mechanical stimulation using a dissecting needle after 1, 2, 3, 4 and 5 hours and worm mortality was evaluated by observation under magnifying glass or after being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, worms confirmed by mortality.

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Statistical Analysis: The dead worms were counted in each experimental set and the percentage of average mortality was calculated in all experiments according the following equation: Average Mortality rate = Total number of dead parasites/Total number of experiment parasites X 100.

- All values were expressed as mean.

RESULTS AND DISCUSSION

Results

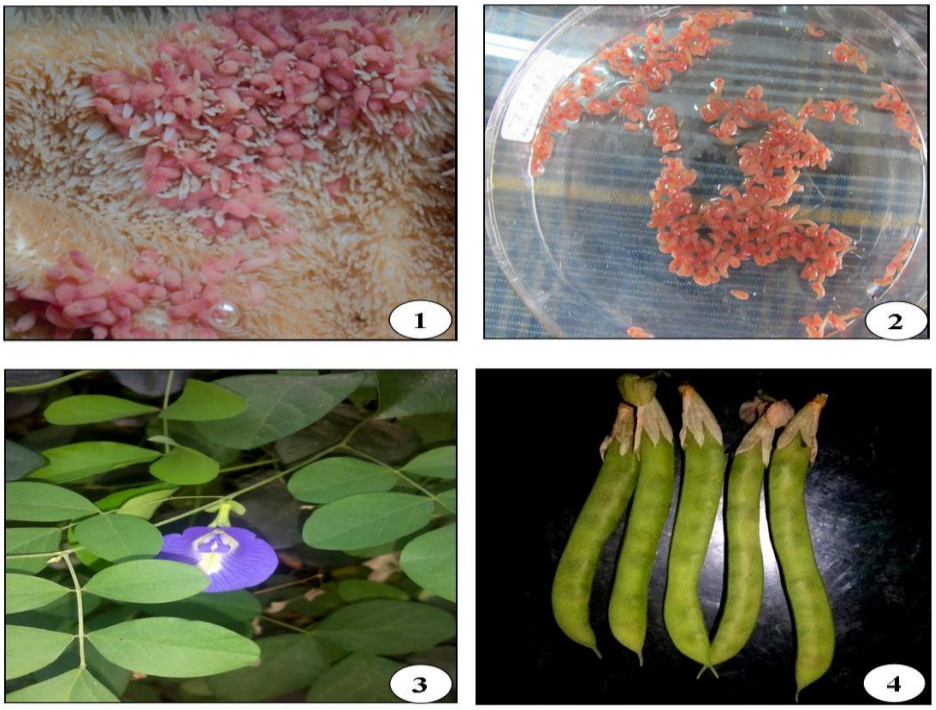


Fig.1. Amphistome *P.cervi* attached with infected rumen of buffalo

Fig.2. Washed *P.cervi* in normal saline

Fig.3. Plant of *Clitoria ternatea*

Fig.4. Mature curved legume with seeds of *C. ternatea*

Control or Untreated Amphistome Paramphistomum Cervi

Paramphistomum cervi were removed from rumen and washed several times in normal saline then they were used as control or untreated group. The control or untreated amphistome *P.cervi* were incubated in saline solution and containing glucose remained actively in mobile condition with whole body movement through the study period 10 to 12 hours (Figure 1 and 2).

In Vitro Anthelmintic Effect of Different Concentrations of Aqueous and Alcoholic Extracts of Clitorea Ternatea on P. Cervi

The anthelmintic effect of aqueous concentrations of *Clitorea ternatea* is shown in Table 1. The aqueous extracts of *Clitorea ternatea* showed high mortality rate of 100% at concentration of 100 mg/ml at 5 hours of exposure time. The lower concentration of aqueous extracts 25 and 50 mg/ml gave mortality rates 84and 88 % respectively at the same exposure time (5 hours).

The effect of alcoholic extracts of *Clitorea ternatea* has been shown in Table 2. The highest mortality rate of 100 % in the experiment was achieved with the alcoholic extracts 100 mg/ml of concentration in 4 and 5 hours exposure time, at 3 hours 96% mortality and The 25 and 50 mg/ml extracts were also exhibited highest mortality (100%) at 5 hours exposure time. Whereas, lowest mortality rates 60 and 76 % was

induced by 25 and 50 mg/ml of alcoholic concentrations respectively at 3 hour exposure time. At 4 hours exposure time concentration of 25 and 50 mg/ml alcoholic extract were showed 84 and 92% mortality rates. Lowest mortality 36% at 25 mg/ml at 1 hour exposure time was exhibited.
Clitorea ternatea alcoholic extract has good anthelminthic activity than aqueous extract of *Clitorea ternatea* and albendazole, graphical representation showing in Graph 1.

Table 1: Anthelminthic Effect of Aqueous Extracts of *Clitorea Ternatea* on *P. Cervi*

Exposure Time in Hrs	Clitorea Aqueous Extracts in mg/ml	No. of Dead Worm		Mortality Rate %	CV %
		Mean	± STD		
	25 mg/ml	0.4	0.54772	**8%**	136.931
1 hour	50 mg/ml	1	0	20%	0
	100 mg/ml	1.6	0.54772	32%	34.2327
	25 mg/ml	1.2	0.44721	24%	37.2678
2 hour	50 mg/ml	2	0.70711	40.00%	35.3553
	100 mg/ml	2.6	0.54772	52%	21.0663
	25 mg/ml	2.2	0.83666	44%	38.03
3 hour	50 mg/ml	2.6	0.54772	52%	21.0663
	100 mg/ml	3.2	0.83666	64.00%	26.1456
	25 mg/ml	3	0.70711	60%	23.5702
4 hours	50 mg/ml	3.6	0.54772	72%	15.2145
	100 mg/ml	4.2	0.44721	**84.00%**	10.6479
	25 mg/ml	4.2	0.44721	**84%**	10.6479
5 hours	50 mg/ml	4.4	0.54772	**88%**	12.4482
	100 mg/ml	5	0	**100.00%**	0

Table 2: Anthelminthic Effect of Alcoholic Extracts of *Clitorea Ternatea* on *P. Cervi*

Exposure Time in Hrs	Clitorea Alcoholic Extracts in mg/ml	No. of Dead Worm		Mortality Rate %	CV %
		Mean	± STD		
	25 mg/ml	1.8	0.83666	**36%**	46.4811
1 hour	50 mg/ml	2.4	0.54772	48%	22.8218
	100 mg/ml	3	0	60%	0
	25 mg/ml	2.4	0.54772	48%	22.8218
2 hour	50 mg/ml	3	0	60.00%	0
	100 mg/ml	4	0	80%	0
	25 mg/ml	3.4	0.54772	68%	16.1095
3 hour	50 mg/ml	3.8	0.83666	76%	22.0174
	100 mg/ml	4.8	0.44721	**96.00%**	9.31695
	25 mg/ml	4.2	0.44721	**84%**	10.6479
4 hours	50 mg/ml	4.6	0.54772	**92%**	11.907
	100 mg/ml	5	0	**100.00%**	0
	25 mg/ml	5	0	**100%**	0
5 hours	50 mg/ml	5	0	**100%**	0
	100 mg/ml	5	0	**100.00%**	0

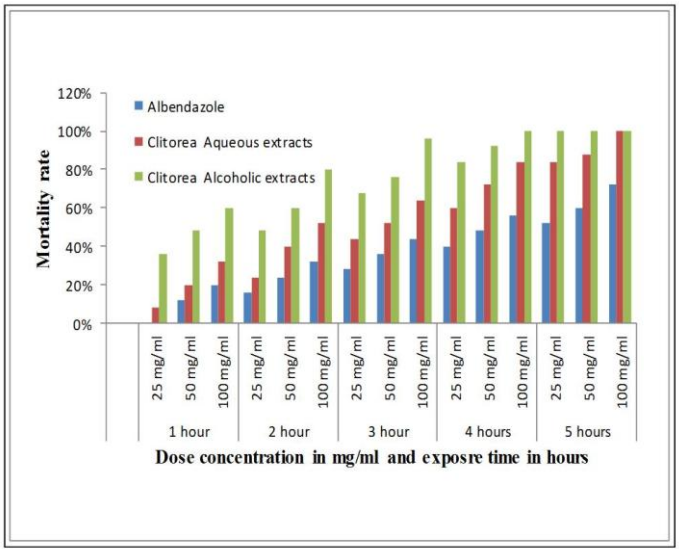
In Vitro Anthelmintic Effect of Different Concentrations of Synthetic Drug Albendazole on Amphistome Paramphistomum Cervi:

Different concentrations of albendazole show anthelmintic effect against *P.cervi* shown in Table 3. Albendazole was less effective against *P.cervi* where it exhibited 72% mortality rate revealed at high concentration 100mg/ml in 5 hour exposure time, than aqueous and alcoholic three of plant extracts. High

concentration of plant extracts showed 100% mortality rate within 3 to 4 hours of exposure time. Lower concentrations of albendazole were allowed to more survive the parasites, where the mortality rates were 52 % and 60% reached at 5 hours with concentrations of 25mg/ml and 50mg/ml shows paralytic condition. Whereas, 25mg/ml and 50mg/ml concentration at 4 hours of exposure time mortality rate exhibited to 40 and 48%. At 1 hour of exposure on lowest concentration did not induce any mortality among *P.cervi*.

Table 3: *In Vitro* Anthelminthic Effect of Albendazole on *P. Cervi*

Exposure Time in hrs.	Albendazole in mg/ml	No. of Dead Worm		Mortality Rate %	CV %
		Mean	± STD		
	25 mg/ml	0	0	**0%**	0
1 hour	50 mg/ml	0.6	0.54772	12%	91.2871
	100 mg/ml	1	0	20%	0
	25 mg/ml	0.8	0.44721	16%	55.9017
2 hour	50 mg/ml	1.2	0.44721	24.00%	37.2678
	100 mg/ml	1.6	0.54772	32%	34.2327
	25 mg/ml	1.4	0.54772	28%	39.123
3 hour	50 mg/ml	1.8	0.44721	36%	24.8452
	100 mg/ml	2.2	0.44721	44.00%	20.3279
	25 mg/ml	2	0	40%	0
4 hours	50 mg/ml	2.4	0.54772	48%	22.8218
	100 mg/ml	2.8	0.44721	**56.00%**	15.9719
	25 mg/ml	2.6	0.54772	52%	21.0663
5 hours	50 mg/ml	3	0.70711	60%	23.5702
	100 mg/ml	3.6	0.54772	**72.00%**	15.2145



Graph 1: Antiparasitic Activity of Alcoholic and Aqueous Extracts of C. Ternatea with Comparision to Albendazole on *P. Cervi*

Discussion

Herbal treatment is one of the most reliable alternative approaches to overcome the side effects of synthetic drugs. Various studies have been done on antiparasitic reports and support this evaluation revealing the herbs are potent anthelminthics against gastrointestinal parasites. In the present study, the

anthelmintic activity of albendazole drug, aqueous and alcoholic concentrations of seeds of *Clitoria ternatea* is evaluated on amphistome *P. cervi.* The *in vitro* study to evaluate the anthelminthic efficacy of alcoholic and aqueous extract of three medicinal plants extracts were evaluated on the basis of reduction in motility or death of *P. cervi.*

Some of medicinal plants alcoholic extract of *Allium sativum* and *Piper longum* on *Gigantocotyle explanatum* shows remarkable paralysis within the ½ hour of incubations and produced significant anthelminthic activity (Ghangale *et al.,* 2009 and Singh *et al.,* 2008), *Zingiber officinale* and *Curcuma longa* at 50 mg/ml concentrations showed paralysis (Singh *et al.,* 2011), Aqueous, methanolic and hydroethanolic extracts of *Azadirachta indica* were tested against *Paramphistomum cervi* showed 100% mortality was observed at 5 min. a post incubation of parasite (Barua *et al.,* 2011). The alcoholic extract of *Morus alba* caused paralysis at 5 minute and time of death at 31 minute while water extract revealed paralysis at 15 min. and death in 40 minutes on *Pheretima posthuma.* Alcoholic extract of *Allium sativum* at 1.0 mg/ml causes high mortality in *Cotylophoron cotylophorum* whereas albendazole induced mortality very low (Nahla *et al*., 2012). Aqueous extract of *Thesperia lampas* at 50 mg/ml concentration has been reported (Kosalge and Fursule, 2009).

Kumar and Singh, (2014) were studied *S. aromaticum* at concentration of 2.95 mg/ml is more effective than *A. sativum* ethanol extract at concentration of 5.48 mg/ml on *Fasciola gigantica*. Islam *et al.,* (2015) evaluate the anthelmintic effect of hydro alcoholic extracts of *Ananas sativus, Erythrina variegata* and *Alocasia indica* against adult *Haemonchus contortus* and *Paramphistomum cervi* at higher concentration. Lokesh and Veerakumari, (2015) revealed sub lethal activity at 0.5 mg/ml concentration of ethanolic extract *Terminalia chebula* on *Cotylophoron cotylophorum* after 8hr exposure time. It has been reported that the presence of Carbohydrates, Alkaloids, Tannins, Saponins and Flavanoids are most responsible chemical constituents for the anthelminthic activity.

T. foenum-graecum and *Balanites aegyptica* was found to effective against trematodes parasite *Gastrothylax crumenifer* and *Paramphistomum cervi* showed 100% mortality (Swarnakar *et al.,* 2014b and Swarnakar *et al.,* 2015b).

Clitoria ternatea alcoholic extract shows anthelminthic activity at 50 mg/ml dose dependant manner on time of paralysis in 4 min and death in 10 min on earthworms as compared to piperazine citrate (Khadatkar *et al.,* 2008).

In previous study Nahar *et al.,* (2010) were reported aparajita at higher concentration of 100mg/ml shows profound anthelminthic activity in comparison with albendazole. Our findings are similar to these studies, and alcoholic extract shows better anthelminthic activity than aqueous extract and albendazole. *Clitoria ternatea* alcoholic extract showed better antiparasitic activity at 100mg/ml concentration, but aqueous extract showed 100% mortality at all concentration only at 5 hours exposure time. Presence of chemical phytoconstitutes tannins in aparajita showed anthelminthic activity (Lijon *et al.,* 2017).

Shaheen and Eman, (2012) revealed *in vitro* effect of mixture of 25µl oxyclozanide and 5mg niclosamide drug were revealed reduced activity of *P. cervi* in 120 min. and death in 240 minutes. In our study albendazole at higher concentration of 100 mg/ml showed high mortality rate 72% after 5 hours of exposure. But in 25mg/ml and 50mg/ml doses did not showed any mortality of parasites.

All above work also compared with albendazole. They found that alcoholic extract of various traditional plants showed significant efficacy at different hours of exposure. Present result shows death of parasites also depends on exposure period, more the exposure time leads more death of parasites. *Clitorea ternatea* alcoholic extract showed better anthelminthic activity than aqueous extract and albendazole. From the result, it conducted that *Clitorea ternatea* showed a potent anthelminthic activity among all extracts. Whereas, extracts of albendazole took longer time to bring death of amphistome *P.cervi.* Hence, our study shown that *Clitorea ternatea* seed alcoholic extract has significantly determined anthelminthic activity. Statistical analysis reveals that significant anthelminthic activity between the concentration and time of exposure of all extracts. The present research work would be significant because it will help to prepare less-costly, eco-friendly anthelmintic and veterinary herbal drugs to replace the synthetic drugs which are currently in use.

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