



Original Research Article

Anthelmintic effect of *Trigonella foenum-graecum* on tegument of *Gastrothylax crumenifer* in cattle of Udaipur, India

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A B S T R A C T

Keywords

Anthelmintic,
Trigonella foenum-graecum,
cattle,
amphistome,
Gastrothylax crumenifer,
and tegument

The prevalence of amphistome parasites is very high in domestic ruminants and spread all over the world, which cause the disease paramphistomiasis. *Trigonella foenum-graecum* is commonly known as methi and Fenugreek. The leaves and seeds of fenugreek are known for their medicinal value. The present study was considered to evaluate *in vitro* efficacy of medicinal plant aqueous extract of *Trigonella foenum-graecum* on *Gastrothylax crumenifer*. 130 mg/ml concentration of aqueous extract gave total mortality at 5 hours. The morphology of control *Gastrothylax crumenifer* was compared with treated animal by light microscopy. *Gastrothylax crumenifer* were treated with *Trigonella foenum-graecum* aqueous extract. *Gastrothylax crumenifer* were showed detachment, discontinuation and blebbing in tegument surface, rupture the parenchyma cells, tegument cells and also observed damaged the musculature cells in sucker. Various veterinary drugs have been used to eliminate parasites from ruminant but these drugs are unaffordable and inaccessible for poor cattle farmers. So the study indicated the possible for developing herbal anthelmintic drug to control *Gastrothylax crumenifer*.

Introduction

The state of Rajasthan is known for cattle wealth. Gastrointestinal parasites infection is one of the major problems in worldwide and causes mortality, morbidity in cattle and economic losses to poor farmers (Perry and Randolph, 1999).

The prevalence of amphistome parasites is very high in domestic ruminants, which cause the disease paramphistomiasis (Qadir *et al.*, 2010 and Swarnakar & Kumawat, 2013).

This disease causes reduces production of milk, meat, skin and loss of life of cattle.

Many scientists have been used various medicinal plants to control helminth parasites in different animals (Veerakumari and Munuswamy 1999; Bashter *et al.*, 2011; Challam *et al.*, 2010; Maharshi *et al.*, 2011; Mohammed & Sulaiman 2013).

Trigonella foenum-graecum is commonly known as methi and Fenugreek (Amri *et al.*, 2009). *Trigonella foenum-graecum* is cultivated in India, Pakistan, China and few other countries and well known medicinal plant having properties of reducing blood sugar level, anthelmintic, antioceptive, antipyretic, antimicrobial and antifertility in human and animals (Ghafghazi *et al.*, 1980-81; Laroubi *et al.*, 2009; Bhalke *et al.*, 2009; Ahirwar *et al.*, 2010; Khadse and Kakde 2010; Zaen Al-abdeen *et al.*, 2010; Chandra *et al.*, 2011 and Moradi Kor & Moradi 2013). Anthelmintic herbal drug are those contain chemical components that eject parasitic worms from the animal body and killing them (Chaturvedi *et al.*, 2009; Ghangale *et al.*, 2009; Jeyathilakan *et al.*, 2012; Ahmed *et al.*, 2013 and Scantlebury *et al.*, 2013). The seeds of the fenugreek herb containing toxic oils so it resist parasites and kill in animal body (Tejaswini *et al.*, 2012 and Moradi Kor & Moradi 2013). The leaves and seeds of fenugreek are known for their medicinal value (Khan *et al.*, 2009; Shaikh *et al.*, 2013; Mohammed & Sulaiman 2013 and Alam *et al.*, 2014). Various veterinary drugs have been used to eliminate parasites from ruminant but these drugs are unaffordable and inaccessible for poor cattle farmers. Herbal drugs of medicinal plants are safer, cheaper and ecofriendly way for poor farmers.

Little research work has been observed on extract of medicinal and indigenous plants tested against different species of amphistome (Tandon, *et al.*, 1997; Nahla *et al.*, 2012; Veerakumari *et al.*, 2012 and Usha *et al.*, 2013). Anthelmintic activity and paralytic effect have shown against *G. explanatum* (Singh *et al.*, 2008).

However, no research work has been

carried out so far to study the effects of *Trigonella foenum-graecum* extracts of indigenous plant on amphistome, *Gastrothylax crumenifer* by light microscope.

There is an urgent need to develop a new, ecofriendly drug to control amphistome infection in cattle. Therefore, it has been decided to undertake the work on "Anthelmintic effect of *Trigonella foenum-graecum* on tegument of *Gastrothylax crumenifer* in cattle of Udaipur".

Materials and Methods

Collection of parasites

The *Gastrothylax crumenifer* were collected from the rumen of freshly slaughtered buffaloes (*Bubalus bubalis*) at the local zoo abattoir in Udaipur. After thorough washing with physiological saline solution (0.7 percent, NaCl) they were divided into three groups. First group of worm were used for identification of species of amphistomes, with the help of whole mount preparation of amphistomes (Dutt, 1980). Second group of the *Gastrothylax crumenifer* were given *in vitro* treatment of aqueous extract of *Trigonella foenum-graecum* at various concentration and *in vitro* treated *Gastrothylax crumenifer* were fixed in Bouin's fixative for histological studies by light microscope. Third group of *Gastrothylax crumenifer* were used as untreated or control amphistomes

Plant collection

Fresh seeds of *Trigonella foenum-graecum* were collected from Udaipur areas for their anthelmintic activity against the amphistomes *Gastrothylax crumenifer* of cattle.

Preparation of plant extract:

Seeds of *Trigonella foenum-graecum* were washed with tap water and distilled water then they were kept in dry in oven at 40 °C for 3-4 days for material become completely solid and dry. The dried Seeds of *Trigonella foenum-graecum* 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.

The seeds *Trigonella foenum-graecum* were extracted by taking 20 g of each sample in 160 ml of water and organic solvent in 250 ml flask. Then continuous shaking with an orbital shaker and an occasional stirring with a glass rod manually at 4 hours interval. After 72 hours the macerates solutions were filtered in separate flasks using a qualitative filter paper (Whatman No 4 filter paper, Whatman Ltd., England). Then centrifuged at x 10000 g for 15 min. and supernatant were used for anthelmintic testing. The filtered supernatant was dried until a constant dry weight of each extract was obtained. Then this dried plant extract were reconstituted in the respective solvent.

Aqueous extract of seeds of *Trigonella foenum-graecum* were reconstituted in 10% DMSO. The extracts were stored in 15 ml black cap bottle, covered with aluminum foil for the prevention of *Trigonella foenum-graecum* extract directly from light. The residues were stored at 4 °C for further used. The extract of seed of *Trigonella foenum-graecum* was tested *in vitro* against *Gastrothylax crumenifer*. Treated parasites with aqueous extract of seeds of *Trigonella foenum-graecum* were fixed in Bouin's fluid for histological studies by light microscope (LM) for 24 hours after they were washed

in running tap water for at least 24 hours. These parasites were dehydrated in ascending series of alcohol, cleared in xylene, blocks were prepared in paraffin wax and sections were cut at 6 μ on rotary microtome. Then sections were stained by Haemotoxylin & Eosin and then mounted with DPX (Bancroft & Stevens, 1977).

Results and Discussion

Present investigation revealed that a number of *in vitro* tests employing *Gastrothylax crumenifer* were carried out to test the activity of aqueous extract of *Trigonella foenum-graecum* in direct contact with *Gastrothylax crumenifer*. Treated worms become slender, shrunken, paralyzed and then finally died after 5 hours at 130 mg/ml concentration of seed of *Trigonella foenum-graecum*.

The effect of aqueous seed extract on the *Gastrothylax crumenifer*, when examined under light microscope observed that the treated worms became small and found shrinkage in tegument. The morphology of control *Gastrothylax crumenifer* was compared with treated animal by light microscopy. Control worms showed smooth spineless tegument followed by surface syncytium, subsyncytial zone, longitudinal and circular muscle. Control *Gastrothylax crumenifer* showed the normal microscopic structure of tegument (Fig. 1). In contrast, aqueous extract of seeds of *Trigonella foenum-graecum* treated *Gastrothylax crumenifer* showed detachment and discontinuation of surface syncytium in tegument (Figs. 2, 3 and 4) and also showed rupturing parenchymatous cell, tegument cells and Surface syncytium of treated *Gastrothylax crumenifer* (Fig. 3). Swelling and blebbing were also observed in tegument of treated *Gastrothylax crumenifer* (Fig. 5).

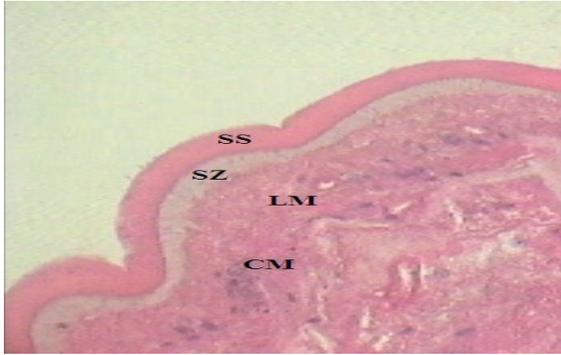


Fig.1 A portion of tegument of control *Gastrothylax crumenifer* showing surface syncytium (SS), subsyncytial zone (SZ), longitudinal muscles (LM) and circular muscles (CM) x 185..

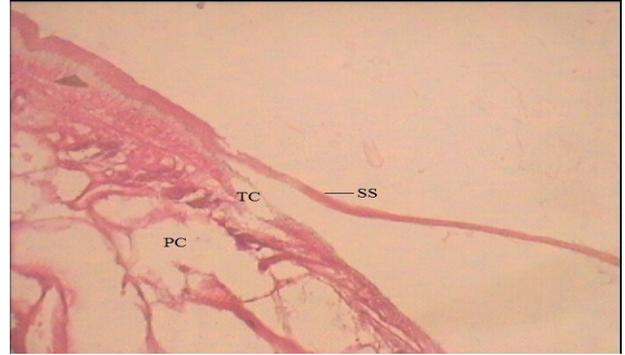


Fig.2 Showing detachment of tegument *Gastrothylax crumenifer* showing surface syncytium (SS), tegumental cell (TC) and parenchymatous cell (PC) x 185.



Fig.3 Photomicrography exhibiting rupture of parenchymatous cell (PC), tegument cells (TC) and surface syncytium (SS) of treated *Gastrothylax crumenifer* x 110.

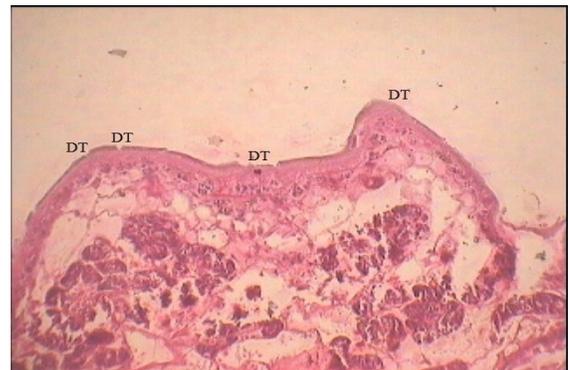


Fig.4 Showing discontinuation in tegument (DT) of treated *Gastrothylax crumenifer* x 110

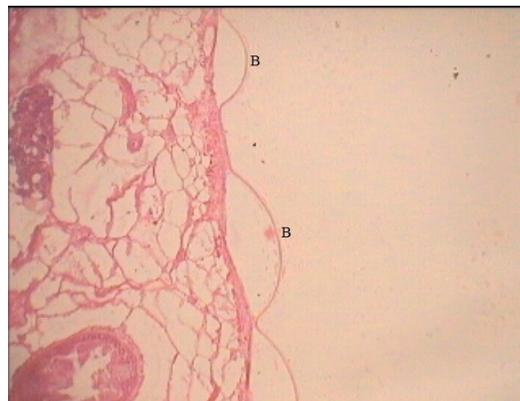


Fig.5 Blebbing (B) of tegument showing in treated *Gastrothylax crumenifer* x 110

Tegument is most important part of the trematode that is in direct contact with the host's tissue along with body fluids. Modification in structure of the tegument is necessary in developing any rational drugs which may damage the parasites through their actions on the tegument (Panyarachun *et al.*, 2010; Shaheen & Eman, 2012 and Saowakon *et al.*, 2013).

Tegumental detachment in treated worms may lead to complete breakdown of cells in the parenchyma leaving vacuolated areas (Veerakumari & Paranthaman 2004 and Veerakumari *et al.*, 2012). Presence of vacuoles in the parenchyma could distort the structure of the *Gastrothylax crumenifer*.

Blebbing is an action in response to anthelmintic treatment, where secretory bodies are rapidly transported towards the tegument and released from the apical plasma membrane in an effort to replace damaged membrane and maintain the integrity of the tegumental surface (McConville *et al.* 2006). Our findings are similar with Buddhachat *et al.*, 2012, showed that anthelmintic plants change the morphology of tegument surface and sucker in trematode.

Trigonella foenum-graecum has many properties to become pathogenic organisms inactive and cause mortality this observations are agreement with Laroubi *et al.*, 2009; Bhalake *et al.*, 2009; Zaen Al-abdeen *et al.*, 2010 Challam *et al.*, 2010 and Alam *et al.*, 2014.

So various studies revealed that *Trigonella foenum-graecum* has no toxic effect for animals and the seeds of *Trigonella foenum-graecum* are very useful plant for treatment of *Gastrothylax crumenifer*. Seed extract of *Trigonella foenum-*

graecum are safer, cheaper and ecofriendly way for poor farmers than costly veterinary medicines. Thus plant-based medicines such as *Trigonella foenum-graecum* could be used as an efficient anthelmintic in treatment of paramphistomiasis.

Acknowledgement

The authors are grateful to Dr. B. Bhardawaj Head of RDDC, Dr. Chandra Shekhar Bhatnagar M. V. Sc. (Veterinary Parasitology) and Ajay kumar salvi, S. V. O. Regional Disease Diagnostic Centre, Dept. of Animal Husbandry, Udaipur, Rajasthan for their valuable suggestions.

References

- Ahirwar D, Ahirwar B and Kharya MD. 2010. Evaluation of antifertility activity of *Trigonella foenum graecum* seeds. *Der Pharmacia Sinica*. 1(3): 33-39.
- Ahmed M, Laing MD and Nsahlai IV. 2013. *In vitro* anthelmintic activity of crude extracts of selected medicinal plants against *Haemonchus contortus* from sheep. *J Helminthol*. 87(02): 174-179.
- Alam MT, Hasan I, Perveen A, Nazamuddin M, Perveen S. 2014. Hulba (*Trigonella foenum-graecum*): The common Indian spice full of medicinal values. *International Journal of Preclinical & Pharmaceutical Research*. 5(1): 41-46.
- Amri M, Sellami F, and Kharrat M. 2009. First Report of the Parasitic Plant *Orobanchefoetida* on Fenugreek (*Trigonella foenum-graecum*) in Tunisia. *Tunisian Journal of Plant Protection*. 4(2): 235-38.
- Bancroft JD and Steven A. 1977. Theory and practice of histological

- techniques. Churchill Living stone. Medical Division of Longman Group Limited. Edinburgh London and New York.
- Bashtar AR, Hassanein M, Abdel-Ghaffar F, Al-Rasheid K, Hassan S, Mehlhorn H, Al-Mahdi M, Morsy K and Al-Ghamdi A. 2011. Studies on monieziasis of sheep I. Prevalence and antihelminthic effects of some plant extracts, a light and electron microscopic study. *Parasitol Res.* 108(1):177-86.
- Bhalke RD, Anarthe SJ, Saspne KD, Satpute SN, Shinde SN and Sangle VS. 2009. Antinociceptive activity of *Trigonella foenum-graecum* leaves and seeds (Fabaceae). *IJPT.*8(2): 57-59.
- Buddhachat K, Chantima K, Chomdej S and Wongsawad C. 2012. *In vitro* effects of Some Thai Anthelmintic Plants on Mortality and Change of Tegumental Surface of *Stellantchasmus falcatus*. *J BacteriolParasitol.* 3(6): 1-3.
- Challam M, Roy B and Tandon V. 2010. Effect of *Lysimachia ramosa* (Primulaceae) on helminth parasites: Motility, Mortality and scanning electron microscopic observation of surface topography. *Vet. Parasitol.*169: 214-218.
- Chandra R, Dwivedi V, Shivam K and Jha AK. 2011. Detection of Antimicrobial Activity of *Oscimum sanctum* (Tulsi) & *Trigonella foenum graecum* (Methi) against some selected bacterial & fungal strains. *Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS).* 2(4): 809 -13.
- Chaturvedi M, Dwivedi S, Dwivedi A, Barpete PK and Sachan R. 2009. Formulation and Evaluation of Polyherbal Anthelmintic Preparation. *Ethnobotanical Leaflets.*13: 329-331.
- Dutt SC. 1980. Paramphistomes and paramphistomiasis of domestic ruminant in India. PAU Press, Ludhiana and published by the joint Director, Communication Center, Punjab Agricultural University, Ludhiana.
- Ghangale GR, Mahale T and Jadhav ND. 2009. *In vitro* anthelmintic activity of alcoholic extracts of *Allium sativum* against rumen amphistome. *Journal of veterinary world.* 2(10): 385-386.
- Ghafghazi T, Farid H and Pourafkari A. 1980-81. *In vitro* study of the anthelmintic action of *Trigonella foenum graecum* L. grown in Iran. *Iranian J. Publ. Hlth.* 9(1-4): 21-24.
- Jeyathilakan N, Murali K, Anandaraj A and Basith SA. 2012. *In vitro* evaluation of anthelmintic property of ethno-veterinary plant extracts against the liver fluke *Fasciola gigantica*. *J Parasit Dis.*36(1): 26–30.
- Khadse CD and Kakde RB. 2010. *In vitro* anthelmintic activity of Fenugreek seeds extract against *Pheritima posthuma*. *Int. J. Res. Pharm. Sci.* 1(3): 267-269.
- Khan FU, Durrani FR, Sultan A, Khan RU and Naz S. 2009. Effect of Fenugreek (*Trigonella foenum graecum*) seed extract on visceral organs of Broiler chicks. *Journal of Agriculture and Biological Science.* 4(1): 58-60.
- Moradi Kor N and Moradi K. 2013. Physiological and Pharmaceutical Effects of Fenugreek (*Trigonella foenum-graecum* L.) as a Multipurpose and Valuable Medicinal Plant. *Global Journal of Medicinal Plant Research.*1(2): 199-206.
- Laroubi A, Farouk L, Aboufatima R, Benharref A, Bagri A and Chait A. 2009. Antinociceptive properties of

- Trigonella foenum graecum* seeds extracts .African Journal of Biochemistry Research (AJBR).3 (2): 017-023.
- Maharshi AK, Swarnkar CP, Singh D, Manohar GS and Ayub M. 2011. Status of anthelmintic resistance in gastrointestinal nematodes of sheep in Rajasthan.Indian Journal of Animal Sciences.81 (2): 105–109.
- McConville M, Brennan G P, McCoy M, Castillo R, Hernandez-Campos A, Ibarra F and Fairweather I. 2006. Adult triclabendazole resistant *Fasciola hepatica*: surface and subsurface tegumental responses to *in vitro* treatment with the sulphoxide metabolite of the experimental fasciocide compound alpha. Parasitology133:195–208.
- Mohammed ST and Sulaiman NM . 2013. Studying the effect of *Trigonella fornum graecum* on *Hymenolepis nana* in mice. Int J Biotec Allied Fields (IJBAF). 1(7): 371-379.
- Nahla AR, Amal IK and Amera EW. 2012. *In vitro* evaluation of anthelmintic activity of *Allium sativum* against adult *Cotylophoron cotylophoron* (*Paramphistomidae*). Parasitological UJ. 5(2): 135-146.
- Panyarachun B, Sobhon P, Tinikul Y, Chotwiwatthanakun C, Anupunpisit V and Anuracpreeda P. 2010. *Paramphistomum cervi*: surface topography of the tegument of adult fluke. Exp Parasitol. 125: 95–99.
- Perry BD and Randolph TF. 1999. Improving the assement of the economic impact of parasite diseases and their control in production animal. Vet. Parasitol. 84: 145-168.
- Qadir S, Dixit AK and Dixit P. 2010. Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants.Veterinary World. 3(11): 515-518.
- Saowakon N, Lorsuwannarat N, Changklungmoa N, Wanichanon C and Sobhon P. 2013.*Paramphistomum cervi*: the *in vitro* effects of *plombagin* on motility, survival and tegument structure. Experimental Parasitology. 133(2): 179-186.
- Scantlebury CE, Peachey L, Hodgkinson J, Matthews JB, Trawford A, Mulugeta G, Tefera G and Pinchbeck GL. 2013. Participatory study of medicinal plants used in the control of gastrointestinal parasites in donkeys in Eastern Shewa and Arsi zones of Oromia region, Ethiopia. BMC Veterinary Research . 9(179): 1-12.
- Shaikh RS, Syed FS, and Tayade DT. 2013. Phytochemical analysis of leaves of *Trigonella foenum-graecum* L. from Anjangaon, Amravati region of Maharashtra state. International Journal of Pharmacy and Pharmaceutical Science Research. 3(3): 94-96.
- Shaheen HM and Eman K. Bazh. 2012. Morphological response of *Paramphistomum cervi* to treatment with oxyclozanide and niclosamide *in vitro*. J.Egypt.vet.med.Assoc. 72(4): 561 – 574.
- Singh TU, Kumar D and Tandan SK. 2008. The paralytic effect of *A. sativum* and *P. longum* on *G. explanatum*. Indian Journal of Pharmacol. 40(2): 64-68.
- Swarnakar G and Kumawat A. 2013. Incidence of Pathogenic Amphistomes *Orthocoelium scoliocoelium* (Trematoda: Digenea) in Udaipur (Rajasthan). International Journal of Scientific Research. 2(3):70-71.
- Tandon V, Pal P, Roy B, Rao HSP and Reddy KS. 1997. *In vitro* anthelmintic activity of root tuber

- extracts of *Flemingia vastita*, an indigenous plant in Shilong. India. Parasitol Res. 83 : 492-498.
- Tejaswini BA, Sireesha CH, Kaladhar DSVGK, Rao DG, Satyanarayana KVVV, Surekha CH. 2012. *In vitro* Antioxidant and Antimicrobial Activities of Crude Extracts of *Trigonella foenum-graecum* Seeds. Asian Journal of Chemistry. 24(11): 5019-5022.
- Usha NP, Dhanya VR and Jose S. 2013. *In vitro* efficacy of *Butea monosperma* against amphistomosis in cattle. J. Vet. Anim.Sci. 44 : 70 – 71.
- Veerakumari L and Munuswamy N. 1999. *In vitro* studies on the effects of some anthelmintics on *Cotylophoron cotylophorum* (Digenea, Paramphistomidae): a structural analysis. Cytobios. 98(387): 39-57.
- Veerakumari L and Paranthaman D. 2004. Light and scanning electron microscopic studies on the effects of niclosamide and oxyclozanide on *Cotylophoron cotylophorum* (Fischoeder,1901). Journal of Veterinary Parasitology.18: 1–12.
- Veerakumari L, Ashwini R and Lalhmingchhuanmawii K. 2012. Light and scanning electron microscopic studies on the effect of *Acacia arabica* against *Cotylophoron cotylophorum*. Indian Journal of Animal Sciences.82 (4): 21–24.
- Zaen Al-abdeen SS, Faraj BM, and Nasrulla OJ. 2010. Antibacterial effects of Fenugreek (*Trigonella foenum-graecum*). Bas.J.Vet.Res. 10(2): 133-40.