Original Research Article

**In vitro** anthelmintic effect of *Citrullus colocynthis* on tegument of amphistome *Orthocoelium scoliocoelium* (Trematoda: Digenea)

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**Abstract**

The alcoholic fruit pulp extract of *Citrullus colocynthis* was tested *in vitro* against amphistome parasites *Orthocoelium scoliocoelium*. The live adult parasites were collected from rumen of freshly slaughtered cattle and exposed to 40mg/ml concentration of plant extract. The treated parasites showed complete loss of activity and paralysis followed by complete mortality at 5 hrs of exposure to 40mg/ml dose of fruit extract. The tegument of treated parasites was compared with controlled parasites by light microscopy. The microscopic observation revealed wide scale deformity in the tegumental architecture of treated parasites with breakage and detachment in surface tegument. During study researchers were also observed vacuolisation in subsyncytial zone and parenchymatous cells. The acetabulum of treated parasite revealed damage, breakage and vacuolisation in musculature of sucker. The overall findings of present research indicated that *Citrullus colocynthis* has potential anthelmintic effect warranting an alternative for commonly used chemical drugs.

**Keywords**

Amphistomes, parasites, *Orthocoelium scoliocoelium*, *Citrullus colocynthis*, fruit pulp extract and tegument.

**Introduction**

Paramphistomiasis is become one of the major problems in the productivity of livestock as well the health of human being throughout the world (WHO 2002). It causes the loss of production through mortality, weight loss, reduced milk, meat, wool production etc., which affect the economy of farmers. Inspite of tremendous development in the field of commercial synthetic drugs during recent era, they are having side effects and unfortunately the high cost of these drugs make them unaffordable to poor farmers (Hammond *et al.*, 1997). Further the development of resistance is also a problem associated with synthetic drugs (Perry *et al.*, 2002 and Waller & Thamsberg, 2004). Therefore, the use of alternative treatment should be the priority. Plants have been used as food and for medicinal purposes for centuries. The traditional plants based medicines holds a great promise as source of easily available effective anthelmintic agent to the people. The plant based medicines are having high percentage of cure with a single therapeutic dose, free from toxicity and should be cost effective.
Various indigenous plants have shown anthelmintic effect against cestodes, trematodes and nematode parasites (Satyavati, 1990; Kushwaha et al., 2004; Temjengmongla & Yadav, 2005; Eguale et al., 2007; Kabore et al., 2009 and Saowakon et al., 2009). Extract of Allium sativum demonstrated activity against Heterakis gallinae, Ascardia galli, Gigantocotyle explanatum, Cotylophoron cotylophorum (Sutton and Haik, 1999, Nagaich et al., 2000; Singh et al., 2008 and Nahla et al., 2012). The sacred Basil (Tulsi) Ocimum sanctum has showed potent in vitro anthelmintic activity against Caenorhabditis elegans (Asha et al., 2001). The legume Serica lepedeza shows remarkable anthelmintic efficacy on flatworms (Min et al., 2004; Shaik et al., 2004 and Lange et al., 2006). The spice Trachyspurrum ammi seed extract was screened for anthelmintic property in sheep and ovicideal activity against Haemonchus contortus (Lateef et al., 2006 and Jabbar et al., 2006a & b). The alcoholic extract of Lysimachia found to effective on Fasciola buski, Ascaris suum and Rallietina echinobrothrida (Challam et al., 2010). Ginger Zingibar officinale have anthelmintic effects on Angiostrongylus simplex and Schistosoma mansoni (Iqbal et al., 2006; Lin et al., 2010 and Osama et al., 2011). The extract of Melia azadarach shows antiparasitic effects on sheep gastrointestinal nematode (Cal, et al., 2012). The fruit extract of Balanitus aegyptica and Artemisia found effective against Fasciola gigantica, Haemonchus contortus, Schistosoma, Trichinella and Toxocara coenorchabditis (Koko et al., 2000; Iqbal et al., 2004; Goula et al., 2007; Shalaby et al., 2009; Doaa et al., 2011 and Shalaby et al., 2012). The methanolic extract of leaves of Bombex malabericum found to be lethal on Paramphistomum explanatum (Hossain et al., 2012). The whole aerial part of Secamone africana and leaves of Vernonia amygdalina found to be anthelmintic against Ascaris suum (Nalule et al., 2013). Leaves of Carissa spp., Azadirachta indica and stem bark of Acacia tortilis were caused mortality in adults of Haemonchus contortus (Mohammed et al., 2013). The seeds extract of Trigonella foenum-graecum caused deformity in the normal tegumental architecture and mortality in Gastrothylax crumenifer (Swarnakar et al., 2014).

The experimental plant Citrullus colocynthis commonly known as bitter apple, bitter cucumber, Gavakshi or Indravaruni is a viny plant, native to Mediterranean basin and Asia especially Turkey, Nubia, Desert area of India and Pakistan. In Rajasthan, this plant occurs in Jaisalmer, Barmer, Shriganganagar. Its fruit extracts have shown antibacterial and antimicrobial effect against Pseudomonas, Staphylococcus, Candida spp., antifungal property against Aspergillus flavus and antihyperglycemic effect on type 2 diabetic patients and rats (Huseini et al., 2009; Murzouk et al., 2009; Dallak, 2011; Amrouche et al., 2011 and Jeyanthi & Christy, 2011).

Further, the plant extract of Citrullus colocynthis found antileishmanial, antitumor agent against Leishmania major (protozoan parasite) and molluscidal action against Biomphalaria arebica (Baloch et al., 2013 and Zaid et al., 2013). The anthelmintic efficacy of Citrullus colocynthis found to be positive on Haemonchus contortus, which caused reduction in egg count and the plant extract paralysed the worm Pheretima posthuma (Ullah et al., 2013 and Talole et al., 2013). The laxative activity of Citrullus colocynthis were observed in Wister rats (Kumar et al., 2014) and also in traditional medicinal practice, seeds of Citrullus colocynthis are used as strong laxative to
treat refractory oedema, amenorrhoea and nerve pain fever. However, no research work has been carried out so far on anthelmintic effects of *Citrullus colocynthis* fruit extracts of indigenous plant on amphistome *Orthocoelium scoliocoelium* by light microscope.

**Materials and Methods**

Live amphistome parasites were collected from the rumen of freshly slaughtered domestic ruminants; buffaloes, Sheep and Goat at the local zoo abattoir in Udaipur. After thorough washing with saline solution (0.7 percent, NaCl), they were divided into three groups. First group of parasite were used for identification of species of amphistomes with the help of whole mount preparation (Dutt, 1980). Second group of the amphistome parasites were untreated used as control amphistomes and third group of the amphistome parasites were given in vitro treatments with fruit extracts of *Citrullus colocynthis*. Control and treated amphistome with the fruit extracts of *Citrullus colocynthis* were fixed in Bouin’s fixative for histological study by light microscope.

**Preparation fruit extracts**

Fresh *Citrullus colocynthis* fruits were collected from the desert area; Shriganganagar, Barmer, Jaisalmer (Rajasthan). Seeds were separate from pulp of fruits. Then pulp was put to dry and pulverize with grinder into a powder. The powder was refluxed in 70% alcohol for 12 hrs at 60°C and the solution filtered through whatman paper no.1. The filtered solution was evaporated and stored at 4°C till further use. Alcoholic extracts were prepared at 40 mg/ml concentration with pulp powder of *Citrullus colocynthis* fruits.

**Histology by Light Microscope (LM)**

Transverse and longitudinal sections of control and treated amphistomes with the fruit extracts of *Citrullus colocynthis* were fixed in Bouin’s fixative (Bancroft & Stevens, 1977), dehydrate, embedded in paraffin wax, sections were cut at 6µ on rotary microtome then dehydrated, stained with Haemotoxylin and Eosin, cleared in xylene and mounted in DPX. Stained sections were examined under light microscope.

**Results and Discussion**

During investigation, number of tests was carried out on *Orthocoelium scoliocoelium* with the fruit extract of *Citrullus colocynthis* to observe the anthelmintic efficacy of plant. The treated worms became agglutinated, shrunken, paralysed and dead after 5 hours at 40 mg/ml concentration of alcoholic fruit extract of *Citrullus colocynthis*.

**Histology of tegument of controlled parasite**

The tegument of controlled *Orthocoelium scoliocoelium* was compared with treated worms. The normal adult *Orthocoelium scoliocoelium* has elongated oval and slightly ventrally curved body. The body of adult is dorsally convex and almost straight at ventral. Anterior end have several rows of cuticular papillae. It has two suckers, an anterior sub terminal oral sucker and a posterior large ventral sucker. The tegumental surface is highly corrugated with transverse folds alternating with grooves and is spineless, which is exceptional character of trematodes. The genital pore is situated at the anterior third of the body. There are two types of bulbous shaped sensory papillae on the surface.
Fig. 1 Tegument of controlled *Orthocoelium scoliocoelium* showing surface syncytium (SS), sub syncytial zone (SZ), longitudinal muscles (LM) and Circular muscles (CM), Parenchymatous cell (PC) and sub tegument (ST)x185.

Fig. 2 A portion of acetabulum of controlled *Orthocoelium scoliocoelium* showing surface tegument (ST), circular muscles (CM), longitudinal muscles (LM) x110.
Fig. 3 Showing detachment (DT) of surface syncytium (SS) from sub syncytial zone (SZ) of tegument and vacuolisation in tegumental muscles (TM) and parenchymatous cells (PC) of treated Orthocoelium scoliocoelium x110.

Fig. 4 This slide shows vacuolisation (V) in tegumental muscles (TM) and sub tegumental cells (STC) of tegument of treated Orthocoelium scoliocoelium x110.
**Fig. 5** Breakage in surface syncytium (SS) of tegument of treated *Orthocoelium scoliocoelium* x110.

**Fig. 6** Separation (S) of muscles in circular muscles (CM) and longitudinal muscles (LM) of acetabulum of treated *Orthocoelium scoliocoelium* x110.
The clusters of papillae on the ventral surface and around the anterior sucker, while there are few on the dorsal surface. The tegument comprises an outer surface syncytium underlined by thick sub syncytial zone and musculature. The surface syncytium is bounded by a basement membrane. This membrane is highly folded and forms finger like projections into the surface layer. The sub syncytial zone is composed of interstitial fibrous connective tissue which surrounds numerous extensions of underlying parenchyma cells. The body musculature is present between the sub syncytial zone and tegumental cells. It comprises bundles of outer circular and inner longitudinal muscles fibres among which pass the trabeculae of tegumental cells which are clearly seen (Fig. 1). The acetabulum of controlled parasite is sub terminally ventral, bordered by ridges with circular muscles beneath the tegument and then the layer of longitudinal muscles exist (Fig. 2).

**Histology of tegumental alteration of treated parasite**

The present investigation revealed that the alcoholic extract of *Citrullus colocynthis* caused destructive alternation and deformity in the tegumental architecture of *Orthocoelium scoliocoelium*. There was detachment of surface syncytium in treated animal seen (Fig. 3) where surface syncytium separated from sub syncytial zone and leave a hollow space and vacuolisation in parenchymatous cells and tegumental musculature were also observed.

Fig. 4 showed distortion of normal tegumental infrastructure, there were vacuolisation and rupturing of parenchymatous cells observed, deformity in the arrangement of musculature of tegument leaving empty spaces between muscle fibres. There were breakages in the surface syncytium of treated parasite examined in Fig. 5.

The anthelmintic efficacy of plant extract of *Citrullus colocynthis* deformed the normal structure of acetabulum. There were separation of muscles in musculature and breakage starting from surface tegument and penetrating into the musculature of acetabulum was seen in Figs. 6 and 7.
As paramphistomiasis becomes a major health problem throughout the world and is responsible for great economic losses to the livestock industries therefore in recent decade there is increase interest in the field of traditional health care practice which led to exponential growth in ethno veterinary research and reinvention of traditional folklore knowledge in the field of animal health. Many *in vitro* studies on biological related trematode parasites have been done by various scientists using different plant based extracts.

The effect of plants extracts like *Allium sativum*, *Azadirachta indica*, *Serica lepedeza*, *Ocimum sanctum*, *Zingibar officinale*, *Secamone africana*, *Vernonia amygdalina*, *Trachyspermum ammi*, *Acacia tortolis*, *Trigonella foenum-graecum* were experimented and their anthelmintic properties have been established through *in vitro* exposure of crude or methanolic/ethanolic extracts against several helminth parasites (Nagaich et al., 2000; Asha et al., 2001; Shaik et al., 2004; Lange et al. 2006; Jabbar et al., 2006; Singh et al., 2008; Lin et al., 2010; Osama et al., 2011; Cala et al., 2012; Nahla et al., 2012; Nalule et al., 2013; Mohammed et al., 2013 and Swarnakar et al., 2014).

Previous survey on *Citrullus colocynthis* suggested antibacterial and antimicrobial effect against *Pseudomonas, staphylococcus* and *candida* spp. and antihyperglycemic activity on type 2 diabetic patients and rats (Murzouk et al., 2009; Huseini et al., 2009; Jayanthi & Christy 2011 and Dallak, 2011). *Citrullus colocynthis* found antileishmanial, antitumor agent against *Leishmania major* (protozoan parasite) and molluscidal action against *Biomphalaria arebica* (Baloch et al., 2013 and Zaid et al., 2013).

The anthelmintic efficacy of *Citrullus colocynthis* found to be positive on *Haemonchus contortus*, which caused reduction in egg count and the plant extract paralysed the worm *Pheretima posthuma* (Ullah et al., 2013 and Talole et al., 2013). In traditional medicinal practice seeds of *Citrullus colocynthis* are used as strong laxative to treat refractory oedema, amenorrhea and nerve pain fever.

In amphistomes, the surface tegument acts as the vital organ of parasites, performing various function like absorption of food materials, protection and osmoregulation and suckers which are modification of tegument, offers organ of anchorage. Modification in normal infra-structure of tegument is necessary to develop any rational drugs which may able to damage the parasite and caused mortality, targeting tegument of parasites.

In this study we have first time assessed the *in vitro* efficacy of alcoholic fruit pulp extract of *C. colocynthis* on tegument of *O.scoliocoelium*. The light microscope used to observe the change in the tegumental surface and our observations are an agreement with the following findings. The crude extract of *Flaminga vastita* caused disorganization of cuticle and body musculature in treated *Ascaris suum* (Yadav et al., 1992) and destruction in tegument and distortion of muscles, vacuolisation in muscles of sucker in different helminth parasites (Tandon et al., 1997) which support our study. The plant extract of *Lasimachia ramosa* caused mortality, shrunken body, destructive surface alteration in *Ascaris suum* (Yadav et al., 1992) and destruction in tegument and distortion of muscles, vacuolisation in muscles of sucker in different helminth parasites (Tandon et al., 1997) which support our study. The plant extract of *Lasimachia ramosa* caused mortality, shrunken body, destructive surface alteration in *Ascaris suum* (Yadav et al., 1992) and destruction in tegument and distortion of muscles, vacuolisation in muscles of sucker in different helminth parasites (Tandon et al., 1997) which support our study.
crumenifer (Swarnakar et al., 2014) which are similar with our findings. In our study the efficacy of fruit pulp extract of Citrullus colocynthis on amphistome Orthocoelium scoliocoelium also showed breakage, detachment in tegument, vacuolisation in parenchymatous cells, separation of muscles of tegument and acetabulum.

Based on the result of present study the alcoholic fruit pulp extract of Citrullus colocynthis could offer a cheaper eco-friendly alternative for the costly chemical drugs. Therefore active principal of plant extract should be identified to establish the actual mode of anthelmintic efficacy and action of plant on parasite.

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