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Original Research Article

In vitro anthelminthic effect of *Balanites aegyptica* on *Paramphistomum cervi* in Buffalo (*Bubalus bubalis*) of Udaipur

G. Swarnakar*, K. Roat and B. Sanger

Parasitology Lab. Department of Zoology, Government P.G. Meera Girls College, MLS University, Udaipur-313001, India

*Corresponding author

ABSTRACT

Keywords

Paramphistomiasis,
Buffalo,
Alcoholic
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Paramphistomum
cervi,
Balanites
aegyptica

Main purpose of this study was the investigation of helminth's prevalence is very high in Buffalos. Paramphistomiasis is one of the major problems in the productivity of buffalo and health of human being throughout the world. This disease causes loss of life of cattle, reduction in milk, meat and wool production. Balanites aegyptica is commonly known as hingot .The fruits, leaves and seeds of hingot are known for their medicinal value. The present study was designed to evaluate the *in vitro* anthelminthic activity of alcoholic extract of Balanites aegyptica on amphistome Paramphistomum cervi. 125 mg/ml concentrations of alcoholic extract gave total mortality at 5 hours. The treated and control Paramphistomum cervi was observe and compared by Light microscopy. The alcoholic extract of hingot showed discontinuous, damaging cells of tegument, vacuolization & breakage in oral sucker and acetabulum of Paramphistomum cervi. This study revealed that the potential role of hingot fruit extract as an anthelminthic activity against Paramphistomum cervi.

Introduction

Helminths are major cause of reduced production in livestock. Rajasthan is the largest state of India having maximum number of livestock or domestic ruminants. The economy of rural people largely depends on domestic ruminants like cow, buffalo, goat and sheep. The prevalence of amphistome parasites is very high in domestic ruminants and spread all over the which world. cause the disease paramphistomiasis (Qadir et al., 2010 and Swarnakar & Kumawat, 2013). The disease

causes high morbidity and mortality resulting in great economic losses through reduced productivity to poor farmers. Chemotherapy is the only efficient and effective tool to cure and control the helminth infection, as efficacious vaccines against helminth have not been developed so far.

Development of resistance in most of the commercially available anthelminthic drugs becomes severe problems worldwide. These drugs are unaffordable, in accessible or inadequately available to resource-poor farmers of developing countries. The use of medicinal plants for the prevention and treatment of gastrointestinal parasitism has its origin in ethno veterinary medicine. Some anthelminthic herbal drugs prepared by the medicinal plants, they are effect on helminths parasites and killing them (Ghangale *et al.*, 2009; Bashtar *et al.*, 2011; Jeyathilakan *et al.*, 2010 & 2012; Nahla *et al.*, 2012; Ahmed *et al.*, 2013 and Scantlebury *et al.*, 2013).

Balanites aegyptica is medicinal plant and commonly known as hingot or desert date belongs to Zygophyllaceae or Balanitaecae family. This plant is an evergreen xerophyte tree distribute in the drier states of India: Rajasthan, Gujrat, and Madhay Pradesh(Yadav & Panghal., 2010; Dubey et al., 2011; Kumawat et al., 2012 and Saboo et al., 2014).

The Fruit of Balanites aegyptica contains many valuable nutrients, is used for preparing beverage, cooked foods & medicines and the seed kernel, which is rich in oil, is used as a source of edible oil (Kumawat et al., 2012). The fruit extract of B. aegyptica and Artemisia found effective against helminth parasites (Koko et al., 2000 and Igbal et al., 2004). The extract of B. aegyptica fruit mesocarp, root bark, leaves seeds and kernels shows larvicidal. vermicidal, antibacterial, wound healing activity and use as an alternative protein source in animal feeding. (Chapagain & Weisman 2005; Dwivedi et al., 2009 and Yadav & Panghal., 2010). B. aegyptica have some properties such as antiinflammatory, anthelminthic, antioxidant, antinoceceptive, antiviral. antimicrobial. anticancer, antidiabetic and antiasthmetic effect in various animals. (Dubey et al., 2011; Suky et al., 2011; Abdallah et al., 2012; Lohlum et al., 2012; Shalaby et al.,

2012; Kommu *et al* 2013; Gajalakshmi *et al* 2013; Intisar *et al.*,2013; Ajayi and Ifedi 2014 and Saboo *et al.*,2014).

Little research work has been reported on extract of few medicinal and indigenous plants tested against different species of amphistomes (Veerakumari and Munuswami, 1999; Singh *et al.*, 2008; Jeyathilakan *et al.*, 2012; Veerakumari *et al.*, 2012 and Saowakon *et al.*, 2013).

However, no research work has been carried out so far to study the effects of *Balanites aegyptica* extracts on *Paramphistomum cervi* by light microscope. So the aims of this study have to test the anthelminthic activity of the alcoholic extract of the fruits of *Balanites aegyptica* against *Paramphistomum cervi* in buffalo.

Material and Methods

Collection of parasites: Live amphistome *Paramphistomum cervi* were collected from the rumen of the freshly slaughtered buffaloes (*Bubalus bubalis*) at local Zoo abattoir in Udaipur. After through washing with physiological saline solution (0.7 percent, NaCl), they were divided into three groups:-

- a) **First group:** Collected *Paramphistomum cervi* were used for identification of species of amphistomes, with the help of whole mount preparation of amphistome as described by Dutt, 1980.
- b) **Second group (Control):** Untreated *Paramphistomum cervi* amphistomes served as Control group.
- c) Third group (*In vitro* treatment with medicinal plant extracts): Third group of *Paramphistomum cervi* amphistomes were incubated in different concentrations of the plant

extracts with a volume of 10 ml in the petri dish for five hours. Then *Paramphistomum cervi* were fixed in Bouin's fixative for histological studies by Light microscopy.

Preparation of fruit extracts

Fresh *Balanites aegyptica* fruits were collected from the desert areas: Udaipur, Jodhpur, Jaisalmer, Bikaner (Rajasthan). Fruits were washed with tap water and distilled water then the fruit was keep in dry (oven at 40 °C for 3-4 days) and pulverize with grinder into a powder. The powder was refluxed in 70% alcohol for 72 hrs. at 60° C and occasional stirring with a glass rod manually at regular intervals.

After 72 hours the macerates solutions were filtered in separate flasks using a Whatman No 4 filter paper. Then centrifuged at x10000 g for 15 min and supernatant was dry until a constant dry weight of each extract was obtained. Then dried plant extracts were reconstituted in the respective solvents (Alcoholic) using 10% DMSO. The extracts were stored in 15 ml black cap bottle, covered with aluminium foil for the prevention of *Balanites aegyptica* fruit extract directly from light. The residues were stored at 4 °C for further used.

Histology by Light Microscopy (LM)

Balanites aegyptica fruit extracts were tested in vitro against Paramphistomum cervi. Treated and control parasites were fixed in Bouins fixative for histological studies by light microscopy (LM) for 24 hours. Then they were washed in running tap water for at least 24 hours. These P. cervi were dehydrated in ascending series of alcohol, cleared in xylene ,blocks were prepared in paraffin wax ,and sections were cut at 6μ on rotary microtome then

dehydrated, stained with Haematoxylin and Eosin, cleared in xylene and mounted in DPX (Bancroft & Stevens, 1977). Sections were examined under light microscope.

Result and Discussion

In this investigation P. cervi were treated with the extract of B. aegyptica. After the treatment body size of P. cervi were decreased, shrunken, paralysed and dead after 5hours at 125 mg/ml concentrations. In study revealed vitro toxicity anthelminthic components of alcoholic fruit extract of B. aegyptica shows good anthelminthic activity caused and deformation of tegument and suckers (Fig. No: 2, 4 & 6).

The control untreated and treated P. cervi were compared by light microscopy. When treated parasites were examined under the light microscopy. Present study revealed that the easy entry routes of fruit extract in the body P. cervi which is ultimately caused paralysis and mortality of the amphistome and many changes were observed that the P. cervi became small, shrunken and also found architectural alteration in tegument and suckers. All morphological structures are plays a very important role in the systematic classifications of Amphistomes. These characters include; body position and shape, presence of tegumental folds, suckers, and tegumental structures.

The tegument acts important role in protection, absorption, excretion, transport and osmoregulation, which is in direct contact with host's tissue along with the body fluids. The anthelminthic activity of treated parasites shows swelling of the body, disruption and detachment of tegument (Veerakumari & Paranthaman 2004 and Veerakumari *et al.*, 2012).

Fig.1 A portion of tegument of control untreated *P. cervi* showing surface syncytium (SS), sub syncytial zone (SZ), longitudinal muscles (LM) and circular muscles (CM) x 110

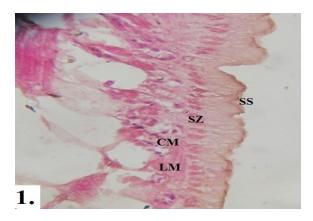


Fig.2 Photograph of tegument of treated *P. cervi s*howing detachment in surface syncytium (SS), breakage in sub syncytial zone (SZ), scattering in parenchymatous cell (SPC) & muscles (M) and discontinuation in tegument (DT) x 110

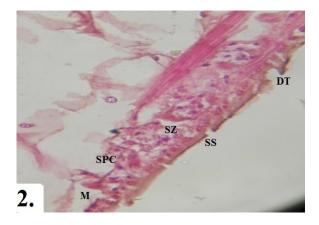


Fig.3 A portion of posterior sucker (Acetabulum) of control untreated *P. cervi* showing sucker cell (SC) and sucker wall (SW) x 110

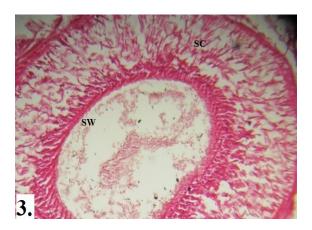


Fig.4 A portion of posterior sucker (Acetabulum) of treated *P. cervi* showing vacualized sucker cell (VSC) and breakage sucker wall (BSW) x 110

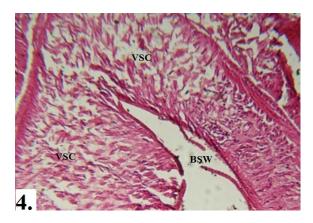


Fig.5 Oral sucker (OS) of control *P. cervi* showing sucker cell (SC) x 110

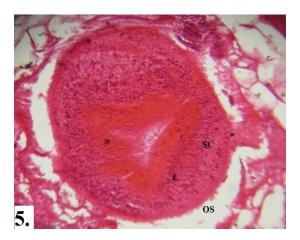
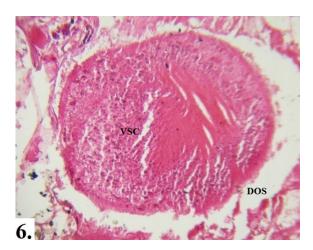


Fig.6 Oral sucker of treated *P. cervi* showing Deformated oral sucker (DOS) and vacuolized sucker cell (VSC) x 110



The control untreated *P. cervi* showed systematically arrangement of tegument, the tegumental surface is highly corrugated with transverse folds, smooth spineless, surface syncytium (SS), subsyncytial zone (SZ), longitudinal muscles (LM), and circular muscles (CM) (Fig.1). *In vitro* effect of fruit extract of *B. aegyptica* on *P. cervi* showing detachment and discontinuation of tegument showing surface syncytium (SS) and also showed vacuolization and breakage in subsyncytial zone (SZ).

Treated Р. cervi showed significant separation of surface syncytium (SS) from the subsyncytial zone (SZ) of tegument. The bundles muscles (M) were also separate and scattered parenchymatous cell (SPC) were observed in present study (Fig.2). Similar observations were also noted in some other helminth parasites that the tegument of treated helminth parasites was wrinkled, vacuolized cells, deformed and shrunken oral & posterior sucker in. (Sharma & Hanna 1988; McConville et al., 2006; Lalchhandama et al., 2007; Ghangale et al., 2009; Dasgupta et al., 2010; Bashtar et al., 2011; Jeyathilakan et al., 2010 & 2012; Nahla et al., 2012; Panyarachun et al., 2010 & 2013; Buddhachat et al., 2012; Shaheen & Eman 2012; Saowakon et al., 2011& 2013; Ahmed et al., 2013 and Scantlebury et al., 2013).

Amphistome parasite *P. cervi* have two suckers; first is oral sucker in circular form present anterior sub terminal region and second one is posterior sucker present in posterior extremity also known as acetabulum. Control posterior sucker (acetabulum) and oral sucker shows sucker cell (SC) and sucker wall (SW), (Fig.3 & 5).

The posterior sucker (acetabulum) and oral sucker of *P. cervi* were distorted and damaged due to extract of *B. aegyptica*.

Acetabulum of *P. cervi* treated shows the vacuolization in sucker cell (VSC) and breakage in sucker wall (BSW) (Fig.4). Treated *P. cervi* also showing deformated oral sucker (DOS) and vacuolized sucker cell (VSC) in oral sucker (Fig.6).

Some studies revealed that *B. aegyptica* is multipurpose plant and it shows antiinflamentory, antioxidant, anti-ulcer, antimicrobial activity using different techniques. (Meda et al., 2010; Chotani and Vaghasiya 2011; Suky et al., 2011; Motaal et al., 2012; Ajayi & Ifedi 2014 and Kant & Gour, 2015). The various anthelminthic activity of B. aegyptica has been found against worms .(Koko et al., 2000; Dwivedi et al., 2009; Ebeid et al., 2011; Shalaby et al., 2012; and Intisar et al., 2013).

In this study the anthelminthic activity of *B*. aegyptica fruit extract on P. cervi also showed breakage, distortion, discontinuation & detachment of tegument, vacuolization in tegumental cells & sucker cell, separation of muscles cells in tegument and oral & posterior sucker (acetabulum). This study suggests the vermifugal activity of these plants extract against Paramphistomum cervi. Based on the present study results the B. aegyptica is a safe and eco-friendly manner drug .Present research work will also provide most important ecologically technique controlling sound for Paramphistomum cervi.

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