A Synopsis

On

ULTRASTRUCTURAL STUDY OF *IN VITRO* ANTHELMINTIC EFFECTS OF *CITRULLUS COLOCYNTHIS* ON AMPHISTOME *ORTHOCOELIUM SCOLIOCOELIUM*

By

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UDAIPUR

1. **Name of Scholar: (In English)**

(In Hindi)

2. **Title of the Research: (In English)**

ULTRASTRUCTURAL STUDY OF *IN VITRO* ANTHELMINTIC EFFECTS OF *CITRULLUS COLOCYNTHIS* ON AMPHISTOME *ORTHOCOELIUM SCOLIOCOELIUM*

(In Hindi)

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3. **Location:**

4. **Importance of proposed Investigation:**

The ruminants have been reported to be infected with amphistome, *Orthocoelium scoliocoelium* parasites, which cause the disease amphistomiasis. Various veterinary drugs have been used to eliminate parasites from ruminant but these drugs are unaffordable and inaccessible for poor cattle farmers. Therefore,

there is an urgent need to develop a new, ecofriendly drug to control helminthes infection.

Importance of proposed investigation:

- Present study is of great importance from medico-veterinary field.
- The study will help in understanding of certain histological, cytological and physiological aspects of amphistome parasites.
- Ultrastructure study by Transmission Electron microscope (TEM) and Scanning Electron Microscope (SEM) of tegument of control and treated amphistome parasites with the fruit extract of *Citrullus colocynthis* would provide knowledge for helminth parasite treatment and chemotherapeutic measures.
- The research may helpful to initiate pharmacological aspect of the fruit extracts of *Citrullus colocynthis* herbal plant to prepare less costly, ecofriendly anthelmintic and veterinary drugs.

5. Scope of the proposed study:

The scope of proposed research work is as below:

- The *C. colocynthis* fruit extract may formulate best alternative herbal preparation to replace the synthetic drugs which are currently in use.
- The infected ruminant will be cared, treated and maintained from *C. colocynthis* fruit extracts which are less costly, ecofriendly anthelmintic and veterinary drugs.

- Socio-economic upliftment of the cattle farmers of Rajasthan by removing of amphistome parasites with the treatment of *C. colocynthis* fruit extracts.

- The present study will be helpful for the documentation of botanical anthelmintic use in the traditional veterinary practices.

6. Review of work already done on the subject:

Various indigenous plants have shown antihelmintic effect against cestodes, trematodes and nematode parasites (Satyavati, 1990; Kushwaha *et al.,* 2004; Temjengmongla & Yadav 2005; Kabore Adama *et al.,* 2009, Eguale *et al.,* 2009 and Saowakon *et al.,* 2009). Extract of *Allium sativum* demonstrated activity against *Heterakis gallinae, Ascardia galli Gigantocotyle explanatum* (Sutton *et al.,* 1999; Nagaich *et al.,* 2000).

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 Ajowan's seed extract was screened for anthelmintic property in sheep and Ovicideal activity against *Haemonchus controtus* (Laleef *et al.,* 2006 and Jabbar *et al.,* 2006 a & 2006b). The alcoholic extract of *Lysimachia* found to be effective on *Fasciola buski*, *Ascaris suum* and *Raillietina echinobrothrida* (Challam *et al.,* 2009). Ginger

have anthelmintic effects on *Angiostronglytus simplex and Schistosoma mansoni* (Iqbal, 2006; Lin *et al.,* 2010 and Osama *et al.,* 2011). The extract of *Melia azadarach* shows antiparasitic effects sheep gastrointestinal nematode (Cala *et al.,* 2012). The fruit extract of *Balanitus aegyptiac* and *Artemisia* found effective against *Fasciola gigantica*, *Hemonchus contortus*, *Schistosoma, Trichinella* and *Toxocara, coenorhabditis* (Koko *et al.,* 2000; Iqbal *et al.,* 2004; Gnoula *et al.,* 2007; Shalaby *et al.,* 2010; Doaa *et al.,* 2011 and Hatem *et al.,* 2012).

Citrullus colocynthis fruit extracts have shown antibacterial and antimicrobial effect against Pseudomonas, Staphylococcus, Candida sps and antihyperglycemic effect on Type 2 diabetic patients & rats (Murzouk *et al.,* 2009, Huseini *et al.,* 2009 and Dallak, 2011).

Little research work has been observed on extract of medicinal and indigenous plants tested against different species of amphistome (Tandon, *et al.,* 1997). Anthelmintic activity and paralytic effect have shown against.

G. explanatum (Singh *et al.,* 2008). Seed extract of *Ricinus communis* was tested against *Paramphistomum cervi* and *Bombex malabericum* leaves against *Paramphistomum explanatum* (Zahir *et al.,* 2009 and Hossain *et al.,* 2012).

However, no research work has been carried out so far to study the effects of *Citrullus colocynthis* fruit extracts of indigenous plant on amphistome, *Orthocoelium scoliocoelium* by light and electron microscope.

Therefore, it has been decided to undertake the work on "Ultrastructural study of *In vitro* anthelmintic effects of *Citrullus colocynthis* on amphistome *Orthocoelium scoliocoelium".*

7. Research gaps identified in the proposed field of the investigation

Although researcher have worked on *Citrullus colocynthis* fruit extracts and they have shown antibacterial and antimicrobial effect against Pseudomonas, Staphylococcus, Candida sps and antihyperglycemic effect on Type 2 diabetic patients & rats (Murzouk *et al.,* 2009, Huseini *et al.,* 2009 and Dallak, 2011), yet none of the scientists have paid attention on anthelmintic property of *C. colocynthis.*

The only effective and efficient tool to cure and control the helminthes infection is chemotherapy, as efficacious vaccines against helminthes have not been developed so far.

8. Objective of the proposed study:

The specific objectives of present investigation are as below:

1. To evaluates the effect of cumulative concentrations of aqueous and alcoholic fruit extracts of *C. colocynthis* on amphistome, O. *scoliocoelium.*
2. To study the histological structure of tegument of control and treated amphistome, *O. scoliocoelium* with *C. colocynthis* fruit extracts by Light microscope (LM).

3. To study the ultrastructural morphology and histology of tegument of control and treated amphistome, *O. scoliocoelium* with *colocynthis* fruit extracts by Transmission Electron Microscope (TEM).

4. To study the ultrastructural morphology and histology of tegument of control and treated amphistome, *O. scoliocoelium* with *C. colocynthis* fruit extracts by Scanning Electron Microscope (SEM).

9. Research methodology:

Hypotheses to be tested

The anthelmintic *In vitro* effect of *Citrullus colocynthis* may produce paralytic effect, mortality, motility distortion and disorganization of the histology of tegument. Ultrastructural changes like swelling, breakage, babble, damage architecture and disorientation of organelles of tegument may be induced by *In vitro* effect of *Citrullus colocynthis extracts on* amphistome, *O. scoliocoelium.*

Sources of Information:

Information and literature related to the present research work will be collected from various sources such as:

- Libraries of Vetenary Hospital, Udaipur; R. N. T Medical College, Udaipur; M. P. U. A. T., Udaipur; M. L. S. University, Udaipur; Pacific University, Udaipur and Rajasthan university Jaipur.

- Various Journals like Veterinary Parasitology, Journal of Helminthology, Journal of Ethnopharmacology, Journal of International Parasitology and Indian Journal of Pharmacology etc.
- Internet.

Tools and Techniques of Research:

Live amphistomes *Orthocoelium scoliocoelium* will be removed 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 will be divided into four groups.

- First group of worm will be used for identification of species of amphistome, *O. scoliocoelium* with the help of whole mount preparation of amphistome (Dutt, 1980).
- Second group of the amphistome, *O. scoliocoelium* will be used for evaluates the effect of cumulative concentrations of aqueous and alcoholic fruit extracts of *Citrullus colocynthis* on amphistome, *O. scoliocoelium.*
- Third group of the amphistome, *O. scoliocoelium* will be untreated and use as control amphistome, *O. scoliocoelium.*
- Fourth group of the amphistome will be given *In vitro* treatments of *Citrullus colocynthis* fruit extracts.

Control and treated amphistomes, *O. scoliocoelium* with the fruit extracts of *Citrullus colocynthis* will be fixed in different fixative for histological study by

light microscope, ultrastructural study by TEM and SEM.

Preparation fruit extracts.

Aqueous and alcoholic extracts will be prepared at different concentration with pulp powder of *Citrullus colocynthis* fruits.

Histology by Light Microscope (LM).

Transverse and longitudinal sections of control and treated amphistome, *O. scoliocoelium* will be fixed in Bouin's fixative (Bancroft and Stevens, 1977).

Ultrastructural study by Transmission and Scanning Electron Microscope (TEM and SEM).

Tissues of control and treated amphistome, *O. scoliocoelium* of *Citrullus colocynthis* fruit extracts will be fixed overnight at 4^{o}C in 3.5% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.2 fixative (Bancroft and Stevens, 1977).

Fixed tissues of amphistomes will be brought to Regional Electron Microscopy Facilities, AIIMS, New Delhi for TEM and SEM process for several times. As Electron Microscope facility is not available in Udaipur.

10. Tentative Chapterization:

Chapter 1 Introduction

Chapter 2 Review of Literature

Chapter 3 Materials and Methodology

Chapter 4 Effects of cumulative concentrations of aqueous and alcoholic fruit extracts of *Citrullus colocynthis* on amphistome, *Orthocoelium*

scoliocoelium.

Chapter 5 Histological observations of tegument of control and treated amphistome, *Orthocoelium scoliocoelium* with *Citrullus colocynthis* fruit extracts by light microscopic.

Chapter 6 Ultrastructural observations of tegument of control and treated amphistome, *Orthocoelium scoliocoelium* with *Citrullus colocynthis* fruit extracts by transmission electron microscope.

Chapter 7 Ultrastructural observations of tegument of control and treated amphistome, *Orthocoelium scoliocoelium* with *Citrullus colocynthis* fruit extracts by scanning electron microscope.

Summary

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