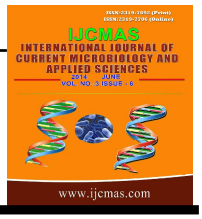
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 571-582**
http://www.ijcmas.com



Original Research Article

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

G.Swarnakar* and A.Kumawat

Parasitology Lab, P.G. Department of zoology,Government Meera Girls College, Udaipur (Raj.)
PAHER University, Udaipur (Raj.), India
*****Corresponding author*

A B S T R A C T

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 *Trachyspurmmum ammi* seed extract was screened for anthelmintic property in sheep and ovicideal activity against *Haemonchus controtus* (Lateef *et al.,* 2006 and Jabbar *et al.,* 2006a & b). The alcoholic extract of *Lysimachia* found to effective on *Fasciola buski, Ascaris suum* and *Raillietina echinobrothrida* (Challam *et al.,* 2010). Ginger *Zingibar officinale* have anthelmintic effects on *Angiostronglytus 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 (Cala *et al.,* 2012). The fruit extract of *Balanitus aegyptica* and *Artemisia* found effective against *Fasciola gigantica, Haemonchus contortus, Schistosoma, Trichinella* and *Toxocara coenorhabditis* (Koko *et al.,* 2000; Iqbal *et al.,* 2004; Gnoula *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 sps., 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 sps.,* 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, amenorrhea 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 vitr*o 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.

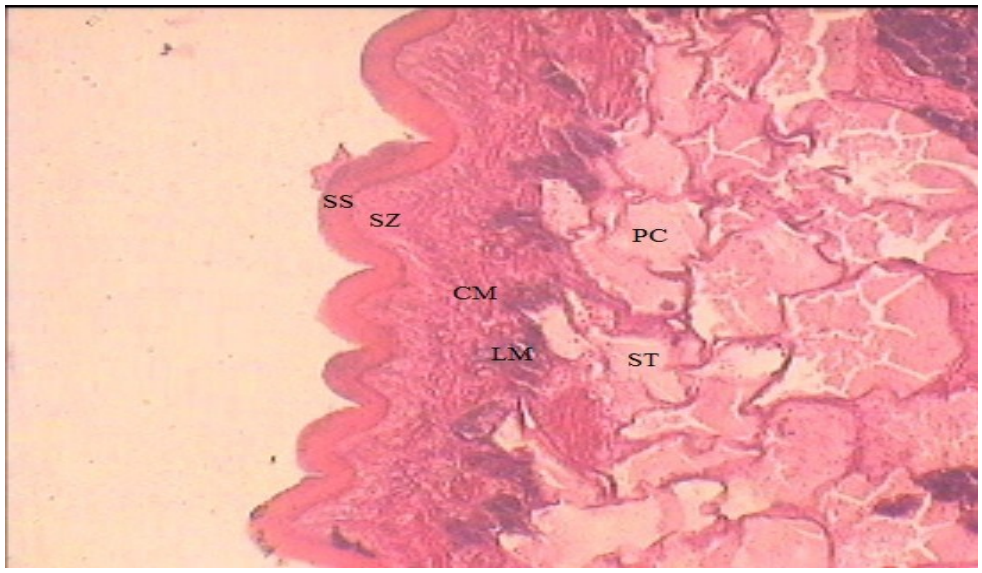


Fig.2 A portion of acetabulum of controlled *Orthocoelium scoliocoelium* showing surface tegument (ST), circular muscles (CM), longitudinal muscles (LM) x110.

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

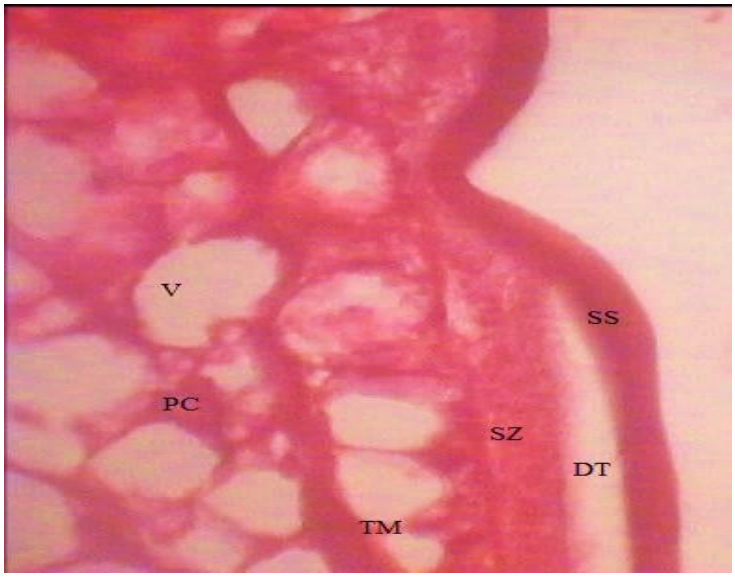


Fig.4 This slide shows vacuolisation (V) in tegumental muscles (TM) and sub tegumental cells (STC) of tegument of treated *Orthocoelium scoliocoelium* x110.

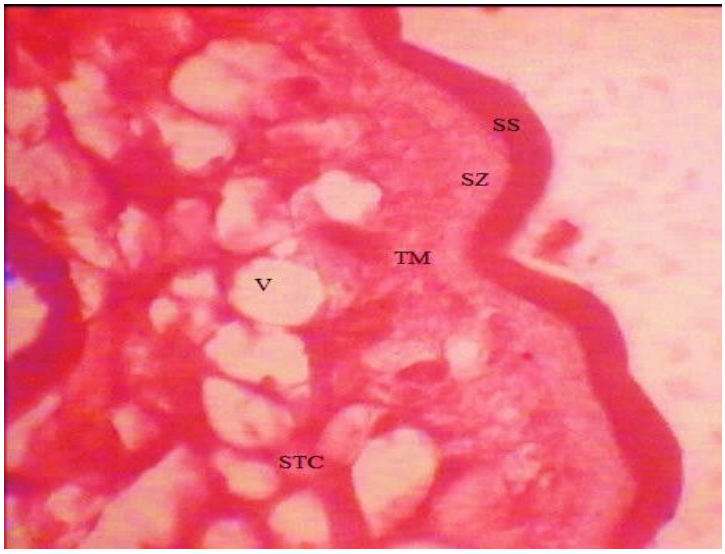


Fig.5 Breakage in surface syncytium (SS) of tegument of treated *Orthocoelium scoliocoelium* x110.

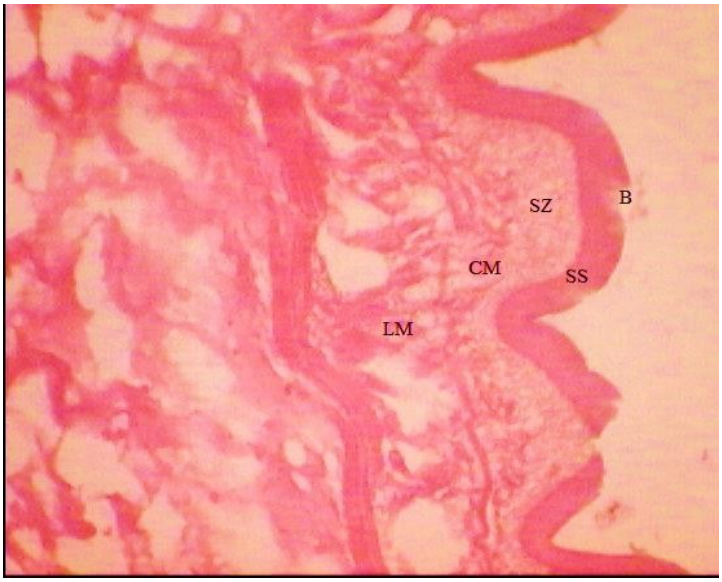


Fig.6 Seperation (S) of muscles in circular muscles (CM) and longitudinal muscles (LM) of acetabulum of treated *Orthocoelium scoliocoelium* x110.

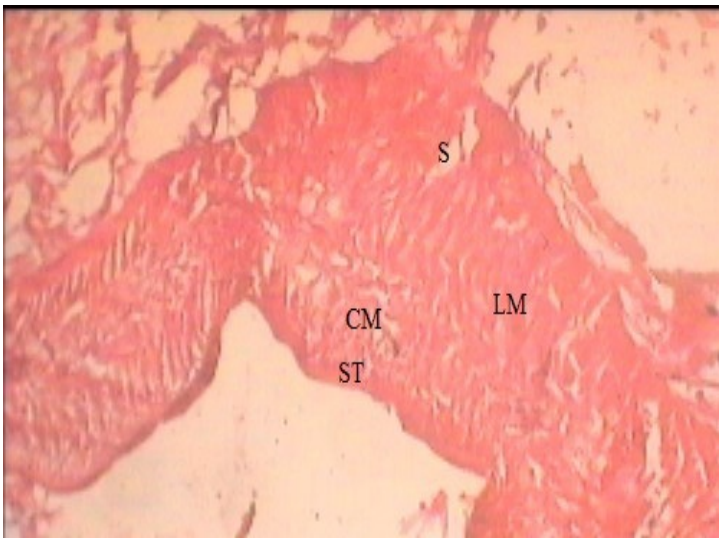
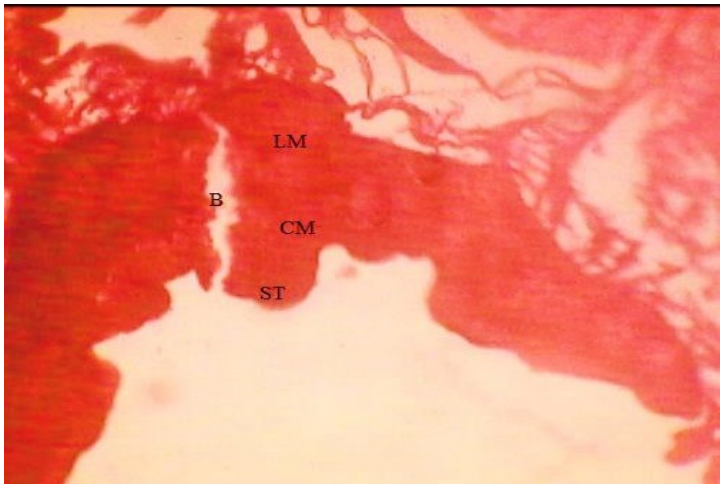


Fig.7 Breakage (B) in the tegument penetrating into the musculature of acetabulum of treated *Orthocoelium scoliocoelium* x110.



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 become 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; Iqbal *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 *C. colocynthis* suggested antibacterial and antimicrobial effect against *Pseudomonas, staphylococcus* and *candida sps.* 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 Fasciola buski* (Challam *et al*., 2010) and the anthelmintic activity of *Trigonella foenum-graecum* produced swelling, detachment, blebbing and discontinuation in tegument of amphistome *Gastrothylax*

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.

Acknowledgement

The second author is grateful to UGC for awarding TRF (F. No: 25-473(12)/2013(FDP/CRO) Dated: 05-07-2013. The authors are also thankful to Miss Bhanupriya Sanger, Miss Kiran Roat, Mr. Hardik Goswami and Mr. Rajnarayan Damor (Research Scholars, Parasitology laboratory, Department of Zoology, Govt. P.G. Meera Girls College, MLSU, Udaipur, Rajasthan) for their valuable suggestions.

References

Amrouche AA, Benmehdi H, Mebarki K, Chaoufi A, Saneba A, Lazouni A and Sari DC. 2011. Evaluation of antifungal activity of some oils from Algerian medicinal plants against *Aspergillus flavus* strain produced aflatoxins. J Appl Pharm Sci. 01(08): 48-53

Asha M, Prashant D, Murali B, Padmaja R and Amit A.2001.Anthelmintic activity of essential oil of *Ocimum sanctum* and Eugenol.Fitoterapia. 72: 669-670.

Baloch N, Nabi S,Kakar AM,Wajid Z and AL-Kahraman YMSA. 2013. *In vitro* antileishmanial, antitumor, cytotoxic activities and phytochemical analysis of *Citrullus colocynthis* fruit extract and its fractions. Int J Med Arom Plants. 3(1): 78-84.

Bancroft JD and Steven A. 1977. Theory and practice of histological techniques. Churchill Livingstone. Medical Division of Longman Group Limited.Edinburgh London and New York.

Cala AC, Chagas AC, Oliviera MC, Matos AP, Borges LM, Sousa LA, Souza FA and Oliviera GP. 2012. *In vitro* anthelmintic effect of *Melia azadarach L.* and *Trichilia claussenii* C. Against sheep gastro intestinal nematodes. Exp Parasitol. 130 (2): 98-102.

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.

Dallak M.2011. *In vivo* hypolipidemic and antioxidant effects *of Citrullus colocynthis* pulp extract in alloxan-induced diabetic rats. Afr J of Biotech. 10(48): 9898-9903.

Doaa,Y, Gamal A,Taher and Zedan Z. Ibraheim. 2011. *In vitro* effect of some herbs used in Egyptian traditional medicine on viability of protoscolices of Hydatid Cysts. Korean J parasitol.49 (3): 255-263.

Dutt SC**.** 1980. Paramphistomes and paramhistomiasis of domestic ruminant in India. PAU Press, Ludhiana and published by the joint

Director, Communication Centre, Punjab Agricultural University, Ludhiana.

Eguale T, Tilahun G, Debella A, Feleka A and Makonnen E**.** 2007.*In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J Ethanopharmacol. 110(3): 428-33.

Gnoula C, Guissou P and Dubois P. 2007. Carboxy urescien diacetateas indicator of *Caenorhabditis elegans* viability for the development of *in vitro* anthelmintic drug assay. Talanta. 71: 1886-1892.

Hammond JA, Fielding D and Bishop SC. 1997. Prospects for plant anthelmintics in tropical veterinary medicine. Vet. Res. Commun.21: 213-18.

Hossain E, Chandra G, Nandy AP, Mandal SC and Gupta JK. 2012. Anthelmintic effect of a methanol extract of *Bombax malabaricum* leaves on *Paramphistomum explanatum.* Parasitol Res. 110(3): 1097-102.

Huseini HF, Darvishzadeh F, Heshmat R, Jafarizar Z and Larijani B. 2009. The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of Type II patients: a randomized, double blind, placebo-controlled trial. Phytother Res. 23(8): 1189-9.

Iqbal Z, Lateef M, Asharaf M and Jabbar A. 2004. Anthelmintic activity of *Artemisia bravifolia* in sheep. J of Ethno-pharmacol. 93: 265-268.

Iqbal Z, Lateef M, Akhtar MS, Ghayur MN and Gilani AH. 2006. *In vivo* anthelmintic activity of ginger against gastrointestinal nematodes of sheep. J Ethanopharmacol. 106 (2): 285-287.

Jabbar A, Iqbal Z and Khan MN.2006a. *In vitro* anthelmintic activity of *Trachyspermum ammi* seeds. Pharmacog mag. 2(6): 126-128.

Jabbar A, Raza MA, Iqbal Z and Khan MN. 2006b. An inventory of the ethnobotanicals used as anthelminthics in the southern Punjab (Pakistan). J Etnopharmacol.108(1): 152-154.

Jeyanthi KA and Mary violet Christy A. 2011. Antioxidant effect of *Citrullus colocynthis* on Alloxan induced Diabetic Rat. Int J Pharm Bio Arch. 2(2): 696-701.

Kabore A, Belem AM, Gaston, Tamboura Hamidow H, Traore A and Sawadogolaya. 2009. *In vitro* anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abosomal nematode of sheep in Burkina Faso. Afr J of Biotechnol. 8 (18): 4690-4695.

Koko R, Galal M and Khalid HS. 2000. Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazol. J Ethanopharmacol. 71: 247-252.

Kumar ABS, Saran G, Bharath KN, Kumar A, Kulashekar KS, Tamoli S and Vamshikrishna N. 2014. Evaluation of Laxative Activity of Easigo (a Polyherbal Formulation). Int J Trad Nat Med. 4(1): 1-5.

Kushwaha DS, Kumar D, Tripathi HC and Tandan SK. 2004. Effect of some indegenous medicinal plant extract on *Fasciola gigantica in vitro*. Indian J Anim Sci. 74: 143-6.

Lange KC, Olcott DD, Miller JE, Mosjidis JA, Terrill TH, Burke JM and Kearney MT. 2006. Effect of *Sericea lespedeza* fed as hay on natural and experimental *Haemonchus contortus* infections in lambs. Vet parasitol.

141: 273-278.

Lateef M, Iqbal Z, Akhtar MS, Jabbar A, Khan MN and Gilani AH. 2006. Priliminary Screening of *Trachyspermum ammi* seed for anthelmintic activity in sheep. Trop Anim health Prod. 38 (6): 491-496.

Lin RJ, Chen CY, Chung LY and Yen CM**.** 2010. Larvicidal activities of ginger (*Zingibar officinale*) against *Angiostrongylus cartonersis*. Acta trop. 115 (1-2): 69-76.

Marzouk B, marzouk Z, Décor R, Edziri H, Haloui E, Fenina N and Aouni M**.** 2009. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthi*s Schrad. From Medenine. J of ethnopharmacol. 2(32): 125, 344-349.

Min BR, Pomroy WE, Hart SP and Sahlu T. 2004. The effect of short term consumption of a forage containing condensed tannins on gastrointestinal nematode parasite infection in grazing wether goats. Small ruminant Res. 51: 279-283

Mohammed A, Wossene A, Giday M, Tilahun G and Kebede N. 2013. *In vitro* anthelminthic activities of four medicinal plants against *Haemonchus contortus*. Afr J of Plant Sci. 7(8): 369-373.

Nagaich SS. 2000. Studies of the anthelmintic activity of *Allium sativum* (Garlic) oil on common poultry worms *Ascardia galli* and *Heterakis gallinae*. J parasitol App Anim biol. 9: 47-52.

Nahla AR, Amal IK and Amera EW. 2012. *In vitro* Evaluation of antihelmintic Activity of *Allium sativum* against Adult *Cotylophoron cotylophorum* (*Paramphistomidae*). PUJ.5(2): 135-146.

Nalule AS, Mbaria JM and Kimenju JW. 2013. *In vitro* anthelmintic potential of *Vernonia amygdalina* and *Secamone africana* on gastrointestinal nematodes. Agri and Biol J of North America. 4(1): 54-66.

Osama MS, Mostafa RA and Eid MA Adly. 2011.Antischistosomal activity of ginger (*Zingibar officinale*) against *Schistosoma mansoni* harbored in 57 mice. Parasitol Res. 109: 395-403.

Perry BD, Randolph TF, Mcdermot JJ, Sones KR and Thornton PK. 2002. Investigating in animal health research to alleviate poverty. International Livestoke Research Institute. 148.

Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V and Sobhon P. 2009. *Fasciola gigantica*: Anthelmintic effect of the aqueous extract of *Artocarpus lakoocha*. Exp Parasitol. 122 (4): 289-98.

Satyavati GV.1990. Use of plants drugs in Indian Traditional System of Medicine and their relevance to primary health care, In Economic and medicinal plant research by Farnworth NR and Wagner (H) Eds. Acedemic Press Ltd., London.190-210.

Shaik SA, Terril TH, Miller JE, Kouakou B, Kannan G, Kallu RK and Mosjidis JA. 2004. Effect of feeding *Sericea lespedeza* hay to goat infected with *Haemonchus contortus*.South Afr J of Anim. Sci. 34: 248-250.

Shalaby HA, Abdel-Shafy S,Abdel-Rahman KA and Derbala AA. 2009. Comparative *in vitro* effect of artemether and albendazole on adult *Toxicara canis*. Parasitol Res. 105: 967-976.

Shalaby HA, Namaky AE, Khalil FA and Kandil OM. 2012. Efficacy of Methanolic extract of *Balanites*

aegyptiaca fruits on *Toxocera vitulorum*. Vet Parasitol. 183: 386-392.

Singh TU, Kumar D and Tandon SK. 2008. The paralytic effect of *A. sativum* and *P. longum* on *G. explanatum*.Ind J of Pharmacol. 40(2): 64-68

Sutton GA and Haik R. 1999. Efficacy of garlic as anthelmintic in donkeys. Israel J.Vet. Med. 54: 23-7.

Swarnakar G, Roat K, Sanger B and Kumawat A. 2014. Anthelminthic effect of *Trigonella foenum-graecum* on tegument of *Gastrothylax crumenifer* in cattle of Udaipur. Int J Curr Microbiol App Sci.3(5): 599-606.

Talole BB, Baheti DG and More PA. 2013. *In vitro* Helmintholytic Activity of Leaves of *Citrullus colocynthis*. IJRPC. 3(2) 240-243.

Tandon V, Pal P, Roy B, Rao HSP and Reddy KS**.** 1997. *In vitro* anthelmintic activity of root tuber extracts of *Flemingia vastita*, an indegenous plant in Shilong. India. Parasitol Res. 83: 492-498.

Temjenmongla and Yadav A. 2005. Anticestodal efficacy of folklore medicinal plants of Naga tribe in North East India. Afr J Trad Cam. 2(2): 129-133.

Ullah S, Khan MS, Sajid MS and Muhammud G. 2013. Comparative Anthelmintic Efficacy of C*urcuma longa, Citrullus colocynthis, Peganum harmala.*Global veterinaria.11(5): 560-567.

Waller PJ and Thamsborg SM. 2004. Nematode control in green ruminant production system. Trend Parasitol. 20: 493-497.

WHO**.**2002. WHO Traditional Medicine Strategy 2002-2005. WHO/EDM/TRM/2002.1: 61.

Yadav AK, Tandon V and Rao HSP. 1992. *In vitro* anthelmintic activityof fresh tuber extract of *Flaminga vestita* against *Ascaris suum.* Fitoterapia. 63:395-398.

Zaid KHA, EL- Wakil H, EL-Hussein A, Jomma S and Shohayeb M. 2013. Evaluation of the Molluscicidal activity of *Punica granatum, Calotropis procera, Solanum incanum* and *Citrullus colocynthis* against *Biomphalaria arabica*. World appl Sci J. 26(7): 873-879.

Zahir AA, Rahuman AA, Kamraj C, Bagavan A, Elangao G, Sangaran A and Kumar BS**.** 2009. Laboratory determination of efficacy of indigeneous plant extracts for parasites control. Parasitol Res. 105(2): 453-61.