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***IN-VITRO* ANTHELMINTIC EFFECT OF *CAPPARIS DECIDUA* (KAIR) ON TEGUMENT OF *GASTROTHYLAX CRUMENIFER* IN GOAT BY LIGHT MICROSCOPY**

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ABSTRACT: Paramphistomiasis is the major problems in the yield of goat and human health. This disease causes loss of life of goat, reduce meat and milk production. The medicinal plant *Capparis decidua* has been used in the treatment of asthma, cough and inflammation. This plant also shows anthelmintic activity. Live amphistome *Gastrothylax crumenifer* were collected from the rumen of the freshly slaughtered goats at local meat market of Chittorgarh. Amphistomes *Gastrothylax crumenifer* was maintained in 0.9% physiological saline and divided into four groups; first group used for identification, second group as control or untreated, third group for treatment with *Capparis decidua* and fourth group for treatment with synthetic drug albendazole. The histological study of treated and control tegument of *Gastrothylax crumenifer* was observed by light microscopy. Extract of *Capparis decidua* caused many topographical changes in *Gastrothylax crumenifer* like discontinuation, distortion, peeling and damaging cells, vacuolization and deformation in the muscles fiber, found blebbings in tegument. Albendazole is less effective than alcoholic extract of *Capparis decidua* (Kair). This study suggests the potential role of fruit extract of *Capparis decidua* (Kair) as an Anthelmintic activity against *Gastrothylax crumenifer*.

INTRODUCTION: Rajasthan is the largest state of India having maximum number of livestock and famous for its cattle wealth. The prevalence of the amphistome parasite in the livestock is very high, that causes disease in cattle called Paramphistomiasis. Paramphistomiasis is the major problems in the yield of goat and human health. This disease causes reduced of meat, milk and wool production in goats.

High mortality and morbidity rate resulting in economic losses due to reduced productivity to poor farmers¹. Chemotherapy is the effective process to control the parasitic infection, as efficacious vaccines against helminth parasites have not been developed so far.

Many effective drugs are available in market but they are expensive, unaffordable, inaccessible to the poor farmers of developing countries like India but synthetic anthelmintic drugs are developed resistance. Therefore medicinal plant based remedies as alternative to the synthetic anthelmintic. Therefore medicinal plant based herbal drug as alternative to the synthetic anthelmintic. Various medicinal plants are used to prepare herbal drugs which having anthelmintic effects against

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nematodes, cestodes and trematode parasites²⁻⁶. Scientists were used herbal drug to treat trematode amphistome parasite *Gigantocotyle explanatum* treated with alcoholic extracts of *A. sativum* and Piper produced significant decrease in the frequency⁷. The *Ricinus communis* leaf extract was investigated against *Paramphistomum cervi* and found to have powerful anthelmintic effect on larva and adult⁸. The extracts of medicinal plants were studied against species of amphistome and they are found to be very harmful and have anthelmintic activity against *Gastrothylax crumenifer*, *Paramphistomum explanatum*, *Cotylophoron cotylophorum* and *Paramphistomum cervi*⁹⁻²¹.

The availability of highly effective and safe drug for cure of parasites was limited and none of drugs is available that show high efficacy on *G. crumenifer*. The plant of *Capparis decidua* (green berries) is commonly known as Kair and the fruit of plant is used in preparations like pickles for over 2000 years. The fruit with seeds of *Capparis decidua* (Kair) have been attributed marvelous phytochemical bioactive compounds grouped as glycosides, flavonoids, alkaloids, phenols, carbohydrates, sterols and fatty acids²².

Capparis decidua possessed many pharmacological activities such as anthelmintic, antibacterial, antifungal, antidiabetic, analgesic, antirheumatic, anti-gout, anti-nociceptive, anti-atherosclerotic, antioxidant, anti-tumor, anti-inflammatory, anticonvulsant, hepatoprotective and useful in the treatment of Asthma, cough²³⁻³².

However, none of scientist was paid attention on anthelmintic study on *Gastrothylax crumenifer* treated with fruit of *Capparis decidua*. So the present investigation determined the histological observation of *Gastrothylax crumenifer* tegument of control and treated with fruit of *Capparis deciduas* against *Gastrothylax crumenifer* with comparison to albendazole under light microscopy.

MATERIAL AND METHODS:

Collection of Parasites: Live amphistome *Gastrothylax crumenifer* were collected from the rumen of the freshly slaughtered goats at local meat market of Chittorgarh. These worms were kept in 0.9 % physiological saline for investigations **Plate 1, Fig. 1** and **2**.

Preparation of Fruit Extracts: Fresh *Capparis decidua* fruits were collected from the desert area Sikar, Rajasthan **Plate 1, Fig. 3** and **4**. The plant was collected from Sikar during May 2021. The plant was identified and authenticated by Dr. Asha Arora, Associate Professor, Department of Botany, B. N. University, Udaipur (Raj.). The herbarium sheet was prepared and deposited in the department for future reference and the accession number is BT/2021-22/C145. Then they were dry and pulverize with a grinder in to fine powder. The powder was refluxed in 70% alcohol for 72 h at 60° C and occasional stirring with a glass rod manually at regular intervals. After 72 h the macerates solutions were filtered in separates flasks using Whatman no. 4 filter paper. Then centrifuged at X 10000 rpm for 15 min. and supernatant was dried until constant dry weights for each extract were obtained. Then dried plant extract were reconstituted in the alcoholic solvents using 10% DMSO. The extract was stored in 15 ml bottles, covered with aluminum foil for the prevention of *Capparis decidua* fruit extract directly from light. The residues were being stored at 4°C for further used.

Experimental Design: Anthelmintic activity was studied by *in-vitro* petri dish method. Amphistomes *Gastrothylax crumenifer* was maintained in 0.9% physiological saline and divided into four groups with five parasites in each group (Three replicate in each group). First group: Collected *G. crumenifer* were used for the identification of species of amphistome, with the help of whole mount preparation of amphistome. Second group: Untreated *G. crumenifer* amphistome served as control group they were fixed for histological studies by light microscope. Third group: *In-vitro* treatment with alcoholic extract of *Capparis deciduas* fruits. *Gastrothylax crumenifer* were incubated in different concentrations of the *Capparis decidua* extracts with a volume of 10 ml in the petri plate for five hours. Treated parasites were fixed for histological studies by light microscope. Fourth group: *Gastrothylax crumenifer* were incubated in different concentrations of the synthetic drug albendazole with a volume of 10 ml in the petri plate for five hours. Treated parasites were fixed for histological studies by light microscope.

Evaluation of the Anthelmintic Activity of Alcoholic *Capparis decidua* Fruit Extract and Albendazole: 10 ml of each concentration of the fruit extracts of *Capparis decidua* and albendazole drug were applied to a group of 5 worms maintained in 10 ml of selected medium and 2 ml of the sterilization solution. The experiment was performed in three replicates at the optimal temperature (37°C) and pH -7.4. Albendazole was obtained from Veterinary Hospital, Udaipur. The motility and mortality of flukes were examined after 1, 2, 3, 4, 5 h. The dead flukes were examined visually and mechanically stimulated using a dissecting needle. Motility was scored using the following criteria:-Score 3 - Movement of the whole body; Score 2 - Movement of only parts of the body; Score 1 - Immobile but not dead and Score 0 - Dead.

Histology by Light Microscope (LM): The control or untreated and *in-vitro* treated immobile but not dead *Gastrothylax crumenifer* with the

alcoholic *Capparis decidua* fruits extract and flukicide drug albendazole were fixed in Bouin's fixative, dehydrated in ascending series of ethanol alcohol, embedded in paraffin wax, sections were cut at 6 μ on rotary microtome then dehydrated, stained with Haemotoxylin and Eosin, cleared in xylene and mounted in DPX. The tegument of adult fluke was studied and photographed using an Olympus CX41 microscope.

RESULTS: Amphistomes parasite from the control group did not show any morphological changes. Control amphistome *Gastrothylax crumenifer* showed densely covered by tegumental folds and smooth spineless tegument. Present study show that the tegument is consist with the syncytium layer, basement membrane and musculature comprises longitudinal and circular musculature and tegumental cells were present in well - arranged manner. Parenchymatous cells found beneath the tegumental cells **Plate 2, Fig. 1.**

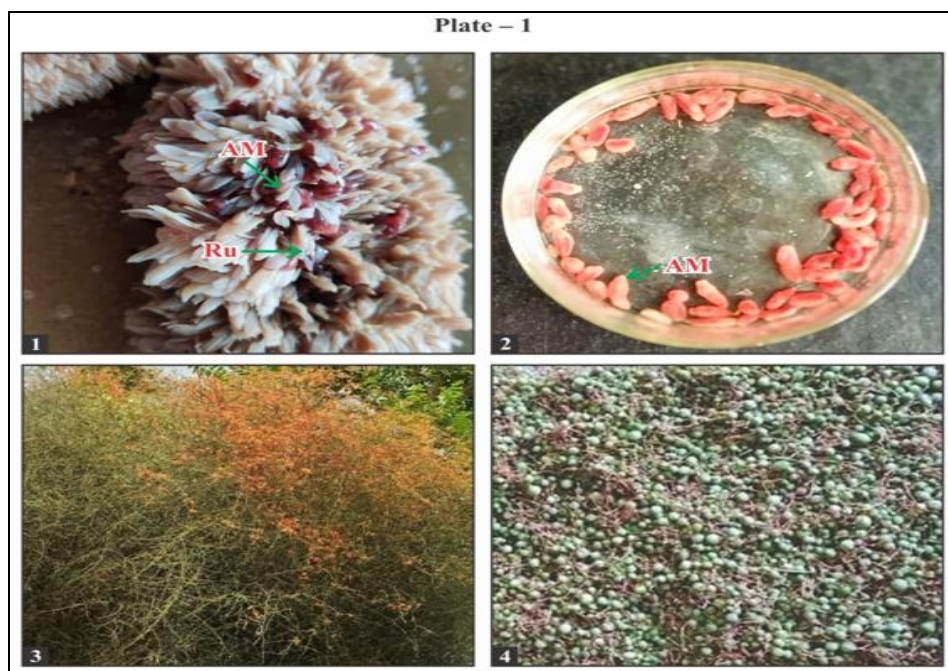


PLATE - 1: FIG. 1 - AMPHISTOME (AM) ATTACHED WITH INFECTED RUMEN (RU), FIG. 2 - AMPHISTOME (AM) *G. CRUMENIFER*, FIG. 3 - PLANT OF *CAPPARIS DECIDUA* FIG. 4 - FRUITS OF *C. DECIDUA*

Gastrothylax crumenifer treated with alcoholic extract of *Capparis decidua* fruits, parasites became paralyzed and dead after 4 to 5 hours exposure time at concentration of 80-100 mg/ml. The present investigation revealed that the alcoholic extract of *Capparis decidua* (Kair) caused highly destructive alternation and deformity in the

tegumental architecture of treated *G. crumenifer*. Alcoholic fruit extract of *Capparis decidua* affects the whole parasite become shrunken and paralyzed. Histological changes were observed in tegumental organization of parasite by light microscope. The tegumental region of treated *G. crumenifer* showed extensive changes in the tegumental grooves, found

disorganization, pronounced detachment, vacuolization, breakages, deformation, obliteration **Plate 2, Fig. 2** and **3**. Treated parasite also shows the highly cuticular disruption, discontinuous, large blebbings and forming large vacuolization in tegument due to blebbing **Plate 2, Fig. 4**. Treated parasite also shows breakage in the ventral pouch wall **Plate 2, Fig. 5**.

In this study the *in-vitro* anthelmintic activity of medicinal plant *Capparis decidua* on *G. crumenifer* is compared with synthetic albendazole drug. Albendazole also showed few of breakage, discontinuation and minor detachment of tegument, few and small vacuoles are seen in tegumental cells **Plate 2, Fig. 6**. Albendazole is less effective than alcoholic extract of *Capparis decidua* (Kair).

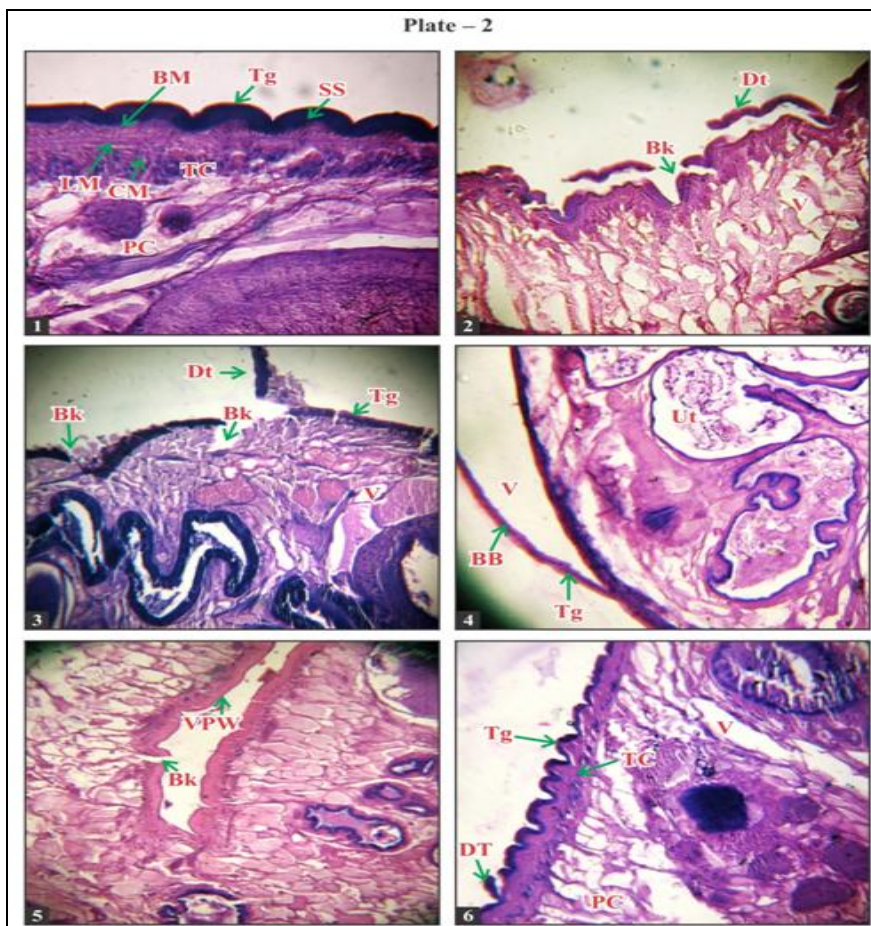


PLATE- 2; FIG. 1 – PHOTOGRAPH OF HISTOLOGY OF TEGUMENT OF *G. CRUMENIFER* BY LIGHT MICROSCOPY SHOWING: FIG. 1 – CONTROL, FIG. 2, 3, 4 AND 5 – TREATED WITH ALCOHOLIC *C. DECIDUA* FRUIT EXTRACT, FIG. 6. – TREATED WITH ALBENDAZOLE X 110

DISCUSSION: In the present study analyzed the anthelmintic efficacy of *Capparis decidua* (Kair) on amphistome *G. crumenifer* under *in-vitro* conditions. The presence of tegumental structures and folds, tegument of oral and posterior suckers, tegumental layer of genital pore and ventral pouch have well develop structure in *G. crumenifer*. The tegument play important role in protection, absorption, excretion, transport and osmoregulation. Tegument of *G. crumenifer* is in direct contact with host's tissues. Modification in structural of the tegument is necessary in developing any rational drugs which may damage

the parasites through their actions on the tegument^{13, 33, 34}. The control and treated *G. crumenifer* were compared histological studies under light microscopic studies revealed the differentiation between control and treated parasite architecture, also found anthelmintic activity of medicinal plant. Detachment in tegument of treated worms may lead to complete breakdown of cells in the parenchyma leaving vacuolated areas. Blebbings are active in response to anthelmintic treatment, where secretory bodies are rapidly transported towards the tegument and released from the apical plasma membrane. Present findings are similar with anthelmintic plants

showed that disorganized the morphology of tegument in parasite^{3, 14 - 21, 35}. In present observations *Capparis decidua* (Kair) alcoholic extract shows many breakages, several blebblings detachment, swelling, vacuolization, deformation and complete dissolution of parenchymatous cells were observed in ruptured tegument of *Gastrothylax crumenifer* and current results are agreements with Swarnakar *et al.*,^{15, 37}. Presence of several vacuoles in the parenchyma could distort the structure of the parasite. Different trematodes, cestodes and nematode were also shown the tegumental deformities with *in-vitro* treatment with different medicinal plants and also observed severe alteration in tegument and parenchymatous cells which shows similarity with current results^{2, 4 - 6, 38, 39}. The *Capparis decidua* (Kair) have been attributed marvelous phytochemical bioactive compound grouped as glycosides, flavonoids, alkaloids, phenols, carbohydrates, sterols and fatty acids²². Perhaps the chemical component in the kair might bring about permeability changes in the tegument of the parasite. Therefore it might be possible that phytochemicals of plants were responsible for anthelmintic activity. Present observations are similar with findings of other scientist who discovered sloughing, eruption, blebs, erosions, breakages and vacuoles in the tegumental structural changes caused by medicinal plants on amphistomes parasites^{10, 13, 15, 19, 36, 40 - 48}. *In-vitro* morphological effect of oxyclozanide and Niclosamides, mixture of both drugs shows few cuticular damages on *Gastrothylax crumenifer*³⁴.

Current observations found that the albendazole shows anthelmintic activity and produce little damages and few small vacuoles on the tegument of *Gastrothylax crumenifer*. Present investigation revealed that *Capparis decidua* (Kair) has most excellent anthelmintic than albendazole. *Capparis decidua* (Kair) plant is very useful to control *G. crumenifer*. Thus plant-based medicines such as *Capparis decidua* (Kair) could be used as an efficient anthelmintic in treatment of amphistomiasis.

CONCLUSION: Present study suggests the potential role of fruit extract of *Capparis decidua* (Kair) as an anthelmintic activity against *Gastrothylax crumenifer*. Albendazole is less effective than alcoholic extract of *Capparis*

decidua (Kair). Medicinal plants are safer, cheaper and ecofriendly way for poor farmers than costly veterinary medicines.

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CONFLICTS OF INTERESTS: The author declares no conflict of interest.

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