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ANTHELMINTIC EFFECT OF *CITRULLUS COLOCYNTHIS* ON THE TEGUMENT OF *COTYLOPHORON COTYLOPHORUM* BY LIGHT MICROSCOPE

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ABSTRACT: The anthelmintic effect of alcoholic *Citrullus colocynthis* fruit extract on the tegument of *Cotylophoron cotylophorum* was studied by light microscopy. Live amphistome *Cotylophoron cotylophorum* flukes were collected from the infected rumen of freshly slaughtered domestic buffaloes (*Bubalus bubalis*) at the local zoo abattoir in Udaipur. The control and anthelmintic treated *C. cotylophorum* with the alcoholic *C. colocynthis* fruit extract, and flukicide drug albendazole were fixed in Bouin's fixative for histological study. The present studies revealed the alcoholic *Citrullus colocynthis* fruits extract effect on the tegument of flukes; showed abundant cuticular disruption, detachments, discontinuous, blebbing, clumping of parenchymal cell and the complete breakdown of cells in the parenchyma leaving vacuolated areas. Albendazole is less effective than alcoholic *Citrullus colocynthis* fruit extract. This study suggests that the alcoholic extract of the fruit of *C. colocynthis* could offer a suitable and cheaper alternative anthelmintic as a comparison to synthetic drugs. The results of this study will help to prepare the eco-friendly, less costly anthelmintic veterinary drug and socio-economic upliftment of cattle farmers.

INTRODUCTION: Paramphistomiasis is one of the most pathogenic diseases caused by the ruman flukes amphistomes which live in rumen and reticulum of domestic ruminants, including cattle, sheep, goats, and buffaloes. Paramphistomiasis is a very common infectious disease in domestic ruminants. The paramphistomiasis is diagnosed by the occurrence of edema, anorexia, and diarrhea in ruminants. The economy of rural people largely depends on domestic ruminants like cows, buffalo, goats, and sheep.

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The incidence of amphistome parasites is very high in domestic ruminants and spread all over the world. The infection of fluke is so severe and acute that the ruminants become debilitated, reduce productivity, shows slow growth of domesticated ruminants, leading to poor production of milk, meat, skin, and wool, causing heavy economic losses to the livestock industry annually.

This disease causes loss of morbidity and mortality in buffalo; also economic losses throughout the world, and the mortality often rises to 90 percent. It has been estimated that domestic ruminants are at risk due to fluke infection. Hence, amphistome flukes have been recognized as an important group of economically important parasites [1-3]. The common flukes amphistomes include *Paramphistomum cervi*, *Gastrothylax crumenifer*,

Cotylophoron cotylophorum, *Orthocoelium scoliocoelium*, *Calicophoron calicoforum* are found in Rajasthan. The infection of *Cotylophoron cotylophorum* is found acute in buffaloes of Udaipur and causes gastrointestinal diseases paramphistomiasis. Amphistome *Cotylophoron cotylophorum* is a digenetic trematode fluke, belongs to the Paramphistomiadae family and differ in body shape, size, and morphological characteristics. Chemotherapy is the only efficient and effective tool to cure and control the amphistome fluke infection.

Effective vaccines against helminth flukes have not been developed so far. The anthelminthic or antihelminthic drugs, albendazole, niclosamide, and Bithionolsulfoxide are recommended for the treatment of paramphistomiasis in ruminants. These drugs are costly, inadequately available to poor farmers of developing countries. However, the resistance to this drug has emerged and may pretense a serious problem as no other effective drug is available. Therefore, new antihelmintic or anthelmintic are urgently required.

The use of medicinal plants for the inhibition and treatment of gastrointestinal parasitism has its origin in ethnoveterinary medicine. The medicinal plant-based remedies as an alternative to synthetic antihelmintic or anthelminthics. Some medicinal plant extracts shown the anthelminthic effect on nematode and cestode parasites [4, 5]. Scientists were used herbal drug to treat trematode parasites; *Fasciola gigantica*, *Gigantocotyle explanatum*, and *Gastrothylax crumenifer* [6-8]. *Plumbago indica* and *Balanites aegyptica* were used to control *Paramphistomum cervi* [9, 10]. The availability of the highly effective and safe drug for the cure of fluke was limited, and none of the drugs is available that show high efficacy on *Cotylophoron cotylophorum*, and no similar studies have been reported.

The experimental plant *Citrullus colocynthis* commonly known as bitter apple, bitter cucumber, Gavakshi or Indravaruni is a vine plant. In Rajasthan, this plant occurs in Jaisalmer, Barmer, and Shriganganagar. Its fruit extracts have shown an antibacterial and antimicrobial effect, antifungal property, antihyperglycemic effect on type 2 diabetic patients found antileishmanial, antitumor agent [11].

C. colocynthis is a very important medicinal plant which used by various tribes for treatment of many diseases in Rajasthan, India [12].

Phytochemical screening of the fruits of *Citrullus colocynthis* has revealed the presence of several bioactive compounds grouped as glycosides, flavonoids, alkaloids, phenols, carbohydrates, fatty acids, and essential oils [13]. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* have been reported [14]. The acaricidal activities of the aqueous methanolic extracts of the fruit of *C. colocynthis* and crude methanol extract were found effective against *Rhipicephalus microplus* larvae of ticks [15]. The crude oil from *C. colocynthis* fruit showed that the major constituents were mainly the triglycerides, free fatty acids, phospholipids, and sterols along with other minor unidentified constituents [16]. The fruit extract of *C. colocynthis* has been used for the treatment of anthelmintic, antimalarial elephantiasis, cancer, diabetes, inflammation, jaundice, leucoderma, microbial, edema, ulcer, and urinary diseases [17, 18]. *In-vitro* anthelmintic effects of fruit extracts of *Citrullus colocynthis* on liver fluke *Fasciola gigantica* in buffaloes [19].

However, no research work has been carried out to study the histopathological changes on the tegument induced by anthelmintic effects of alcoholic *Citrullus colocynthis* fruit extract on the tegument of *Cotylophoron cotylophorum* by light microscope.

MATERIALS AND METHODS:

Collection of Parasites: Live *Cotylophoron cotylophorum* flukes were collected from the rumen of freshly slaughtered domestic buffaloes (*Bubalus bubalis*) at the slaughterhouse of local zoo abattoir and meat market in Udaipur (Rajasthan). These flukes or worms or parasites were kept in 0.9% physiological saline for observations **Fig. 1A,** and **B**.

Collection of Plant Material: The plant *Citrullus colocynthis* with fruits **Fig. 1C** and **D** were collected from the Barmer (Rajasthan). The plant was identified and authenticated by Dr. Asha Arora, Associate Professor, Department of Botany, B. N. University, Udaipur (Rajasthan). The herbarium sheet was prepared and deposited in the

department for future reference, and an accession number BNC/11-12/021123 was assigned.

Preparation of Fruit Extract: The pulp was separated from the fruit, then dry and crush with a grinder into a powder form. The powder was refluxed in 70% alcohol for 72 h at 60 °C, and occasional stirring with the help of a glass rod at regular intervals. After 72 h the macerates solutions were filtered in a separate beaker using a Whatman no. 4 filter paper. Than, centrifuged at x10000 g for 15 min, and the supernatant was dried until a constant dry weight of each extract was found. Then dried *C. colocynthis* fruit extract was reconstituted in the respective solvents (alcoholic) using 10% DMSO.

Experimental Design: Anthelmintic activity was studied by the *in-vitro* petri dish method. Flukes were maintained in 0.9% physiological saline and divided into three groups, with seven flukes in each group. The first group of the flukes was untreated or controlled *C. cotylophorum*, the Second group of flukes was treated with drug albendazole, the third group of flukes was given in vitro treatments with alcoholic *C. colocynthis* fruit extract. Albendazole was obtained from the pharmaceutical department of Veterinary Hospital, Udaipur.

Evaluation of the Anthelmintic Activity of Alcoholic *Citrullus colocynthis* Fruit Extract and Albendazole: 10 ml of each the concentration of the fruit extracts of *Citrullus colocynthis* and the synthetic drug was applied to a group of 7 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 ºC) and pH 7.4. The motility and mortality of flukes were examined after 1, 2, 3, 4, 5 h. The dead flukes were examined mechanically and visually stimulated by a dissecting needle, and worm mortality was evaluated by observation under a magnifying glass or after being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, worms confirmed by mortality.

Motility of the Fluke was Scored by 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.

Statistical Analysis: The dead worms were counted in each experimental set, and the percentage of average mortality was calculated in all experiments according to the following formula:

Average Mortality rate = Total number of dead flukes × 100 / Total number of experimental flukes

All the values were expressed as mean ± standard deviation (SD).

Histology of Tegument by Light Microscopy: Control and treated Score 1 - immobile but not dead *C. cotylophorum* after incubation in sub-lethal dose (LC92) for 4 h at 60 mg/ml concentration of alcoholic *Citrullus colocynthis* fruit extract and after incubation in sub-lethal dose (LC76) for 5 hours at 100 mg/ml, 100% albendazole were fixed in Bouin's fixative for 24 h. Then treated flukes were dehydrated in ascending series of alcohol, embedded in paraffin wax, and sections were cut at 6µ on a rotary microtome. Sections of flukes were dehydrated in ascending series of alcohol, stained with Haemotoxylin & Eosin, cleared in xylene, and mounted in DPX. The tegument of adult fluke was studied and photographed by using an Olympus microscope.

RESULTS: The anthelmintic effect of different concentrations of alcoholic *Citrullus colocynthis* fruit extract and drug albendazole against *Cotylophoron cotylophorum* is shown in **Table 1** and **2**. Due to the effects of fruit extracts and albendazole, the flukes were paralyzed.

Anthelmintic Effect of Alcoholic *C. colocynthis* Fruit Extract on *C. cotylophorum*: The effect of different concentrations of alcoholic *Citrullus colocynthis* fruit extracts on *Cotylophoron cotylophorum* has been shown in **Table 1**. The highest mortality rate of 100% in the experiment carried out with the alcoholic extracts 80 and 100 mg/ml of concentration in 4 h exposure time. However, the same highest mortality rate (100%) was also found on 20 to 100 mg/ml concentrations at 5 h exposure time. Whereas, lowest mortality rates 40% was induced by 20 mg/ml of alcoholic concentrations at 1 h exposure time.

These results indicate the highest toxic effect of alcoholic extracts is better in comparison to albendazole extracts at lower concentrations and lesser exposure time. The treated worms became

agglutinated, shrunken, and paralyzed after incubation in sub-lethal dose (LC92) incubated for 4 h at 60 mg/ml concentration of alcoholic fruit extract of *Citrullus colocynthis*.

A perusal of coefficients of variation (CV%) of mortality (15.20% in 1hr at 100 mg/ml and 9.32% at 100 mg/ml in 3 h) indicates a consistency in the observation. The efficacy of alcoholic extracts of *Citrullus colocynthis* has been found most effective on 100 mg/ml at 1 h (CV% 15.20%), 100 mg/ml at 2 h (CV% 12.45%), 100 mg/ml at 3 h (CV 9.32%) and 60 mg/ml at 4 h (CV 11.91%).

TABLE 1: ANTHELMINTIC EFFECT OF ALCOHOLIC *C. COLOCYNTHIS* FRUIT EXTRACT *C. COTYLOPHORUM*

Exposure time in h	*C. colocynthis* alcoholic extract in mg/ml	No. of dead worm		Mortality rate %	CV %
		Mean	± STD		
1 hour	20 mg/ml	2	0.71	40%	35.36
	40 mg/ml	2.4	0.55	48%	22.82
	60 mg/ml	2.8	0.45	56%	15.97
	80 mg/ml	3.2	0.45	64%	13.98
	100 mg/ml	3.6	0.55	72.00%	15.21
2 hour	20 mg/ml	2.8	0.45	56%	15.97
	40 mg/ml	3	0	60%	0
	60 mg/ml	3.4	0.55	68%	16.11
	80 mg/ml	3.8	0.45	76%	11.77
	100 mg/ml	4.4	0.55	88.00%	12.45
3 hour	20 mg/ml	3.2	0.45	64%	13.98
	40 mg/ml	3.6	0.55	72%	15.21
	60 mg/ml	4	0	80%	0
	80 mg/ml	4.4	0.55	88%	12.45
	100 mg/ml	4.8	0.45	96.00%	9.32
4 hour	20 mg/ml	4.2	0.45	84%	10.65
	40 mg/ml	4.4	0.55	88%	12.45
	60 mg/ml	4.6	0.55	92%	11.91
	80 mg/ml	5	0	100%	0
	100 mg/ml	5	0	100.00%	0
5 hour	20 mg/ml	5	0	100%	0
	40 mg/ml	5	0	100%	0
	60 mg/ml	5	0	100%	0
	80 mg/ml	5	0	100%	0
	100 mg/ml	5	0	100.00%	0

Anthelmintic Effect of Albendazole on *Cotylophoron cotylophorum*: The anthelmintic effect of different concentrations of albendazole on *Cotylophoron cotylophorum* is shown in **Table 2**.

The high concentration of albendazole of 100 mg/ml displayed a 76% mortality rate after 5 h of exposure on *Cotylophoron cotylophorum* parasites. Whereas 20 mg/ml concentration did not induce any mortality among test parasites. Whereas, lowest mortality rates 8% was induced by 40 mg/ml of alcoholic concentrations respectively at 1 h exposure time. The treated worms were developed agglutinated, shrunken, paralyzed after incubation in sub-lethal dose (LC_{76}) incubated for 5 h at 100 mg/ml, 100% concentration of albendazole on *C. cotylophorum*.

A look at the coefficients of variation (CV %) indicates the results of mortality at 40 mg/ml 1 h. the exposure period is highly variable (CV 136.93%); therefore, the results cannot be considered as consistent.

TABLE 2: ANTHELMINTIC EFFECT OF ALBENDAZOLE ON C. *COTYLOPHORUM*

Exposure time in hrs.	Albendazole in mg/ml	No. of dead worm		Mortality rate %	CV %
		Mean	± STD		
1 hour	20 mg/ml	0	0	0%	0
	40 mg/ml	0.4	0.55	8%	136.93
	60 mg/ml	0.6	0.55	12%	91.29
	80 mg/ml	0.8	0.45	16%	55.9

	100 mg/ml	1	0	20.00%	0
2 hour	20 mg/ml	0.6	0.55	12%	91.29
	40 mg/ml	0.8	0.45	16%	55.9
	60 mg/ml	1	0	20%	0
	80 mg/ml	1.2	0.45	24%	37.27
	100 mg/ml	1.6	0.55	32.00%	34.23
3 hour	20 mg/ml	1.4	0.55	28%	39.12
	40 mg/ml	1.6	0.55	32%	34.23
	60 mg/ml	2	0	40%	0
	80 mg/ml	2.2	0.45	44%	20.33
	100 mg/ml	2.6	0.55	52.00%	21.07
4 hour	20 mg/ml	1.6	0.55	32%	34.23
	40 mg/ml	2	0	40%	0
	60 mg/ml	2.2	0.45	44%	20.33
	80 mg/ml	2.6	0.55	52%	21.07
	100 mg/ml	3	0.71	60.00%	23.57
5 hour	20 mg/ml	2.2	0.45	44%	20.33
	40 mg/ml	2.4	0.55	48%	22.82
	60 mg/ml	2.6	0.55	52%	21.07
	80 mg/ml	3.2	0.45	64%	13.98
	100 mg/ml	3.8	0.45	76.00%	11.77

During investigation number of tests was carried out on *Cotylophoron cotylophorum* **Fig. 1A** and **B** with the fruit extract of *Citrullus colocynthis* **Fig. 1C** and **D** to observe the anthelmintic efficacy.

FIG. 1: A - AMPHISTOME (AM) ATTACHED WITH INFECTED RUMEN (RU), B - AMPHISTOME (AM) *C. COTYLOPHORUM*, C - VINE OF *C. COLOCYNTHIS* WITH FRUITS, D - FRUIT OF *C. COLOCYNTHIS* SHOWING PULP AND SEEDS

The control and treated tegument of *C. cotylophorum* were observed by light microscopy. *C. cotylophorum* was the control and treated with alcoholic *Citrullus colocynthis* fruit extract and compared with drug albendazole.

Histological Study of the Tegument of Control *Cotylophoron cotylophorum*: The outer surface *C. cotylophorum* covered by the tegument. The body of the adult is dorsally convex and almost straight at ventral. The anterior end has several rows of cuticular papillae. This fluke was having three distinct suckers; an anterior subterminal oral sucker and a posterior large ventral sucker. The genital pore present in genital sucker which is situated at the anterior third of the body. Oral, posterior, and genital suckers, their opening is present on the outer side of the body surface, and they are enveloped by the tegument of the fluke. The musculature of oral, posterior, and genital suckers of the control parasite was well developed.

Control amphistome *Cotylophoron cotylophorum* showed densely covered by tegumental folds with grooves and smooth, spineless tegument. Light microscopic study shows that the tegument is consists of the surface syncytium layer, basement layer, and musculature comprises longitudinal and circular musculature, and tegumental cells were present and well-arranged form. The outer surface of the tegument of the fluke is formed a thick layer of the surface syncytium. The surface syncytium layer rested on the basement membrane is made up of a very thin layer. The basement membrane is highly folded and forms finger-like projections into the surface layer. The body musculature is present between the parenchymatous cells and tegumental cells. Tegumental muscles comprise bundles of external circular and internal longitudinal muscles, among which pass the tegumental cells, which are seen. The tegumental cells are located deep among the parenchymal tissues of treated fluke **Fig. 2A**.

Histological Study of the Tegument of *C. cotylophorum* Treated with Alcoholic *C. colocynthis* Fruit Extract: The anthelminthic activities were carried out on adult *Cotylophoron cotylophorum* treated with alcoholic *Citrullus colocynthis* fruit extract. Treated flukes became clumped, paralyzed and dead after 4 h of exposure time at a concentration of 60 and 80 mg/ml alcoholic *C. colocynthis* fruit extract. Alcoholic extract of *Citrullus colocynthis* fruit effect the whole parasite become shrunken or elongated and paralyzed.

Histological changes were observed in the tegumental organization of parasite by light microscopy. Alcoholic *Citrullus colocynthis* fruit extracts direct contact with the tegument of parasite showed many changes. Extensive disorganization and alteration, pronounced detachment and blebbing, breakages in the tegument, damage and big values in the basal membrane, circular & longitudinal muscles and tegumental cells, massive vacuolization in the musculature of the tegumental region and parenchymatous cells were observed in the treated flukes. The present investigation revealed that the alcoholic *C. colocynthis* fruit extract highly damaging alternation, breakages, and deformity in the tegumental architecture of treated *C. cotylophorum* **Fig. 2B, C, D,** and **E**.

The extract of *Citrullus colocynthis* found an alteration in the tegument of oral sucker architecture in the form of damage, disturbance, breakage, separation of tegument, muscle fibers, and vacuolization. The shrinkage in the normal diameter of the oral sucker was also observed in **Fig. 2F**.

The anthelmintic efficacy of extract of *Citrullus colocynthis* deformed the normal structure of acetabulum or posterior sucker. There was damage in the sucker wall, separation of muscles in musculature and the breakage started from surface tegument, and penetrating the musculature of the acetabulum was seen. The observation also showed the damage and breakage in the internal lining of the tegument covering the acetabulum **Fig. 2G**.

Genital suckers of *Cotylophoron cotylophorum* is enveloped by tegument. Numerous abundant vacuoles, damage and disturbance in longitudinal and circular musculature and damage and disturbance in the tegument were observed in the treated fluke **Fig. 2H**.

Histological Observation of the Tegument of *C. cotylophorum* Treated with Albendazole: In this study, the anthelminthic activity of alcoholic *C. colocynthis* fruit extract on the tegument of *C. cotylophorum* is compared with albendazole drug. Albendazole also showed few breakage, discontinuation, and detachment of tegument, rare vacuoles are seen in the tegumental and parenchymal cells **Fig. 2I**. Albendazole is less effective than alcoholic *Citrullus colocynthis* fruit extract.

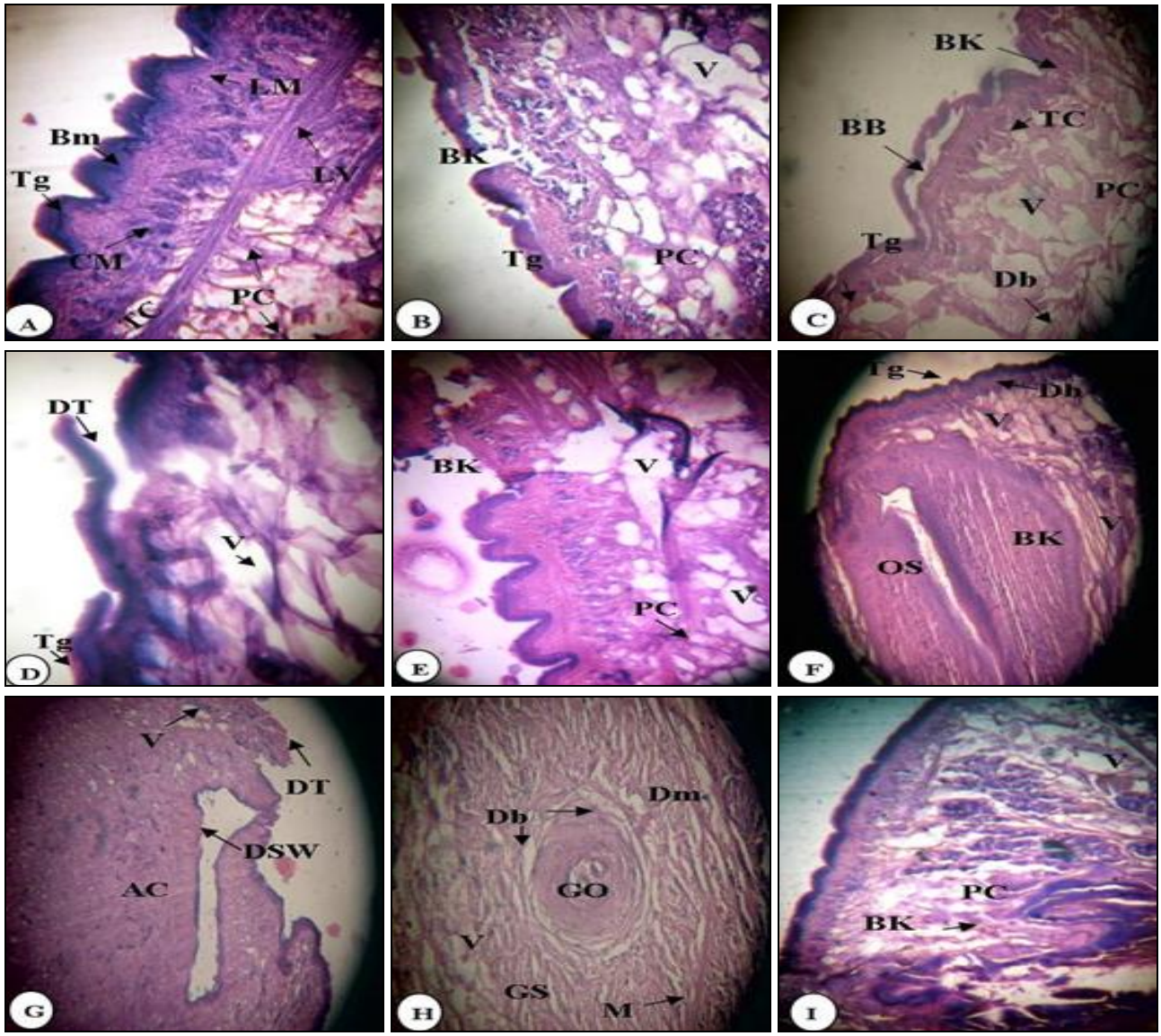

FIG. 2: PHOTOGRAPH OF HISTOLOGY OF TEGUMENT OF *C. COTYLOPHORUM* BY LIGHT MICROSCOPY SHOWING: A - CONTROL, B, C, D, H - TREATED WITH ALCOHOLIC *C. COLOCYNTHIS* FRUIT EXTRACT, I - TREATED WITH ALBENDAZOLE X 110. Abbreviations: (AC) Acetabulum, (Bm) Basal membrane, (BB) Blebbing, (BK) Breakages, (CM) Circular muscles, (Db) disturbance, (Dm) Damage, (DSW) Damage sucker wall, (DT) Damage tegument, (GO) Genital opening, (GS) Genital sucker, (LM) Longitudinal muscles, (LV) Lymphatic vessels, (M) Musculature, (OS) Oral sucker, (PC) Parenchymatous cells, (Tg) Tegument, (TC) Tegumental cells and (V) Vacuolization.

DISCUSSION: In this study we have assessed, for the first time, the effect of alcoholic *Citrullus colocynthis* fruit extract against *Cotylophoron cotylophorum* elucidated the anthelmintic potential of *Citrullus colocynthis*. From the visual and physical touch by a dissecting needle and worm, mortality was evaluated by observation under a magnifying glass or after being removed from the experimental medium and dipped in slightly warm water and on gentle stimulation, worms confirmed by mortality. The observations on the mortality of parasite, it was found that *C. colocynthis* was effective against *C. cotylophorum*. The study exhibited that alcoholic *C. colocynthis* fruit extract caused more mortality than synthetic drug albendazole, and higher concentration is more effective than lower concentration. These results indicate the highest toxic effect of alcoholic extracts in comparison to albendazole extract at lower concentrations and lesser exposure time.

Some other scientists also worked on the anthelmintic activity of *C. colocynthis* on *Fasciola gigantic* [19] and *Orthocoelium scoliocoelium* [20]. The current observations also convinced with other findings on different species of amphistome showed the anthelmintic effect of different medicinal plants.

The *in-vitro* antihelminthic activity of *Trigonella foenum-graecum*, *Allium sativum*, and *Piper longum* evaluated comparison with albendazole and niclosamide against *Cotylophoron cotylophorum*, and *Gigantocotyle explanatum* showed remarkable paralysis in these parasites [12, 22]. Our findings showed significant similarity with the above observations.

The present study demonstrated the histological study of the efficacy of alcoholic *Citrullus colocynthis* fruit extract on the tegument of *Cotylophoron cotylophorum* under light microscopic study. The tegument is the most important part of the trematode *C. cotylophorum* that is in direct contact with the host's tissue along with body fluids. The tegument plays an important role in protection, absorption, excretion, transport, and osmoregulation, which is in direct contact with the host's tissue along with the body fluids. Modification in the structure of the tegument is necessary for developing any rational drugs which may damage the parasites through their actions on the tegument. Damage and disturbance in the tegument were certainly disrupted its physiological functions were stopped; then definitely parasite was dead [6-10].

The light microscopic changes in the tegument of the parasite have long been studied as an important parameter for the determination of the efficacy of anthelminthic drugs. The detachment of the tegumentin treated worms may lead to the whole breakdown of cells in the parenchyma, leaving vacuolated areas. Blebbing's are active in response to anthelminthic treatment, where secretory bodies are rapidly transported towards the tegument and released from the apical plasma membrane to replace damaged membranes and maintain the integrity of the tegumental surface. Present findings are similar to anthelminthic medicinal plants showed that disorganized the morphology of tegument, tegument of genital, anterior (oral), and posterior suckers (acetabulum) in the parasite [9, 22].

The presence of several vacuoles in the parenchyma could distort the structure of the parasite. Different trematodes (*Faciolopsis buski* and *Fasciola gigantica*), cestodes (*Raillietina echinobothrida*) and nematode (*Ascari diagalli* and *Haemonchus contortus*) were also shown tegumental deformities with *in-vitro* treatment with different medicinal plants; *Stephania glabra*, *Ocimum sanctum* Linn, *Trichosanthes multiloba*, *Lysimachia ramose* and *Citrullus colocynthis* also examined severe distortion in suckers and coupling of parenchymatous cells. The treated flukes showed a deformed body with the shrunken and wrinkled tegumental surface with pit formations, and the sucker region is also deformed [24, 30].

The extract of *C. colocynthis* used severe deformity and extensive alteration in the normal tegumental architecture, numerous blebbing damage, and detachment of tegument layer, vacuolization in parenchymatous cells of a treated fluke. Surface blebbing was a common feature of a medicinal plant treated parasites and had been described for other trematodes and flatworm parasites after exposure to anthelmintics. It had been suggested that the blebbing occurred because of increased efforts on the part of the parasite to shed and replace the outer tegumental membrane damaged by the action of the medicinal plant on fluke [22]. It might be significant, then, that the process of the tegumental layer to their rupture.

Some researchers were found similar observations; shape and many structures deformed, alteration in the tegument, breakage, and vacuolization in the musculature of oral, posterior, and genital suckers in *Orthocoelium scolocoelium* and other worms treated with alcoholic *C. colocynthis* fruit extract [26]. This damage would undoubtedly disrupt many of the physiological processes associated with the tegument, including protection, osmoregulation, secretion, and synthesis. Besides, the damage of the tegumental folds of the posterior sucker (acetabular) the region might disrupt its function in drawing the rumen wall tissue of the host into the acetabular cavity resulting in a weak hold for the *C. cotylophorum* fluke.

Surface changes observed in the study resembled that demonstrated on *P. explanatum* treated with methanolic extracts of leaves of *Dregea rolubilis*. Besides, a similar sequence of tegumental changes occurred in *P. cervi* treated with plumbagin; a compound that was rich in the roots of *Plumbago indica* rosea [10]. Moreover, the tegumental surface alterations induced by *C. colocynthis* extract, in the present study, had also been observed in specimens

of a biologically related trematode, *Fasciola gigantica*, following incubation with several anthelmintics [19]. Current research work has an agreement with the previous observation of different scientists that *in-vitro* anthelmintic activity of the extracts of medicinal plants against adult different trematode parasites [20-23, 27].

The present results are similar with the previous study that the researchers were observed disruptions, disarrangement, and detachment of tegument from the basal membrane, eruption, blebs, erosions and vacuoles in the tegumental structure, swelled deformed body, retracted sucker, morphological and structural changes caused by *Balanites aegyptica*, *Plumbago indica*, *Dregea volubilis* and the oil of *Nigella sativa* treated on adult *Paramphistomum microbothrium*, *Paramphistomum cervi*, *Gastrothylax crumenifer*, *Paramphistomum explanatum* and *Fasciola gigantic* [8, 10, 25, 28, 29]. In the present study synthetic drug albendazole also shows mild anthelminthic activity and serves few damages and vacuoles on the tegument of *Cotylophoron cotylophorum*.

Phytochemical screening of the fruits of *Citrullus colocynthis* has revealed the presence of several bioactive compounds grouped as glycosides, flavonoids, alkaloids, phenols, carbohydrates, fatty acids, and essential oils. *C. colocynthis* has been used for the treatment of various diseases [13, 14].

The treated *C. cotylophorum* with alcoholic *C. colocynthis* fruit extract were studied under light microscopic; this study revealed various extensive alteration in treated fluke tegumental architecture, also found the anthelminthic activity of *C. colocynthis* fruit. As evident from the present results, biochemical compounds of plants study chemotherapeutic potential since they have caused significant inhibition of parasite mortality serves disruption of the tegumental surface. The literature survey on *C. colocynthis* suggested that alcoholic extracts of the plant's tissues had anthelmintic activity [19, 20, 26, 30].

This study suggests that the alcoholic extract of fruit pulp of *C. colocynthis* could offer a suitable and cheaper alternative anthelmintic as a comparison to synthetic drugs. Consequently, it might help to reduce the occurrence of the parasite in the host environment after treatments.

The results of this study will help to prepare the eco-friendly, less costly the anthelmintic veterinary drug, socio-economic upliftment of cattle farmers, and scientific documentation of traditional veterinary practices.

CONCLUSION: Alcoholic *C. colocynthis* fruit extract has a powerful and progressive effect on the tegument of *C. cotylophorum*. The medicinal plant *Citrullus colocynthis* has been used for the treatment of various diseases. The tegumental alterations induced by this plant *in-vitro* against in many cestodes, nematode and trematode flukes. The use of Alcoholic *C. colocynthis* fruit extract offers new aspect and potential for control of such an ignored infectious disease in ruminants, at a time when paramhistomosis has arisen as an important cause of economic loss. Thus plant-based medicines such as *Citrullus colocynthis* could be used as an efficient anthelminthic in the treatment of paramphistomiasis.

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