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IN-VITRO ANTHELMINTIC EFFECTS OF MEDICINAL PLANT *CASSIA FISTULA* EXTRACT ON THE TEGUMENT OF *FASCIOLA GIGANTICA* BY LIGHT MICROSCOPE

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ABSTRACT: The efficacy of alcoholic *Cassia fistula* leaves extract on the tegument of *Fasciola gigantica* was studied by light microscopy. Live liver fluke *Fasciola gigantica* parasites were collected from the infected liver and bile duct of freshly slaughtered domestic goat (*Capra hircus*) at the local zoo abattoir in Udaipur. Liver flukes *F. gigantica* were maintained in 0.9% physiological saline and divided into three groups. The first group of the parasite was untreated used as control liver fluke, the Second group of parasites was treated with synthetic drug albendazole and the third group of parasites was given *in-vitro* treatments with alcoholic extracts of *C. fistula* leaves. The control and *in-vitro* treated *F. gigantica* with the alcoholic *C. fistula* leaves extract, and flukicide drug albendazole were fixed in Bouin's fixative for histological study. The present studies revealed that the alcoholic extract of *C. fistula* leaves was an effect on the tegument of parasite; showed abounded cuticular disruption, detachments, discontinuous, babblings, damage in tegument and in parenchymal cell and the complete breakdown of cells in the parenchyma, leaving vacuolated areas. Albendazole is less effective than alcoholic *C. fistula* leaves extract. This study suggests that the alcoholic extract of the leaves of *Cassia fistula* could offer a suitable and cheaper alternative anthelmintic as a comparison to synthetic drugs. The results of this study will help prepare the eco-friendly, less costly anthelmintic veterinary drug, socio-economic upliftment of cattle farmers.

INTRODUCTION: Fasciolosis is caused by trematode *Fasciola gigantica*, which occurs in the liver and bile duct of ruminants. Fasciolosis is a disease that causes low productivity and a low population of ruminants, which contribute to excessive economic loss.

The control of fasciolosis mainly by anthelmintic or anthelmintic chemical drugs; however, the effectiveness of the drugs is reduced due to indiscriminate use, which leads to parasite resistance.

Furthermore, chemical drugs may be toxic to ruminants and also have chemical residue problems that essentials to be resolved^{1, 2}. *In-vitro* Anthelmintic or anthelmintic activities of the extracts of medicinal plants were evaluated against adult *F. gigantica*. Their instigation proved that medicinal plants are produced safer, cheaper, and effective remedies for fasciolosis in ruminants³⁻¹³.

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One of the plants used as medicine is *Cassia fistula* Linn. and also known as a rich source of tannins, flavonoids, and glycosides. These plants have medicinally important values. *C. fistula* was investigated for their anthelmintic, antifungal, antibacterial, ovicidal, and larvicidal activities on the gastrointestinal parasites of goats and sheep¹⁴⁻²⁰. However, none of the scientists paid attention to the anthelmintic or anthelmintic study on *Fasciola gigantica* treated with *Cassia fistula*. So the present investigation determined the histology of tegument of control and *in-vitro* treated *Fasciola gigantica* with medicinal plant *Cassia fistula* leaves extract and compare with the chemical drug by light microscopic.

MATERIALS AND METHODS:

Collection of Parasites: Live liver fluke *Fasciola gigantica* parasites were collected from the liver and bile duct of freshly slaughtered domestic ruminant domestic goat (*Capra hircus*) at the local zoo abattoir in Udaipur. These flukes were kept in 0.9 % physiological saline for investigations.

Preparation of Extracts: Fresh *Cassia fistula* leaves were collected from the Udaipur (Rajasthan). The medicinal plant *Cassia fistula* (Amaltas) was authenticated and identified by Dr. Asha Arora, Associated 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 BNU/BIOT/2021-22/1136 was assigned. The leaves were put to dry and pulverized with a grinder into a powder. The powder was refluxed in 70% alcohol for 72 h at 60 °C and occasionally stirred with a glass rod manually at regular intervals.

After 72 h the macerates solutions were filtered in separate flasks using a Whatman no. 4 filter paper. Then centrifuged at x10000 g for 15 min, and the supernatant was dried until a constant dry weight of each extract was obtained. Then dried plant leaves extracts were reconstituted in the respective solvents (Aqueous and alcoholic) using 10% DMSO.

Experimental Design: Anthelmintic activity was studied by *in-vitro* petri dish method²¹. *Fasciola gigantica* were maintained in 0.9% physiological

saline and divided into three groups with five parasites in each group (Three replicate in each group). The first group of parasites was untreated used as control Liver fluke. The second group of parasites were treated with the chemical/ synthetic drug albendazole. The third group of parasites was given *in-vitro* treatments with alcoholic extracts of *Cassia fistula* leaves.

Evaluation of Anthelmintic Activity of Alcoholic Extracts of *Cassia fistula* Leaves and Albendazole: 10 ml of each concentration of the leaves extracts of *C. fistula* leaves and synthetic 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 optimal temperature (37°C) and pH 7.4. Albendazole was obtained from Veterinary Hospital, Udaipur. The motility and mortality of flukes were examined after 15 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 of Tegument by Light Microscopy: The control or untreated and *in-vitro* treated immobile but not dead *Fasciola gigantica* with the alcoholic *Cassia fistula* leaves extract, and flukicide drug albendazole were fixed in Bouin's fixative, dehydrated in ascending series of alcohol, 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 DPX²². The tegument of adult fluke was studied and photographed using an Olympus CX41 microscope.

RESULTS: *Fasciola gigantica* parasites were treated with alcoholic plant extracts leaves of *Cassia fistula* (Amaltas) and compared with synthetic drug albendazole. The control and treated tegument of liver fluke *Fasciola gigantica* were observed by light microscopy. The present study showed that aqueous extract is less effective than alcoholic extract. Based on this, the alcoholic extract was used for further studies.

Histological Study of Tegument of Control *Fasciola gigantica*: Control liver fluke *Fasciola gigantica* covered by tegument containing ridges, pits with pointed and comb-like spines. The light microscopic study shows that the tegument consists of the surface syncytium layer, basement layer, musculature comprises longitudinal and circular musculature, spines and tegumental cells are observed in well-arranged form. The outer surface of the tegument of the parasite is formed a thick layer of the surface syncytium.

The surface syncytium layer rested on the basement layer; it is a very thin layer. The basement layer is highly folded and forms finger-like projections into the surface layer. The tegumental musculature is present between the parenchymatous cells and tegumental cells. Tegumental muscles comprise bundles of external circular and internal longitudinal muscles passing the tegumental cells that are clearly seen. The tegumental cells are located deep among the parenchymal cells **Fig. 1 and 2**.

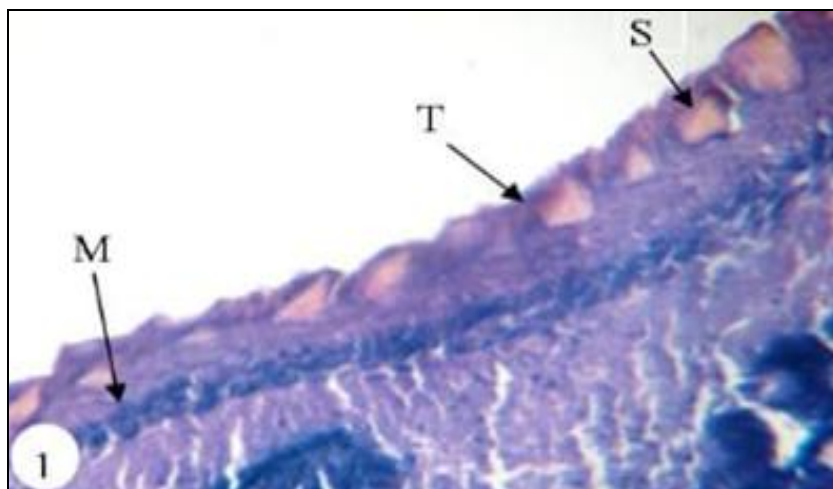


FIG. 1: PHOTOGRAPH OF HISTOLOGY OF CONTROL TEGUMENT OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING X110

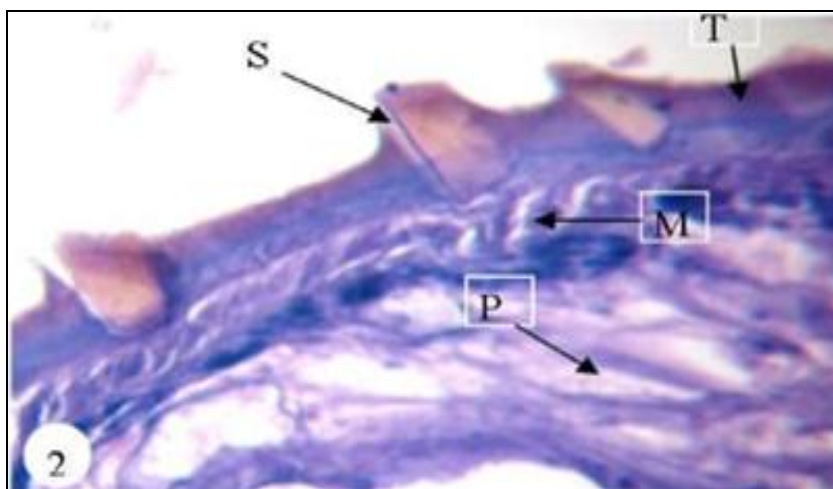


FIG. 2: HIGH MEGNIFIED PHOTOGRAPH OF HISTOLOGY OF CONTROL TEGUMENT OF *F. GIGANTICA* BY LIGHT MICROSCOPY X110

Tegumental Variations in *Fasciola gigantica* Treated with Synthetic Drug Albendazole: In the present study, the *in-vitro* anthelmintic activities of *Cassia fistula* leaves on *Fasciola gigantica* are compared with synthetic albendazole drug. Albendazole also showed only a few and minute damages, detachment and minor

deformation are present in longitudinal and circular muscles of tegument. Few small vacuoles are seen in parenchymal cells and muscles. The spines are still present in the tegument after treatment of albendazole drug **Fig. 3**. Albendazole is less effective than alcoholic leaves extract of *Cassia fistula* (Amaltas).

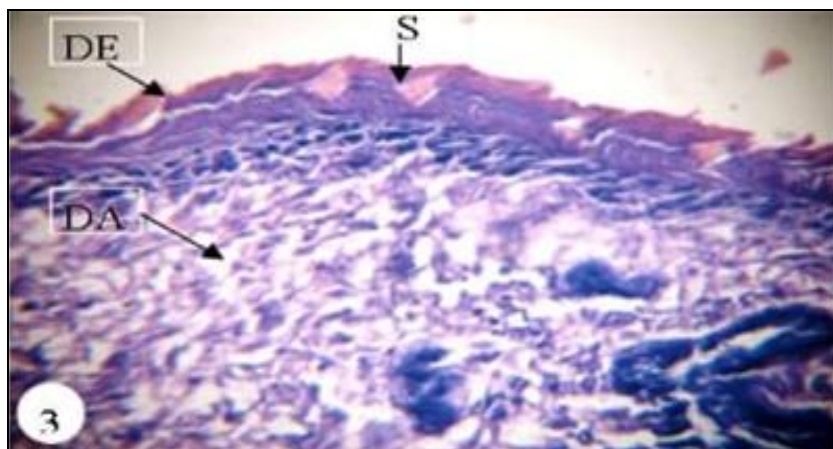


FIG. 3: HISTOLOGY OF TEGUMENT TREATED WITH ALBENDAZOLE OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING; DAMAGE (DA), DADETACHMENT (DE) AND SPINES (S) X 110

Histology of Tegument of *In-vitro* Treated *Fasciola gigantica* with Alcoholic Leaves Extract of *Cassia fistula* by Light Microscopic (LM): *Fasciola gigantica* parasites are treated with alcoholic extracts of leaves extract of *Cassia fistula*. Treated parasites became clumped, paralyzed and died after 15 h exposure time at a concentration of 50 mg/ml alcoholic extracts of *C. fistula* leaves.

The present investigation revealed that the alcoholic extract of medicinal plants caused highly destructive alternation such as vocalization, detachment, babbling, breakage and damage in the tegumental architecture of treated *F. gigantica* parasites. *Cassia fistula* alcoholic leaves extract on treated *F. gigantica* parasites shows several alterations and changes the integumental structure. Tegument of the anterior region and oral sucker of treated *F. gigantica* showing highly damaged, breakage, vacuolization, detachment of different

cell in different layers of tegument and many & large-sized, deep holes are visible due to the removal of spine; sometimes outer layers are totally removed from the body surface. Vacuolization is exhibited in the longitudinal and circular muscle of the tegument and parenchymal cells **Fig. 4** and **5**. The tegument of the middle region of treated *F. gigantica* also shows the extensive removal of the outer layer with spins of surface syncytium layer.

Also observe wide damages, large vacuolization, detachment and babbling of basal layer from circular and longitudinal muscles of sub tegumental layer of tegument **Fig. 6** and **7**.

Large vacuolization and detachment are present in circular and longitudinal muscles of the tegument of the posterior region of treated *F. gigantica* showing. The tegument is highly damaged due to removal and damage of spins. Vacuolization and damage also observed in parenchymal cells **Fig. 8**.

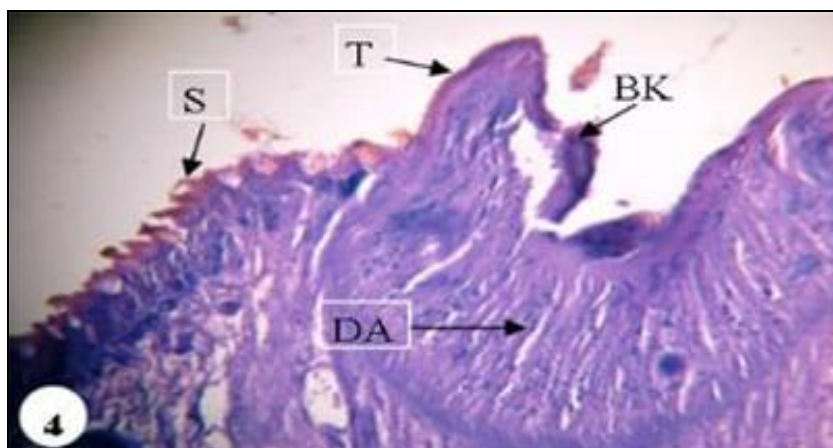


FIG. 4: HISTOLOGY OF TEGUMENT TREATED WITH ALCOHOLIC *C. FISTULA* OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING; BREAKAGES (BK), DAMAGES (DA), SPINES (S) AND TEGUMENT (T) X 110

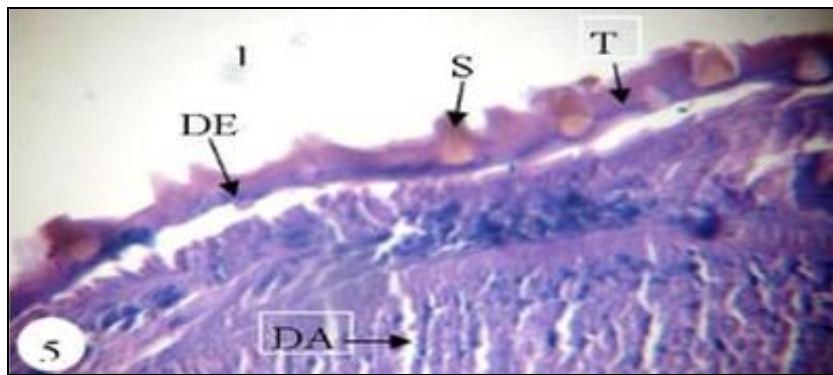


FIG. 5: HISTOLOGY OF TEGUMENT TREATED WITH ALCOHOLIC *C. FISTULA* OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING, DAMAGES (DA), DETACHMENT (DE), SPINES (S) AND TEGUMENT (T) X 110

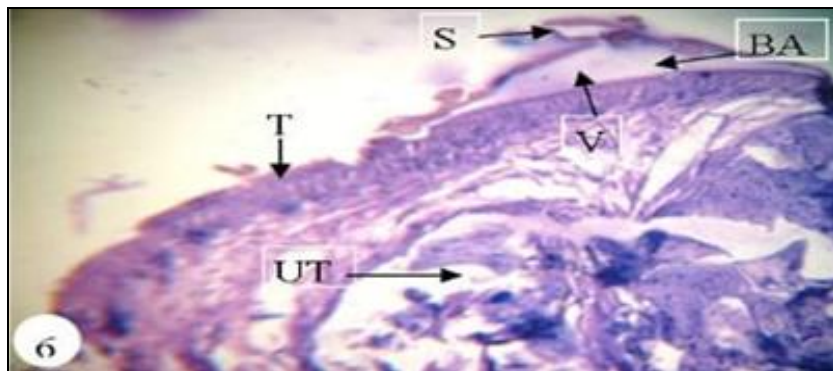


FIG. 6: HISTOLOGY OF TEGUMENT TREATED WITH ALCOHOLIC *C. FISTULA* OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING; BLEBBING (BA), SPINES (S), TEGUMENT (T), UTERUS (UT) AND VACUOLIZATION (V) X 110

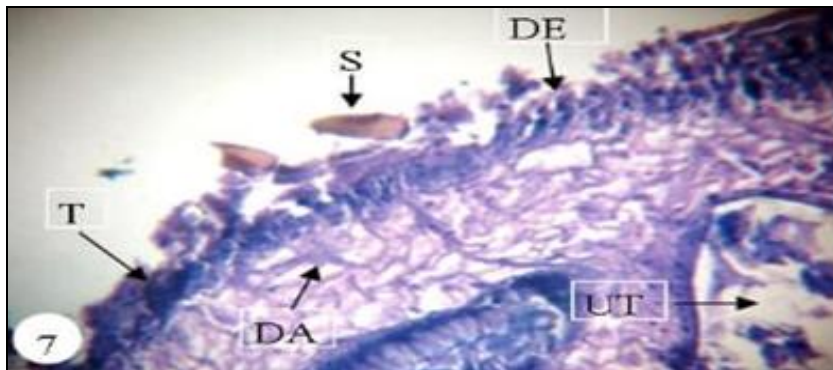


FIG. 7: HISTOLOGY OF TEGUMENT TREATED WITH ALCOHOLIC *C. FISTULA* OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING; DAMAGES (DA), DETACHMENT (DE), SPINES (S), TEGUMENT (T) AND UTERUS (UT) X 110

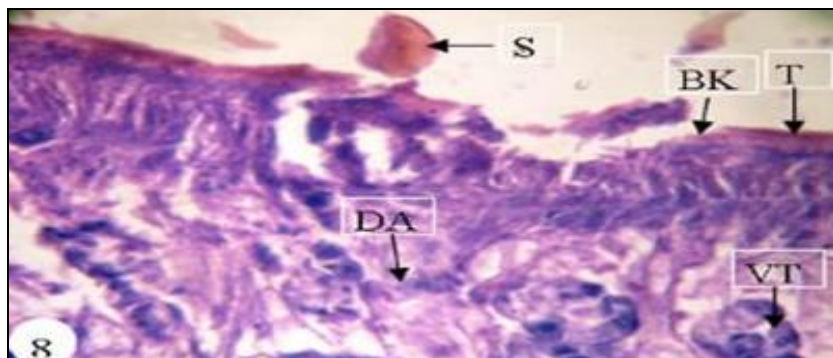


FIG. 8: HISTOLOGY OF TEGUMENT TREATED WITH ALCOHOLIC *C. FISTULA* OF *F. GIGANTICA* BY LIGHT MICROSCOPY SHOWING; BREAKAGES (BK), DAMAGES (DA), SPINES (S), TEGUMENT (T) AND VITELLINE FOLLICLES (VF) X 110

DISCUSSION: Syncytial epithelium contains abundant spines embedded throughout its matrix. The outer borders of the tegument and tips portions of the spines were intensely stained. The tegument rested on a layer of connective tissue with parenchymal cells called reticular lamina which connected the former to the underlying and intensely stained two muscular layers. Tegument cells locate beneath the muscles and send their processes between the muscle cells outwardly to join up with the tegument. No significant histological differences were observed between control liver fluke *F. gigantica* and treated fluke incubated for 15 hrs in solvent 50 mg/ml of synthetic drug albendazole. After *in-vitro* treated with alcoholic leaves extract of *Cassia fistula* (Amaltas) and compare with synthetic drug 50 mg/ml concentration at 15 h.

Incubated produced several histological alterations in the tegument of *F. gigantica*. Tegumental grooves, swelling, and babbling were observed in the tegument of the spines. The underlying structures still appeared normal. These changes became more severe with spines dislodged from their sockets, and others showed extensive cracking towards the base following 15 h incubation with 50 mg/ml leaves extract of *Cassia fistula*. Besides appearances of numerous vacuoles in the tegument syncytium and the tegument, it was partly sloughed off, but muscle underlying the tegument still exhibited normal appearances. Severe swelling of the tegument between the spines was apparent after 15 h. Incubation with 50 mg/ml. in these specimens, the spines had enlarged and appeared sunken as the tegument had swollen to engulf them, and sometimes spines were totally removed from the tegument and produced holes integument. The surface syncytium, muscle bundles, and tegumental cells identified tissues' flooding in the intercellular spaces, which appeared to be entirely separated from the surrounding tissue.

The tegument of digenean trematodes *Fasciola gigantica*, *Cotylophoron cotylophorum*, *Gigantocotyle explanatum*, and *Gastrothylax crumenifer* has several significant roles, including protection, osmoregulation, secretion, and synthesis hence represents a primary drug target^{6, 9, 23, 24, 26 - 28}. Present research work has an agreement with the previous observation of different scientist that *in-*

vitro anthelmintic activity of the extracts of medicinal plants; *Merytadenhami*, *Artocarpus lakoocha*, *Cymbopog annardus*, *Areca catechu*, *Erythrina indica*, *Zingiber officinale*, *Azadirachta indica* and triclabendazole against adult *F. gigantica*^{7, 8, 10-13}. The characteristics of the tegument of *Fasciola* spp. have numerous transporting epithelium functions that were helped in ion and water controls. The swelling of tegumental syncytium indicated that trouble of the apical membrane and its associated ion pumps had led to perturbation of the liver fluke osmoregulatory system and the entry of water the swelling of the basal infolds would make possible the sloughing of the tegument as a whole, by causing the detachment of the basal plasma membrane from the underlying basal lamina. Breakages and swelling were a particular characteristic of drug action with flukicide.

The tegumental changes induced by synthetic drug albendazole were less severe than those observed after 15 hrs exposure with alcoholic leaves extract of *Cassia fistula*. Fluke *F. gigantica* showed extensive swelling, vacuolization, and loss of spines were observed in tegument after treatments of medicinal plants. Vacuolization of the tegument in the present study raised from dilation of the basal infolds due to a water and ion imbalance. Similar vacuolization of the tegument had been described in a number of anthelmintic treated schistosomes, *opisthorchiasis viverrini*, and also in *F. hepatica*². Phytochemicals constituents phenolic antioxidants such as anthraquinones, terpenoids, flavonoids, 3-ol derivatives, alkaloid, tannins, Saponins, and glycosides, reducing sugar and steroids of *Cassia fistula* leaves were investigated and effective on trematode parasites²⁵. The present study confirmed the fasciocidal properties of leaves extract of *Cassia fistula* which caused interruption to the tegument of *F. gigantica* after *in-vitro* incubation. Extensive tegumental alterations were observed alcoholic leaves extract of *Cassia fistula* in comparison with synthetic drug albendazole.

CONCLUSION: Alcoholic *Cassia fistula* leaves extract has a powerful and progressive effect on the tegument of *Fasciola gigantica*. The medicinal plant *C. fistula* 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. fistula* leaves extract offers new aspects and potential for control of such an ignored infectious disease in ruminants, when liver flukes have arisen as an important cause of economic loss. Thus plant-based medicines such as *Cassia fistula* could be used as an efficient anthelmintic or anthelminthic in treating liver fluke.

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