Chapter 5

Histology of tegument of control and *in vitro* treated *Faschiola gigantica* with three selected medicinal plant extracts and synthetic drug by light microscope
HISTOLOGY OF TEGUMENT OF CONTROL AND IN VITRO TREATED FASCIOLA GIGANTICA WITH THREE SELECTED MEDICINAL PLANT EXTRACTS AND SYNTHETIC DRUG BY LIGHT MICROSCOPE

Introduction

Fasciolosis is a major parasitic disease of livestock with over 700 million production animals at risk of the infection. Fasciolosis is a zoonotic helminth infection and causes of an important economic loss. Fasciolosis disease belongs to the plant-borne trematode zoonoses. A definite host of trematode parasite is very broad and includes many herbivore mammals, including animal and human. Fasciolosis, mainly caused by *F. gigantica* is one of the most prevalent helminth infections of livestock, also known as a human disease of large public health importance and persistent constraint on their growth and production in different part of the world inducing significant morbidity and mortality (WHO, 1995; El-Shazly et al., 2009; and Tansatit et al., 2012).

Anthelmintic drugs are currently used as the most effective tool for the treatment of fasciolosis. Synthetic drugs are not easily available in some of the remotest rural areas of developing countries or have some serious disadvantages such as the development of drug resistance in continuous use adverse drug residual effects. Control of fasciolosis is challenged for researchers a likely increase in disease and the spread of resistance to, the most potent flukicide, triclabendazole. Use of common spices as anthelmintics offer an alternative source can solve these problems and more acceptable to the native users. Some medicinal plant extracts shown the anthelminthic effect on tegument of nematode and cestode parasites (Abdul-Ghani et al., 2009; Challam et al., 2012; Decinea et al., 2012; Shalaby et al., 2012a; Shalaby and El-Moghazy 2013 and Balqis et al., 2017).

Few scientists used the herbal drug to treat trematode parasites; *Fasciola gigantica, Cotylophoron cotylophorum, Gigantocotyle explanatum* and *Gastrothylax crumenifer* (Jeyathilakan et al., 2012; Veerakumari et al., 2012; Khan et al., 2015 and Rajesh et al., 2016).
Indian traditional several medicinal plants have different properties such as antioxidant, antiviral, antibacterial, antifungal, molluscicidal, antihelminthic and larvicidal the in vitro toxicity on the liver fluke *F. gigantica*. In vitro anthelmintic activity of the extracts of medicinal plants; Meryta denhami, *Artocarpus lakoocha*, *Cymbopogan nardus*, *Areca catechu*, *Erythrina indica*, *Zingiber officinale*, *Azadirachta indica*, and triclabendazole were evaluated against adult *F. gigantica*. (Massoud *et al.*, 2001, 2012; Kumar *et al.*, 2014; Mahardika *et al.*, 2017 and Ullah *et al.*, 2017).

The few research work has been observed on tegumental alteration of *F. gigantica* due to in vitro treated with synthetic drugs and extracts from indigenous medicinal plants viz. *Carica papaya*, *Mallotus philippinensis* Mirazid and myrrh volatile oil and *Azadirachta indica* by light microscopy. Resin extract of these plant used as Schistosomicide, fasciolicide, heterophycide and molluscicide have been reviewed in detail and provided suitable evidence for uses of medicinal plant as antiparasitic agent Their instigation proved that medicinal plants are produced safer, cheaper and effective remedy for fasciolosis in ruminants The morphological variation and histopathological changes in treated and control fluke was studied by light and electron microscopy (Meaney *et al.*, 2002; Rivera *et al.*, 2004; Kushwaha *et al.*, 2005; Shehab *et al.*, 2009; Saowakon *et al.*, 2009; Jenthilakhan *et al.*, 2010 a and b; Toner *et al.*, 2008 and 2010 b).

*Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) are very important medicinal plants which are used by various tribes for treatment of many diseases in Rajasthan, India. These medicinal plants have shown antimicrobial, paralytic, antinociceptive, antioxidant and wound healing activity of different fungi, bacteria, and animals. Herbal plants investigated for used in the treatment of ophthalmic, neuralgia, migraine bronchitis, abortifacient, cathartic, purgative, fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, tumor, leishmaniasis, cytotoxic activities and phytochemical analysis. The extract and fractions were
tested against major parasites (Hamayun et al., 2006; Chamundeewari et al., 2007 and Mathew et al., 2008; Sumi & Oommen, 2011; Irshad et al., 2012 and Sahoo et al., 2012; Patel et al., 2012; Baloch et al., 2013; Elgerwi et al., 2013; Paydar et al., 2013; Javad et al., 2013; Talole et al., 2013 and Ullah et al., 2013; Meena et al., 2014 a and b; Negi et al., 2014; Bhatia and Paliwal, 2015; Gopikrishna et al., 2016; Mehta et al., 2016).

However, none of the scientists paid attention to the anthelminthic study on *Fasciola gigantica* treated with Kalijiri, Indrayan or Kharatumba and Ajwain. So the present investigation determined the histology of tegument of control and *in vitro* treated *Fasciola gigantica* with three selected medicinal plant extracts; *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) and compare with the synthetic drug by light microscopic.
Result

The control and treated tegument of liver fluke *Fasciola gigantica* were observed by light microscopy. *Fasciola gigantica* parasites were treated with three alcoholic plants extracts seeds of *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) and compared with synthetic drug albendazole. The present study showed that aqueous extract is less effective than the alcoholic extract. On the basis of this alcoholic extract was used for further studies.

*F. gigantica* have a dorso-ventrally flat body, leaf-like in shape with the sharp pointed anterior end and blunt posterior end, the body of the worm divided into three regions such as anterior, middle and posterior regions, its tegumental surface appears rough due to the occurrence of many different size, shape, and arrangement of spines. Oral sucker (anterior sucker), genital pore and ventral sucker (Acetabulum) are present on ventral side of the body. Both oral and ventral suckers are spineless and covered thick rims of transverse folds. Oral sucker opens into the pharynx. The genital pore is located between oral and ventral suckers and near to the ventral sucker (acetabulum). The body of *F. gigantica* can be divided into three regions viz., anterior, middle and posterior regions (Plate 1, Fig. 1 A, 1B and 1C & Diagram 1).

**Histological study of tegument of control *Fasciola gigantica***

Control liver fluke *Fasciola gigantica* covered by tegument containing rids, 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 tegument of the parasite is formed a thick layer of the surface syncytium. The surface syncytium layer rested on basement layer, it is made up of the very thin layer. 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 among which pass the tegumental cells which are clearly seen. The tegumental cells are located deep among the parenchymal cells (Plate 2, Fig. 2A, 2B, 2C, 2D, 2E, and 2F).

**Histology of tegument of in vitro treated *Fasciola gigantica* with alcoholic seeds extract of *Centratherum anthelminticum*, fruits of *Citrullus colocynthis* and seeds of *Trachyspermum ammi* by light microscopic (LM)**

*Fasciola gigantica* parasites are treated with alcoholic extracts of seeds of *Centratherum anthelminticum*, fruits of *Citrullus colocynthis* and seeds of *Trachyspermum ammi*. Treated parasites became clumped, paralyzed and died after 15 hours exposure time at a concentration of 50mg/ml alcoholic extracts of these three of plants. The present investigation revealed that the alcoholic extract of medicinal plants caused highly destructive alternation such as vocalization, detachment, shrinkage, and damage in the tegumental architecture of treated *Fasciola gigantica* parasites.

1. **Tegumental variations in *Fasciola gigantica* treated with seeds of *Centratherum anthelminticum***

*Centratherum anthelminticum* alcoholic seed extract on treated *F. gigantica* parasites shows several alterations and changes the integumental structure. Tegument of the anterior region 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 and shrinkage are exhibited in the longitudinal and circular muscle of tegument, intestinal caeca and parenchymal cells (Plate 3, Fig. 3A and 3B).

Tegument of the middle region of treated *F. gigantica* also shows the extensive removal of outer layer with spins of surface syncytium layer. Also observe wide damages, large vacuolization, and detachment of basal layer from circular and longitudinal muscles of sub tegumental layer of tegument (Plate 3, Fig. 3 C and 3D). Large vacuolization and detachment are present in circular and longitudinal muscles of tegument of the posterior region of treated *F. gigantica* showing. Tegument is highly damaged due removal and damage of spins. Vacuolization also observes in parenchymal cells (Plate 3 Fig. 3E and 3F).
2. **Tegumental variations in *Fasciola gigantica* treated with alcoholic fruits extract of *Citrullus colocynthis***

Tegument of the anterior region of treated *F. gigantica* parasites shows highly damages in surface syncytium and spines are removed. Breakages are present in all layers of tegument Large vacuoles are present in parenchymal cells and muscles due to damages (Plate 4 Fig. 4A and 4B). Tegument of the middle region of treated *F. gigantica* shows extensive vacuolization, alterations in musculature; remove spines and outer layer. Extensive detachment, shrinkage and highly damaging to tegumental tissues are observed (Plate 4, Fig. C and D). Large vacuolization present in various tissues, and damages are an observer in a different layer of tegument of the posterior region of treated *F. gigantica*. It is also investigated that spines are completely removed from tegument and damages in muscles (Plate 4 Fig. 4E and 4F).

3. **Tegumental variations in *Fasciola gigantica* treated with fruits extract of *Trachyspermum ammi***

Detachment and babbling in the outer layer, damages in circular and longitudinal muscles are visible in the tegument of the anterior region of treated with alcoholic seed extract of *Trachyspermum ammi* on *F. gigantica*. Many spins are removed from tegument and induced breakages and vacuolization in the outer layer of tegument. Numerous breakage is observed in various tissues. Shrinkage and large vacuolization are also present in the epithelial membrane of intestinal caeca, in sub tegumental layer and parenchymal cells (Plate 5 Fig. 5A and 5B). Tegument of the middle region of treated *F. gigantica* showing detachment, shrinkage, damaged and removed the outer layer of tegument with spines, large vacuole formation due to damaged parenchymal cells and muscle (Plate 5 Fig. 5C and 5D). Numerous spins are removed from the tegument of the posterior region of treated *F. gigantica*. It is also observed that shrinkage, breakage and damages integument, vacuolization in parenchymal cells and intestinal caeca present in treated tegument (Plate 5 Fig. 5E and 5F).
4. Tegumental variations in *Fasciola gigantica* treated with synthetic drug albendazole

In the present study, the *in vitro* anthelminthic activities of three selected medicinal plants on *Fasciola gigantica* are compared with synthetic albendazole drug. Albendazole also showed only a few and little breakage, damages and detachment of tegument, moderate vacuoles are seen in parenchymal cells and muscles. Whereas, few spins are still present in tegument after treatment of synthetic drug albendazole (Plate 6 Fig. 6A, 6B and 6C). Treated tegument shows moderate damages in their tegumental structure. Few damages, shrinkage, detachment, and deformation are present in longitudinal and circular muscles of tegument (Plate 6, Fig. 6D, 6E and 6F). Albendazole is less effective than alcoholic seeds extracts of *Centratherum anthelminticum* (Kalijiri), fruits of *Citrullus colocynthis* (Indrayan or Kharatumba) and seeds of *Trachyspermum ammi* (Ajwain).
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 sent 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 extracts of *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) and compare with synthetic drug 50 mg/ml concentration at 15 hrs incubated produced several histological alterations in the tegument of *F. gigantica*.

Tegumental swelling and furrows were seen as well as the spines appeared to be surrounded by the tegument. The underlying structures still appeared normal. These changes became more severe with spines dislodged from their sockets and other showed extensive cracking towards the base following 15hrs incubation with 50 mg/ml *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain). Besides appearances of numerous vacuoles in the tegument syncytium and the tegument itself 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 hrs 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 tegument and produce holes integument. Flooding of tissues was identified in the intercellular spaces between surface syncytium, muscle bundles and tegumental cells which appeared to be entirely separated from the surrounding tissue.
The tegument of digenean trematodes *Fasciola gigantica*, *Cotylphoron cotylophorum*, *Gigantocotyle explanatum* and *Gastrothylax crumenifer* has a number of significant roles, including osmoregulation, secretion, synthesis, protection hence represents a primary drug target (Jeyathilakan et al., 2012; Veerakumari et al., 2012; Khan et al., 2015 and Rajesh et al., 2016). Present research work has an agreement with the previous observation of different scientist that *in vitro* anthelmintic activity of the extracts of medicinal plants; *Meryta denhami*, *Artocarpus lakoocha*, *Cymbopogan nardus*, *areca catechu*, *Erythrina indica*, *Zingiber officinale*, *Azadirachta indica* and triclabendazole against adult *F. gigantica*. (Massoud et al., 2001, 2012 and 2013; Kumar et al., 2014; Mahardika et al., 2017 and Ullah et al., 2017).

The tegument of *Fasciola* spp. had many of the characteristics of a transporting epithelium that was involved 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 was a particular characteristic of drug action with flukicides. The tegumental changes induced by synthetic drug albendazole were less severe than those observed after 15hrs exposure with alcoholic extracts of *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain). 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 present study raised from dilation of the basal infolds as a result of 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* (Tansatit et al., 2012). Phytochemicals present in these three plants had been investigated previously and resulted in a series of metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates and long chain aliphatic alcohol derivatives isolated and identified from Commiphora species, these
secondary metabolites of the Commiphora species exhibited diverse biological activities, such as cytotoxic effects). The present study confirmed the fasciocidal properties of Kalijiri, Indrayan and Ajwain alcoholic extract which caused interruption to the tegument of *F. gigantica* after *in vitro* incubation. Extensive tegumental alterations were observed alcoholic extract of medicinal plants *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) in comparison with synthetic drug albendazole.
Diagram 1. Representation of *Fasciola gigantica*
Explanation of Photographic Plate 1

Photograph of whole mount liver fluke *Fasciola gigantica*

Fig. 1 A. *Fasciola gigantica* showing oral sucker (OS), genital pore (GP), ventral sucker (VS), tegument (T), intestinal caeca (IC), testis (TE) and excretory pore (EXP).

Fig. 1 B. Anterior region of *F. gigantica* showing oral sucker (OS), genital pore (GP), ventral sucker (VS), tegument (T), intestinal caeca (IC) and uterus (U).

Fig. 1 C. Posterior region of *F. gigantica* showing excretory pore (EXP) and intestinal caeca (IC).
Explanation of Photographic Plate 2

Photograph of tegument of control *Fasciola gigantica* by light microscope

Fig. 2 A. Anterior region of *F. gigantica* showing spines (S) and muscles (M), tegument (T), vitelline follicles (VF) intestinal caeca (IC) and parenchyma (P) X 110.

Fig. 2 B. Spines (S), muscles (M) and parenchyma (P) are present integument of anterior region of *F. gigantica* X 110.

Fig. 2 C. High magnification of tegument showing tegument (T), muscles (M) and developing spines (S) of *F. gigantica* X 185.

Fig. 2 D. Developed single spine (S) and muscles (M) are present in tegument (T) of middle region of fluke X 140.

Fig. 2 E. Muscles (M) and developing spines (s) exhibiting in tegument (T) and parenchyma (P) are also visible in posterior region of the worm X 110.

Fig. 2 F. High magnification of tegument (T) of posterior region of worm showing muscles (M) and developing spines (S) X 185.
Explanation of Photographic Plate 3

Photographs of in vitro treated with seeds extract of *Centratherum anthelminticum* tegument of *Fasciola gigantica* by light microscope (LM)

Fig. 3 A. Anterior region of treated *F. gigantica* showing highly damaged (DA) and brakeage (BR) and vacuolization (V) in tegumental X 110.

Fig.3 B. High magnification of anterior region of treated *F. gigantica* showing highly damaged (DA), vacuolization (V) and detachment (DE) in genital apparatus and tegument X 110.

Fig. 3 C. Middle region of treated *F. gigantica* showing detachment (DE) and vacuolization (V) in tegument X 110.

Fig. 3 D. High magnification of middle region of treated *F. gigantica* showing large amount of vacuoles (V), vacuolization (V) and damages (DA) in tegument and parenchyma cells X 110.

Fig. 3 E. Posterior region of treated *F. gigantica* showing detachment (DE), damaged (DA) and vacuolization (V) in parenchymal cells and tegument X 140.

Fig. 3 F. High magnification of posterior region of treated *F. gigantica* showing removed outer layer of tegument with detachment (DE) and vacuolization (V) X 145.
Explanation of Photographic Plate 4

Photographs of in vitro treated with fruits extract of Citrullus colocynthis tegument of Fasciola gigantica by light microscope (LM)

Fig. 4 A. Anterior region tegument of treated F. gigantica showing tissues are damages (DA) in tegument and highly vacuolization (V) in parenchymal cells X 110.

Fig. 4 B. High magnification of anterior region tegument of treated F. gigantica showing removed outer layer, brakeage (BK) in tegument and large vacuolization (V) due to damages (DA) in parenchymal cells X 110.

Fig. 4 C. Middle region of treated F. gigantica showing removed outer layer of tegument and vacuolization (V) present due to damages (DA) and detachment (DE) in tegument and parenchymetic cell X 110.

Fig. 4 D. Treated F. gigantica showing large vacuole (V), highly damaged (DA) and shrinkage (SH) in tegument and parenchymetic cell X 110.

Fig. 4 E. Posterior region of treated F. gigantica showing completely removed outer layer with spines, shrinkage (SH) in muscles of tegument, large vacuole (V) and damages (DA) in intestinal caeca X 110.

Fig. 4 F. High magnification of posterior region of tegument of treated F. gigantica showing removed outer layer of tegument. Shrinkage (SH), large vacuole (V) and damages (DA) present in tegumental cell X 145.
Photograph of *in vitro* treated with seeds extract of *Trachyspermum ammi* tegument of *Fasciola gigantica* by light microscope (LM)

Fig. 5 A. Anterior region tegument of treated *F. gigantica* showing removed outer layer of tegument, breakage (BK) in epithelial membrane of intestinal caeca and vacuolization (V) and damages (DA) in sub tegumental layer and parenchymal cells X 110.

Fig. 5 B. High magnification of tegument of treated *F. gigantica* exhibiting detachment (DE), vacuolization (V) and shrinkage (SH) in outer layer of tegument X 110.

Fig. 5 C. Middle region tegument of treated *F. gigantica* showing damaged (DA) and removed outer layer of tegument, large vacuole (V) formation due to damaged (DA) parenchymal cells X 110.

Fig. 5 D. High magnification of tegument of treated *F. gigantica* showing removed outer layer of tegument with spines, shrinkage (SH) and detachment (DE) and vacuolization (V) in parenchymal cells X 140.

Fig. 5 E. Posterior region of treated *F. gigantica* observing vacuolization (V) in parenchymal cells, shrinkage (SH) and damaged (DA) in tegument X 110.

Fig. 5 F. High magnification of tegumental of posterior region of treated *F. gigantica* showing highly damaged (DA) in tegument and vacuolization (V) in parenchymal cells X 110.
Explanation of Photographic Plate 6

Photograph of tegument of *Fasciola gigantica in vitro* treated with synthetic drug albendazole by light microscope (LM)

Fig. 6 A. Anterior region of treated *F. gigantica* showing little shrinkage (SH) in epithelial wall of intestinal caeca (IC) and few damaged (DA) in tegument (T) and small and few vacuolization (V) in parenchyma (P) cells X110.

Fig. 6 B. High magnification of tegument of treated *F. gigantica* showing few vacuolization (V), little shrinkage (SH) and few spines are attached with tegument. Also present vacuolization (V) in parenchymal (P) cells X 110.

Fig. 6 C. High magnification of middle region of treated *F. gigantica* showing few vacuoles (V) and small breakage (BK) due to detachments (DE) of spines (S) in tegument X 140.

Fig. 6 D. Middle region of treated *F. gigantica* showing little shrinkage (SH) in outer layer of tegument (T) and few small vacuoles (V) are present in parenchymal cell and on outer side of intestinal caeca (IC) X 110.

Fig. 6 E. Posterior region of treated *F. gigantica* showing spines (S) vacuoles (V) present due to damages in tegumental cells and muscles (M) X 110.

Fig. 6 F. High magnification of posterior region tegument of treated *F. gigantica* showing little shrinkage (SH), detachment (DE), and few vacuoles (V) present in muscles (M) X 145.