Chapter 7

Ultrastructural observations of surface topography of *in vitro* treated *Fasciola gigantica* with three selected medicinal plant extracts by scanning electron microscope
ULTRASTRUCTURAL OBSERVATIONS OF THE SURFACE TOPOGRAPHY OF IN VITRO TREATED FASCIOLA GIGANTICA WITH THREE SELECTED MEDICINAL PLANTS EXTRACTS BY SCANNING ELECTRON MICROSCOPE

Introduction

Fascioliasis has been recognized as an important helminthic disease of livestock causing significant loses to livestock owners, on account of poor growth and lower productivity of domestic ruminants. Liver flukes Fasciola gigantica and Fasciola hepatica are very dangerous because they cause various mechanical and biochemical damages to buffaloes. Fasciola gigantica is trematode parasites; it is one of the most abundant and damaging flatworms of buffaloes. F. gigantica causes fascioliasis disease, this disease shows some external and internal symptoms such as; traumatic hepatitis, hepatic fibrosis, hyperplastic cholangitis, jaundice, anemia and edema (bottle jaw) in domestic buffaloes. Fascioliasis is a significant livestock problem; weight loss, severe reductions in milk and meat yield as well as losses due to decreased fertility in production animals (Bennet, 1975a and Ramajo et al., 2001). Due to the high level of prevalence and intensity of natural infection, Fasciola appears to be endemic in this geographical region and probably represent one of the most important animal health problems.

F. gigantica has been reported in the worldwide. In India, the treatment of Fasciolosis is very costly and unaffordable to the owner of the livestock. Ultrastructure of tegument of Fasciola gigantica under light microscope, scanning electron microscope were studied by some scientists in various part of the world (Sobhona et al., 2000; Srimuzipo et al., 2000; Ashour et al., 2001; Meaney et al., 2002 and Swarnakar and Damor, 2016).

Control of trematodes infection in domestic ruminants is widely based on anthelmintic synthetic drugs. Its continuous uses have led to the additional problem of appearance of anthelmintic resistant in livestock. According to world health organization, medicinal plants would be the best source to obtain a variety of drugs. Time-dependent tegumental surface changes in juvenile F. gigantica in
response to triclabendazole treatment in goat. Therefore, such plants should be investigated to better understand its safety and efficacy (Nascimento et al., 2000; Kundu et al., 2014 and Shareefa et al., 2017).

The origin of many effective drugs is found in traditional medicinal practices and has made several researchers undertake studies for evaluating folklore medicinal plants on their declared anthelmintic efficacy. Hence, investigations into the efficacy of new drugs based on traditional knowledge and traditionally used medicinal plants as an alternative remedies diseases is important (Waller, 1997; Geerts and Gryseels, 2001; Mali and Mehta, 2008; Mehlhorn et al., 2011; Tandon et al., 2011 and Hossain et al., 2012).

In vitro, anthelmintic activity of the extracts of stem and leaves of Meryta denhami and Artocarpus lakoocha, essential oils of Cymbopogan nardus, areca catechu, Erythrina indica, Zingiber officinale and Azadirachta indica and triclabendazole were evaluated against adult F. gigantica were studied. The morphological variation and histopathological changes in treated and control fluke were studied by light and scanning electron microscopy (Shehab et al., 2009; Saowakon et al., 2009; Jenthilakhan et al., 2010a and b).

The tegumental alterations in adult Fasciola hepatica induced by the experimental fasciolide OZ78 were investigated utilizing scanning electron microscopy (Keiser and Morson, 2008). Ultrastructural tegumental changes of trematode parasites due to herbal medicinal plants and synthetic drugs have been investigated by some scientist by Scanning electron microscopy (Stitt and Fairweather 1993; Veerakumari and Paranthaman, 2004; Keiser et al., 2006a and b; Keiser and Morson, 2008a and b; Halferty et al., 2009; Shalby et al., 2009; Ebedi et al., 2011; Soliman and Taha, 2011; Veerakumari et al., 2012; Tansatit et al., 2012; Swarnakar et al., 2015; Shalaby et al., 2016 and Devi et al., 2018).

However, no research work has been carried out so far to study the effects of Centratherum anthelminticum, Citrullus colocynthis and Trachyspermum ammi medicinal plant on liver fluke Fasciola gigantica scanning electron microscope.
The present study aimed to determine the ultrastructural observations of tegument of \textit{in vitro} treated \textit{Fasciola gigantica} with an alcoholic extract of seeds of \textit{Cenratherum anthelminticum} (Kalijiri), the fruit of \textit{Citrullus colocynthis} (Indrayan or Kharatumba) and seeds of \textit{Trachyspermum ammi} and compared with albendazole by scanning electron microscope.

The present research work would be significant because it will provide knowledge for morphological and ultrastructural characteristics of \textit{F. gigantica} for fascioliasis treatment and chemotherapeutic as well as phytotherapeutic measures. Findings of the study will improve the socio-economic condition of the cattle farmers of Udaipur by removing pathogenic liver fluke \textit{Fasciola gigantica} parasites.
Result

The tegumental ultrastructural of liver fluke *Fasciola gigantica* trematode parasites were studied by scanning electron microscopy (SEM) first time in Udaipur. *Fasciola gigantica* is commonly occurring in the bile ducts and liver of buffaloes. Adult liver flukes *Fasciola gigantica* have a flat body and leaf-like in shape with knife-like and tapering anterior and blunt posterior ends. *Fasciola gigantica* are more elongated 6-7 cm than *Fasciola hepatica* 3.5 cm in length and width of *F. gigantica* 1-1.5 cm whereas in the *F. hepatica* width is larger 1.5 – 1.8 cm in the middle region.

Ultrastructural observations of the surface topography of control *Fasciola gigantica* by scanning electron microscope (SEM)

**Anterior region:** Spines are small sized, increasing in numbers and closely-packed present in the anterior region of the ventral and dorsal surface of the worm. The top surface of spines appears pointed and comb-like edges. Between the spines, the surface area of the shows alternates groove and transverse folds. Type 1 Papilles are observed around the oral and ventral suckers but they are bulbous, numerous, large in size with a smooth surface and present in the cluster form. Sensory papillae are bulbous in shape with nipples-like tips without cilia observed in the clustered form in the anterior region and ventral side but dorsal surfaces have few papillae (Plate 1 Figs. 1A, 1B, 1C, and 1D).

**Middle region:** Large sized spines with increasing in number are observed in the ventral and dorsal side in the middle region of the liver fluke. The large size spines with sharp comb-like edges are examined on the middle region of the body. Sensory papillae are in clustered form, larger in numeral and short size sensory papillae and spins are revealed on the dorsal side of the middle region of the body (Plate 1 Fig. 1E & 1F and Plate 2 Fig. 2A and 2B).

**Posterior region:** The spines are gradually decreased in size and number. Spins are observed in scattered form, small in size, covered with the tegumental surface, they are not comb-like and pointed. Dome-shaped sensory papillae but they are not in clustered form are present in the dorsal and ventral side of the
posterior region of the worm. Prominent excretory pore present on the blunt posterior tip and few spines are also observed the posterior end of the body of *F. gigantica* (Plate 2 Fig. 2C and 2D).

Ultrastructural observations of the surface topography of *in vitro* treated with an alcoholic extract of seeds of *Centratherum anthelminticum*, fruits of *Citrullus colocynthis* and seeds of *Trachyspermum ammi* *Fasciola gigantica* by scanning electron microscope (SEM)

*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 and distortion in the tegumental architecture of treated *Fasciola gigantica* parasites.

1. Surface topographic alterations in *Fasciola gigantica* treated with seeds of *Centratherum anthelminticum*

Anterior region of *F. gigantica* treated with *C. anthelminticum* showing shrinkage and damages in opening mouth part of oral sucker. Highly shrinkage and damages in the tegumental surface of genital apparatus, ventral sucker, and all spines are removed from (Plate 2, Fig. 2A and 2B). Dorsal middle region surface tegument and spines of treated *F. gigantica* showing large amount size of holes are present by removed spines and swelling, shrinkage and fold in the tegumental surface. The ventral middle region of the tegumental surface also exhibited swelling, shrinkage, furrows, and folds in the tegument and holes are present due to removed spines (Plate 2, Fig. 2C and 2D). Posterior region of treated *F. gigantica* exhibited extensive sloughing, shrinkage and folds present in the tegument and all spines are removed (Plate 2, Fig. 2E and 2F).
2. **Surface topographic alterations in *Fasciola gigantica* treated with alcoholic fruits extract of *Citrullus colocynthis***

Anterior region of *F. gigantica* treated with *C. Colocynthis* showing highly shrinkage and damages in the tegument of an oral sucker, genital apparatus, ventral sucker and removed all spines (Plate 3 Fig. 3A and 3B). Dorsal mid region surface tegument and spines of *F. gigantica* treated with *C. colocynthis* showing numerous and large sized holes due to removed and damaged spines. The ventral middle region of the tegumental surface also observing shrinkage furrows and big holes and spins are absent (Plate 3 Fig. 3C and 3D). Posterior region of *F. gigantica* treated with *C. colocynthis* showing extensive sloughing, shrinkage and folds present in the tegument and all spines are removed with damage tegumental surface (Plate 3 Fig. 3E and 3F).

3. **Surface topographic alterations in *Fasciola gigantica* treated with fruits extract of *Trachyspermum ammi***

Surface topography of the anterior region of treated *F. gigantica* showing highly injuries, contractions and folds on the oral sucker, genital apparatus, ventral sucker and all spines are removed (Plate 4 Fig. 4A and 4B). Middle region of surface tegument of treated *F. gigantica* exhibiting numerous and large sized holes due to removed spines, swelling and shrinkage & furrows and folds (Plate 4 Fig. 4C and 4D). Posterior region of treated *F. gigantica* also exhibited extensive sloughing, shrinkage, swelling and folds on the tegument surface and all spines are removed with the damaged posterior marginal region of tegument surface (Plate 4 Fig. 4E and 4F).

4. **Surface topographic alterations in *Fasciola gigantica* treated with synthetic drug *albendazole***

Anterior region of *F. gigantica* treated with synthetic drug albendazole showing few dames & shrinkage in opening the mouth of oral sucker and ventral sucker. Also present numerous pointed spins near to surface tegument of oral sucker and ventral sucker. Folds are present on the rim of oral sucker and ventral sucker (Plate 5 Fig. 5A and 5B). Middle region and posterior region of *F. gigantica* exhibited few damages and folds in surface tegument and some spines are pointed on middle region whereas submerged spines are present on the surface tegument posterior region (Plate 5 Fig. 5C, 5D, 5E, and 5F).
Discussion

Ultrastructural study of the surface topography of control *F. gigantica* showed same general patterns as that described in the *F. hepatica* by other scantiest in all over the world. Whereas, few different ultrastructural variation were observed in the present study that all three types of sensory papillae such as; type 1. Papillae are bulbous and smaller in size with a smooth surface, type 2. Papillae are bulbous in shape with nipple like tips without cilia and type 3. Papillae are bulbous shape have nipple-like tips with short cilia were present in the anterior region of the body of *F. gigantica* found in the buffaloes of Udaipur. The presence of three types sensory papillae was indicated specialization of the tegumental functions like improving excretion, absorptive capacity and increase ionic and osmoregulation in the tegumental surface. This type of structural characters also reported in some other digenean trematodes and helminths (Threadgold, 1963; Smyth and Halton, 1982; Aminhasmit *et al.*, 1993; Sobhan and Upatham, 1990; Sobhon *et al.*, 1994; Dalton *et al.*, 2004; Balasubramanian and Ramasamy, 2010; Naem and Budke, 2012, Alsaqabi, 2014 and Swarnakar and Damor, 2016).

Spines are small and more in numbers in the same region were indicted that this spine helpful in movement and attachment to the liver of buffaloes. Whereas, spines are absent on the oral and ventral suckers but papillae are observed around the oral and ventral suckers but they are bulbous, numerous, large in size and present in the cluster form. These papillae provide smooth sealing with mucous liver and bile duct of buffaloes. Presence of spines on the surface of *F. gigantica* and other trematodes were reasonably suggested to play significant roles in the advantage of the worm; they serve as attachment function, helping the parasite to anchor to the host surface to maintain good contact in his surrounding environment which in turn protects it from being washed out with the host secretions (Srisawangwonk *et al.*, 1989). Moreover, the spines could function as a rough apparatus to host epithelium producing debris for feeding (Hong *et al.*, 1991; Bennett, 1975a, b; Ashoue *et al.*, 1999; Fairweather *et al.*, 1999; Sobhon *et al.*, 2000; Srimuzipo *et al.*, 2000; Dangprasert *et al.*, 2001 & Lotfy and Hillyer, 2003).
The present study has shown series of tegumental drastic changes of the adult *Fasciola gigantica* when incubated in the alcoholic extract of *Centratherum anthelminticum*, *Citrullus colocynthis*, and *Trachyspermum ammi*. The SEM result also showed blabbed, corrugated and bulbous, indicating damage in the tegument. But the dose rate of 50 mg/ml alcoholic extract of *Centratherum anthelminticum*, *Citrullus colocynthis* and *Trachyspermum ammi* produced severe changes in the surface tegument. These changes occurred in definite sequences, where the swelling was the first sign observed followed by blebbing, then dislodgement of spines with disruption of their covering tegument. Although changes of alcoholic extract of three medicinal plants concentration and time-dependent were more than synthetic. The tegumental changes were considered to be an indicator of stress reactions by the fluke in an attempt to replace damaged surface (Stitt and Fairweather, 1993). Moreover, damage of the tegument is not only exposed deeper lying tissues and even internal organs to be attacked by the extract causing more damage to the fluke, but also undoubtedly disrupted many of the physiological processes associated with the tegument including osmoregulation, synthesis of secretary materials and nutrient uptake secretion (Keiser and Morson 2008b and Haltom, 2004). Present the result was in accordance with many other similar works on *Fasciola gigantica* (Jeyathilakan et al., 2010, Veerakumari et al., 2012; Swarnakar et al., 2015; Shalaby et al., 2016 and Devi et al., 2018).

Treated with an alcoholic extract of *Centratherum anthelminticum*, *Citrullus colocynthis* and *Trachyspermum ammi* the tegument observed by SEM. the tegumental surface changes were observed as holes are present due to removed spines, swelling, fibrous network formation between major and minor folds, blebbing, which later ruptured, leading to erosion and desquamation of the tegument, resulting in the rough and distorted surface tegument. The tegument is an important structure of parasite because it provides covering and protection of the body of the parasite and supports internal organs. It also controls the secretion, synthesis, sensitivity of sensory stimuli and osmoregulation. It was demonstrated here that tegument a major target of alcoholic extract of *Centratherum anthelminticum*, *Citrullus colocynthis* and *Trachyspermum ammi*, which was
probably absorbed by the tegument. Present research work is confirmation with the study of Saowkon et al., (2009) and demonstrated that depending on dose, an alcoholic extract of *Centratherum anthelminticum*, *Citrullus colocynthis* and *Trachyspermum ammi* can reduce motility and cause the death of adult *Fasciola gigantica* on varying time mode. The effects of three selected medicinal plants alcoholic extracts were more severe than synthetic drug albendazole. It is apparent that synthetic drug takes longer time and higher dose to inactive or paralyzes and kills the trematodes parasites. Once the surface layer is totally destroyed, the drug could penetrate deeper into the muscular layer and caused motility reduction and stop that lead finally to death (Skuce et al., 1987 and Hossain et al., 2012). Regional difference of responses to the medicinal plants *Centratherum anthelminticum*, *Citrullus colocynthis* and *Trachyspermum ammi* was also observed with the ventral side being more severely affected than the dorsal surface and the anterior and middle third regions, as well as the lateral margins of the flukes, were generally more affected than the posterior region. The tegument of trematode comprises an outer surface syncytium underlined by a thick sub syncytial zone and musculature (Sharma and Hanna, 1988). Medicinal plants might be applied its effect on the tegument first then permeated through the underlying muscle, which exhibited drastically decreased motility. The gross distraction of tegument clearly noticeable to the naked eye was observed in all specimens at the higher doses. The *F. gigantica* surface appeared dark with tegumental desquamation.

Tegumental changes observed during the present work resemble those reported by Meaney et al., (2002), who studied the effect of the active sulphoxide metabolite of triclabendazole Fasciolicide of choice on the surface morphology of *F. gigantica* in vitro. He observed that changes became progressively severe with higher concentrations and longer durations of exposure. Moreover, several studies have documented extensive tegumental damage in triclabendazole-susceptible strains of *Fasciola gigantica*, whereas triclabendazole resistant ones showed only localized and relatively minor disruption of the tegument. Swelling of tegument with slight loss of spines reported with lower concentrations, while severe tegumental ulcerations and sloughing, many furrows, extreme swelling and
distortion, swollen suckers and severe loss of spines leaving empty holes occurred with increased extract concentration and incubation period (Robinson et al., 2002 and McConile et al., 2006; Keiser et al., 2006 and Shalaby et al., 2009). It was speculated that artemisinins might act against tested worms via multiple mechanisms, as the formation of free radicals which possibly react with different targets as proteins and proteinase (Meshnick, 2002).

Other medicinal plants like Flemingia vestita, C. molmol, and Meryta denhamii were examined using electron microscopy for their in vitro fasciolicide activity against adult Fasciola gigantica. Their investigations were reported that shrinkage of tubercles, severe tegumental edema, extensive disruption in inter papillary areas, damage in sensory papillae, loss of spines and several changes in tubercles of surface tegument of Fasciola gigantica. (Badria et al., 2001; Hassan et al., 2003; Massoud et al., 2004; Fathy et al., 2005; Mohamed et al., 2005; Toner et al., 2008).

Large and numerous sensory papillae were present on oral and ventral suckers which are responsible for the act as rheo-receptors, sensing the currents of fluids in their environment, also they are tango receptors, sensing contact with the surface epithelium. Moreover, chemoreceptor function of sensory papillae was also considered as produce pressure on tegument. Whereas other types of sensory papillae were distributed all over the body surface they act as tango receptors. It is found that minor and severe destruction of spines and sensory papillae were deprived the parasite of their vital function, facilitating its clearance (Woo et al., 1998; Aminhasmit et al., 1993; Lotfy and Hillyer, 2003). Type 1 papillae bulbous, Comb-like and pointed spines of the tegument and ventral sucker may help to make strong connection and attachment with the bile ducts during the flow of bile juice and helpful in the movement in the liver of host. The presence of spines and sensory papillae type 2 bulbous in shape with nipple like tips without cilia and type 3 papillary are bulbous shape have nipple-like tips with short cilia were present on genital apparatus averted cirrus of F. gigantica. Dome-shaped papillae are commonly present on trematodes tegument surface and they are believed to have a sensory function (Tandon and Maitra, 1982) associated to feeding at the oral aperture reception around the genital pore (Benneft, 1975). The papillae were
also disturbed and damaged by treated medicinal plants, which caused the loss of sensory functions. Besides, the damages of the ventral sucker might affect the holding onto the host tissues (Shalaby et al., 2010). These papillae and spines may help in successful transport of sperms and cross-fertilization. Tegumental folds of trematode parasites helpful for an increase of surface area and improve the absorption of nutrition, important chemicals and exchange of micro-molecules from the liver host. A similar structure also recognized by other researchers in some trematodes (Sobhon et al., 1994; Dalton et al., 2004; Naem et al., 2014; Swarnakar and Sanger, 2014; Mary et al., 2015 and Pandaya et al., 2015).

The present study suggests that Centratherum anthelminticum, Citrullus colocynthis, and Trachyspermum ammi extract have a reliable source of antihelmintic property hence it is found that these medicinal plants can be used as a potent antihelmintic drug, cost-effective and to overcome anthelmintic resistance against synthetic drug and other flukicidal drugs. Present study might be an outcome initiation for further detailed study as in vivo animal models to discover toxicity. Thus knowledge and understanding gained in vitro trematode model could promote in vivo controlled studies in an animal model and an array of bioactive molecules could be discovered for further clinical applications in human and veterinary parasitology.
**Explanation of Photographic Plate 1**

Ultraphotomicrographs of control *Fasciola gigantica* showing surface topographic structure by scanning electron microscope (SEM)

Fig. 1 A. Ultramicrograph of surface topography of control *Fasciola gigantica* showing tegument (T) and spines (S) of anterior region of *F. gigantica* with oral sucker (OS), genital apparatus (GA) and ventral sucker (VS) x 200µm.

Fig. 1 B. High magnification of oral sucker (OS) showing mouth opening of esophagus (ES), rounded sensory papillaes are present on rim of oral sucker, spines (S) are present on tegument (T) of anterior region or near to the oral sucker of *F. gigantica* X100µm.

Fig. 1 C. High magnification of genital apparatus (GA), spines (S) and tegument (T) of *F. gigantica* X100µm.

Fig. 1 D. High magnification of ventral sucker (VS) and spines (S) of *F. gigantica* X100µm.

Fig. 1 E. Showing tegument (T) and comb like spines (CS) of anterior region of control *F. gigantica* X20µm.

Fig. 1 F. Showing tegument (T) and comb like spines (CS) of middle region of *F. gigantica* X20µm.
Explanation of Photographic Plate 2

Ultraphotomicrographs of control *Fasciola gigantica* showing surface topographic structure by scanning electron microscope (SEM)

Fig. 2 A. High magnified micrograph exhibiting single comb like spine (CS) with finger like projections of control *F. gigantica* X 2µm.

Fig. 2 B. High magnified micrograph showing single pointed spine (PS) and sensory papillae of control *F. gigantica* X 2µm.

Fig. 2 C. Tegument (T) and spines (S) of posterior region of *F. gigantica* X 200µm.

Fig. 2 D. High magnification of posterior region of control *F. gigantica* showing tegument (T) and spines (S) are rounded, smooth and lower density than anterior region X 20µm.
Explanations of Photographic Plate 3

Ultraphotomicrographs of *in vitro* treated *Fasciolagigantica* with seeds extract of *Centratherum anthelminticum* showing surface topographic alterations by scanning electron microscope (SEM)

Fig. 3 A. High magnification of oral sucker of *F. gigantica* treated with *C. anthelminticum* showing shrinkage (SH) and damages (DA) in opening mouth part X100µm.

Fig. 3 B. High shrinkage (SH) and damages (DA) present in genital apparatus (GA) and ventral Sucker (VS) X 200µm.

Fig. 3 C. Surface tegument of dorsal middle region showing swelling (SW), shrinkage (SH) and folds (FO) and numerous and large sized holes due to removed spines  X 20µm.

Fig. 3 D. Surface tegument of ventral middle region of *F. gigantica* depicting swelling (SW), shrinkage (SH) and folds (FO) and holes (HO) due to removed spines X 20µm.

Fig. 3 E. Posterior region of treated *F. gigantica* showing extensive sloughing (SL), shrinkage (SH) and folds (FO) present on the surface tegument with all removed spines X 200µm.

Fig. 3 F. High magnified photomicrograph of posterior region of treated *F. gigantica* showing extensive swelling (SW), shrinkage (SH) and damage (DA) and all spines are removed on the tegumental surface X 20µm.
Explanations of Photographic Plate 4

Ultraphotomicrographs of *in vitro* treated *Fasciola gigantica* with fruits extract of *Citrullus colocynthis* showing surface topographic alterations by scanning electron microscope (SEM).

Fig. 4 A. High shrinkage (SH), folds (FO) and damages (DA) exhibiting in tegument of oralsucker (OS). Spins are absent on genital apparatus and ventral sucker of treated *F. gigantica* X500µm.

Fig. 4 B. High magnification of oral sucker (OS) of treated *F. gigantica* showing removed spines, shrinkage (SH) and damages (DA) in tegment X 50µm.

Fig. 4 C. Surface tegument of dorsal middle region of treated *F. gigantica* showing shrinkage (SH), folds (FO) and numerous & large sized holes (HO) due to removed spines X 100µm.

Fig. 4 D. Ventral middle region of tegument of treated *F. gigantica* showing shrinkage (SH), folds (FO), swelling (SW) and holes (HO) are present by removed spines X 50µm.

Fig. 4 E. Posterior region of *F. gigantica* treated with *C. colocynthis* showing extensive sloughing (SL), shrinkage (SH) and folds (FO) present in the tegument and all spines are removed X 500µm.

Fig. 4 F. High magnified posterior region of treated *F. gigantica* showing extensive tegumental shrinkage (SH) and folds (FO), all spines are removed with damaged tegumental surface X 200µm.
Explanation of Photographic Plate 5

Ultraphotomicrographs of *in vitro* treated *Fasciola gigantica* with seeds extract of *Trachyspermum ammi* showing surface topographic alterations by scanning electron microscope (SEM)

Fig. 5 A. High damages (DA) and shrinkage (SH) in tegument of oral sucker (OS), genital apparatus (GA) and ventral sucker (VS) in anterior region of treated *F. gigantica* X 200µm.

Fig. 5 B. High magnification of ventral sucker (VS) of treated *F. gigantica* showing damages (DA) in tegument X 100 µm.

Fig. 5 C. Middle region tegumental surface of treated *F. gigantica* showing large amount size of holes (HO) are present due to removed spines and damages in tegument X 100µm.

Fig. 5 D. High magnification of middle region tegument of treated *F. gigantica* showing single holes (HO) are present due to removed spines in the tegument X20µm.

Fig. 5 E. Posterior region of *F. gigantica* treated with *T. ammi* showing extensive damages (DA) and shrinkage (SH) in tegument and all spines are removed X100µm.

Fig. 5 F. High magnified posterior region of *F. gigantica* treated with *T. ammi* showing extensive damaged (DA) posterior tail region and all spines are removed from tegumental surface X 20µm.
Explanations of Photographic Plate 6

Ultraphotomicrograph of *in vitro* treated *Fasciola gigantica* with synthetic drug albendazole showing surface topographic alterations by scanning electron microscope (SEM)

Fig. 6 A. Few damages (DA) are present on opening of oral sucker (OS) of treated *F. gigantica* X20µm.

Fig. 6 B. Ventral sucker of treated *F. gigantica* showing little shrinkage (SH) in tegument (T) and spines (S) are present near to the ventral sucker X 100µm.

Fig. 6 C. Middle region of treated *F. gigantica* showing few damages (DA) in tegument (T) and spines (S) X 100µm.

Fig. 6 D. Posterior region of treated *F. gigantica* showing few damages (DA) and shrinkage (SH) in tegument (T) X 100µm.

Fig. 6 E. Posterior region tegument of treated *F. gigantica* showing few damages in tegument (T) and spines (S) are submerged X 100µm.

Fig. 6 F. High magnification of posterior region of treated *F. gigantica* showing damaged (DA) and submerged spines (S) in tegument (T) X 20µm.