Chapter 6

Histology of oral sucker, genital pore, ventral sucker and reproductive organs of *in vitro* treated *Fasciola gigantica* with three selected medicinal plant extracts and synthetic drug by light microscope
HISTOLOGY OF ORAL SUCKER, GENITAL PORE, VENTRAL SUCKER AND REPRODUCTIVE ORGANS OF IN VITRO TREATED *FASCIOLA GIGANTICA* WITH THREE SELECTED MEDICINAL PLANT EXTRACTS AND SYNTHETIC DRUG BY LIGHT MICROSCOPE

**Introduction**

The reproductive organs, genital pore, oral sucker and ventral sucker of the Liver fluke *Fasciola gigantica* have highly complex structure. Reproductive organs testis and ovary are responsible for the development of gametes sperm and ova. Genital pore is very important for fertilization process. Oral and ventral sucker is the specially modified structure of tegument or body wall of *Fasciola gigantica*. Oral sucker of this worm is responsible for taking the nutrition from liver and the ventral sucker is used in attachment with liver of the host of animals and human.

Male reproductive apparatus has a highly complex structure. The reproductive system is very important since they are engaged in fertilization, development, and storage of the sperm. Among digenetic trematodes liver fluke *Fasciola gigantica* is of paramount importance due to the pathogenicity caused by them in domestic animals. The histology of testes by light and electron microscope has been studied in a number of trematodes (Burton, 1972) Threadgold, 1975; Halton and Hardcastle, 1977; Rees, 1979; Lots of investigations have been made on histology of the female reproductive system in trematodes *Aporocotyle simplex*, fasciola species, *Pharyngostomoides procyonis*, *Paragonimus Ohirai Paramphistomum Cervi Schistosoma margrebowiel* Lung fluk at light microscopy as well as electron microscopy (Thorsell and Bjorkman, 1965; Threadgold and Irwin, 1970; Madhavi, 1971; Spence and Silk, 1971 a and b; Erasmus, 1973; Grant et al., 1977; 1980; Thulin, 1982; Gupta *et al.*, 1983, 1985; Popiel *et al.*, 1984 and Orido, 1990).
The histological changes by synthetic drug were observed on trematode and suggested the apoptosis in cells undergoing mitosis or meiosis (spermatogonia and spermatocytes in the testis; oogonia and primary oocytes in the ovary and stem cells in the vitelline follicles (Kumar et al., 2005; Hanna et al., 2008 and 2010; McConville 2010; Hanna et al., 2011 and Hanna et al., 2012 a & b and 2013).

Many of the anthelmintic drugs are available to control Liver fluke *Fasciola gigantica* parasites infection have developed resistance. Therefore medicinal plant-based remedies as an alternative to the synthetic anthelmintics. Some medicinal plant extracts shown the anthelmintic effect on nematode and cestode parasites (Shalaby et al., 2012; Shaheen and Eman 2012; Saowakon et al., 2013; Shalaby and El-Moghazy 2013 and Balqis et al., 2017).

Few scientists used herbal drug to treat trematode parasites; *Fasciola gigantica, Cotylophoron caryophyllophorum, Gigantocotyle explanatum* and *Gastrothylax crumenifer* (Jeyathilakan et al., 2012; Veerakumari et al., 2012; Saowakon et al., 2013; Khan et al., 2015 and Rajesh et al., 2016).

The availability of highly effective and safe drug for the cure of parasites was limited and none of the drugs is available that show high efficacy on *Fasciola gigantica. Centratherum anthelminticum, Citrullus colocynthis, and Trachyspermum ammi* medicinal plants were used in the traditional medicoveterinary field, they are used to treat worms and found antiparasitic activity.

Ethanolic extract of *Centratherum anthelminticum* seeds have good antibacterial, antioxidant, antidiabetic, antimicrobial, anti-urolithiasis, anti-inflammatory, analgesic and anticancer property (Patel et al., 2012; Paydar et al., 2013 and Javad et al., 2013; Ashok et al., 2013; Bhatia and Paliwal, 2014; Galani and Panchal, 2014; Negi et al., 2014; Bhatia and Paliwal, 2015; Mudassir et al., 2015; Gopikrishna et al., 2016 and Mehta et al., 2016).

The alcoholic extracts of *Citrullus colocynthis* possess good antimicrobial, antidiabetic, antirheumatic and anti-hemorrhoids activity against selected test bacteria, fungi, and helminth parasites. *C. colocynthis* plant 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. (Menghani et al., 2012; Prasanthreddy et al., 2012; Soufane et al., 2013; Bnyan et al., 2013; Khalid et al., 2013; Baloch et al., 2013 and Talole et al., 2013. C. colocynthis is a very important medicinal plant which is used by various tribes for treatment of many diseases in Rajasthan, India. Seventeen recipes are based on root, twelve on fruits, five in seeds, two on the whole plant, three on leaf and in two recipes both roots and fruits are used (Meena et al., 2014 a and b; and Dash et al., 2015). Swarnakar et al., (2015) reported the in vitro studies anthelmintic activity of an aqueous and alcoholic extract of C. colocynthis on amphistome Orthocoelium scoliocoelium. Aqueous and alcoholic extract of C. colocynthis found to be potential sources for novel anthelmintic and justify their ethnoveterinary use. Extensively recorded and discussed and investigation of morphological and biological detailed information of C. colocynthis aspects of important plants from India (Rani et al., 2017).

Seeds of Trachyspermum ammi possess antiseptic, stimulant, carminative, diuretic, anesthetic, antimicrobial, antiviral, nematicidal, antiulcer, antihypertensive, antitussive, bronchodilatory, antiplatelet, hepatoprotective and antihyperlipidemic effects Abbasi et al., 2013 and Rajput et al., 2013; Jan et al., 2015 and Reji et al., 2015; Hassan et al., 2016; Omidpanah et al., 2016 and Chahal et al., 2017).

However, none of the scientists was paid attention to anthelmintic effects of Citrullus colocynthis, Centratherum anthropmaticum and Trachyspermum ammi medicinal plants on Fasciola gigantica. Therefore, it was decided to carry out the histology of oral sucker, genital pore, ventral sucker and reproductive organ of in vitro treated Fasciola gigantica with fruits of Citrullus colocynthis, seeds of Centratherum anthelminticum and seeds of Trachyspermum ammi medicinal plant extracts and compare with the synthetic drug by light microscopic.
Result

The control and treated reproductive organ 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.

**Histological study of oral sucker, genital pore, ventral sucker and reproductive organs of control *Fasciola gigantica***

Anterior region of *F. gigantica* showed surrounded by tegument with numerous, different sized and shaped spins, circular and longitudinal tegumental and parenchymal cells. *Fasciola gigantica* anterior region has an oral sucker, genital pore and ventral sucker are present in the anterior region.

Oral sucker is triangular form, present at the anterior terminal region of the liver fluke *Fasciola gigantica*. Oral sucker surrounded by thick tegument with surface syncytium, the mass of tightly packed circular, longitudinal, redial and oblique muscles and tegumental cells. Spins are not present on the surface of oral (Plate 1 Fig. 1A). The genital pore is the last joint opening of male and female reproductive ducts present on the anterior region and on the ventral surface of the worm. The terminal end of male ejaculatory duct fused with the terminal end of metraterm of the uterus that formed ducts hermophroditicus. The terminal opening of ducts hermophroditicus is surrounded by a modified tegument or body wall is known as a genital pore. The opening of genital pore encloses a space called genital atrium. Histological it is found that genital pore is surrounded by surface syncytium, basal layer, circular, longitudinal and oblique muscles. The inner wall of genital pore *F. gigantica* parasites is composed of syncytial epithelium which bears a striated luminal border towards the lumen (Plate 1 Fig. 1B).

Ventral sucker present in rounded or cup form also known as acetabulum. Ventral sucker of controlled *Fasciola gigantica* parasite consists of thick tegumental architecture and comprises tightly packed numerous layers of longitudinal, redial, oblique and circular muscles. Spins are not present on the surface of ventral sucker (Plate 1 Fig. 1C.).
Reproductive organs namely ovary and testis are well developed and complex in *F. gigantica* parasites. The ovary is single, tubular, highly branched and present at the anterior of the testes at the anterior region of the body. Two testes are present behind of the ovary. Testes are ramified tubular and placed tandem form or one behind the other. Testes are spreads middle to the posterior region of the body. Vitelline gland present in branched from with vitelline follicles from anterior to the posterior region. Mehlis gland and prostate gland are also present at the anterior side of *F. gigantica* showing elongated tubules with S1 secretory cells and S2 secretory cells (Plate 1 Fig. 1D, 1E and 1F.).

Middle region of *F. gigantica* showed branched and tubular form testes vitelline follicles, intestinal caeca and parenchymal cells. vitelline follicles of *F. gigantica* observing undifferentiated vitelline cells, intermediate vitelline cells, mature vitelline cells, and parenchyma.

The posterior region of the *F. gigantica* is surrounded by tegument with circular and longitudinal muscles and many spins. Also part of the worm exhibited end part of intestinal caeca, vitelline follicles, few branches of tubular testes and excretory pore.

**Histology of oral sucker, genital pore, ventral sucker and reproductive organs 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)**

In vitro anthelmintic activities were carried out on liver fluke *Fasciola gigantica* treated with seeds of *Centratherum anthelminticum*, fruits of *Citrullus colocynthis* and seeds of *Trachyspermum ammi* alcoholic extracts. Treated parasites became clumped, paralyzed and died after 15 hours exposure time at a concentration of 50mg/ml alcoholic extracts of 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. **Oral sucker, genital pore, ventral sucker and reproductive organs alterations in *Fasciola gigantica* treated with seeds extract of *Centratherum anthelminticum***

Treated *F. gigantica* exhibited detachment, shrinkage, and vacuolization in genital apparatus and parenchymal cells. Ventral sucker of treated *F. gigantica* showing large vacuolization are present due to highly damaged parenchymal cells (Plate 2, Fig. 2A and 2B). Ovary of treated *F. gigantica* observing damaged outer membrane of the ovary and vacuolization in parenchymal cells. Mehlis gland of treated *F. gigantica* shown shrinks in ootype and vacuolization due to removed S1 and S2 secretory cells and secretory cells (Plate, 2 Fig. 2C and 2D). Testis of treated *F. gigantica* showing highly vacuolization and damages present in testis due to removed spermatocyte. Intestinal caeca of treated *F. gigantica* showing highly vacuolization and damages are present due to removed epithelial cells (Plate, 2 Fig. E and F).

2. **Oral sucker, genital pore, ventral sucker and reproductive organs alterations in *Fasciola gigantica* treated with fruits extract of *Citrullus colocynthis***

Genital apparatus and ventral sucker of treated *F. gigantica* observed highly damages and vacuolization in parenchymal cells. Genital apparatus of treated *F. gigantica* showing vacuolization and damaged in the outer membrane and circular, longitudinal, oblique and radial muscles (Plate 3, Fig. 3A and 3B). Testis of treated *F. gigantica* exhibiting large vacuoles present in testis due to removed germinal epithelial membrane, spermatogonia and different spermatocyte cells and vacuolization are also present in parenchymal cells. The uterus of treated *F. gigantica* showing shrinks in the uterus and parenchymal cells (Plate 3 Fig. 3C and 3D). Ovary of treated *F. gigantica* observing large vacuole present due to removed eggs and extremely damaged parenchymal cells and ovary membrane. Treated *F. gigantica* also observed vacuolization and damages in the ovary (Plate 3, Fig. 3E and3 F).
3. Oral sucker, genital apparatus, ventral sucker and reproductive organs alterations in *Fasciola gigantica* treated with fruits extract of *Trachyspermum ammi*

Anterior region of *F. gigantica* showing highly damaged oral sucker muscles and tegument, detachment and vacuolization in genital apparatus and parenchymal cells, shrinks in the ventral sucker. Genital apparatus of treated *F. gigantica* observed detachment of genital apparatus membrane, damages and vacuolization in parenchymal cells (Plate 4, Fig. 4A and 4B). Ventral sucker of treated *F. gigantica* showed large vacuolization. The uterus of treated *F. gigantica* showed damage and large vacuole present in uterus and vacuolization in parenchymal cells (Plate 4, Fig. 4C and 4D). Ovary of treated *F. gigantica* exhibited damages and vacuolization. Testis of treated *F. gigantica* showed shrinks and vacuolization in testis due to damages in germinal epithelium wall and different stages of spermatogonia and spermatocytes (Plate 4, Fig. 4E and 4F).

4. Oral sucker, genital apparatus, ventral sucker and reproductive organs alterations in *Fasciola gigantica* treated with synthetic drug *albendazole*

Oral sucker of treated *F. gigantica* showed few damages integument and vacuolization in parenchymal cells. Ventral sucker of treated *F. gigantica* observed several damages and vacuolization presence in parenchyma cells, detachment in ventral sucker muscles (Plate 5, Fig. 5A and 5B). Testis of treated *F. gigantica* observing little damaged in testis, several vacuolizations in intestinal caeca and parenchymal cells. Ovary of treated *F. gigantica* exhibited little shrinkage and damages, vacuolization in parenchymal cells (Plate 5, Fig. 5C and 5D). Treated *F. gigantica* observing vacuolization and few damaged in intestinal caeca. Vitelline follicles of treated *F. gigantica* observed several damages and vacuolization (Plate 5, Fig. 5E and 5F).
Discussion

The light microscopic study clearly revealed that there are several cellular alterations produced in oral sucker, genital pore, ventral sucker and reproductive organ of *in vitro* treated *Fasciola gigantica* with fruits of *Citrullus colocynthis*, seeds of *Centratherum anthelminticum* and seeds of *Trachyspermum ammi* medicinal plant extracts. The effect of these medicinal plant extracts against rumen *Fasciola gigantica* tested and it was found that complete paralysis and death of the worm occurred at a higher concentration with least incubation time. Gross microscopically changes under the light microscope showed drug-treated worms became small with the shrunken tegumental structure of reproductive organs, genital pore, oral and ventral sucker. Therefore, the present research work is a great import from the medico-veterinary standpoint.

Several investigations have been identified on *in vitro* anti-trematodal activity of medicinal plants *viz*; alcoholic extract of *Allium sativum* and *Piper logum* (Singh et al., 2015), *Balanites aegyptica* alcoholic extract (Swarnakar et al., 2015), bark of *Prosopis cineraria* (Manigandan and Veerakumari 2015), Plumbagin on newly excysted and 4 week old juvenile parasite of *Fasciola gigantica* (Natcha et al., 2014) and Plumbagin on motility, survival and histology of tegument structure of *Paramphistomum cervi* (Saowkon et al., 2013) have been carried out. Similarly, *an in vivo* study in natural fasciolosis infected cattle (Shokier et al., 2013) has been reported.

Histopathological examination in the present study also suggested affection of the tegument and parenchymatous cells, vascular degenerative changes and apoptosis cells of testes, ovary, Mehlis, vitelline & prostate gland, genital pore, oral and ventral sucker which may be depicting an action similar to that of oxyclozanide. However, the control flukes showed the normal microscopic structure of various organs and tegument the result was in accordance with many other similar works on amphistomes (Jeyathilakan et al., 2010, 2012; Veerakumari et al., 2012; Swarnakar et al., 2014).
Histological changes were observed in the genital pore, oral and ventral sucker and reproductive organs of *F. gigantica* treated with three medicinal plants which exhibited vacuolization, damage, distorted and swollen presence with scattered blebs along their rims. Surface changes observed in the present study resemble that demonstrated in *F. gigantica* treated with aqueous extract of *Artocarpus lakopcha* (Saowkon et al., 2009), and on artemether (Shalaby et al., 2010).

Dome-shaped papillae are commonly present on trematodes tegument surface and they are believed to have a sensory function (Tandon and Maitra, 1982) related to feeding at the oral aperture reception around the genital pore (Benneft, 1975a and b). Similarly, Veerakumari and Paranthaman (2004) reported prominent changes in the oral sucker, ventral sucker and on the ventral surface of *Fasciola gigantica* treated with synthetic drug albendazole. The thickening of the folds is due to the swelling of the basal in the fold of the tegumental syncytium (Shalaby et al., 2009). Ventral sucker of *Fasciola gigantica* helps the parasite to adhere to the liver rumen and prevent the parasite shift from the host (Balasubramanian and Ramasamy, 2010). The papillae were also wounded by antihelminthic medicinal plants, which produced the loss of sensory functions. Besides, the damages of the acetabulum might affect the holding onto the host tissues. Distortions in the ventral sucker lead to dislodgement of the flukes from the liver wall. The present study revealed the anthelmintic efficacy of *Citrullus colocynthis*, seeds of *Centratherum anthelminticum* and seeds of *Trachyspermum ammi* medicinal plants on *Fasciola gigantica*. Structural analysis of the control and treated flukes confirmed various degrees of degeneration, vacuolization and swollen detachment of cell and tissues. It may be concluded that *Citrullus colocynthis*, seeds of *Centratherum anthelminticum* and seeds of *Trachyspermum ammi* medicinal plants during their absorption, affect the structural conformation of the flukes. Thus three medicinal plants based medicines such as *Centratherum anthelminticum*, *Citrullus colocynthis*, and *Trachyspermum ammi* could be used as an effective anthelmintic to treat fasciolosis.
Histological changes consistent with apoptosis were described in the reproductive organs; testis and ovary. Specifically many of reproductive cells that normally undergo mitosis or meiosis in the spermatogonia and spermatocytes in the testis, the oogonia and oocytes in the ovary and the stem cells in the vitelline follicles and they become rounded or partially isolated from the surrounding, developing pyknotic or karyorrhectic nuclei and eosinophilic cytoplasm. At the same time, the cell populations in these organs decline due to a failure to replace the maturing cell types (spermatids and spermatozoa, primary oocytes and mature vitelline cells in the testis, ovary and vitelline follicles, respectively. It was found that normal primary spermatocytes in control *F. gigantica*, particularly those close to the periphery of the tubules. Irregular primary spermatocytes were noted in the peripheral zone of the testis tubules. It has been shown that sustentacular tissue is located in the peripheral zone of the testis tubules in *F. hepatica* (Hanna et al., 2012). This is carried out by a process of heterophagic digestion using lysosomal enzymes generated in the cytoplasm of the sustentacular tissue.

*In vitro* treated *Fasciola gigantica* with fruits of *Citrullus colocynthis*, seeds of *Centratherum anthelminticum* and seeds of *Trachyspermum ammi* medicinal plant extracts showed extensive damage, vacuolization, swollen and apoptosis in cells and tissues in the oogonia and primary oocytes of ovarian tubule and uterus. The layer of nurse cells are also distorted, nurse cells believed to have supportive and nutritive functions for the germinal layer of ovary and testis cells is interposed between the ovarian walls (Bjokman and Thorsell 1964). Primary oocytes move out of the ovary at the end of prophase of the first meiotic division, completing their development in the proximal coils of the Uterus (Gresson, 1964). In the triploid Cullompton isolate flukes, development of the ova appears to proceed parthenogenetically without a reductive division, so in untreated Cullompton flukes apoptosis in the oocytes is not triggered by abortive attempts at meiosis unlike the situation with primary spermatocytes (Hanna et al., 2008).

In the vitelline cells of *in vitro* antihelminthic medicinal plants, treated flukes resulted in the arrest of mitosis in stem cells at the periphery of each follicle. Thereafter, there was progressive depletion of the cell population as preexisting vitelline cells moved through, firstly synthesis of shell protein.
globules and finally glycogen accumulation before moving away from the follicle. The present study has an agreement with Irwin and Threadgold (1970) and (Threadgold, 1982) that many of the peripherally located stem cells of vitelline follicle cells displayed morphological changes consistent with apoptosis at 15 hrs treatment and their apoptotic was found confirmed. Many complexes network of cytoplasmic nurse cells branches and extensions surrounds, supports and nourishes to the developing vitelline cells were identified. Similar observations were also identified by previous researchers (Irwin and Threadgold 1970; Fairweather and Boray, 1999; Kumar et al., 2005; Fairweather. 2005, 2009, 2011; Toner et al., 2010a & b; Hanna et al., 2012 and Veerakumari et al., 2012).
Explanation of Photographic Plate 1

Photographs of control *Fasciola gigantica* by light microscope (LM)

Fig. 1 A. Anterior region of *F. gigantica* exhibiting (OS), esophagus (ES), tegument (T) and parenchymal cells (P) X 110.

Fig. 1 B. High magnification of genital apparatus (GA) and parenchymal (P) cell of *F. gigantica* X 190.

Fig. 1 C. High magnification of ventral sucker (VS) of *F. gigantica* X 190.

Fig. 1 D. High magnification of vitelline follicles (VF), parenchymal cells (P) and intestinal caeca (IC) of *F. gigantica* X 185.

Fig. 1 E. Uterus (U) of *F. gigantica* with secretory cells (S1) and secretory cells (S2) X 190.

Fig. 1 F. Testis (TE) of *F. gigantica* X 190.
Explanation of Photographic Plate 2

Photograph of *in vitro* treated *F. gigantica* with seeds extract of *Centratherum anthelminticum* by light microscopy (LM)

Fig. 2 A. Treated *F. gigantica* exhibiting detachment (DE), shrinks (SH) and vacuolization (V) in genital apparatus and parenchymal cells X 190.

Fig. 2 B. Ventral sucker of treated *F. gigantica* showing large vacuolization (V) are present due to highly damaged (DA) parenchymal cells X 185.

Fig. 2 C. Ovary of treated *F. gigantica* observing damaged (DA) outer membrane of ovary and vacuolization (V) in parenchymal cells X 195.

Fig. 2 D. Mehlis gland of treated *F. gigantica* showing shrinks (SH) in ootype, vacuolization (V) due to removed secretory cells and secretory cells X 185.

Fig. 2 E. Testis of treated *F. gigantica* showing highly vacuolization (V) and damages present in testis due to removed spermatocyte X 190.

Fig. 2 F. Intestinal caeca of treated *F. gigantica* showing highly vacuolization (V) and damages (DA) are present due to removed epithelial cells X 165.
Explanation of Photographic Plate 3

Photograph of *in vitro* treated *F. gigantica* with fruit extract of *Citrullus colocynthis* by light microscopy (LM)

Fig. 3 A. Genital apparatus and ventral sucker (VS) of treated *F. gigantica* observing highly damaged (DA), vacuolization (V) in parenchymal (P) cells X 185.

Fig. 3 B. High magnification of genital apparatus of treated *F. gigantica* showing vacuolization (V) and damaged (DA) in outer membrane X 190.

Fig. 3 C. Testis of treated *F. gigantica* exhibiting large vacuoles (V) present in testis due to removed spermatocyte cells and vacuolization are also present in parenchymal (P) cells X 190.

Fig. 3 D. Uterus of treated *F. gigantica* showing shrinks (SH) in uterus and parenchymal (P) cells X 185.

Fig. 3 E. Ovary of treated *F. gigantica* observing large vacuole (V) present due to removed eggs and extremely damaged (DA) parenchymal cells and ovary membrane X 190.

Fig. 3 F. High magnification of treated *F. gigantica* showing vacuolization (V) and damages (D) in ovary X 195.
Explanation of Photographic Plate 4

Photograph of *in vitro* treated *F. gigantica* with seeds extract of *Trachyspermum ammi* by light microscopy (LM)

Fig. 4 A. Anterior region of *F. gigantica* showing highly damaged (DA) oral sucker muscles and tegument, detachment (DE) and vacuolization (V) in genital apparatus and parenchymal cells, shrinks (SH) in ventral sucker X 110.

Fig. 4 B. High magnification of genital apparatus of treated *F. gigantica* observing detachment (DE) of genital apparatus membrane, damages (DA) and vacuolization (V) in parenchymal cells X 190.

Fig. 4 C. High magnification of ventral sucker of treated *F. gigantica* showing large vacuolization (V) X 195.

Fig. 4 D. Uterus of treated *F. gigantica* showing damage (DA) and large vacuole (V) present in uterus and vacuolization (V) in parenchymal cells X 140.

Fig. 4 E. Ovary of treated *F. gigantica* showing damages (DA) and vacuolization (V) X 145.

Fig. 4 F. Testis of treated *F. gigantica* showing shrinks (SH) and vacuolization (V) in testis X 165.
Explanation of Photographic Plate 5

Photograph of *in vitro* treated *F. gigantica* with synthetic drug albendazole by light microscopy (LM)

Fig. 5 A. Oral sucker of treated *F. gigantica* showing few damages in tegument and vacuolization (V) in parenchymal cells X 185.

Fig. 5 B. High magnification of ventral sucker of treated *F. gigantica* observing several damages (DA) and vacuolization presence in parenchyma cells, detachment (DE) in ventral sucker muscles X 195.

Fig. 5 C. Testis of treated *F. gigantica* observing little damaged (DA) in testis, several vacuolization (V) in intestinal caeca and parenchymal cells X185.

Fig. 5 D. High magnification of ovary of treated *F. gigantica* exhibiting few shrinkage (SH) and damages, vacuolization (V) in parenchymal cells X 195.

Fig. 5 E. Treated *F. gigantica* observing vacuolization (V) and few damaged (DA) in intestinal caeca X 180.

Fig. 5 F. High magnification of vitelline follicles of treated *F. gigantica* observing several damages (DA) and vacuolization (V) X 195.