Chapter 9

Conclusion
CONCLUSION

Fasciolosis 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. Fasciolosis is mainly caused by *Fasciola gigantica* in buffaloes. Effective control of this parasite includes the strategic use of anthelmintic drugs. However, problems have emerged with the use of chemical drugs, notably the development of resistance in the parasite, chemical residues and toxicity problem as well as the cost of drugs for treatment in cattle. The synthetic anthelmintic drug is commonly used against liver flukes. Various veterinary drugs have been used to eliminate parasites from buffaloes. The continued use of drugs is also posing a major problem of drug resistance in several parasitic species as well as unwanted adverse effect such as abdominal discomfort, nausea, vomiting, diarrhea, drowsiness vertigo, rashes is common and they are also indicated in certain groups of patients. Traditional medicinal plants have been used by many researchers to complete remove of helminth parasites from the ruminants. Traditional medicinal plants have no side effect, easily available to poor cattle farmer and develop the new herbal anthelmintic drug. It was decided to undertake the work on “studies on anthelmintic effects of fruits of *Citrullus colocynthis* (Indrayan or Kharatumba), seeds of *Centratherum antheminticum* (Kalijiri) and seeds of *Trachyspermum ammi* (Ajwain) medicinal plants on *Fasciola gigantica* in buffaloes (*Bubalus bubalis*)” by light and electron microscope. The Present result establishes on following facts:

1. Three medicinal plants were selected namely fruits of *Citrullus colocynthis* (Indrayan or Kharatumba), seeds of *Centratherum antheminticum* (Kalijiri) and seeds of *Trachyspermum ammi* (Ajwain) for their antihelminthic activity against *Fasciola gigantica* in buffaloes (*Bubalus bubalis*) by light and electron microscope.

2. Fruits *C. colocynthis* (Kharatumba) were collected from the desert area; Shriganganagar, Barmer, Jaisalmer (Rajasthan) and seeds of *C. antheminticum* and *T. ammi* were procured from local market of Udaipur.
3. Three plant materials were extracted and prepared aqueous and alcoholic extracts. A preliminary study showed that aqueous extract is less effective than the alcoholic extract. On the basis of this alcoholic extract was used for further studies.

4. Live liver fluke *Fasciola gigantica* parasites were collected from the liver of freshly slaughtered domestic buffaloes (*Bubalus bubalis*), at the local zoo abattoir in Udaipur.

5. After removing the parasites carefully, from the liver, they were again washed several times in physiological saline to remove debris and mucus, etc. These worms were kept in 0.9% physiological saline for investigations.

6. After thorough washing with a physiological saline solution (0.7 percent, NaCl), they were divided into five groups. The first group of the worm was used for identification of species of *Fasciola gigantica*. The second group of the *Fasciola gigantica* was untreated and used as control *Fasciola gigantica*. The third group of the *Fasciola gigantica* was used for evaluating the anthelmintic effect of different increasing concentrations of aqueous or alcoholic extracts of three selected medicinal plants on *Fasciola gigantica*. The fourth group of the *Fasciola gigantica* was used to evaluate the anthelmintic effect of commonly used synthetic drug albendazole on *Fasciola gigantica*. The fifth group of the *Fasciola gigantica* was given *in vitro* treatments of extracts of three selected medicinal plants at increasing concentrations and tissues of *in vitro* treated *Fasciola gigantica* were fixed in different fixative for histological and ultrastructural studies by SEM and TEM.

7. Histology of tegument, oral and ventral sucker, genital pore, reproductive organs; testes and ovary of control and *in vitro* treated *Fasciola gigantica* with three selected medicinal plant extracts and the synthetic drug was observed by light microscopic (LM).
8. No significant histological differences were observed between control liver fluke *F. gigantica* and treated fluke with synthetic drug albendazole by LM.

9. Several histological alterations were observed in the tegument, oral and ventral sucker, genital pore, reproductive organs; testes and ovary of *F. gigantica in vitro* treated with alcoholic extracts of *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) by LM.

10. Ultrastructural observations of the surface topography of *in vitro* treated *Fasciola gigantica* with three selected medicinal plants extracts; Kalijiri, Kharatumba, Ajwain, and compared with albendazole were exhibited by scanning electron microscope (SEM).

11. The present SEM studies showed drastic changes such 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 in the surface topography of *in vitro* treated *Fasciola gigantica* with three selected medicinal plants extracts. Also produced severe 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.

12. Ultrastructural observations of tegument of *in vitro* treated *Fasciola gigantica* with an alcoholic extract of Kalijiri, Kharatumba, Ajwain and compared with albendazole by transmission electron microscope.

13. Several disruption and vacuolization, erosion in basal lamina, depletion of the parenchymal cell, stripping of tegument, clumping of chromatin in the nucleus and fuzzy form mitochondria are common destructions in the tegumental cytoplasm were observed by TEM.
14. Numerous tegumental changes were characterized by swelling, loss of spines, cytoplasm vacillations, the disappearance of secretory granules, degeneration of nuclear materials and sunken sensory papillae in the swollen tegument. Cytoplasmic and cell organelles variations in the tegument induced by medicinal plants seeds of Kalijiri, kharatumba, and seeds of Ajwain extract could use a profound effect upon the parasites metabolic activities due to alteration effect upon the parasites surface rich in glycoproteins.

15. Extensive tegumental alterations were observed on fluke treated with analcoholic extract of medicinal plants *Centratherum anthelminticum* (Kalijiri), *Citrullus colocynthis* (Indrayan or Kharatumba) and *Trachyspermum ammi* (Ajwain) in comparison with synthetic drug albendazole.

16. Present research work is much significant because medicinal plants *Centratherum anthelminticum*, *C. colocynthis* and *T. ammi* will offer a suitable and cheaper anthelmintic herbal drugs alternative for the most expensive anthelmintic synthetic drugs. It showed a potent and progressive effect on the parasites.

17. Consequently, it will help to reduce the occurrence of the parasite in the host environment after treatments. The results of this study will be helpful in preparing eco-friendly and less costly anthelmintic veterinary drug.

18. The current research work provides knowledge for fasciolosis treatment and chemotherapeutic as well as phytotherapeutic measures. Findings of the study would improve the socio-economic condition of the cattle farmers of Udaipur by removing pathogenic liver fluke *Fasciola gigantica* parasites with the treatment of medicinal plants.