7. SUMMARY

1. Paramphistomosis is one of the major parasitic disease severely limiting the animal productivity in dairy animals. Chemotherapy is a major treatment modality used for the control of helminths infections in livestock. However, due to increasing development of anthelmintic resistance and the limited availability of commercial drugs to the rural people as well as the high cost of such synthetic medicines, a growing interest in the ethno-veterinary approach to examine the anthelmintic properties of plants traditionally used by local farmers in different parts of the globe is emerging. Medicinal plants are resources of new drugs and are known to provide a rich source of botanical anthelmintics. Phytotherapeutic drugs are safe, non-toxic, biodegradable and do not leave residues in animal’s products. The present study demonstrates the anthelmintic efficacy of Adhatoda vasica and Piper betle against the paramphistome, C. cotylophorum.

2. C. cotylophorum were collected from the rumen of the sheep and were maintained in vitro in Hedon–Fleig solution (pH 7.0) at 37°C. The parasites were incubated in various solvent and aqueous extracts of A. vasica and P. betle.
3. Gross visual observations on the motility of the parasites exposed to *A. vasica* and *P. betle* revealed that ethyl acetate extracts of *A. vasica* and *P. betle* were effective against *C. cotylophorum*. Based on the motility and viability of parasites in five different sub-lethal concentrations of AvEaE and PbEaE were selected for further *in vitro* studies.

4. Quantitative measure of motility of AvEaE and PbEaE-treated parasites recorded with the aid of Electronic Micromotility Meter (EMM) confirmed the inhibitory effects of AvEaE and PbEaE on *C. cotylophorum*. The degree of inhibition is directly proportional to the concentration of the extract and period of incubation.

5. Histopathological studies on the control and drug–treated flukes were performed using light, scanning and transmission electron microscope. Pathological changes include severe lesions and vacuolization in the tegument, parenchyma, gastrodermis, testis and eggs in the drug-treated parasites. Scanning electron micrographs of the tegument, oral sucker and ventral view of the drug-treated flukes revealed pathological changes. Intense structural variations were observed in the tegument, parenchyma and in the cellular organelles viz. mitochondria and nucleus under transmission electron microscope.
6. Both AvEaE and PbEaE inhibited the activity of the enzymes involved in the carbohydrate metabolism such as pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), fumarate reductase (FR) and succinate dehydrogenase (SDH). The inhibition of PK activity results in reduced production of pyruvate. The inhibition of PEPCK apprehends the PEP-succinate pathway and switches the PEP towards the formation of pyruvate. This results in reduced production of malate, which serves as the main substrate for mitochondrial phosphorylation. Inhibition in the LDH activity catalysing both the oxidation of lactate to pyruvate and reduction of pyruvate to lactate was observed. Further, significant inhibition of MDH, FR and SDH was also observed. The inhibition of the enzymes of carbohydrate metabolism affects the energy generation process of the parasites and leads to reduced production of ATP. Decreased production of energy ultimately results in the death of the parasites.

7. Both AvEaE and PbEaE significantly reduced the level of AcPase and AlPase. Inhibition of acid and alkaline phosphatase levels were dose dependent and it impairs glucose uptake. Impairment of glucose uptake resulted in depletion of glycogen in *C. cotylophorum*.

8. Significant inhibition in the activity of acetylcholinesterase (AChE), the enzyme involved in neurotransmission, was observed in drug-treated parasites. Inhibition of AChE affects the
neurotransmission by accumulation of endogenous acetylcholine result in muscular paralysis and may result in expulsion of the parasites from the host.

9. *AvEaE* and *PbEaE* significantly inhibited the glutathione S-transferase (GST) activity of *C. cotylophorum*. Inhibition of GST activity results in the accumulation of toxic metabolites, which might be lethal to the parasites.

10. Trace elements are cofactors of enzymes and therefore have pivotal roles in biochemical reactions that can have widespread repercussions in animal metabolism and physiology. The effect of *AvEaE* and *PbEaE* extracts on trace elements of *C. cotylophorum* was studied. Prepared sample was directly used for quantitative measurement of the trace elements using PERKIN ELMER OPTIMA 5300 DV ICP-OES at specific wavelengths for specific minerals. Alterations in the level of vital trace elements viz., Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na and Zn present in *C. cotylophorum* treated with *AvEaE* and *PbEaE* was estimated.

11. *A. vasica* and *P. betle* were further explored for their phytochemical profile to identify the active constituent responsible for anthelmintic activity. Steroid, flavonoid, triterpenoid, sugar, saponin, acid, tannin, phenol and alkaloids are present in *A. vasica*; whereas, steroid,
triterpenoid, quinone, coumarin, sugar, saponin, acid, tannin, phenol and alkaloids are the phytochemicals identified in *P. betle*.

12. Fractions obtained from *AvEaE* and *PbEaE* using column chromatography were pooled based on Rf value obtained in TLC and the fractions were tested for its efficacy against *C. cotylophorum*. Gross visual observations revealed that *AvCEaF*-5 and *PbCEaF*-4 were the most effective in inhibiting the motility of *C. cotylophorum*. Further, quantitative measure on the motility of the treated parasites using Electronic Micromotility Meter (EMM) confirmed the efficacy of *AvCEaF*-5 and *PbCEaF*-4 on *C. cotylophorum*.

13. GC-MS analysis revealed the presence of 4 compounds santonin, linoleic acid methyl ester, palmitoleic acid and octadecenoic acid in *AvCEaF*-5 and 5 compounds 4-isobutylhydratropic acid, oleic acid, methyl stearate, 4-0- methylonokiol and 2-methylpalmitic acid in *PbCEaF*-4. The anthelmintic activity of *AvEaE* and *PbEaE* may be attributed to the presence of the phytoconstituents in the extract that interferes with the energy metabolism of the parasites by uncoupling oxidative phosphorylation and finally causes death. The presence of active components in *AvEaE* and *PbEaE* suggests that these phytochemicals have individual or combined effect on *C. cotylophorum* thereby leading to their death.
14. In the *in vivo* studies, *PbEaE* was tested for its anthelmintic efficacy in naturally paramphistome-infected sheep. A significant reduction in faecal egg count was observed in sheep treated with two various concentrations of *PbEaE*. The egg counts reduced on day 7 post-treatment and continued to decline further till day 21 post-treatment.

15. Haematological and biochemical profiles were observed in treated sheep. Haemoglobin, packed cell volume, total erythrocytes, total leucocytes, neutrophils, lymphocytes, eosinophils, total serum protein, albumin, globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of infected sheep showed remarkable improvement in sheep treated with *PbEaE* and restored to normal levels on 21-day post treatment.

16. Both *AvEaE* and *PbEaE* have multiple mode of action and are capable of causing a number of detrimental effects which altogether accounts for their effectiveness in combating the paramphistome, *C. cotylophorum*. Phytochemical evaluation of *A. vasica* and *P. betle* showed that the plants bear the phytoconstituents which are responsible for the antiparasitic activity. *In vivo* studies entrenched the ameliorative role of *PbEaE* against paramphistome infection by promoting the health status of the host. *A. vasica* and *P. betle* are highly effective against paramphistomosis. The discovery and
development of noval substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important source of bioactive substances. Hence *A. vasica* and *P. betle* could be used to treat paramphistomosis. This study paves the way for designing integrated solutions for the control of ruminal paramphistomosis.