Introduction
1. INTRODUCTION

The global livestock sector is rapidly changing in response to globalization and growing demand for animal-source foods, driven by population growth and increasing wealth in much of the developing world. Animal husbandry will be a lucrative occupation for alleviating poverty, unemployment and rural transformation besides reducing the migration of human population to urban areas from rural areas. The rapid rate of urbanization seen in many countries is not only linked to growing affluence but also gives rise to changes in people’s food preferences; usually tending towards greater convenience and higher standards of safety (Robinson et al., 2011).

Animal Husbandry plays a vital role in Indian economy and livestock occupies an important place in the life of rural people. Beside the improved production potentials, our livestock and poultry farming have become economically viable and remunerative. Due to this, animal husbandry, which was all along a subsidiary occupation, has now become a main source of income for many rural, poor farmers. Livestock sector contributes substantially to the gross domestic product (GDP) of the country. India holds one of the largest livestock populations of the globe and the predominance of the mixed crop livestock system is one of the characteristics of Indian agrarian economy. Apart from yielding priority products like milk, egg and
meat, livestock sector paves way for sustainable livelihood of the rural folk, eminently the small and marginal farmers and landless labourers. With emerging demands for milk, meat, chicken, pork, fish and egg this sector is poised for greater growth in the years to come. This sector also has a strong backward and forward linkage, which in turn boosts livestock based food processing, leather and wool industries that earn foreign exchange.

Tamil Nadu is showing an impressive growth in livestock production. Tamil Nadu ranks fourth in India with a total sheep population of 7.9 million which constitute 11.17 percent of the total sheep population of the country. The sheep population of Tamil Nadu has increased from 5.53 million in 1982 to 7.9 million in 2007. The estimate of mutton production of the state is 15 thousand tones which is 5.01 percent of the total meat production of the country. The gross value of output from livestock in the state is 22,017.59 crores in the year 2010 - 2011 which contributes 2.58 per cent of State’s GDP and 24.80 percent of the agriculture and allied sector output. The state contributes 18.27 percent of egg, 8.78 percent of meat and 5.61 percent of milk production and ranks second, fifth and eighth position in the country respectively (Tamil Nadu Veterinary and Animal Sciences University, 24th Annual Report, 2012-2013).

Parasitic diseases reduce the production potential of livestock. Outbreaks of diseases cause huge economic loss to the farming community. An estimated livestock output worth Rs. 50 billion is lost annually due to
diseases. Generally parasitism is the single important cause of production losses in large ruminants (Chauhan et al., 1997) and small ruminants (Jithendran et al., 1998). Parasites form a genetically heterogeneous group of organisms consisting of ecto and endoparasites. Some of these parasitic infections are lethal causing severe damage to livestock and human population. Parasitic infections have become a key issue of social and economic problem in developed and developing countries. Globally parasitic diseases continue to be a major constraint for poor developing countries. They are rarely associated with high mortality and their effects are usually characterised by lower outputs of animal products, by-products, manure and traction all contributing to assure food security.

Paramphistomes, the parasites of ruminants which particularly affect the cattle, sheep and goats, causing the disease paramphistomosis, are digenean trematodes belong to the family Paramphistomidae, parasitizing the rumen of ruminants globally (Kanwal et al., 2014). The amphistome species responsible for the majority of outbreaks of paramphistomosis in ruminants are *Cotylophoron ctylophorum*, *Cotylophoron jacksoni*, *Calicophoron calicophorum*, *Calicophoron raja*, *Calicophoron microbothrium*, *Calicophoron clavula*, *Calicophoron sukumum*, *Calicophoron phillerouxi* and *Paramphistomum cervi* (Madzingira et al., 2002). Among these species *Cotylophoron ctylophorum* has a wide distribution in cattle, sheep and goats of India (Lone et al., 2013).
Cotylophoron ctylophorum (Fischoeder, 1901) is commonly known as rumen fluke or stomach fluke, they are pear-shaped flukes with small tegument surface papillae. The parasites appear as reddish or pink clusters between the papillae of the rumen and reticulum. The tegument is often penetrated by many deep pits and variously interrupted by cytoplasmic projections of gland cells, by openings of excretory pores and by nerve endings. The distal cytoplasm usually contains vesicular inclusions. Mitochondria occur in the distal cytoplasm. Muscles fibers are smooth and most prominent in the anterior parts of the body.

C. ctylophorum is characterised by two distinct suckers. The oral sucker leads to the muscular pharynx. The posterior sucker (acetabulum) is situated at the posterior end of rumen flukes and is well developed. The flukes are hermaphroditic, and the genital pore is located ventro-medially in the anterior third, serving as a common opening for male and female sex organs. Testes are deeply lobed, diagonal and occur in posterior half of the body. Ovary is sub spherical, lies posterior to testes and dorsal to acetabulum.

C. ctylophorum have more complex life-cycle where larval stages undergo asexual amplification in intermediate hosts, snails. The fresh water snails viz. Indoplanorbis exustus, Gyraulus convexicculus, Lymnaea luteola and L. auricularia are known to act as intermediate host. These snails are extremely adaptable, prolific breeder and play significant role in the transmission of disease. The distribution of naturally infested snails depends
entirely upon the distribution of infested carriers of the adult flukes. Adult flukes reside in the rumen and reticulum of the sheep and the un-embryonated eggs are passed in the host’s faeces. After being freed from the faeces, the miracidia hatch from the eggs in water within 12 to 16 days, miracidia larva leads free swimming life for some time, later penetrates inside the snail.

*Indoplanorbis exutus* is the most commonly found intermediate host in Asia (Liu *et al.*, 2010). The miracidium penetrates the mantle, head and foot of *I. exutus*, loses its ciliated epidermal cells, and transforms into a sporocyst. The sporocyst develops rapidly produces rediae. A number of generations of rediae may occur resulting in the production of free swimming cercariae. Each redia produces approximately 25 cercariae. Cercariae released from snails, after a short free swimming period encyst on vegetation to form metacercariae. Encysted metacercariae on plants are ingested by the ruminants and become excysted as young flukes in the duodenum of the final host. Young flukes in the duodenum attach to the mucous membrane of the intestine with their posterior sucker (Vorster and Mapham, 2012). The immature flukes penetrate the mucosa of the small intestine deeply and attaches with a plug of mucosa drawn into their acetabulum. This causes strangulation and eventual necrosis of the mucosa, leading to its erosions. These lesions cause intestinal discomfort and decreased appetite to the host. Clinical signs of infection vary considerably depending on the site and duration of infection.
The control of gastrointestinal parasites has traditionally relies on grazing management and anthelmintic drugs treatment. Grazing management schemes are often impractical due to expense or the hardiness of infective larvae on pasture and limitations in communal grazing system in the tropic areas. Anthelmintic drugs have been used either prophylactically or curatively to control gastrointestinal parasites. Current large scale sheep and goat production relies heavily on the application of chemical anthelmintics. The compulsory and often excessive use of chemotherapeutics (Hein and Harrison, 2005), often in combination with poor management practices (Wolstenholme et al., 2004), has resulted in development of resistance to various synthetic drugs. Although synthetic molecules are effective in the treatment/management of parasitic infections, they suffer from limitations of side effects or toxicity.

Albendazole produces few side effects when used for short-term therapy of gastrointestinal helminthiasis; however, the long-term treatment of echinococcosis or cysticercosis with high-dose albendazole accounted for most of the adverse drug reactions attributed to anthelmintic therapy (Bagheri et al., 2004). Mebendazole transient symptoms of abdominal pain, distention and diarrhoea have occurred in cases of massive infestation and expulsion of gastrointestinal worms and may be associated with occipital seizures (Wilmshurst and Robb, 1998). In neurocysticercosis, inflammatory reactions to praziquantel may produce meninges, seizures, mental changes and
cerebrospinal fluid pleocytosis (Adam et al., 2004). Usage of very high doses of ivermectin causes signs of central nervous system toxicity, including lethargy, ataxia, mydriasis, tremors and eventually death in animals (Campbell, 1993). Piperazine salts cause side effects like nausea, intestinal disturbances and giddiness (Liu and Weller, 1996). The frequent use of broad spectrum of anthelmintics such as albendazole, benzimidazole, imidazothiazole and ivermectin, over many years has led to the development of drug resistance among various groups of gastrointestinal parasites.

Helminths affect millions of livestock resulting in considerable economic losses in domestic and farmyard animals. The gastrointestinal helminths become resistant to currently available anthelmintic drugs which is a foremost problem in treatment of helminth diseases (Dash et al., 2002), the widespread development of anthelmintic resistance and increasing concern about the environmental impact of anthelmintic use necessitate the need for alternative control programs (Millar et al., 1998). The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people as well as animals, particularly in tropical developing countries, including India. Hence there is an increasing demand towards natural anthelmintics.

Several plants or plant derived formulations are used to cure helminth infections in man and animals (Akerele, 1990; Satyavati, 1990). The origin of many effective drugs is found in the traditional medicine practices
and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic efficacy (Kozan et al., 2006; Veerakumari and Priya, 2006; Lyndem et al., 2008; Roy et al., 2008; Al-Shaibani et al., 2009; Navaneetha and Veerakumari, 2009; Kosalge and Fursule, 2009; Challam et al., 2010; Manolaraki et al., 2010; Veerakumari et al., 2012; Priya et al., 2013; Veerakumari, 2015).

Etewa et al. (2011) stated in their review the active components of different herbs that could be used as drug targets in parasitic diseases. They can be derived from any part of the plant like bark, leaves, flowers, seeds, etc. An integral component of drug development includes the selection of plants on the basis of traditional reputation for efficacy in the treatment. The products which are derived from primary or secondary metabolism of plants are bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids, these phytochemicals may serve as a good source of therapeutic agents against various parasitic infections.

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy. In the present study, anthelmintic efficacy of Adhatoda vasica and Piper betle was investigated against the paramphistome, C. cotylophorum.
Adhatoda vasica Nees (family: Acanthaceae), commonly known as vasaka or arusha, is a well-known herb in indigenous systems of medicine for its beneficial effects. A. vasica is a shrub 1-2.5 m high with opposite ascending branches. The leaves are simple, opposite, 7-19 cm long and 4-7 cm wide. The flowers are white, pink or purple. The plant grows throughout the Indian peninsula up to an altitude of 1300 m (Claeson et al., 2000). A. vasica is a perennial, evergreen shrub and bitter in taste. The plant lives for multiple seasons and retains its leaves throughout the year (Frankel, 1995; Abhyankar and Reddy, 2007).

A. vasica is an ayurvedic medicinal plant which is a home remedy for several diseases and human requirements. It is mentioned in Vedas as an herbal remedy for treating cold, cough, whooping cough, chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic. It is an official drug and is mentioned in the India Pharmacopoeia. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaves extract of A. vasica used to treat fever, rheumatism, bronchitis, asthma, cough, cold, menorrhagia, leprosy, jaundice, stomachic, wound healing, malaria, mumps, heart trouble and delivery complaints (Jayaweera, 1981; Atta et al., 1986; Manandhar, 1991; Hussain and Hore, 2007; Ahmad and Javed, 2007; Rahman et al., 2008; Khan and Yadav, 2010).
The leaves of *A. vasica* have been found to be a rich source of alkaloids of which vasicine and vasicinone are bioactive compounds (Chowdhury and Bhattacharyya, 1987; Singh, 1997). A non-nitrogenous neutral principle, vasakin, vasicinone, adhvasinone, desmethoxyaniflorine and 7 methoxyvasicinone were also identified from the ethanolic extract of the leaves (Thappa and Agarwal, 1996). The uterotonic activity of vasicine is seemed to be similar to that of oxytocin and methyl ergometrine (Claeson et al., 2000). Vasicinone isolated from the leaves of *A. vasica* possess bronchodilator action (Gandhi, 2005; Gupta, 2006) ethanolic extract of the leaves have hypoglycaemic (Ilango et al., 2009), anti-inflammatory, antimicrobial (Panthi and Chaudhary, 2006; Karthikeyan et al., 2009), antiviral (Kumar et al., 2013), anthelmintic (D’cruz et al., 1980; Al-Shaibani, 2008; Arun and Vareishang, 2008), insecticidal and hepatoprotective activity (Srivastav et al., 1965; Bhaduri et al., 1985). Acute and chronic toxicity studies proved the use of vasicine and vasicinone comparatively safe (D’cruz et al., 1980). Clinical trials of a drug containing vasicine and vasicinone have not revealed any side effects while treating bronchial asthma (Wakhlo et al., 1980; Pahwa et al., 1987).

*Piper betle* L. (betel vine, Pan) a member of the piperaceae an indigenous medicinal plant, has a folk reputation in the rural areas of southern India. The plant is dioecious, shade loving perennial root climber. *P. betle* is traditionally known to be useful for the treatment of various diseases like bad
breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries (Agarwal et al., 2012). Fresh juice of P. betle is used in many ayurvedic preparations (Sharma, 1991). In ayurveda betel leaf extract is frequently used as an adjuvant and mixed with different medicines possibly for better effects beside its independent use as medicine. P. betle leaves are considered being useful in treating bronchitis and dyspnea (Mula et al., 2008).

Phytochemical investigation on P. betle leaves revealed the presence of alkaloids, carbohydrate, amino acids, tannins and steroidal components (Sugumaran et al., 2011). Leaves contained caryophyllene, cadinene, γ-lactone, allyl catechol, p-cymene and eugenol methyl ether in varying amounts (Rastogi and Medhrotra, 1993). 3β-acetyl ursolic acid and ursonic acid was isolated from the roots of P. betle (Saeed et al., 1993). Cepharadione A, dotriacontanoic acid and tritriacontane were also isolated from the petrol extract of leaves; piperine and piper longuminine were isolated from petrol and dichloromethane extract of stems and β-sitosteryl palmitate from petrol and dichloromethane extract of root parts of P. betle (Parmar et al., 1997). The literature cited that essential oil constituents in P. betle are the sources of many pharmacological activities (Sharma et al., 1987). P. betle leaf has a significant antifilarial (Meghna et al., 2009), antifungal (Ali et al., 2010), anti-inflammatory (Pin et al., 2010), anti-larvicial (Arambewela et al., 2011)
and antimicrobial activity (Devjani and Barkha Shah, 2011; Jesonbabu et al., 2012).

Both the plants *A. vasica* and *P. betle* possess antibacterial and antiviral activities and no side effects have been reported. As there is paucity of information related to anthelmintic property of *A. vasica* and *P. betle* in the present investigation, solvent extracts of leaves of *A. vasica* and *P. betle* were assessed for their anthelmintic efficacy against the trematodes parasite *C. cotylophorum*. Anthelmintic properties of plant products are scientifically validated mainly through *in vitro* studies, followed by *in vivo* field trials. The effect of the plant extracts on the morphology, internal architecture and physiology of the parasite was studied *in vitro*. Based on the results the effective plant extract was used for *in vivo* studies.

The motility and survival strategy of helminths are controlled by proper neuromuscular coordination system and any interference to this system could lead to the paralysis and expulsion from the host body or even death of the parasite (Mansour, 2002). Hence, the most common parameter used to assess the anthelmintic property of a drug is its impact on the motility of the parasites *in vitro* condition. Visual assessment of motility involved, observation for free spontaneous movement of the parasites in the medium of incubation (Bowen and Vitayavirasak, 2005). In many investigations, efficacy of drugs has been evaluated by gross visual observation on the motility response of parasites which demands defined skill and experience
(Behnke et al., 2008; Challam et al., 2010; Roy et al., 2012). Electronic Micromotility meter (EMM) provides quantitative measure of the motor activity of drug-treated parasites and could be used to assess motility of the flukes more conveniently and authentically (Veerakumari, 2003).

The tegument of the parasite, which is the interface between the parasite and its micro environment inside the host, seems to be the foremost potential target for action of many anthelmintics (Tandon et al., 1997). Parasites have an external body surface covered with tough and strong tegument. It protects the parasites from the adverse conditions inside the host body and also plays many important functions like evasion of host immune system, absorption of certain nutrients, excretion, control of motility and osmotic gradients. In vitro treatments of the parasites with different plant extracts altered the structure and composition in their tegumental architecture (Roy and Tandon, 1996; Veerakumari and Munuswamy, 1999) resulted in reduced intake of nutrition and irregular osmoregulation (Dasgupta et al., 2010; Roy et al., 2010). Hence the structural deformities have been studied as an inevitable parameter for evaluation of drug action.

The efficacy of an anthelmintic drug should be tested on various biochemical aspects of the parasites, such as neurotransmission, carbohydrate metabolism, surface transport mechanism and detoxification. Enzymes involved in these biochemical processes could have been serving as vital targets for action of anthelmintics. Hence the anthelmintic efficacy of
A. vasica and P. betle was studied against the key enzymes involved in various biochemical processes.

Many parasites rely on neuromuscular coordination for resisting host’s intestinal propulsive forces and for absorption and distribution of food for them (Maule et al., 2002). During the transmission of impulse acetylcholine esterase (AChE) hydrolyzes acetylcholine to choline and acetate at the synaptic cleft (Moczon and Swietlikowska, 2005). Because of its physiological significance in cholinergic transmission (Selkirk et al., 2005; Hewitson et al., 2009), AChE has been considered as a target of many drugs and poisons, both natural and synthetic (Martin, 1997).

The inhibition in the activity of enzymes involved in carbohydrate metabolism affects the energy generating process resulting in decreased production of energy (Pallewad et al., 2015). Consequently, the energy deprived parasite unable to sustain themselves in situ may be expelled from the host (Priya and Veerakumari, 2012). Carbohydrate metabolism of the helminth parasites resembles their host animals, until the formation of phosphoenol pyruvate (PEP). PEP obtained from glycolysis can either be carboxylated to oxaloacetate (OAA) by phosphoenolpyruvate carboxykinase (PEPCK), or dephosphorylated to pyruvate by pyruvate kinase (PK). Later this pyruvate further reduced to lactate by lactate dehydrogenase (LDH) and OAA is reduced to malate by malate dehydrogenase (MDH). Malate permeates into the mitochondrion where it undergoes dismutation in which
one-half of malate is oxidized to pyruvate by malic enzyme (ME) and the other half is dehydrated to fumarate by fumarase (FM), which is further reduced to succinate by fumarate reductase (FR). Succinate is oxidized to fumarate by succinate dehydrogenase (SDH).

Glucose is absorbed from the host via the glucose transporters located in the tegument and intestinal epithelium of trematodes (Skelly et al., 1994; Thompson and Geary, 1995). Inhibition of glucose uptake results in energy crisis and reduced muscular activity. It also reduces the glycogen content, which suggests utilization of glycogen as a result of inhibition of glucose uptake (Kushwaha et al., 2004). Inhibition of glucose uptake results in reduced ATP production and decrease in glycogen levels that ultimately lead to state of starvation and energy deprivation. The energy deprived worms unable to sustain themselves in situ are expelled by the host system. Hence the study of glucose and glycogen in drug treated parasites are significant parameters to evaluate the efficacy of anthelmintics.

Phosphatases are known to play a variety of important roles at the transporting surfaces in extracellular digestion and phosphorylation of nutrients transported, secreted and excreted (Lumsden, 1975; Pappas and Read, 1975; Maki and Yanagisawa, 1980). In a number of helminth parasites, acid phosphatase (AcPase) and alkaline phosphatase (AlPase) have been detected histochemically and found to be closely associated with the tegument, sub tegument, somatic musculature, gut and cuticle (Pappas, 1988;
Fetterer and Rhoads, 2000). AcPase and AlPase are found to be involved in the uptake of certain nutrients, glycogen and lipoprotein and play an important role in hydrolysisation and active transport of metabolites in helminth parasites (Pappas and Read, 1975; Sharma, 1976; Parshad and Guraya, 1978; Smith et al., 2000). Anthelmintics may alter these enzymes and modify the normal metabolism of the helminth parasites. Tegumental enzymes play a very important role in maintaining the tissue homeostasis within the parasite. In this context the present study was designed to analyse the level of AcPase and AlPase in drug-treated parasites.

Glutathione-s-transferase (GST) is an important antioxidant in plants, animals, fungi and some bacteria and archaea, preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals (Pompella et al., 2003). The antioxidant molecule glutathione plays an important role in parasite defenses via the activity of GSTs (EC 2.5.1.18). These enzymes carry out a wide range of functions from the detoxification of exogenous electrophilic compounds (xenobiotics), the inactivation of endogenous metabolite products of oxidative stress (lipid hydro peroxide and reactive carbonyl), the biosynthesis of signaling molecules (prostaglandins, leukotrienes), to the transport of ligands. GSTs have been detected in all helminths and are the most extensively characterized enzymes of detoxification pathways. Because
parasitic helminths appear to lack or have very low levels of the phase I detoxification enzymes (cytochromes P450, cytochrome b5) (Precious and Barrett, 1989). GSTs have been widely investigated because of their immunogenic character. Hence, GSTs are likely to be the major enzymes involved in detoxification and also serve as a potential drug targets (Brophy and Barrett, 1990; Cvilink, et al., 2009). Therefore, this study has raised real interest for the development of drugs against various helminth infections.

Minerals act as co-factors for enzyme reactions. They maintain the pH balance and vitality within the body. Minerals actually facilitate the transfer of nutrients across the cell membranes. Minerals play important role in muscle contraction, tissue growth and provide structural and functional support for the body. There are two categories of minerals, macro-minerals and micro-minerals. Macro-minerals are calcium, phosphorous, potassium, magnesium, sulfur, sodium and chloride. Micro-minerals are iron, boron, chromium, iodine, manganese, molybdenum, selenium, silicon, vanadium, zinc, lithium, germanium, rubidium, cobalt and copper. Intestinal parasites use carbohydrates, lipids, minerals, vitamins and other food sources of the host in order to obtain essential energy of the life cycle (Hellard et al., 2000). Minerals are inevitable for proper functioning of cell metabolism as well as reproduction of parasites. Hence the mineral survey in drug-treated parasites may serve as a good parameter to test the anthelmintic efficiency of the drug.
Plants have been proven as promising sources of new and biologically active natural products exhibiting higher activity in medicinal applications. The usage of natural products and active plant extracts has been increased recently and new drugs are discovered using new technological advancements. These drugs are widely used in the treatment of different ailments Indian system of medicine. Most of the medicinal plants are rich for phytoconstituents viz. alkaloids, saponins, flavonoids, and tannins. Anthelmintic effect of tannin rich plants against gastro intestinal helminth parasites was recorded by many investigators (Hoste et al., 2006; Alonso Diaz et al., 2008; Calderon-Quintal et al., 2010). Noedl et al. (2003) stated that the extraction of pure bioactive compounds from crude plant extracts has become necessary to study its action against parasites. Hence the isolation and characterization of the bioactive compounds present in effective fractions of plants extracts becomes an essential requirement to further progress in drug discovery.

The use of medicinal plants for the prevention and treatment of gastro intestinal parasitism has its origin in ethno veterinary medicine. Although until recently the majority of the evidence on the antiparasitic activity of medicinal plants was anecdotal and lacked scientific validity, there is currently an increasing number of controlled experimental studies that aim to verify and quantify such plant activity. There are indeed a large number of
plants whose anthelmintic activity has been demonstrated under controlled experimentation, either through feeding the whole plant or administering plant extracts to parasitised hosts. Bioactive components that are candidates for therapeutic application will still have to undergo extensive clinical and toxicological studies before can be prescribed as medicine. Concentrations of potentially active substances used in vitro do not always correspond to in vivo bioavailability. Therefore, in vitro assays should always be accompanied by in vivo studies when used to validate the anthelmintic properties of medicinal plants (Githiori et al., 2006). The in vivo assay such as the faecal egg count reduction test (FECRT) is suitable for the evaluation of all types of anthelmintics (Verma et al., 2006). Furthermore, in vivo observations on the haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential count (DC), serum glucose (SG), total serum protein (TSP), albumin (A), globulin (G), aspartate aminotransferase (AST) and alanine amino transferase (ALT) of the naturally infected animals before and after the treatment with drug will give clear understanding about its anthelmintic efficacy. Hence, in the present investigation in vivo studies were carried out to substantiate the results obtained in vitro.
Objectives of the present investigation are the following:

- To evaluate the efficacy of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *Adhatoda vasica* and *Piper betle* against *Cotylophoron cotylophorum*.

- To study the mode of action of effective extract of *Adhatoda vasica* and *Piper betle* on *Cotylophoron cotylophorum* based on the morphological, physiological and biochemical aspects.

- To study the effect of *Adhatoda vasica* and *Piper betle* on the mineral composition in *Cotylophoron cotylophorum* using ICP-MS.

- To isolate, and identify the phytochemicals present in *Adhatoda vasica* and *Piper betle* using column chromatography, TLC and GC-MS.

- *In vivo* assessments of the anthelmintic property of effective plant extract using faecal egg count and several haematological and biochemical parameters of the host, sheep.