CHAPTER - IV

IN VITRO ANTIPARASITIC ACTIVITY OF SELECTED GASTROPOD EXTRACTS ON PHERETIMA POSTHUMA AND RHIPICEPHALUS SANGUINEUS

INTRODUCTION

Parasitic diseases are illness caused by infestation with parasites such as endo and ectoparasites. Endoparasites or Helminths are multicelled organisms that can be either free-living or parasitic in nature. The term ectoparasites can broadly include blood-sucking arthropods such as ticks, fleas, lice and mites. Parasites live everywhere but they particularly thrive in warm and moist climates. So they are most common in the tropics and sub tropics regions such as the Indian subcontinent, Sub-Saharan Africa, Southeastern Asia, Central and South America. Nearly 6 billion people are infected with parasites (Van and Basson, 1989).

ANTHELMINTHIC ACTIVITY

Helminthic infections are among the most common infection in human beings affecting many people in the world. More than one third of the world’s population is infected with helminths (Sharmin et al., 2013). They probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Helminths are classified as eukaryotic endoparasites because they live inside the body. There are many different types, but
the most common are soil-transmitted helminths viz., roundworm, whipworm and hookworm.

They can live in humans or animals and are usually transmitted through contaminated food, water, feces, unwashed hands or contact with any contaminated objects. It can be transferred to human through a process called zoonosis. Immature forms of the parasite invade human beings via the skin or gastrointestinal tract and evolve into well differentiated adult worms that have characteristic tissue distribution (Bairagi et al., 2011).

Various clinical symptoms arises due to this infection include dysentery, diarrhea, nausea, vomiting, loss of appetite and weight, acidity and sometimes anaemia (Tripathi, 2008). Other manifestation of helminthic infections includes respiratory symptoms, dermatological consequences and epilepsy as a result of neurostercerosis. Helminthic infections may also subvert immune response to pathogens of other diseases such as tuberculosis, HIV and malaria (Hossain et al., 2012). Moreover children in developing nations are at higher risks for helminthic infections. Many helminth infections occur in poverty-stricken and developing countries with warm, moist environments with poor sanitary conditions.

Over the years, helminthiasis had been controlled by the synthetic anthelminthic drugs. Anthelminthics are drugs that expel parasitic worms (helminths) from the intestinal tract or tissues of the body by either stunnig or killing them (Sharma, 2007). Most of these parasitic control programs are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control, vaccination and ethnoveterinary treatment
The major anthelmintic drugs commonly used for the control of helminth parasites in small ruminants are benzimidazoles (albendazole, triclabendazole and fenbendazole) and levamisole (tetramisole) and Oxyclozanide.

Most of these existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea (Bundy, 1994). Moreover, the continuous and long-term applications of these compounds had led to the development of drug resistance in many helminthic strains. Some anthelmintic drugs, such as praziquantel and albendazole are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age (El- Halawany et al., 2007). To overcome the development of drug resistance it is crucial to synthesize a new class of compounds possessing different chemical properties from those of used commonly.

The intestinal roundworm *Ascaris lumbricoides* causes ascariosis estimated to infect one billion people. Several *in vivo* and *in vitro* techniques have been developed to detect anthelmintic resistance in nematodes (Craven et al., 1999). *In vitro* techniques had advantage to evaluate anthelmintic activities of claimed natural products over *in vivo* studies due to simplicity and cost effectiveness of this technique. Different species of worms *viz.*, *Ascaris, Nippostrongylus* and *Heterolus* has been used in the screening of anthelmintic agents. But of all the worms, earthworms have been widely accepted for *in vitro* studies mainly because of their resemblance to intestinal round worms in reaction to anthelmintics due to their easy availability. It is observed that all anthelmintics that are toxic to earthworms are worthy to be investigated upon as potent anthelmintic agents (Vandana et al.,
Hence in the present study, Indian earthworm was selected as the model organisms.

**ACARICIDAL ACTIVITY**

The diseases caused by ectoparasites are of global concern and are considered as a major obstacle in the health and product performance of animals. Ectoparasites may live on the skin and feed on the blood of humans or other animals. All the ectoparasites may require single larger blood meal or regular small blood meals to complete their life cycles. In addition to disease transmission their bites cause lesions in the skin that they prove slow to heal may become secondarily infected by bacteria.

Among all the parasites, ticks are the most important vector of diseases affecting both humans and animals worldwide and transmit a greater variety of protozoan, bacterial, rickettsial and viral pathogens than any other arthropod vector (Ahmed et al., 2007). Ticks are also responsible for transmission of various tick borne diseases such as lyme disease, rocky mountain spotted fever, relapsing fever, tularemia, meningoencephalitis, Colorado tick fever, Crimeancongo hemorrhagic fever, babesiosis, cytauxzoonosis etc., in animals and man.

These ticks cause damage directly due to herd irritability, blood spoliation, hide and udder injuries and inoculation of toxins, and indirectly by transmission of *Babesia* sp and *Anaplasma marginale* to the cattle (FAO, 2004). Problem of ectoparasites in animals has been quite alarming leading to enormous economic losses through affecting meat and milk production, health status, innumerable skin diseases and transmission of infectious diseases.
The tick *Rhipicephalus sanguineus* is the species currently with the most worldwide spread, due to the wide distribution of its natural host (dog) and also due to its nidicolous habits (Pegram *et al*., 1987). The parasite can also be identified as an important vector that transmitting several *Rickettsia* species to man such as *R. rickettsii* and *R. conorii* the causative agents of rocky mountain spotted fever in Mexico and South America and boutonous fever in the Mediterranean region of South Europe and Northern Africa (Swango *et al*., 1989).

Only 5% of *R. sanguineus* are found on dogs and the other 95% are free in the environment. Therefore, its effective elimination will require an integrated control strategy, aimed at both the canine population and the environment (Miller *et al*., 2001 and Roma *et al*., 2010). The currently available tools for tick control consist of chemical acaricides used with different application methods and various formulations. Various acaricidal compounds have been used for the control of tick *viz.*, arsenical compounds, chlorinated hydrocarbon, organophosphorous compounds and pyrethroids etc. In India alone, the cost of tick and tick-borne diseases and tick worry in animals has been estimated in the tune of 498.7 million US $ and 57.2 million US $ per annum, respectively [Minjauw and McLeod, 2003].

However, the repeated use of acaricides has led to the development of resistance in these ticks, because they have a very short life cycle and abundant progeny. It is therefore necessary to look for alternative measures which are adaptable and less expensive, especially for the subsistence of farmers with limited capital who constitute the majority of animal rearers in the developing countries, including India. In recent years plants with acaricidal properties could be used as an alternative which would help to limit the well-documented damage that the synthetic
acaricides can cause to the environment. Some studies have used herbal medicine for the control of the ticks (Kaaya et al., 1995; Fernandes and Freita, 2007; Ribeiro et al., 2007 and Pirali-Kheirabadi and DaSilva, 2011).

Marine gastropods contain potential bioactive compounds which have not yet been characterized and can be prove as potent drug against parasitic infections. Considering this fact, the present investigation have been designed to found out the *in vitro* antiparasitic activity of three marine gastropods against an endoparasite (Indian adult earthworm- *P. posthuma*) and the ectoparasite (dog tick- *R. sanguineus*)
MATERIAL AND METHODS

ANTHELMINTHIC ACTIVITY

Indian adult earthworm, *P. posthuma* (Phylum- Annelida; Family- Megascolecida) earthworms were collected from Vivekananda Kendra (Natural Resource Development Project), Kanyakumari. Worms were washed with normal saline to remove all faecal and waste matters. The earthworms of 8-10 cm length and 0.3-0.4 mm in width were used for all the experiments.

Experimental setup

The anthelminthic activity was performed according to the method of Ajaiyeoba *et al.* (2001) with slight modification. Approximately equal size adult Indian earthworm (n= 10) were placed in 9 cm petridishes containing the shell and soft tissue methanolic extract of marine gastropods (*D. aspera, T. radiatus* and *T. brunneus*) at the concentrations of 0.25 to 2.50 ml/dl. Albendazole (0.05 to 0.5 mg/ml) used as positive control and normal saline served as negative control. Observations were made for the time taken to paralysis and death of individual worm upto 4 hrs of the test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body colours (Dash *et al*., 2002).

STATISTICAL ANALYSIS

The experiment was designed in three replicates. The data obtained were expressed as mean ± SD.
ACARICIDAL ACTIVITY

Adult ticks, *R. sanguineus* (Phylum– Arthropoda; Family– Ixodidae) were collected from naturally infested dog. The dog has not been treated with acaricides for 50 days before collection of ticks. Male ticks were selected morphologically. Ticks were taken to the laboratory within 3-4 hrs to perform subsequent experiments.

Experimental setup

Acaricidal activity has been successfully used previously by Pumnuan et al., 2010. *R. sanguineus* ticks were divided into treatment and control ticks (n= 10). Square pieces of Whatman No.1 filter paper (9.8 cm$^2$) were used for the test. Each paper were soaked in gastropod extracts (*D. aspera* shell - 0.75 to 3.25ml/dl; *D. aspera* soft tissue- 0.25 to 2.50ml/dl; *T. radiatus* shell- 0.25 to 2.75 ml/dl; *T. radiatus* soft tissue- 0.45 to 1.45ml/dl; *T. brunneus* shell- 1.25 to 3.75ml/dl and *T. brunneus* soft tissue- 0.25 to 2.75ml/dl) for 1 hr. After drying for 2 hrs, each filter paper was attached to the bottom of a round petri dish (14 cm$^2$) using double sided cellophane tape. Vaseline was applied at the immediate edges of the filter paper to prevent escape of ticks. Ivermectin (0.24 to 0.44 ml/dl) used as positive control and distilled water used as negative control. Twenty adult ticks were placed onto each treated filter paper. Treatment and control petri dishes were held at 75% relative humidity and 25°C inside a closed air tight glass chamber. Mortalities were determined after 24 hours post- treatment. Ticks were considered as dead if their appendages do not move when prodded with a pin. Mortality rates of groups were recorded at 24 to 48 hrs of inoculation by counting dead ticks.
STATISTICAL ANALYSIS

The mean concentration dose (LC$_{50}$) values were calculated using a test probit analysis.
RESULTS

ANTHELMINTHIC ACTIVITY

The present investigation revealed that the methanolic extract of three marine gastropods showed considerable dose dependent anthelminthic activity against *P. posthuma* as compared to the standard drug albendazole.

The mean paralyzing time of *P. posthuma* with *D. aspera* shell extract at the dose of 1.25 to 2.50 ml/dl were found to be 54.2 ± 1.00, 50.2 ± 0.80, 45.78 ± 1.41, 40.11 ± 0.95, 34.65 ± 1.05 and 28.01 ± 1.40 mins respectively. The *D. aspera* shell extract showing a mean death time of 100.9 ± 1.19, 99.21 ± 0.85, 84.05 ± 1.02, 77.52 ± 0.81, 62.13 ± 0.98 and 53.98 ± 1.58 mins respectively with concentrations ranging from 1.25 to 2.50 ml/dl against *P. posthuma*.

*D. aspera* soft tissue extract causes paralysis in the helminths in 47.25 ± 1.28, 40.53 ± 0.64, 37.4 ± 0.57, 34.21 ± 1.10, 29.55 ± 0.87, 23.95 ± 1.35, 19.15 ± 1.10 and 19.18 ± 0.81 mins respectively at the dose of 0.75 to 2.50 ml/dl. The mean death time of *D. aspera* soft tissue extract with the same dose were found to be 87.8 ± 0.61, 79.27 ± 0.08, 72.8 ± 0.61, 70.23 ± 0.78, 61.15 ± 1.15, 51.17 ± 1.16, 48.9 ± 1.26 and 42.78 ± 0.64 mins respectively (Table 4.1 and Figure 4.1 and 4.2).

Methanolic extract of *T. radiatus* shell and soft tissue showed short time of paralysis and death in *P. posthuma* (Table 4.2 and Figure 4.3 and 4.4). At the doses of 1.00 to 2.50 ml/dl the *T. radiatus* shell exhibited paralysis after 44.76 ± 0.59, 41.20 ± 1.24, 32.67 ± 0.70, 29.50 ± 0.66, 21.90 ± 1.40, 20.20 ± 0.21 and 13.78 ± 1.58 mins of exposure, whereas with the same doses, the death occurred at 74.66 ±
0.73, 70.31 ± 1.10, 61.17 ± 1.03, 60.6 ± 0.70, 51.28 ± 0.75, 42.81 ± 0.70 and 34.05 ± 1.00 mins respectively. The *T. radiatus* soft tissue extract exhibited higher paralytic effect after 31.82 ± 0.62, 30.35 ± 1.05, 31.55 ± 0.95, 24.25 ± 1.15, 20.2 ± 1.07, 14.57 ± 1.36, 13.17 ± 0.16, 10.58 ± 1.47 and 6.87 ± 0.72 mins of exposure and death after 60.13 ± 1.07, 53.46 ± 1.49, 47.1 ± 1.03, 31.18 ± 1.10, 27.17 ± 1.22, 21.05 ± 1.02, 17.85 ± 0.66, 13.93 ± 1.17 and 10.17 ± 0.16 mins of exposure at the concentrations of 0.50 to 2.50 ml/dl respectively.

The table 4.3 and Figure 4.5 and 4.6 showed anthelminthic activity of the methanolic extracts of shell and soft tissue of *T. brunneus*. At the concentrations of 1.50 to 2.50 ml/dl caused paralysis at 61.41 ± 1.18, 59.11 ± 0.98, 56.26 ± 0.85, 53.83 ± 1.07 and 50.77 ± 0.67 mins of inoculation and death after 100.77 ± 1.28, 98 ± 1.00, 84.37 ± 0.78, 77.55 ± 0.58 and 71.4 ± 1.21 mins. Whereas with 1.00 to 2.50 ml/dl concentration of soft tissue extract, the paralysis occurred at 51.55 ± 0.60, 50.18 ± 1.12, 48.06 ± 1.01, 44.81 ± 1.61, 43.42 ± 2.96, 42.11 ± 0.90 and 40.98 ± 1.35 mins and death after 97.3 ± 0.72, 89.07 ± 1.05, 75.9 ± 1.08, 71.78 ± 1.61, 62.05 ± 1.78, 58.57 ± 1.42 and 48.33 ± 1.52 mins of introduction.

The standard drug albendazole causes paralysis in the helminths in 74.67 ± 1.08, 62.67 ± 1.78, 33.67 ± 2.27 and 25 ± 0.71 mins respectively at the dose of 0.05 to 0.5mg/ml. The mean death time of albendazole with the same dose were found to be 152.3 ± 1.78, 122.67 ± 1.78, 102.33 ± 1.78 and 83 ± 2.12 mins respectively. The negative control did not show any anthelminthic activity.
ACARICIDAL ACTIVITY

The methanolic extracts of three marine gastropods were evaluated for their acaricidal properties. *R. sanguineus* ticks were exposed to 11 different concentrations of *D. aspera* shell extract ranging from 0.75 ml/dl to 3.25 ml/dl. At 1.00 ml/dl concentration 10% mortality was observed after 96 hrs exposures. All the exposed ticks were died within 24 hrs with 3.25 ml/dl concentration and 96 hrs at 2.25 ml/dl concentration. 90% mortality was observed after 48 hrs at 2.75 ml/dl concentration (Table 4.5).

Based on the probit analysis, the 24 hrs, 48 hrs and 96 hrs LC$_{50}$ values of the *D. aspera* shell extract against *R. sanguineus* was found to be 2.548, 2.165 and 1.533 with significant upper (2.729, 2.355 and 1.703) and lower (2.376, 1.989 and 1.379) fiducial limits (Table 4.9 and Figure 4.8).

With soft tissue extract of *D. aspera* higher mortality was observed in *R. sanguineus* at 0.50 ml/dl concentration, 10% mortality was observed after 96 hrs exposure. 100% mortality was observed within 24 hrs at 2.50 ml/dl, 48 hrs at 2.25 ml/dl and 96 hrs at 2 ml/dl concentrations (Table 4.10).

The estimated LC$_{50}$, LCL and UCL values for *D. aspera* soft tissue extract was 1.787, 1.671 and 1.973 for 24 hrs, 1.484, 1.324 and 1.663 for 48 hrs and 0.894, 0.758 and 1.052 for 96 hrs respectively (Table 4.14 and Figure 4.8).

The results indicated that *T. radiatus* shell extract at 11 different concentrations (0.25 ml/dl to 2.75 ml/dl) could significantly kill the ticks. At 0.50 ml/dl concentration 10% mortality was observed after 96 hrs exposures. All the
exposed ticks were died within 24 hrs with 2.75 ml/dl concentration and 96 hrs in 1.75ml/dl concentration. 90% mortality was observed after 48 hrs at 2.25ml/dl concentration (Table 4.15).

*T. radiatus* shell extract recorded the LC$_{50}$ values of 2.020, 1.695 and 0.962 for 24, 48 and 96 hrs with significant LCL (24 hrs= 1.864, 48 hrs= 1.503 and 96 hrs= 0.806) and UCL (24 hrs= 2.188, 48 hrs= 1.911 and 96 hrs= 1.147) values (Table 4.19 and Figure 4.9).

*R. sanguineus* ticks were exposed to 11 different concentrations of *T. radiatus* soft tissue extract ranging from 0.45 ml/dl to 1.45 ml/dl showed significant mortality response. At 0.55 ml/dl concentration 10% mortality was observed after 96 hrs exposures. All the exposed ticks were died within 24 hrs in 1.45 ml/dl concentration and 96 hrs at 1.05 ml/dl concentration. 90% mortality was observed after 48 hrs at 1.25 ml/dl concentration (Table 4.20).

With *T. radiatus* soft tissue extract, LC$_{50}$ values of 1.200, 1.064 and 0.738 for 24, 48 and 96 hrs was recorded with significant upper (24 hrs = 1.271; 48 hrs = 1.137 and 96 hrs = 0.797) and lower (24 hrs = 1.132; 48 hrs = 0.995 and 96 hrs = 0.682) fiducidal limits (Table 4.24 and Figure 4.9).

The methanolic extract of *T. brunneus* shell extract at 11 different concentrations (1.25 ml/dl to 3.75 ml/dl) was effective against ticks. At 1.50 ml/dl concentration 10% mortality was observed after 96 hrs exposures. All the exposed ticks were died within 24 hrs with 3.75 ml/dl concentration and 96 hrs in 2.75 ml/dl concentration. 90% mortality was observed after 48 hrs at 3.25 ml/dl concentration (Table 4.25).
Significant probit results for *R. sanguineus* mortality were recorded with *T. brunneus* shell extract (LC<sub>50</sub>= 3.077, 2.922 and 1.923; LCL= 2.894, 2.568 and 1.779 and UCL= 3.265, 3.324 and 2.076 for 24, 48 and 96 hrs respectively) (Table 4.29 and Figure 4.10).

Similarly, *R. sanguineus* ticks were exposed to 11 different concentrations of *T. brunneus* soft tissue extract ranging from 0.25 ml/dl to 2.75 ml/dl. At 0.25 ml/dl concentration 10% mortality was observed after 96 hrs exposures. All the exposed ticks were died within 24 hrs with 2.75 ml/dl concentration and 96 hrs at 1.50 ml/dl concentration. 90% mortality was observed after 48 hrs at 2.25 ml/dl concentration (Table 4.30).

*T. brunneus* soft tissue extract recorded the LC<sub>50</sub> values of 1.994, 1.548 and 0.685 for 24, 48 and 96 hrs with significant LCL (24 hrs= 1.831, 48 hrs= 1.375 and 96 hrs= 0.539) and UCL (24 hrs= 2.170, 48 hrs= 1.742 and 96 hrs= 0.870) values (Table 4.34 and Figure 4.10).

Eleven different concentrations of ivermectin (0.24 ml/dl to 0.44 ml/dl) were tested against *R. sanguineus*. At 0.26 ml/dl concentration, 10% mortality was observed after 96 hrs exposure. All the exposed ticks were died within 24 hrs with 0.44 ml/dl concentration, 90% mortality was observed after 48 hrs at 0.40 ml/dl concentration. All the ticks were died after 96 hrs at 0.36 ml/dl concentration (Table 4.35).

Ivermectin recorded the LC<sub>50</sub> values of 0.382, 0.357 and 0.307 for 24, 48 and 96 hrs with significant LCL (24 hrs= 0.366, 48 hrs= 0.344 and 96 hrs= 0.293) and
UCL (24 hrs= 0.397, 48 hrs= 0.369 and 96 hrs= 0.320) values (Table 4.39 and Figure 4.8 to 4.10). No mortality was observed with negative control.
DISCUSSION

ANTHELMINTHIC ACTIVITY

Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. In the present study when the shell and soft tissue extracts of the three marine gastropods were administered to the test parasities, paralytic symptoms appeared within 40 mins and mortality occurred after three such paralytic attacks. Most of the extracts in the current study inhibited 100% mortality at low concentration (0.25 to 1.00 ml/dl) as compared to the other natural products studied previously (Agrahari et al., 2011; Dey and Pal, 2011 and Durga et al., 2013).

The crude methanolic extracts of *D. aspera* shell produced a significant anthelminthic activity on *P. posthuma* followed by soft tissue extract. This potent anthelminthic activity may be due to the fact that it contains 1- [(2- trimethylsiloxy) vinyl- 4- trimethylsiloxy- 2, 6- dideuteriobenzene. The *in vitro* anthelminthic activity of indole derivatives was determined by Davyt et al. (1998).

In the present investigation *T. radiatus* extracts exhibited good anthelminthic activity and this is compared with the effect produced by the standard drug albendazole. Considering the time of paralysis and death of earthworms the soft tissue extract of *T. radiatus* was more potent than the shell extract. GC-MS reports on *T. radiatus* shell and soft tissue had revealed the presence of 3- (4'-Bromophenyl)- 5, 6- diphenylimidazo [2, 1- b] thiazole and 6- (4- chlorophenyl)- 2, 5, 5-triphenyl- 5, 8- dihydro- 6H- azeto [1, 2- a] [1, 3] thiazolo [4, 5- d] pyrimidine respectively. The anthelminthic potential of thiazole derivatives was proved by
Shetty et al. (2010). Manojkumar et al. (2013) stated that the amides of pyrimidine derivatives exhibited significant anthelminthic activity.

Similar to *D. aspera* and *T. radiatus*, the *T. brunneus* soft tissue also exhibit high anthelminthic activity than its shell extract. This may be due to the presence of the pyridazine and pyridine derivatives such as 6- (Diphenylphosphoryl)- 3, 4- bis (diiso propylamino)- 5-pyrrolidino pyridazine and 3, 3, 4, 4- Tetracyano- 5, 6- diphenyl- 2- (cyclohexylimino)- 2, 3, 4, 5- tetrahydropyridine. Ali et al. (2011) reported the anthelminthic activity of pyridazine derivatives. Some of the pyridine compounds have been proved as good anthelminthics (Fisher and Lusi, 1972; Bochis et al., 1981 and Novak and Blackburn, 1985). Phillips and Burrows (1961) reported that the pyridine derivatives are highly effective against certain nematode parasites of mammals. Pyridines inhibit many enzymes, especially acetylcholinesterase by phosphorylating esterification sites. This phosphorylation blocks cholinergic nerve transmission in the parasite, resulting in spastic paralysis (Vercruysse and Claerebout, 2014).

Anthelminthic activity exhibited by gastropod extracts in the present study was more or less equivalent to the anthelminthic activity exhibited by commercial drug Albendazole. The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole increases chloride ion conductance of worm muscle membrane and produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Also albendazole has been reported to cause paralysis in worm by disrupting the microfilaments, microtubules and β-tubulins component of their cytoskeletal structure (Nikesh et al., 2011). The extracts of gastropod might have
exerted their anthelminthic effects on the test worms in a similar manner to albendazole.

Generally death of parasite was observed after three attacks of paralysis. Low mortality rate at lower extract concentration and higher mortality at higher extract concentration, in the present study indicate the dose dependent response of helminths against the gastropod extracts. The mechanism of the anthelminthic activity of gastropod extracts could possibly be due to the disruption of the cell membrane permeability of earthworm causing vacuolization and disintegration of the integuments.

Another possible suggestion for the observed paralysis and death of the worms is the binding of the bioactive principles of the extract to glycoproteins on the cuticle of the worms causing disruption of cell membrane integrity. Earlier researchers reported that anthelminthic agents act by binding to the free proteins in the gastrointestinal tract of the host animal or glycoproteins on the cuticle thereby causing disruption of cell membrane integrity and disruption of the metabolic pathways of the worms (Thompson and Geary, 1995; Goodman and Gilman, 2001 and Singh et al., 2002).

**ACARICIDAL ACTIVITY**

Throughout the world tick control is based mainly on the repeated use of chemical acaricides. The current results reported the acaricidal bioactivities of three marine gastropods and indicated that shell and soft tissue extracts showed contact toxicity against the common dog tick *R. sanguineus*. 
Methanolic extracts of *D. aspera* shell and soft tissue were tested for acaricidal activity. Among them, the soft tissue extract exhibited higher activity than the shell extract. The shell extract contain Ethyl 4- (chloromethylene)- 2, 2-diphenyl- 3- oxazoline- 5- carboxylate. The acaricidal activity of oxazoline derivatives were reported by Li *et al.* (2014). The high activity of the soft tissue extract may be due to the presence of 15- Hydroxydehydroabietic acid, methyl ester. Normally, the abietic acids are potentially toxic and inhibit growth (Alvarez-Manzaneda *et al.*, 2006). The shell extract also contain bioactive acaricidal compounds such as Octadecamethyl cyclononasiloxane (Dai *et al.*, 2014).

The results of this study also suggested that the crude methanolic extract of *T. radiatus* soft tissue also highly effective in killing the ticks than its shell extract. The presence of octadecanic acid in the soft tissue extract enhances its acaricidal activity. Romeh (2013) reported that the octadecanic acid from *Ficus sycomorus* L. leaves act as insecticidal and acaricidal agent against certain important insects and mites. Cristina *et al.* (2008) studied the acaricidal activity of *E. cyparissias* extract containing hexadecanic acid and octadecanic acid against *R. microplus*. Chagas *et al.* (2012) studied the *in vitro* efficacy of plant extracts against *R. (Boophilus) microplus*.

In the present study, the *T. brunneus* soft tissue extract showed stronger acaricidal activity than shell extract. The high toxicity of soft tissue extract may due to the presence of bioactive compound such as 6- (Diphenylphosphoryl)- 3, 4 bis (diisopropylamino)- 5- pyrrolidino pyridazine. It possess diverse biological activities includes, herbicides, anti- inflammatory, acaricidal, antiparasitic, insecticidal, antioxidant, analgesic and antimicrobial activity (Wu *et al.*, 2012a).
Ivermectin is a broad spectrum antiparasitic drug in the avermectin family. Field studies have demonstrated that the animals treated with ivermectin supports a significantly reduced diversity of invertebrates and the drug persists for longer durations (Iglesias et al., 2006). The acaricidal activity of *T. radiatus* extract was comparable to that of ivermectin, which showed the best response followed by *D. aspera* and *T. brunneus*. From the result, it could be speculated that gastropod extracts have potent acaricidal action against the brown dog tick.

The three marine gastropod extracts look selective on *R. sanguineus*, although high concentrations were required for high mortality rates of the ticks. The mode of action of the anthelminths and acaricides in marine gastropods are unknown, but the extracts exhibited both anthelminthic and acaricidal properties. Further studies are needed to focus the actual mechanisms of acaricidal activity exhibited by the gastropod extracts against *R. sanguineus*. 