CHAPTER-VII - ANTHELMENTIC ACITIVITY

7.1 INTRODUCTION TO HELMENTHES

Helmentic infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmentic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmentics 1. A large number of medicinal plants are claimed to possess anthelmentic activity in traditional systems of medicine and also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants and isolated phyto principles have been scrutinized for their anthelmentic activity to achieve lead molecules in the search of novel anthelmentic drugs.

7.1.1 Soil-transmitted helmenthiasis 2,3:

Soil-transmitted helmenthiasis (STH) is a group of parasitic infections of the intestine caused by nematode worms usually transmitted by soil. STH is the most prevalent of neglected tropical diseases and is responsible for significant morbidity and, indirectly, mortality in poor developing countries.

STH contributes to general weakness, malnutrition, iron-deficiency anemia, and impaired physical and intellectual development in school-age children.
• **Signs and symptoms:** The common symptoms of STH are: Abdominal pain and enlargement, anemia, weight loss, malnutrition and loss of appetite.

• **Effects:** The important side effects of soil transmitted helminthiasis in children are
  o Decreased physical development decreased physical activities, decreased mental growth in children.

• **Mode of transmission:**
  o The Fecal - oral route for Ascaris, Trichuris and Hookworm *(Necator americanus).*
  o The skin penetration for hookworms *(Necator americanus* and *Ancylostoma duodenale).*

• **Diagnosis:**
  o The kato Katz technique- determines the intensity of infection.
  o The direct fecal smear.
  o The concentration method.

• **Prevention:**
  o Maintenance of good personal hygiene like washing hands before eating and after using the toilet, clean and safe preparation of food, use of slippers or shoes, use of proper toilet facilities, providing of proper environmental sanitation facilities.
Soil-transmitted helminthiasis, commonly known as infections with intestinal worms, are the most common infections worldwide affecting the most deprived communities. The three main causative worms of soil-transmitted helminthiasis of public health importance are:

a) **Whipworm:**

The human whipworm (*Trichuris trichiura* [T. trichiura] or *Trichocephalus trichiuris*), is the third most common roundworm found in humans. The name "whipworm" refers to the shape of the worm; the worms look like whips with wider "handles" at the posterior end. There is an estimated 800 million people infected worldwide. It is also highly prevalent in children. Co-infection of whipworm with *Giardia, Entamoeba histolytica, Ascaris lumbricoides*, and hookworm is common.

Symptoms range from asymptomatic through vague digestive tract distress for light infestations to emaciation with dry skin and diarrhea (usually mucoid and/or bloody) for heavy infestations. In children, heavy infections could lead to growth retardation. Additionally, long- standing bloody diarrhea could lead to iron-deficiency anemia in many individuals. In the most severe cases, individuals could experience rectal prolapse.

The finger clubbing is the best clinical predictor of the intensity of infection. Infection can be avoided by proper disposal of human feces, not eating dirt, and not eating crops fertilized with night soil.

Whipworm infestation is detectable by stool examination, which can detect eggs and charcot-leyden crystals. The Kato-Katz is the technique of choice for diagnosis and quantification of infection with *T. trichiura*. Adult worms may be seen in a prolapsed rectal mucosa.
b) **Hookworm**

Human hookworm infection is a soil-transmitted helminthiasis infection caused by nematode parasites *Necator americanus* (*N. americanus*), *Ancylostoma duodenale* (*A. duodenale*), or both. Mild infections with hookworm cause mild diarrhea and abdominal pain. More severe infections with hookworm can create serious health problems for newborns, children, pregnant women, and persons who are malnourished. Hookworm infection is the leading cause of anemia and protein malnutrition in developing nations, afflicting an estimated 740 million people.

Hookworm infection may be associated with dermatitis, eosinophilia, pulmonary infiltrates, pneumonitis, and urticarial rash. Gastrointestinal symptoms would include mild abdominal pain, nausea, vomiting, and anorexia. Iron-deficiency anemia due to blood loss is often associated with hookworm infestation. Cutaneous larva migrans (CLM) condition can be found in humans infected with animal hookworms. Increased maternal and neonatal mortality have been found to be associated with hookworm iron-deficiency anemia.

In general, CLM is clinically diagnosed. Hookworm infection definitive diagnostic is established by identifying hookworm eggs in feces under light microscopy. In the case of humans infected by some animal hookworms, definitive diagnostic is based on the identification of parasite by endoscopy. Regiments with Mebendazole and Albendazole are currently the treatment of choice for adult hookworms. Associated-iron deficiency anemia should be detected and treated adequately.
c) **Round worm (Ascaris):**

*Ascariasis*, one of the most common human helmentic infections, is caused by the intestinal parasite *Ascaris lumbricoides* (*A. lumbricoides* [large roundworm], affecting an estimated one billion persons at any one time worldwide. It affects 50 percent of populations in tropical and subtropical areas. Globally, *Ascariasis* causes an estimated 20,000 deaths per year.

The clinical effects include a wide range of manifestations. Most potential and common complications comprise pneumonitis due to passage of worms in the lungs, with pulmonary eosinophilia (Loeffler’s syndrome); intestinal obstruction by mass of worms; biliary obstruction and pancreatic obstruction by worms. Infection with *A. lumbricoides* may contribute substantially to child morbidity when associated with malnutrition, pneumonia, enteric diseases and vitamin A deficiency. *Ascariasis* adversely affects children's growth and development.

Ultrasonography and radiology are the most appropriate tools to diagnose of intestinal and biliary obstruction due to *A. lumbricoides* as well as to detect other abdominal localization of the worms. Mebendazole or Albendazole are currently the drugs of choice to treat adult worms.

### 7.1.2 Anti-Helmenthic Drug Therapy:

Anthelmentic drugs are to treat these infestations functions either by destroying the worms on contact or by paralyzing them, or by altering the permeability of their plasma membranes. The dead worms then pass out of the body in the feces. Anthelmentic drugs are medicines that rid the body of parasitic worms. Each type of anthelmentic
drug is effective against particular kinds of worms. For example, Niclosamide is effective against tapeworms, but will not work for treating pinworm or roundworm infestations. Antihelmentic drugs are available only with a physician's prescription. They are sold as liquids and tablets (regular and chewable). Commonly used antihelmentsics are Mebendazole (Vermox), Niclosamide (Niclocide), Praziquantel (Biltricide), Pyrantel (Antiminth), and Thiabendazole (Mintezol).

Table 7.01: List of some synthetic anthelmentic drugs.

<table>
<thead>
<tr>
<th>Worm</th>
<th>First choice drugs</th>
<th>Alternative drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round worms</td>
<td>Mebendazole, Albendazole</td>
<td>Piperazine, Levamisole</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>Pyrantel</td>
<td></td>
</tr>
<tr>
<td>Hook worm</td>
<td>Pyrantel, Mebendazole, Albendazole</td>
<td>Levamisole</td>
</tr>
<tr>
<td><em>Ancylostoma duodenale</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thread worm</td>
<td>Pyrantel, Mebendazole</td>
<td>Piperazine</td>
</tr>
<tr>
<td><em>Enterobius vermicularis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>Ivermectin</td>
<td>Albendazole</td>
</tr>
<tr>
<td>Whipworm</td>
<td>Mebendazole</td>
<td>Albendazole</td>
</tr>
<tr>
<td><em>Trichuris trichura</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>Albendazole</td>
<td>Mebendazole</td>
</tr>
<tr>
<td>Filarial worm</td>
<td>Diethylcarbamazine, Ivermectin</td>
<td>Albendazole</td>
</tr>
<tr>
<td><em>Wuchereria bancrofti</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea worm</td>
<td>Metronidazole</td>
<td></td>
</tr>
<tr>
<td><em>Dracunculus medinensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapeworm</td>
<td>Praziquantel, Niclosamide</td>
<td>Albendazole</td>
</tr>
<tr>
<td><em>Taenia solium</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydatid disease</td>
<td>Albendazole</td>
<td>Mebendazole</td>
</tr>
<tr>
<td><em>Echinococcus granulosus</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7.1.3 *Pheretima Posthuma*:

Earthworm is the common name for the largest members of Oligochaeta in the phylum Annelida. *Pheretima* is placed in the suborder Lumbricina of the order Haplotaxida, Folk names for the earthworm include "dew-worm", "rainworm", "night crawler" and "angleworm" (due to its use as fishing bait).

Earthworms are also called megadriles (or big worms), as opposed to the microdriles (or small worms) in the family’s Tubificidae, Lumbriculidae, and Enchytraeidae, among others. The megadriles are characterized by having a distinct clitellum and a vascular system with true capillaries.

**Anatomy:**

The basic body plan of an earthworm is a tube, with in a tube, the muscular slimy, moist outer body. The body is annular, formed of segments that are most specialized in the anterior. Earthworms have a simple circulatory system. They have two main blood vessels that extend through the length of their body: A ventral blood vessel which leads the blood to the posterior end, and a dorsal blood vessel which leads to the anterior end. The blood is distributed from the ventral vessel into capillaries on the body wall and other organs and into a vascular sinus in the gut wall, where gases and nutrients are exchanged. This arrangement may be complicated in the various groups by suboesophageal, supraoesophageal, parietal and neural vessels, but the basic arrangement holds in all earthworms.

**Locomotion:**

Earthworms travel underground by the means of waves of muscular contraction which alternately shorten and lengthen the body. The shortened part is anchored to the
surrounding soil by tiny claw-like bristles (setae) set along its segmented length. (In all the body segments except the first, last and clitellum, there is ring of S-shaped setae, embedded in the epidermal pit of each segment, perichaetine). The whole burrowing process is aided by the secretion of lubrication mucus. Worms can make gurgling noises underground when disturbed as a result of the worm moving through its lubricated tunnels. Thus earthworm activity aerates and mixes the soil, and is constructive to mineralization and nutrient uptake by vegetation.

- **Special anatomical features:**

  The soft & necked body of Earthworm is metamerically divided into a series of about 100 to 120 segments. The external transverse groove of the body corresponds to the internal segmentation, by septum. The earthworm has no distonet head. The anterior first segment is known as peristomium. On the ventral side of Peristomium, there is a semilunar aperture, the mouth. The peristomium is prolonged anteriorly into a fleshy lobe, known as prosotomium, which overhangs the mouth. In a mature Earthworm, the 14th, 15th & 16th segments do not show any segmentation but completely & permanently surrounded by a thick & distinct band of glandular tissue which is called as clitellum. This offers the formation of cocoon or egg-capule. The body of Earthworm can be region & clitellum to last segment is the post-clitellar region. About the middle of each segment there is a hing of tiny curved, rod-shaped, chitineous bristles called as setae are the main locomontory organs. Setae are not found in first segment, clitellum (14, 15 &16 segments) & the last segment.
The different apertures present in the body of an Earthworm are:\(^ {12} \):

Mouth is a crescentric anterior aperture lying on the ventral side of pheristomium overhung by the prostomium. Anus is a median circular aperture situated terminally at the last anal segment. Female Genital Pores are a pair of crescentric apertures of spermathecae in the grooves of 5/6, 6/7, 7/8 & 8/9 segments, one on each side in ventro-lateral position. Male Genital Pores are a pair of crescentric apertures of the common prostatic & spermatic ducts open on the ventral surface of the 18\(^{th} \) segment, one on each side.

7.2 MATERIALS AND METHODS:

Adult Indian earth worms (*Pheretima Posthuma*)

Albendazole – standard (10mg/ml)

5% aq. Dimethyl fluoride (DMF)

A.S.H.E  - *Annona squamosa* Hexane extract (100,200 mg/ml)

A.S.E.A.E  - *Annona squamosa* Ethyl acetate extract (100,200 mg/ml)

A.S.E.E  - *Annona squamosa* Ethanol extract (100,200 mg/ml)

A.R.H.E  - *Annona reticulata* Hexane extract (100,200 mg/ml)

A.R.E.A.E  - *Annona reticulata* Ethyl acetate extract (100,200 mg/ml)

A.R.E.E  - *Annona reticulata* Ethanol extract (100,200 mg/ml)

A.M.H.E  - *Annona muricata* Hexane extract (100,200 mg/ml)
A.M.E.A.E - *Annona muricata* Ethyl acetate extract (100,200 mg/ml)

A.M.E.E - *Annona muricata* Ethanolic extract. (100,200 mg/ml)

### 7.3 EXPERIMENTAL WORK:

Evaluation of Anthelmentic activity:


**Worm collection and Authentication:** Adult earthworms (*Pheretima Posthuma*), were used to evaluate anthelmentic activity *In vitro*. The Indian earthworm *Pheretima posthuma* was collected from the S.V.University, Zoolgy department, Tirupati. The average size of earthworm was 8- 10 cm and was authenticated by Dr. P. K. Sanyal, Head, Dept of Parasitology, College of Veterinary Sciences and Animal husbandry.

![Fig:7.01 Pheretima Posthuma](image1)

![Fig:7.02 : stored earth worms in tyrode](image2)

(Indian earth worm)

The different concentrations (100 mg/ml, 200 mg/ml) of selected plant extracts *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn were prepared by triturating the sample in 5% aqueous DMF. Albendazole at a dose of 10mg/ml was
prepared using 5% aqueous DMF. Control was maintained using 5% aqueous DMF. All the test solution and standard drug solution were prepared freshly before starting the experiments.

Six petridishes of equal size were taken for each group and labeled as control, standard (Albendazole10mg/ml) test extracts of Test group-III A.S.H.E-50mg/ml, A.S.H.E-100mg/ml, A.S.E.A.E -50mg/ml, A.S.E.A.E -100mg/ml, A.S.E.E-50mg/ml,A.S.E.E-100mg/ml and numbered. 50ml of formulation of different concentrations of the extracts were placed in six petridishes of each group. Control was maintained in one petridish. All petridishes were placed at room temperature.

**Table: 7.02 The protocol study of Anthelmentic activity of leaf extracts of *Annona squamosa* Linn on earth worms (*Pheretima posthumas*)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Aqueous 5% DMF</td>
</tr>
<tr>
<td>Standard group</td>
<td>Albendazole in 5% Aqueous DMF</td>
</tr>
<tr>
<td>Test group-III</td>
<td>A.S.H.E -100mg/ml in 5% Aqueous DMF.</td>
</tr>
<tr>
<td>Test group-IV</td>
<td>A.S.H.E -200mg/ml in 5% Aqueous DMF.</td>
</tr>
<tr>
<td>Test group-V</td>
<td>A.S.E.A.E - 100mg/ml in 5% Aqueous DMF.</td>
</tr>
<tr>
<td>Test group-VI</td>
<td>A.S.E.A.E -200mg/ml in 5% Aqueous DMF.</td>
</tr>
<tr>
<td>Test group-VII</td>
<td>A.S.E.E -100mg/ml in 5% Aqueous DMF.</td>
</tr>
<tr>
<td>Test group-VIII</td>
<td>A.S.E.E -200mg/ml in 5% Aqueous DMF.</td>
</tr>
</tbody>
</table>
Test group- IX  A.R.H.E -100mg/ml in 5% Aqueous DMF.
Test group-X  A.R.H.E -200mg/ml in 5% Aqueous DMF.
Test group-XI  A.R.E.A.E -100mg/ml in 5% Aqueous DMF.
Test group-XII  A.R.E.A.E -200mg/ml in 5% Aqueous DMF.
Test group-XIII  A.R.E.E -100mg/ml in 5% Aqueous DMF.
Test group-XIV  A.R.E.E -200mg/ml in 5% Aqueous DMF.
Test group-XV  A.M.H.E -100mg/ml in 5% Aqueous DMF.
Test group-XVI  A.M.H.E -200mg/ml in 5% Aqueous DMF.
Test group-XVII  A.M.E.A.E -100mg/ml in 5% Aqueous DMF.
Test group-XVIII  A.M.E.A.E -200mg/ml in 5% Aqueous DMF.
Test group-XIX  A.M.E.E -100mg/ml in 5% Aqueous DMF.
Test group-XX  A.M.E.E -200mg/ml in 5% Aqueous DMF.

7.3.1 Analysis:

Observations were made for the time taken for paralysis was noted when no movement or loss of movement\textsuperscript{16} (Not retrieve even in normal saline). Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water and fading away the colour of worm. All the results are shown in Table-7.03--7.05, and expressed as a mean of the selected worms in each group.
7.4 RESULTS AND DISCUSSION:

The anthelmentic activity was screened by taking *Pheretima Posthuma* as experimental model. The extracts were showing dose dependent anthelmentic activity. The anthelmentic activity was performed on the Indian earth worms (*Pheretima Posthuma*) and the results are reported in Table: 7.03 for the leaf extracts of *Annona squamosa* Linn. Table: 7.04 for the leaf extracts of *Annona reticulata* Linn. Table: 7.05 for the leaf extracts of *Annona muricata* Linn. In all the three plants Ethylacetate extract has shown highly significant action as compare to other two extracts.

Earth worms present in Control group has not shown any paralysis or death even after a period of 24 h.

Being a standard drug albendazole has shown very high significant Anthelmentic activity, paralysis was found to be 2.55±0.76mins , and death time was found to be 4.37±0.66 min.

A.S.H.E at a dose of 100mg/ml concentration has shown 25.56±0.56 mins paralysis time and death time is 27.62±0.14.

A.S.H.E at a dose of 200mg/ml concentration has shown 21.43±0.64 mins paralysis time and death time is 23.11±0.26

A.S.E.A.E at a dose of 100mg/ml concentration has shown 4.27±0.02 mins paralysis time and death time is 6.43±0.07.

A.S.E.A.E at a dose of 200mg/ml concentration has shown 2.19±0.11 mins paralysis time and death time is 4.11±0.09.
A.S.E.E at a dose of 100mg/ml concentration has shown 12.43±0.088 mins paralysis time and death time is 16.12±0.45.

A.S.E.E at a dose of 200mg/ml concentration has shown 9.16±0.066 mins paralysis time and death time is 12.17±0.16.

A.R.H.E at a dose of 100mg/ml concentration has shown 12.53±0.16 mins paralysis time and death time is 14.16±0.05.

A.R.H.E at a dose of 200mg/ml concentration has shown 10.43±0.11 mins paralysis time and death time is 13.19±0.25.

A.R.E.A.E at a dose of 100mg/ml concentration has shown 4.55±0.23 mins paralysis time and death time is 6.53±0.22.

A.R.E.A.E at a dose of 200mg/ml concentration has shown 2.12±0.54 mins paralysis time and death time is 4.11±0.03.

A.R.E.E at a dose of 100mg/ml concentration has shown 5.41±0.01 mins paralysis time and death time is 7.23±0.41.

A.R.E.E at a dose of 200mg/ml concentration has shown 3.12±0.04 mins paralysis time and death time is 5.41±0.03.

A.M.H.E at a dose of 100mg/ml concentration has shown 11.42±0.03 mins paralysis time and death time is 14.53±0.09.

A.M.H.E at a dose of 200mg/ml concentration has shown 9.34±0.41 mins paralysis time and death time is 12.44±0.17.
A.M.E.A.E at a dose of 100mg/ml concentration has shown 5.76± 0.42 mins paralysis time and death time is 6.12± 0.12.

A.M.E.A.E at a dose of 200mg/ml concentration has shown 3.41± 0.11 mins paralysis time and death time is 5.41± 0.65.

A.M.E.E at a dose of 100mg/ml concentration has shown 10.44± 0.46 mins paralysis time and death time is 12.41± 0.31.

A.M.E.E at a dose of 200mg/ml concentration has shown 7.31± 0.22 mins paralysis time and death time is 10.32± 0.18.

Further results can be supported by the graphical representation given in (Graph 7.01 to 7.02).

Table :7.03 Anthelmentic effects of *Annona squamosa* Linn leaf extracts on Indian earth worms (*Pheretima Posthuma*)

<table>
<thead>
<tr>
<th>s.no</th>
<th>Treatment</th>
<th>Paralysis time</th>
<th>Death time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole- 10mg/ml</td>
<td>2.55±0.76</td>
<td>4.37±0.66</td>
</tr>
<tr>
<td>3</td>
<td>A.S.H.E -100mg/ml</td>
<td>25.56±0.56</td>
<td>27.62±0.14</td>
</tr>
<tr>
<td>4</td>
<td>A.S.H.E -200mg/ml</td>
<td>21.43±0.64</td>
<td>23.11±0.26</td>
</tr>
<tr>
<td>5</td>
<td>A.S.E.A.E of -100mg/ml</td>
<td>4.27±0.02</td>
<td>6.43±0.07</td>
</tr>
<tr>
<td>6</td>
<td>A.S.E.A.E -200mg/ml</td>
<td>2.19±0.11</td>
<td>4.11±0.09</td>
</tr>
<tr>
<td>7</td>
<td>A.S.E.E -100mg/ml</td>
<td>12.43±0.88</td>
<td>16.12±0.45</td>
</tr>
<tr>
<td>8</td>
<td>A.S.E.E -200mg/ml</td>
<td>9.16±0.66</td>
<td>12.17±0.16</td>
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</tbody>
</table>

Results are expressed as Mean ± SEM, n=6, student ‘t’ test vs control
Table 7.04 Anthelmentic effects of *Annona reticulata* Linn leaf extracts on Indian earthworms (*Pheretima Posthuma*)

Results are expressed as Mean ± SEM, n=6, student ‘t’ test vs control

<table>
<thead>
<tr>
<th>s.no</th>
<th>Treatment</th>
<th>Paralysis time</th>
<th>Death time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole</td>
<td>2.55±0.76</td>
<td>4.37±0.66</td>
</tr>
<tr>
<td>3</td>
<td>A.S.H.E -100mg/ml</td>
<td>12.53±0.16</td>
<td>14.16±0.05</td>
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<tr>
<td>4</td>
<td>A.S.H.E -200mg/ml</td>
<td>10.43±0.11</td>
<td>13.19±0.25</td>
</tr>
<tr>
<td>5</td>
<td>A.S.E.A.E of -100mg/ml</td>
<td>4.55±0.23</td>
<td>6.53±0.22</td>
</tr>
<tr>
<td>6</td>
<td>A.S.E.A.E -200mg/ml</td>
<td>2.12±0.54</td>
<td>4.11±0.03</td>
</tr>
<tr>
<td>7</td>
<td>A.S.E.E -100mg/ml</td>
<td>5.41±0.01</td>
<td>7.23±0.41</td>
</tr>
<tr>
<td>8</td>
<td>A.S.E.E -200mg/ml</td>
<td>3.12±0.04</td>
<td>5.41±0.03</td>
</tr>
</tbody>
</table>

Table 7.05 Anthelmentic effects of *Annona muricata* Linn leaf extracts on Indian earthworms (*Pheretima Posthuma*)

Results are expressed as Mean ± SEM, n=6, student ‘t’ test vs control

<table>
<thead>
<tr>
<th>s.no</th>
<th>Treatment</th>
<th>Paralysis time</th>
<th>Death time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
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<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albendazole</td>
<td>2.55±0.76</td>
<td>4.37±0.66</td>
</tr>
<tr>
<td>3</td>
<td>A.S.H.E -100mg/ml</td>
<td>11.42±0.03</td>
<td>14.53±0.09</td>
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<tr>
<td>4</td>
<td>A.S.H.E -200mg/ml</td>
<td>9.34±0.41</td>
<td>12.44±0.17</td>
</tr>
<tr>
<td>5</td>
<td>A.S.E.A.E of -100mg/ml</td>
<td>5.76±0.42</td>
<td>6.12±0.12</td>
</tr>
<tr>
<td>6</td>
<td>A.S.E.A.E -200mg/ml</td>
<td>3.41±0.11</td>
<td>5.41±0.65</td>
</tr>
<tr>
<td>7</td>
<td>A.S.E.E -100mg/ml</td>
<td>10.44±0.46</td>
<td>12.41±0.31</td>
</tr>
<tr>
<td>8</td>
<td>A.S.E.E -200mg/ml</td>
<td>7.31±0.22</td>
<td>10.32±0.18</td>
</tr>
</tbody>
</table>
Graph: 7.01 Graphical representation of *Annona squamosa* Linn leaf extracts Anthelmintic action

Graph: 7.02 Graphical representation of *Annona reticulata* Linn leaf extracts Anthelmintic action
Graph: 7.03 Graphical representation of *Annona muricata* Linn leaf extracts Anthelmentic action

7.5 CONCLUSION:

From the results we conclude that traditional usage of Annonaceae family plants for treating helmentic infection has been validated. From the results we conclude that the extracts were exhibiting dose dependent action, higher is the concentration of the extract less time is taken for death of the organism. As less is the time taken for paralysis and death of the *phbritima posthuma* higher is activity. The order of activity was found to Albendazole > A.R.E.A.E -200mg/ml > A.S.E.A.E -200mg/ml > A.M.E.A.E- 200mg/ml > A.S.E.A.E-100mg/ml > A.R.E.A.E-100mg/ml > A.M.E.A.E-100mg/ml > A.R.E.E 200mg/ml > A.R.E.E-100mg/ml > A.M.E.E-200 mg/ml > A.S.E.E-200 mg/ml > A.M.E.E-100 mg/ml > A.S.E.E-100mg/ml. action shown by the ethyl acetate extracts of the all the three plants were almost equal with that of the standard drug Albendazole. Flavanoids
present in ethyl acetate extract could be responsible for the highly significant action against helmenthes\textsuperscript{17}.

7. 6 REFERENCES:


8) Rouse G. The Annelida and their close relatives in Anderson D.T. \textit{Invertebrate Zoology}. 4\textsuperscript{th} Edn, Oxford University Press. 2007:186.


CHAPTER-VIII SUMMARY AND CONCLUSION

The thesis represents the results of the Pharmacognostical, Gastro protective, Antioxidant and Anthelmintic studies on the three selected Annonaceae plants, is a study of Pharmacognostical characterization, pharmacological screening of its gastroprotective, in correlation with In vitro antioxidant studies and anthelmintic studies of Annona squamosa (Annonaceae), Annona reticulata (Annonaceae) and Annona muricata (Annonaceae) which are having traditional usage for curing liver disorders, Gastro intestinal infections, for microbial infection, lice killing, helmientic infestations in animals, for curing inflammatory diseases etc.

Chapter- I

This chapter was divided into two parts

In first part of the chapter, the author described the general introduction to the plants and natural products obtained from various sources and a brief review on the species utilized by humans given by graphical representation of the percentage of medicinal plant used in various herbal treatments. Medicinal plants already proved for therapy were also described in this chapter.

In second part of the chapter author described about the aim, objective and plan of work.

CHAPTER-II

In this chapter author carried a study on Annonaceae family plants, various genera comes under the Annonaceae family were selected, depending upon folklore usages, plants belonging to Annona genus were selected for the study are Annona squamosa
Linn, *Annona reticulata* Linn, *Annona muricata* Linn. A brief literature review on the selected plants was given in this chapter.

**CHAPTER-III**

This chapter describes about the collection, authetification and extraction of the selected plants with schematic representation of the extraction process. Pharmacognostic parameters were studied to identify the specific characters present in the selected plants. Microscopical characters were found through transverse section and powder microscopic studies. The results revealed that identifying characters like unicellular head, unicellular stalk glandular trichomes, unicellular covering trichomes, multicellular covering trichomes, paracytic stomata, prism shape, rosette shape calcium oxalate crystals, spiral, pitted vascular bundles. Quantitative microscopy like leaf constants like stomatal index, stomatal number, palisade ratio, veinislet number, vein termination number were determined, which can be another identifying tool for Annonaceae family plants. Physicochemical characterization like Foreign organic matter, Ash value, Swelling index, Foaming index, Extractive values were determined for the selected plants.

**CHAPTER- IV**

This chapter deals with the phytochemical testing of the obtained extracts for the identification of the metabolites which revealed the presence of phytosterols, glycosides, flavonoids, alkaloids, phenolic compounds.
CHAPTER-V

This chapter is divided into two parts

First part describes about the preliminary pharmacological screening of selected plant extract on adult Swiss albino mice.

The experimental protocol was approved by the Institutional animal ethical committee and by the regulatory body of the government (Regd No: 516/01/A/CPCSEA).

Acute toxicity studies were performed for the extracts of selected three Annonaceae plants according to the toxic classic method as per guidelines 423 prescribed by OECD. None of these extracts showed mortality even at dose of 2000mg/kg and therefore considered safe.

The doses selected for the extracts of Annona squamosa Linn, Annona reticulata Linn, Annona muricata Linn were about 1/10th and 1/20th of the maximum tolerated safe dose found from acute toxicity studies i.e., 2000mg/kg b.w.

Second part deals with the gastroprotective activity screening on wistar albino rats using pyloric ligation method. Omeprazole 20mg/kg b.w is taken as a standard drug for comparing test extracts towards ulcer healing action. Volume of acid secreted, pH, Total acidity, Free acidity were tested and results were supported by photomicrographs of the animals stomach and by histopathological studies.

The comparative efficacy of the extracts tested for their gastroprotective acivity, and the relationship between dose and other gastric parameters were depicted in the form of a bar diagram as shown in (Graphs 5.01- 5.12).
As pH increases acidity decreases and protection increases towards the ulcer. The standard group treated with Omeprazole (20mg/kg b.w) drug significantly increased the pH, from 1.1±0.07 to 5.1±0.09 when compare with the control group showed regeneration and prevents formation of hemorrhagic condition of stomach.

pH shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be (1.9±0.23, 2.07±0.54) respectively.

pH shown by the A.S.E.A.E treated group (100, 200mg/kg b.w) was found to be (4.1±0.65, 4.8±0.54) respectively.

pH shown by the A.S.E.E treated group (100, 200mg/kg b.w) was found to be (2.8±0.21, 3.9±0.11) respectively.

A.R.H.E treated group (100, 200mg/kg b.w) exhibited pH of (2.02±0.56, 2.9±0.02) respectively.

A.R.E.A.E treated group (100, 200mg/kg b.w) exhibited pH of (3.8±0.71, 4.6±0.06) respectively.

A.R.E.E treated group (100, 200mg/kg b.w) exhibited pH of (3.21±0.16, 4.0±0.08) respectively.

A.M.H.E treated group at a dose of 100, 200mg/kg b.w exhibited pH of (1.9±0.23, 2.7±0.04) respectively.

A.M.E.A.E treated group at a dose of 100, 200mg/kg b.w exhibited pH of (4.1±0.01, 4.7±0.09) respectively.

A.M.H.E treated group at a dose of 100,200mg/kg b.w exhibited pH of (3.3±0.52, 3.9±0.06) respectively.
As there is increase in the total acidity and free acidity content the chances of ulcer formation will also be high.

Total acidity exhibited by the standard group was significantly less 30.4±2.6 when compared to the total acidity exhibited by the control group 96.7±2.5.

Total acidity shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be (91.2±0.51, 85.2±4.6) respectively.

Total acidity shown by the A.S.E.A.E treated group (100, 200 mg/kg b.w) was found to be (45.2±0.55, 39.1±0.23) respectively.

Total acidity shown by the A.S.E.E treated group (100,200mg/kg b.w) was found to be (65.9±0.45, 59.5±0.12) respectively.

Total acidity shown by the A.R.H.E treated group (100,200mg/kg b.w) was found to be (79.4±3.6, 49.6±3.4) respectively.

Total acidity shown by the A.R.E.A.E treated group (100,200mg/kg b.w) was found to be (39.6±1.92, 31.4±2.1) respectively.

Total acidity shown by the A.R.E.E treated group (100,200mg/kg b.w) was found to be (61.4±4.5, 39.5±2.9) respectively.

Total acidity shown by the A.M.H.E treated group (100,200mg/kg b.w) was found to be (82.1±1.4, 50.2±3.3) respectively.

Total acidity shown by the A.M.E.A.E treated group (100,200mg/kg b.w) was found to be (43.4±1.8, 33.3±2.1) respectively.

Total acidity shown by the A.M.E.E treated group (100,200mg/kg b.w) was found to be (63.2±3.8, 41.1±3.1) respectively.
Ulcerc index is the index which indicates the severity of ulcers. Increase in the ulcer index more is the severe condition of ulcer.

Standard drug Omeprazole treated group showed ulcer index of 0.18±0.04 which is significantly less as compare to the ulcer index of control group 0.65±0.06.

Ulcer index shown by the A.S.H.E treated group (100, 200mg/kg b.w) was found to be (0.31±0.54, 0.28±0.01) respectively.

Ulcer index shown by the A.S.E.A.E treated group (100, 200mg/kg b.w) was found to be (0.25±0.11, 0.19±0.06) respectively.

Ulcer index shown by the A.S.E.E treated group (100, 200mg/kg b.w) was found to be (0.26±0.06, 0.23±0.05) respectively.

Ulcer index shown by the A.R.H.E treated group (100, 200mg/kg b.w) was found to be (0.45±0.54, 0.36±0.01) respectively.

Ulcer index shown by the A.R.E.A.E treated group (100, 200mg/kg b.w) was found to be (0.24±0.15, 0.21±0.45) respectively.

Ulcer index shown by the A.R.E.E treated group (100, 200mg/kg b.w) was found to be (0.36±0.15, 0.30±0.05) respectively.

Ulcer index shown by the A.M.H.E treated group (100,200mg/kg b.w) was found to be (0.35±0.09, 0.29±0.05) respectively.

Ulcer index shown by the A.M.E.A.E treated group (100,200mg/kg b.w) was found to be (0.26±0.12, 0.20±0.05) respectively.

Ulcer index shown by the A.M.E.E treated group (100, 200mg/kg b.w) was found to be (0.28±0.03, 0.25±0.04) respectively.
Standard drug Omeprazole treated group has shown the percentage protection of 72%.

Whereas, A.S.H.E at a dose of 100,200mg/kg b.w has shown percentage protection of 52%, 56% respectively.

A.S.E.A.E at a dose of 100,200mg/kg b.w has shown percentage protection of 61%, 70% respectively.

A.S.E.E at a dose of 100, 200mg/kg b.w has shown protection of 60%, 64% respectively.

A.R.H.E at a dose of 100, 200mg/kg b.w has shown protection of 30%,44% respectively.

A.R.E.A.E at a dose of 100, 200mg/kg b.w has shown protection of 63%, 67% respectively.

Whereas, A.R.E.E at a dose of 100,200mg/kg b.w has shown percentage protection of 44%, 53% respectively.

A.M.H.E at a dose of 100,200mg/kg b.w has shown protection of 46%, 55% respectively.

A.M.E.A.E at a dose of 100,200mg/kg b.w has shown protection of 60%, 69% respectively.

Whereas, A.M.E.E at a dose of 100,200mg/kg b.w has shown protection of 56%, 61% respectively.

Photographs of rats stomach treated with selected plant extracts were given in the Fig:5.06-5.08
Histopathological changes on pylorus ligation model showed the degeneration, hemorrhage, edematous appearance of the gastric tissue in control group, whereas ethyl acetate at a dose of 200mg, 100mg/kg b.w and Omeprazole (20 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in (Fig:5a,5b,5c).

The gastric tissue of ethyl acetate, ethanol treated group in all three selected plants showed regains of cellular structure, less hemorrhage condition, and with less edema which is almost equal to that of the standard group, exhibiting high significant action. whereas, in hexane treated groups it was not showing significant effect and it exhibits high degeneration of tissue, red patches, lessions, hemorrhage, edema condition was observed at a higher dose of hexane extract treated group shows less hemorrhagic condition.

Gastroprotective action of certain phytoconstituents like flavonoids, alkaloids, Tannins have been well documented in the literature. The phytoconstituents alone or in combination may be responsible for the gastroprotective activity of the selected plants.

**CHAPTER-VI:**

This chapter is divided into two parts

The first part deals with the introduction to antioxidants, types of antioxidants, their mechanism, therapeutic uses of antioxidants in treating various diseases.

The second part deals with the experimental procedure for *In vitro* antioxidant activity of the extracts of the selected *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn and known antioxidant Ascorbic acid used as a standard in scavenging free radicals of DPPH, Nitric oxide, hydroxyl radical and super oxide radical,
followed by their results and discussion. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner.

In DPPH Model (Graph: 6.13) the mean concentration of Ascorbic acid for 50% inhibition ($IC_{50}$) was found to be 65 µg/ml.

A.S.H.E needed for 50% inhibition was found to be, 496 µg/ml where as A.S.E.A.E, A.S.E.E were found to be 78.5, 396 µg/ml respectively.

The concentration of A.R.H.E needed for 50% inhibition was found to be 497, µg/ml where as A.R.E.A.E, A.R.E.E were found to be 74, 115 µg/ml respectively.

The concentration of A.M.H.E needed for 50% inhibition was found to be 495 µg/ml where as A.M.E.A.E, A.M.E.E were found to be 72, 94 µg/ml respectively.

In NO Model (Graph: 6.14) mean concentration of Ascorbic acid for 50% inhibition ($IC_{50}$) was found to be 66 µg/ml.

A.S.H.E needed for 50% inhibition was found to be, 491µg/ml where as A.S.E.A.E, A.S.E.E were found to be 82, 99.95 µg/ml respectively.

The concentration of A.R.H.E needed for 50% inhibition was found to be 495 µg/ml where as A.R.E.A.E, A.R.E.E were found to be 73, 82 µg/ml respectively.

The concentration of A.M.H.E needed for 50% inhibition was found to be 498 µg/ml where as A.M.E.A.E, A.M.E.E were found to be 71.2, 74.98 µg/ml respectively.

In hydroxyl radical scavenging model (Graph: 6.15) mean concentration of Ascorbic acid for 50% inhibition ($IC_{50}$) was found to be 81 µg/ml.
A.S.H.E needed for 50% inhibition was found to be, 500µg/ml where as A.S.E.A.E, A.S.E.E were found to be 100,396 µg/ml respectively.

The concentration of A.R.H.E needed for 50% inhibition was found to be 500 µg/ml where as A.R.E.A.E, A.R.E.E were found to be 95,496 µg/ml respectively.

The concentration of A.M.H.E needed for 50% inhibition was found to be 498 µg/ml where as A.M.E.A.E, A.M.E.E were found to be 82,396 µg/ml respectively.

In superoxide radical scavenging model (Graph: 6.15) mean concentration of Ascorbic acid for 50% inhibition (IC$_{50}$) was found to be 160 µg/ml.

A.S.H.E needed for 50% inhibition was found to be, 497 µg/ml where as A.S.E.A.E, A.S.E.E were found to be 394,506 µg/ml respectively.

The concentration of A.R.H.E needed for 50% inhibition was found to be 499 µg/ml where as A.R.E.A.E, A.R.E.E were found to be 400,480 µg/ml respectively.

The concentration of A.M.H.E needed for 50% inhibition was found to be 499.9 µg/ml where as A.M.E.A.E, A.M.E.E were found to be 394,297 µg/ml respectively.

In DPPH model the free radical scavenging capacity was found to be highly significant when compared to other three models. In all the three selected plants ethyl acetate extract was found to have high scavenging activity than ethanolic, hexane extracts. Scavenging activity of ethyl acetate extracts may be due to presence of the flavonoids$^{20}$, phenolics$^{21}$. 
CHAPTER-VII

This chapter deals with the anthelmentic study of the selected leaf extracts were taken in 2 different doses of 100mg/ml and 200mg/ml on adult earth worm *Pheritima posthumas* using Albendazole as a standard drug at a dose of 10mg/ml concentration from the results it was found that all the plant extracts were exhibiting dose dependent activity. In all the three plants Ethyl acetate extract has shown highly significant action as compared to other two extracts.

The order of activity was found to Albendazole > A.R.E.A.E. -200mg/ml > A.S.E.A.E -200mg/ml > A.M.E.A.E - 200mg/ml > A.S.E.A.E-100mg/ml > A.R.E.A.E-100mg/ml > A.M.E.A.E-100mg/ml > A.R.E.E-200mg/ml > A.R.E.E-200mg/ml > A.M.E.E-200mg/ml > A.S.E.E-200mg/ml > A.M.E.E-100mg/ml > A.S.E.E-100mg/ml. Action shown by the ethyl acetate extracts of the all the three plants were almost equal with that of the standard drug Albendazole. Flavonoids present in ethyl acetate extract could be responsible for the highly significant action against helmenthes.

CHAPTER-VIII

This chapter deals with the summary of all the chapters.
CONCLUSION

Present data obtained from the pharmacognostic studies of three medicinal plants of Annonacea, *Annona squamosa* Linn, *Annona reticulata* Linn, *Annona muricata* Linn can be an important identification tool for standardization of these plants. The pharmacological studies carried on these plants are validating the traditional usages and can be concluded to use these plants leaf extracts for gastroprotective action such as in the treatment of ulcer, in treating worm infestations and the results could be supported by the *In vitro* antioxidant studies by scavenging free radicals of DPPH, Nitric oxide, hydroxyl radical and superoxide radical.

FUTURE SCOPE:

Future scope demands that there is a need for the isolation of the constituents responsible for the pharmacological action and to screen the exact mechanism of action for the curative purpose.