CHAPTER - EIGHT : ANTHELMINTIC STUDIES.

Helminthic infections are now being recognised as the cause of chronic illness and inefficiency among tropical people. Helminths in the tissues may cause several damages in the alimentary tract. They cause malnutrition by robbing the host of a major portion of food. The extent of their diversion of food stuff from the vertebrate to the invertebrate kingdom can be gauged from the calculation made by Stoll\(^1\) who estimated the total weight of round worm in the Chinese bowel in that year to be equal to the weight of an army of 200,000 men. The annual output of eggs by these worms, largely composed of protein, was about 8000 tonnes, causing a direct loss of it from the host. More than half of the population of the world carries worm infections of one kind or another and many people harbour several species at the same time. Domestic animals also carry large burdens of parasitic worms which make further inroads upon human food supplies.

A brief account of the different types of common helminth diseases and drugs used\(^2-4\) for their treatment are given below:
**Ancylostomiasis (Hook Worm Disease)**

It is caused by the hook worm *Ancylostoma duodenal* or *Neactor americanus* in man. The disease is found in tropical countries. The man usually acquires this infection by walking barefoot on the infected soil. In contact with skin, the fully developed larvae enter the body and find their way into the lungs by passing through lymphatic and small blood vessels. Then they find their way into small intestine where they mature within 4-7 weeks. The adult worm is cylindrical in shape and 10 mm in diameter. Severe infection causes indigestion, eructation, headache, anorexia and vertigo. In advance stage it causes epigastric pain, anaemia and ultimately diarrhoea.

Drugs used - Tetrachloroethylene, Thymol, etc.

**Ascariasis (Round Worm Disease)**

It is caused by *Ascaris lumbricoides*. The female worm is 15-30 cm long and male worms are shorter than female and have curved tail. The man is infected by eating food, contaminated fully with embryonated ova. Larvae hatch out in duodenum and make their way by blood stream to lungs where they develop further. After several days they ascend bronchi and trachea and swallowed. Finally larvae develop in the intestine.

Drugs used - Piperazine salts are most useful.
Trechuriasis (Whip Worm Infestation)

It is caused by Trechuris trichuria, a common intestinal nematode. The mature worm is 3-5 cm long and has a thin coiled anterior end resembling a whip. Man is infected by eating food contaminated with soil containing mature ova. Whip worms usually do not give rise to symptoms but in heavy infection the symptoms are indigestion, anaemia and utricularia.

Drugs used - Dithiazine is the drug of choice. Hexyl resorcinol and piperazine are also useful.

Enterobiasis (Thread Worm Infestation)

The worms look like white thread, 0.5-1.0 cm in length. They widely invade the small intestine. Female worms cause intense itching at night during her egg laying period. Abdominal pain, weight loss and nervousness are also the symptoms of the infection.

Drugs used - Piperazine.

Tapeworm Infestation

Taenia saginata, the beef tapeworm is the only tapeworm commonly found in man. Infection is acquired when insufficiently cooked beef, infected with cystic larval stage of the worm is eaten. The head of the worm
is about the size of a large pin head and has four suckers. From scolex arises series of progressively larger segments, so that mature worm is a white ribbon shaped creature 5-10 m in length. Mature segments measure about 20x7 mm. The presence of adult worms seldom produces intestinal symptoms but the discovery of segments in the stool may alarm the patient.

Drugs used - Many tape worms infestation respond to antimalarials.

The drugs, known as anthelmintics rid the hosts of parasitic worms. An effective and successful anthelmintic drug should confirm the following requirements. 5

1. The drug should reach that portion of the intestine where the worm infestation occur with a minimal degree of absorption.

2. It should be of minimal toxicity to the mucous membrane of the gastro-intestinal tract.

3. If absorbed from alimentary tract, the drug's systematic toxicity should be minimal.

4. The drug should be tolerable orally without producing side effects.

5. It should be chemically stable and inexpensive.

A survey of the existing anthelmintics show that these drugs have not developed to perfection. Among the
most widely used, piperazine salts have been reported to produce various undesirable side effects like nausea, vomiting and giddiness in some cases. Hence the screening of anthelmintic drugs either of plant products or synthetic compounds is always beneficial.

Evaluation of Technique

It is a great problem that very few helminths of man infect laboratory animals. Hence species which are natural parasite of small rodents and related ecologically in their habitat to the respective human parasite are used in the study of anthelmintic activity of new substances. As a matter of fact, condition of alimentary tract of different experimental animals cannot be the same. Therefore, screening of anthelmintic activity may be done by exposing the worms to the solution of anthelmintic substances.

In vitro techniques involve *Ascaris lumbricoides, Uncinara stencephala, Trichostruquis cakaratus* and some other species of worms. It has been reported by Sollmann\(^6\) that all clinical anthelmintics are toxic to earthworms. Tandelburg\(^7\) proved that various species of ascaris which are the major helminths causing ascariasis and various other helminth infestation in man and animal have, remarkable anatomical similarities with common earthworm. Larson et al.\(^8\) proposed that toxicity of anthelmintic drugs to
the earthworm don't correspond to the pig's ascaris. The substances non-toxic to earthworm are not worth for further study, while positive results show that it has possibilities as an anthelmintic. Furthermore Watkins⁹ and a number of other workers¹⁰-¹⁶ have used earthworms instead of other helminths for preliminary in vitro evaluation of anthelmintic activity of new substances.

Due to the availability of earthworms and their acceptance as substitute of various ascaris species, qualitative in vitro anthelmintic evaluation of the synthesized compounds and plant extracts was done using earthworms and adopting the techniques described by 'Watkins'.

**Experimental**

For the present study 4% solution of all the test samples (chalcones, S-triazines and alcoholic extract of the seeds) and standard anthelmintic drug 'Piperazine phosphate' were made in ethylene glycol and they were diluted to a concentration of 2% with ethylene glycol. Normal saline¹⁷ solution was prepared in distilled water.

25 ml of normal saline solution and 2 ml of test sample solutions in ethylene glycol was transferred to the petridishes of four inches diameter. Two earthworms of same size were washed with the normal saline solution and placed in each petridish. The movement of earthworms were stimulated and became more marked, worms tried to
get out of the solution. Thereafter, they became progressively sluggish until death supervened. The same experiment was also performed with the control (piperazine phosphate) under the same conditions. The experiments were performed in duplicate (with two earthworms) and average paralytic time and lethal time in minutes are given in table I-III and are represented in bar graphs (I-IIIA). The time taken by the earthworm to become motionless was noted as paralytic time. To ascertain the death of motionless worms, one or more worms were frequently transferred to hot water at 50°C, which stimulate and induce movements in the worms, if alive. Observations were taken to confirm the findings.

**TABLE I**

*In vitro* Anthelmintic Activity of the Alcoholic Extracts of the seeds.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the alcoholic extract</th>
<th>Concentration 4%</th>
<th>Concentration 2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paralytic time (min)</td>
<td>Lethal time (min)</td>
</tr>
<tr>
<td>1.</td>
<td><em>Cassia nodosa</em></td>
<td>2.5</td>
<td>22.0</td>
</tr>
<tr>
<td>2.</td>
<td><em>Cassia renigera</em></td>
<td>2.4</td>
<td>22.0</td>
</tr>
<tr>
<td>3.</td>
<td><em>Taxodium mucronatum</em></td>
<td>1.8</td>
<td>18.0</td>
</tr>
<tr>
<td>4.</td>
<td>Piperazine phosphate</td>
<td>1.7</td>
<td>17.0</td>
</tr>
</tbody>
</table>
### Table II

**In vivo** Anthelmintic Activity of Chalcones Derived from 2',4' - dihydroxy Acetophenone

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the compound</th>
<th>Concentration</th>
<th>4%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralytic time (min)</td>
<td>Lethal time (min)</td>
</tr>
<tr>
<td>1.</td>
<td>2,2',4'-tri hydroxy chalcone</td>
<td></td>
<td>2.0</td>
<td>19.0</td>
</tr>
<tr>
<td>2.</td>
<td>4-methoxy-2',4'-dihydroxy chalcone</td>
<td></td>
<td>3.2</td>
<td>30.0</td>
</tr>
<tr>
<td>3.</td>
<td>2-nitro-2',4',-dihydroxy chalcone</td>
<td></td>
<td>1.4</td>
<td>15.5</td>
</tr>
<tr>
<td>4.</td>
<td>3,4,2',4'-tetrahydroxy chalcone</td>
<td></td>
<td>1.6</td>
<td>16.0</td>
</tr>
<tr>
<td>5.</td>
<td>3,4-dimethoxy-2',4'-dihydroxy chalcone</td>
<td></td>
<td>1.3</td>
<td>14.0</td>
</tr>
<tr>
<td>6.</td>
<td>4-chloro-2',4'-dihydroxy chalcone</td>
<td></td>
<td>3.8</td>
<td>32.0</td>
</tr>
<tr>
<td>7.</td>
<td>2',4'-dihydroxy chalcone</td>
<td></td>
<td>3.0</td>
<td>28.0</td>
</tr>
<tr>
<td>8.</td>
<td>3-methoxy-4',2',4'-tri hydroxy chalcone</td>
<td></td>
<td>1.5</td>
<td>17.2</td>
</tr>
<tr>
<td>9.</td>
<td>Piperazine phosphate</td>
<td></td>
<td>1.7</td>
<td>17.0</td>
</tr>
</tbody>
</table>
**TABLE III**

*In vitro* Anthelmintic Activity of 4',6'-bis (anilino) -2'-aryl/alkyl amino-S-triazine

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the compound</th>
<th>Concentration</th>
<th>4%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paralytic time (min)</td>
<td>Lethal time (min)</td>
<td>Paralytic time (min)</td>
</tr>
<tr>
<td>1.</td>
<td>4',6'-b(a)-2'-chloro-S-T</td>
<td>1.5</td>
<td>17.0</td>
<td>4.0</td>
</tr>
<tr>
<td>2.</td>
<td>4',6'-b(a)-2',2'-toluidino-S-T</td>
<td>2.0</td>
<td>21.0</td>
<td>4.5</td>
</tr>
<tr>
<td>3.</td>
<td>4',6'-b(a)-2',4'-toluidino-S-T</td>
<td>2.5</td>
<td>23.0</td>
<td>5.3</td>
</tr>
<tr>
<td>4.</td>
<td>4',6'-b(a)-2',1'-naphthylamino-S-T</td>
<td>2.5</td>
<td>17.0</td>
<td>5.3</td>
</tr>
<tr>
<td>5.</td>
<td>4',6'-b(a)-2',2'-naphthylamino-S-T</td>
<td>2.8</td>
<td>19.0</td>
<td>6.0</td>
</tr>
<tr>
<td>6.</td>
<td>4',6'-b(a)-2',2'-nitroanilino-S-T</td>
<td>1.5</td>
<td>12.0</td>
<td>3.0</td>
</tr>
<tr>
<td>7.</td>
<td>4',6'-b(a)-2',3'-nitroanilino-S-T</td>
<td>1.5</td>
<td>10.0</td>
<td>4.0</td>
</tr>
<tr>
<td>8.</td>
<td>4',6'-b(a)-2',4'-nitroanilino-S-T</td>
<td>1.3</td>
<td>9.0</td>
<td>2.0</td>
</tr>
<tr>
<td>9.</td>
<td>4',6'-b(a)-2',-diphenylylnino-S-T</td>
<td>3.0</td>
<td>18.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10.</td>
<td>4',6'-b(a)-2'-ethylenediamino S-T</td>
<td>2.0</td>
<td>17.0</td>
<td>4.5</td>
</tr>
<tr>
<td>11.</td>
<td>4',6',-b(a)-2',-propylenediamino-S-T</td>
<td>2.3</td>
<td>15.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Piperazine phosphate  
1.7 17.0 2.2 23.0

4',6'-b(a) stands for 4',6'-bis-(anilino) and S-T stands for S-Triazine.
### Table III

**In vitro Anthelmintic Activity of 4',6'-bis (anilino) -2'-aryl/alkyl amino-S-triazine**

| S. No. | Name of the compound | Concentration 4% | | Concentration 2% | |
|--------|---------------------|------------------|------------------|------------------|
|        |                     | Paralytic time (min) | Lethal time (min) | Paralytic time (min) | Lethal time (min) |
| 1.     | 4',6'-b(a)-2'-chloro-S-T | 1.5               | 17.0             | 4.0               | 38.5             |
| 2.     | 4',6'-b(a)-2',2'-toluidino-S-T | 2.0             | 21.0             | 4.5               | 34.0             |
| 3.     | 4',6'-b(a)-2',4'-toluidino-S-T | 2.5             | 23.0             | 5.3               | 37.0             |
| 4.     | 4',6'-b(a)-2',1'-naphthylamino-S-T | 2.5             | 17.0             | 5.3               | 32.0             |
| 5.     | 4',6'-b(a)-2',2'-naphthylamino-S-T | 2.8             | 19.0             | 6.0               | 35.0             |
| 6.     | 4',6'-b(a)-2',2'-nitroanilino-S-T | 1.5             | 12.0             | 3.0               | 30.0             |
| 7.     | 4',6'-b(a)-2',3'-nitroanilino-S-T | 1.5             | 10.0             | 4.0               | 19.2             |
| 8.     | 4',6'-b(a)-2',4'-nitroanilino-S-T | 1.3             | 9.0              | 2.0               | 18.0             |
| 9.     | 4',6'-b(a)-2',-diphenylamino-S-T | 3.0             | 18.0             | 5.0               | 26.0             |
| 10.    | 4',6'-b(a)-2'-ethylene diamino S-T | 2.0             | 17.0             | 4.5               | 25.5             |
| 11.    | 4',6'-b(a)-2',-propylenediamino-S-T | 2.3             | 15.0             | 5.0               | 26.0             |
|        | Piperazine phosphate | 1.7             | 17.0             | 2.2               | 23.0             |

4',6'-b(a) stands for 4',6'-bis-(anilino) and S-T stands for S-Triazine.
DISCUSSION

On going through the results of anthelmintic activity of the alcoholic extracts at different concentrations, 4% and 2% in ethylene glycol and comparing their activity with the solution of piperazine phosphate at the same concentration, it has been observed that (taking only lethal time into account for discussion), the alcoholic extract of *Taxodium mucronatum* possess almost equal activity as piperazine phosphate. The alcoholic extracts of *Cassia nodosa* and *Cassia renigera* have also shown fairly considerable activity as anthelmintics.

On comparing the anthelmintic activity of chalcones derived from 2',4'-dihydroxyaceto phenone with the standard, the anthelmintic property of 3,4-dimethoxy-2',4'-dihydroxy chalcone was found better than the standard anthelmintic drug piperazine phosphate. The activity of 2-nitro-2',4'-dihydroxy chalcone and 3,4,2',4'-tetrahydroxy chalcone has been found to be equal to the anthelmintic activity of the standard drug. Among the chalcones, 4-chloro-2',4'-dihydroxy chalcone possess the least anthelmintic activity.

On going through the results of anthelmintic activity of 4',6'-bis-(anilino)-2'-aryl/alkyl-S-triazines : 4',6'-bis-(anilino)-2', 2 - nitroanilino-S-triazine; 4',6'-bis (anilino)- 2',4-nitroanilino-S-triazine and 4',6'-bis-(anilino)-2'-diphenyl-amino-S-triazine possess better
anthelmintic property than the standard drug. The anthelmintic activity of 4',6'-bis(anilino)-2'-ethylene diamino-S-triazine and 4',6'-bis(anilino)-2'-propylene diamino-S-trianine is almost equal to the anthelmintic activity of piperazine phosphate. Among the S-triazines, 2-chloro-4',6'-bis(anilino)-S-triazine possess the least anthelmintic activity.

CONCLUSION

These studies suggest that S-triazines, alcoholic extract of Taxodium mucronatum and chalcones possess considerable anthelmintic property and they may find use for expelling the worms.
FIG. 1: ANTHELMINTIC ACTIVITY OF ALCOHOLIC EXTRACTS IN 4 % DILUTION

INDEX

LETHAL TIME
PARALYTIC TIME

TIME (IN MINUTES)

ALCOHOLIC EXTRACT
FIG. 1A: ANTHELMINIC ACTIVITY OF THE ALCOHOLIC EXTRACTS IN 2% DILUTION

INDEX

LETHAL TIME

PARALYTIC TIME

TIME (IN MINUTES)

ALCOHOLIC EXTRACTS

1  2  3  P
FIG. II: ANTHELMINTIC ACTIVITY OF CHALCONES IN 4% DILUTION

INDEX

LETHAL TIME

PARALYTIC TIME

TIME (IN MINUTES)

1  2  3  4  5  6  7  8  P

COMPOUNDS
FIG. II A: ANTHELMINTIC ACTIVITY OF CHALCONEs IN 2% DILUTION

INDEX

LETHAL TIME
PARALYTIC TIME

TIME (IN MINUTES)

1 2 3 4 5 6 7 8 P

COMPOUNDS
FIG. III: ANTHELMINTIC ACTIVITY OF S-TRIAZINE IN 4% DILUTION

INDEX

TIME (IN MINUTES)

LETHAL TIME
PARALYTIC TIME

COMPONENTS
FIG. III A: ANTHELMINTIC ACTIVITY OF S-TRIAZINES IN 2% DILUTION.

INDEX

LETHAL TIME

PARALYTIC TIME

TIME (IN MINUTES)

COMPOUNDS

1 2 3 4 5 6 7 8 9 10 11 P
REFERENCES


3. Ibid., p. 7249.

4. Ibid., p. 9140.


