Anthelmintic Activity

Chapter VIII
Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.

Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines. There is thus a pitifully small repertoire of chemotherapeutic agents available for treatment (see Table 1). In some respects, this situation has been exacerbated by the remarkable success of ivermectin over the last twenty years which has decreased motivation for anthelmintic drug discovery programmes. This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only be a matter of time before this phenomenon occurs in parasites of humans.
Table 1. Key drugs registered for the treatment of parasitic worms in humans.

<table>
<thead>
<tr>
<th>Schistosomiasis (blood fluke)</th>
<th>Intestinal round worms</th>
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<tbody>
<tr>
<td>Antimonials</td>
<td>Piperazine</td>
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<tr>
<td>Metrifonate</td>
<td>Benzimidazoles</td>
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<tr>
<td>Oxamnaquine</td>
<td>Morantel</td>
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<td>Praziquantel</td>
<td>Pyrantel</td>
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<td></td>
<td>Levamisole</td>
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<tr>
<td>Cestodiasis (tape worm)</td>
<td>Avermectins and milbemycins</td>
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<tr>
<td>Niclosamide</td>
<td>Closantel (and halogenated salicylamides)</td>
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<td>Benzimidazoles</td>
<td>Emodepside</td>
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<td>Praziquantel</td>
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<tr>
<td>Fasciolasis (liver fluke)</td>
<td>Filaria (tissue round worms)</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>Diethylcarbimazine</td>
</tr>
<tr>
<td>Closantel</td>
<td>Suramin</td>
</tr>
<tr>
<td>(and halogenated salicylamides)</td>
<td>Ivermectin</td>
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</table>

Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes. However, the majority of drugs are more limited in their action, e.g., praziquantel, a drug used in the treatment of schistosomiasis and thought to act by disrupting calcium homeostasis, has no activity against nematodes (Table 1). For the purpose of this review we will focus on drugs used in human and veterinary medicine to treat parasitic nematode infection.
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Now a days man and animal are affected with infections caused by various helminthes or worms. These infections pose a major health problem. Multiple infections are common in men. A large population is affected by serious diseases, thereby causing ill health, inefficiency, malnutrition. The disease caused by helminthes is known as helminthiasis. Man acquires it by contact, eating infected food, via mosquitoes (filarial worms) soil and water. The worms enter the body in the form of eggs or larvae.

The term worm\(^1\) is applied to an assemblage of organisms with elongated bodies and more or less creeping habitat. It has a precise zoological meaning. The parasitic helminthes fall under the four different phyla\(^2\) namely platyhelminth, acanthocephala, nematohelminth, and annelida. These phyla occupy an important position in the animal kingdom.

In 1947, Stoll\(^3\) estimated that around 400 million helminths occur in among some 200 million people. There is enough reason to believe that the number of people with helminthes infection has increased considerably.

Helminthes cause mechanical damage by biting intestinal wall and causing heamorrhages (hook worms), tumours (schistosomes, spirurata and peporate) and cause peritonitis of walls of digestive tract. Tissue damage and inflammation due to burrowing is caused by lung flukes and guinea worms. Some cause loss of blood, anaemia, and vitamin deficiency. Some block passage and cause obstruction ex. ascaris, liver flukes and ban croifs filarial and they interfere with the normal flow of lymph, bile etc. Some cause eosinophilia, carry pathogenic bacteria, allergic reactions (migrating larvae, guinea worms etc.). These allergic reactions give rise to the symptoms of helminth
infections. Helminths\(^4\) cause a variety of disease. Domestic animals also carry the burden of parasitic worms. The infection of helminths are wide and cause number of diseases in men and animals. The main parasitic worms found in India are round worms, hook worms, thread worms, tape worms, filaria worms, guinea worms, and flukes which fall under the categories of nematodes, cestodes. The brief account of the different type of common helminth diseases and drugs\(^5-8\) used for them are given below:

1. **Ancylostomiasis**:

   It is caused by an intestinal infection in man by two hook worms *Ancylostoma duodenals* and *Neactor americanus*. The disease is found in tropical and subtropical countries. Man acquires infection when the filarial worm larval penetrates through the skin of hand and feet. They develop in small intestine. Infection results in gastrointestinal disturbances like anemia and nervous disorders.

   **Drug**: Tetrachloroethylene, and carbon tetrachloride are effective.

2. **Ascariasis**:

   It is roundworms infection\(^9\) caused by *Ascaris lumbricoides*. Infection causes with contaminated food and water. It lives in small intestine of man, certain apes and pigs. About 17% of the population of country is affected by ascariasis worms in intestine cause abdominal pains, headache and nausea.

   **Drug**: piperazine salts are most useful.

3. **Trichuriasis**:

   It is a whipworm infection caused by *Trichuris trichiura*, which occurs in man and pigs. It resides in the large intestine. Infection in
human results from the ingestion of ova from faecally polluted soil. It was common in worm climate, loss of appetite and diarrhoea may occur.

4. Enterobiasis:

   It is commonly called pinworm infection caused by Enterobius vermicularis. This kind of infection is most common in children. Its larval forms mature in the ileum. The worms cause irritation, loss of appetite, sleeplessness and nervousness.

   **Drug**: Piperazine is the drug of choice in enterobiasis.

5. Strongyloides:

   This is most frequently acquired by penetration of the skin by the larva of Strongyloides stercoralis, commonly called thread worms. These invade the lining of the alimentary canal.

   **Drug**: Drugs used are dithiazine.

6. Trichinosis:

   Trichinosis is caused by Trichinella spiralis. These occur in small intestine of man, pig, rat and many other mammals, infection is acquired by eating raw or improperly cooked pork.

   **Drugs**: Piperazine citrate.

7. Taeniasis:

   It is a tapeworm. Infection is caused by the species belonging to the genus Taenia which included mainly Taenia solium and T. saginata. Infection is acquired by eating improperly cooked pork.

   **Drug**: Common antimalarial drugs are used.
8. Schistosomiasis:

It is caused by three species of blood flukes. Schistosoma mansoni, S. japonicum and S. haematobium. These live in streams.

**Drug**: Antimony compounds are used.

9. Fasciolopsiasis:

It is caused by intestinal fluke Fasciolopsis foelleborni. It caused erosion of intestinal living, resulting in bleeding and pain.

**Drug**: Hexylresorcinol is helpful.

10. Fascioliasis:

It is caused by liver fluke Fasciola hepatica. It usually inhibits the liver and bile duct of cattle, sheep, rabbit and also other vertebrates. It damages the liver as well as bile duct of the host and causes the disease called liver-rot or Fascioliasis.

**Drug**: Hexachloroethen and CCl₄ are effective.

11. Paragonimiasis:

This disease is caused by lungs fluke, Paragonimus westerman and results in chest pain and shortness of breath.

**Drug**: Emetine hydrochloride and sulphur drugs are effective.

12. Filariasis:

It is caused by filarial worms Wuchereria bancrofti and W. malayi. These worms live in the lymphatic vessel and connective tissues of the body. Infection is acquired through the bites of culex mosquito. This disease disfigure legs and other parts of the body and are enormously enlargement called elephantiosis.

**Drug**: Hetrazan.
In this way, these parasitic worms cause so much harm to men and animals. Therefore methods are being tried to suppress the disease in men and animals. For this purpose, the search is on for chemicals which have the properties to remove or kill these pathogenic worms or helminths. These substances or chemicals are known as anthelmintic agents.

Many compounds have shown good activity against worm infections. Carbon tetrachloride, tetrachloroethylene, hexachlororesorcinol, were earlier known anthelmintics. Lomson and his co-workers reported various phenolic anthelmintics.

Mc. Donogh and Christopherson reported the properties of antimony compounds for curing schistosomiasis. Later piperazine for enterobius and dithiazone for skin fluke (Strongyloides) and chloroquine for clonorchis were discovered in 1938. Phenothiazone and hexachloroethen were reported by Harwood. Later various drugs atabrin for tapeworm, antimony and arsenic compounds for filariosis and hetrazon were introduced as anthelmintics.

Earlier number of workers\textsuperscript{10-12} have reported the antihelmintic activities of a large number of plant as well as synthetic compounds and found many of them possessed good anthelmintic activities. Recently some compounds like triazolothiadiazines\textsuperscript{13,14} quinozolines\textsuperscript{15}, heterocyclic compounds\textsuperscript{16}, and S-substituted phenothiazone\textsuperscript{17} and piperazine have been suggested as potential anthelmintic agents.

Anthelmintic may act in two ways. The drugs which directly or indirectly kill the worms are called vermicides ex. dichlorophene, hexyl resorcinol etc. Those which expel worms from the body of host usually by paralyzing them are known as vermifuges eg. piperazine, santonin,
chenopodium oil, etc. The chemotherapeutic anthelmintics, destroy the parasitic worm or remove them from hosts either the way of

i. Direct action on the worm causing paralysis or death.

ii. By irritating tissue of the parasite.

iii. Antimetabolic interfering with the metabolism of parasite.

An ideal anthelmintic\(^{18}\) should have a broad spectrum of action. It should first paralyse the worms and then expel them. It should achieve a high percentage of cure with a single therapeutic dose. It should not be toxic to the host and should be cheap.

**Classification**: Anthelmintic may be classified into two different ways.

a. According to their mode of action. \(^{(19-21)}\)

(I) Vermicides are drugs that paralyse or kill parasites.

(II) Vermifuges are drugs that weaken the worms so that they can no longer attach themselves to the intestinal mucosa and thus can be expelled.

b. According to their chemical structure.

(I) Chlorinated hydro carbons CCl₄, tetrachloroethane etc.

(II) Phenols and related compounds – thymol (oil, of thyme), hexyl resorcinol.

(III) Antimonials and arsenicals stibophen, tartar emetic.

(IV) Piprazine derivatives-hetrazan, antepar.

(V) Triphenyl methane, cyanine dyes-gentian violet, crystal violet.

(VI) Phenothiazines, certain antimalariaals (azacrine), certain xanthones, carbamates.

(VII) Natural products-pelletierine, santonin, oil of chenopodium, aspidium and emetin.
Mode of Action:

Anthelmintics are the drugs used in the treatment of helminthiasis. They are used to kill or remove the parasitic worm and rid the host of them. An ideal and effective anthelmintic drug\(^{22-24}\) is one that causes minimum toxicity to the mucous membrane of gastrointestinal tract and alimentary tract. If absorbed from it, drug should be chemically stable, inexpensive and tolerable orally without producing symptoms. Most important is that the drug should reach that portion of the intestine where the worm infestation occurs with minimum degree of absorption. It should be immediately lethal. Anthelmintic caused death or remove the worm by stimulating paralysis or nacrosis. This is accomplished by gaining access into the body via cutile or ingestion and interfering with worms metabolisms. It should be specific.

Vegetable remedies like molefern, cusso, arecanut (tapeworms) and santonin, (nematodes) are the earliest known anthelmintics. In 1880, a landmark was set when the value of thymol for hook worms was established by some Italian workers. Other anthelmintics were oil of chenopodium [1913], CCl\(_4\) (1921), tetrachloroethylene (1925), hexyl resorcinol (1930), replaced oil of chenopodium in ascariasis. Mc. Donagh and christopherson established the value of antimony compounds for schistosomiasis. Gentain violet (1927) was replaced by chloroquine for clororchis, piperazine for enterobius and dithiazine for strongyloides. Phenothiazine (1938) by Harwood and hexachloroethene (1926) for fasciola were discovered. After world was II, aterbin (for tape worms), antimony and arsenic compounds and hetrazan (for filariasis) were introduced. After world war II piperazine salts played vital role as anthelmintic (i.e.) hetrazan is useful against a number of helminths. Several workers have illustrated the anthelmintic activity of a large number of compounds of synthetic origin. They are given in table, below:
### Literature Survey

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<th>Group</th>
<th>Activity</th>
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<td>Pyrazoline derivatives</td>
<td>Anthelmintic activity</td>
<td>Xiaohui Oa, (30)</td>
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<td></td>
<td>Pyrazoline derivatives</td>
<td>Anthelmintic activity</td>
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<td>Pyrazoline derivatives</td>
<td>Anthelmintic activity</td>
<td>Chun Guo, et al., (29)</td>
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<tr>
<td>Heterocyclic compound</td>
<td>Benzothiazole derivative</td>
<td>Anthelmintic activity</td>
<td>Kallusaya B, et al., (16)</td>
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<tr>
<td></td>
<td>Azetidinones derivatives</td>
<td>Anthelmintic activity</td>
<td>N.P. Karyriva et al., (28)</td>
</tr>
<tr>
<td>Heterocyclic compound</td>
<td>Isoxazole 3-aryl-5-halomethyl isoazole</td>
<td>Anthelmintic (Pinworm)</td>
<td>H.G. Sen et al., (25)</td>
</tr>
<tr>
<td></td>
<td>Isoxazole dihydro isoazole</td>
<td>Anthelmintic (Trypanosomol)</td>
<td>Donila Davyt et al., (26)</td>
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<td></td>
<td>3- oxy-substituted isoazoles</td>
<td>Anthelmintic (Humigera, hemigera )</td>
<td>Wofua, Hassan et al., (27)</td>
</tr>
</tbody>
</table>

### Evaluation of Technique:

In vitro techniques involve *A. lumbricoides, Unicara strencomnepholia*, and some other species of worms. Very few helminths of man infect the laboratory animals. The condition of alimentary canal of different experimental animals may not be the same. So the screening of anthelmintic activity may be done by exposing the worm to the solution of anthelmintic substances.

It has been reported by Sollman (31) that all clinical anthelmintics are toxic to earthworms. Trandelberg proved that various species of *Ascaris* have remarkable anatomical similarities with common earthworms. Furthermore, a number of workers (32-39) have used...
earthworms for preliminary in vitro evaluation of anthelmintic activity of new substances. Due to the availability of earthworms and their acceptance as substitute, qualitative in vitro anthelmintic screening of synthesized heterocyclic compounds, was done using earthworms by adopting the technique given by Watkins\textsuperscript{40-41}.

**MATERIALS AND METHODS\textsuperscript{42-43}**:  

4 and 2 percent solution of pyrazolines, thiazolidinone and their 5-arylidene, azetidinone and isoxazole derivatives were prepared in ethyleneglycol. Same concentration (4% and 2%) of standard drug piperazine hydrochloride was also prepared in ethylene glycol.

In the petridish, 25 ml normal saline solution and 2 ml of test sample solution were poured. Two living earthworms of nearly equal size, washed with normal saline solution, were transferred into the petridish. Same experiment was performed with the standard.

The time taken by earthworm to become motionless was noted as paralytic time. The time of death is noted as lethal time. Death of motionless earthworm was ascertained by placing the earthworm in lukewarm water, which stimulates movement if the worm is alive, Experiment were carried out of duplicate and average values are shown in the form of graphs.

Data of anthelmintic activity of synthesized compounds are represented by bar graphs.
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Anthelmintic Activity of pyrazoline derivatives (2% solution)

Paralytic time  Lethal Time

Anthelmintic Activity of Pyrazoline derivatives (4% solution)

Paralytic time  Lethal Time
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Anthelmintic Activity of 4-thiazolidinone derivatives (2% solution)

Time in minutes

Compound Code

Paralytic Time = Lethal Time

Anthelmintic Activity of 4-thiazolidinone derivatives (4% solution)

Time in minutes

Compound Code

Paralytic Time = Lethal Time
RESULT AND DISCUSSION:

Comparing the lethal time of synthesized compounds with standard drug, in the pyrazoline derivatives observed that chloro phenyl, nitro phenyl and methoxy phenyls are more potent. In the 4-thiazolidinone series, the chloro phenyl and 4-hydroxy-3-methoxy phenyl derivatives exhibited more potency. In the 5-arylidene -4 thiazolidinone series the chlorophenyl, 4-nitro phenyl, 4-hydroxy, 3-methoxy phenyl derivatives exhibit more potency. 2-Azetidinone derivatives almost exhibited promising anthelmintic activity. The isoxazole series showed good activity.

SUMMARY:

4 and 2 percent solutions of synthesized compounds were prepared in ethylene glycol same concentration of standard drug piperazine hydrochloride was also prepared in ethylene glycol. The test sample solution was poured into petridish containing earthworm. The time taken by the earthworm to become motionless was noted as paralytic time. The time of death is noted as lethal time. Experiments were performed in duplicate and average values are noted.