Anthelmintics are therapeutic agents that are used to destroy parasitic worms or remove them from the infected host. The majority of helminth infections are acquired by contact with:

(a) Infected animals
(b) Ground contaminated by human or animals' excrement.
(c) Water infected with cercariae.
(d) Ingestion of infected meat.

Parasitic worms are dependent upon the host for permanent existence. They must therefore have some method of gaining access to the body of the host and their offspring (either eggs or larvae) must have a means of escaping from the host's body to perpetuate the species; helminth eggs or larvae are generally not able to produce an immediate infection in a new host.

Some time period varying from few hours in the case of oxyurids to months in the case of other parasites is necessary before the infective stage to again reach, before, preventive measures against the spread of parasitic infections can be taken. An exact knowledge of this critical period in the life cycle of the helminth is essential.

Surveys have shown that one third of the human race suffers from helminth diseases, of which a large number are multiple infection. Although helminth infections are usually
associated with tropical reasons and more than 40 million Americans are also victims. In additions, these diseases a serious economic problem to animal industry wherein every class of domestic animal is vulnerable to a large number of parasitic warming infections.

Many highly effective and selective anthelmintics are available, but such compounds must be used correctly and judiciously to obtain a favourable clinical response and to minimize selection for anthelmintic resistance. It is impossible to list all claims and precautions regarding all drugs in all countries; the label should always be read before using any drug. Additional information is found under relevant disease headings. Modern drugs have a wide margin of safety. Considerable activity against immature or larval stages of parasites and a broad spectrum of activity. Nonetheless, the usefulness of any anthelmintic is limited by the inherent efficacy of the drug itself, its mechanism of action, its pharmacokinetics properties, features relating to the host animal (e.g. operation of the oesophageal groove reflex,) or features relating to the parasite, (e.g. its location in the body, its degree of hypobiosis, or whether it has developed anthelmintic resistance).

The "ideal" anthelmintic should have a broad spectrum of activity against mature and immature parasites (including hypobiotic larvae), be easy to administer to a
large number of animals, have a wide margin of safety and be compatible with other compounds, not require long.

- Mechanisms of Action
- Cellular Integrity
- Inhibitors of Tubulin polymerisation
- Uncouplers of Oxidative Phosphorylation
- Inhibitors of Enzymes in the Glycolytic pathway
- Neuromuscular co-ordination
- Cholinesterase Inhibitors
- Cholinergic Agonists
- Muscle Hyperpolarisation
- Potentiation of Inhibitory Transmitters

Anthelmintics\textsuperscript{2-8} must be selectively toxic to the parasite. This is usually achieved either by inhibiting metabolic processes vital to the parasite but which are not vital or are absent in the host, or by inherent pharmacokinetic properties of the compound that cause the parasite to be exposed to higher concentrations of the anthelmintic than are the host cells. While the physiologic mode of action of anthelmintic is not fully understood, the sites of action and biochemical mechanisms of many of them are known. Parasitic helminths must maintain an appropriate feeding site, and nematodes and trematodes must actively ingest and move food through their digestive
tracts to maintain an appropriate energy state; this and reproductive processes require proper neuromuscular coordination. Parasites must also maintain homeostasis in the face of host immune reactions. The pharmacologic basic of the treatment for helminths generally involves interference with the integrity of parasite cells, neuromuscular coordination, or protective mechanisms against host immunity, which lead to starvation, paralysis, and expulsion of the parasite.

CELLULAR INTEGRITY

There are several classes of anthelmintics that impair cell structure, integrity, or metabolism.

INHIBITORS OF TUBULIN POLYMERIZATION

Benzimidazoles and probenzimidazoles (which are metabolized in-vivo to active benzimidazoles and thus act in the same manner).

UNCOPPLERS OF OXIDATIVE PHOSPHORYLATION

Salicylanilides and substituted phenols.

INHIBITORS OF ENZYMES IN THE GLYCOLYTIC PATHWAY

The benzimidazoles inhibit tubulin polymerisation; it is believed that the other observed effects, including inhibition of cellular transport and energy metabolism, are consequences of the depolymerisation of microtubules. Inhibition of these secondary events appears to play an essential role in the lethal effect on worms. Because they
progressively deplete energy reserves and inhibit excretion of waste products and protective factors from parasite cells, an important factor from parasite cells, an important factor in efficacy of the benzimidazoles is prolongation of contact time between drug and parasite. These compounds have a broad spectrum of activity and are often effective against adults, larvae and eggs. Cross-resistance can exist all members of this group because they act on the same receptor protein, β-tubulin, which is altered in resistant organisms such that none of the benzimidazoles can bind to the receptor with high affinity.

The anthelmintic modes of action are closely related to the life-support requirements of the parasites. Differential toxicity between host and parasite is based on a unique parasite receptor for the anthelmintic or on an effective concentration of drug that inhibits the parasite's system without interfering with the host's comparable system (selective toxicity).

** ADSORPTION AND DISTRIBUTION **

After administration, anthelmintics are usually absorbed into the blood stream and transported to different part of the body, including the liver where they are metabolized and eventually excreted in the feces and urine. With some anthelmintics (e.g. probenzimidazoles), antiparasitic activity lies not with the original compound but with its metabolites. The speed with which an anthelmintics is metabolites. The
speed with which an anthelmintics is metabolized and excreted determines the length of the withdrawal time. This speed can vary among species and can also be effected by the route of administration and the dose. While many gastrointestinal parasites reside in the lumen or close to the mucosa, others live at other sites e.g. liver and lungs; for action against these, absorption of drug from the gastrointestinal tract, injection sites, or skin is essential. Intestinal parasites come in contact not only with the unabsorbed drug passing through the gastrointestinal tract but also with the absorbed fraction in the blood as they feed on the intestinal mucosa and with any that is recycled into the gut.

The route of administration influences persistence in the body and thus efficacy. The ruminants, administration directly into the abomasum via the oesophageal groove, may increase the rate of excretion in the feces, which may reduce efficacy.

Operation of the ruminal bypass acts to reduce the efficacy of certain benzimidazole anthelmintics. (For example, immediate arrival of oxefendazole in the abomasum after dosing reduces its efficacy from 91% to 45% against thiabendazole - resistant strains of haemonchus contortus).

The rumen acts as a drug reservoir from which plasma concentrations can be sustained for long periods; it
also shows the passage of unabsorbed drug through the GI (gastro intestinal tract). In general, the benzimidazoles are most effective if deposited directly into the rumen, less so if injected into the rumen, less so if injected into the abomasum.

The absorption of levamisole is not affected by the route of administration because it is highly soluble and is unaffected by ruminal bypass.

The development of resistance by nematodes to various chemical groups of anthelmintics is recognized as a major problem. Until recently, resistance to anthelmintics in nematodes had been shown to develop under field condition (in comparison with antibiotic resistance in bacteria). However, resistance is becoming wide spread because relatively few chemically dissimilar groups of anthelmintics have been introduced over the past decades. Most of the commonly used anthelmintics belonging to two or three chemical classes, within which all individual compounds act in a similar fashion. Thus, resistance to one particular compound may be accompanied by resistance to other members of the group.

Continued application of a highly effective anthelmintic selectively removes most susceptible genotypes, with the resultant progeny of succeeding generations being composed of resistant strains. Resistance to an anthelmintic
is expressed by passage of increased numbers of parasite eggs, higher establishment rates of adults in the host, and greater numbers of larvae on the parasites after treatment than would occur if the parasites were susceptible to the drug. Resistance of *Haemonchus* species is becoming a global problem. Resistance to *Terichostrongylus* and *ostertagia* spp. in sheep and goats is also becoming common in all parts of the world where small ruminants are treated frequently. Resistance of small strongyles in horses is also a problem in many areas. Resistance to benzimidazole and levamisole has been reported in nematodes of swine. Although resistance to benzimidazoles, levamisole, and recently to macrocyclic lactones have all been reported for nematodes of cattle, resistant is less of a problem in cattle than in sheep, goats and horses.

Detection of significant levels of resistance seems to require 9-10 generation of helminths exposed to the same class of anthelmintic. However, evidence suggests that genes for resistance are already present, at a low frequency, when anthelmintics are introduced. Selection for resistance simply requires the preferential killing of the susceptible parasites and survival of the parasites with the resistance genes.

Anthelmintic\(^9\)-\(^{16}\) of different chemical grouping or of differing modes of action should be used in alternate years to prolong their worthwhile therapeutic existence. Care
should be taken to use the anthelmintic no more often than is needed to control the parasites; emphasis should be placed on husbandry methods to minimize exposure to the helminths.

Cross-resistance is frequently seen between members of benzimidazole group because of their similar mechanisms of action. Control of benzimidazole-resistant parasites by levamisole can be expected because of its different mode of action. Although there is no cross-resistance between levamisole and benzimidazole, this does not mean that worms resistant to both kinds of drugs will not evolve if both types of anthelmintics are used frequently. Nematodes resistant to levamisole are cross-resistant to mechanisms of actions.

In summary, emphasis should be placed on management practices designed to reduce exposure to parasites and to minimize the frequency of anthelmintic use. The development of an anthelmintic resistance may be delayed by using chemicals with different modes of action. The current recommendation to delay the onset of resistance when it is not already apparent is for a slow rotation of the different chemical groups. Because, with important exceptions, such as Haemonchus contortus, there are usually only one or two generations of parasites per years in temperate zones, anthelmintics from different groups probably should be rotated annually between dosing seasons. In the control of parasites, there is no doubt that
economic benefit is best obtained by planned treatment of a whole flock or herd and considering the biology of the parasite(s). Results should be good provided that correct control measures are directed against the parasite phase in the body of the host at the appropriate time and that attention is given to the free-living, non-parasitic stages in the miscellaneous anthelmintics phenothiazine was used extensively in livestock for years but has been largely replaced by drugs with broader spectra of activity. It is still used, primarily in ruminants, in prophylactic, low level, in-feed programs; in horses, it is used in mixtures with other drugs. It is not used in pigs, dogs, or cats. The mode of action is not understood. Toxicity within host species is variable, but the safety margin is narrow in comparison with most of the newer anthelmintics. Its efficacy is best against *haemonchus* and *oesophagostomum* species in rumintants and against small *strongyles* in horses.

Piperazine\(^{10-17}\) is rapidly absorbed from the gastrointestinal tract, and piperazine base can be detected in the urine as early as 30 min. after administration. The excretion rate is maximum at 1-8 hr and excretion is practically complete within 24 hr. Piperazine acts to block neuromuscular transmission in the parasite by hyperpolarizing in the nerve membrane, which leads to flaccid paralysis. It also blocks succinate production by the worm. The parasites, paralyzed and depleted of energy, are
expelled by peristalsis. The spectrum of activity of piperazine is largely against ascarid parasites in all species and also *Oesophagostomum* species. There is variable activity against hookworms and strongytes and little effect against whipworms or flatworms, the safety margin is wide.

Diethylcarbamazine (DEC), a derivative of piperazine, also acts to paralyze nematodes by interfering with nerve function. It is used for heartworm prevention in dogs. In existing infections, the dogs must first be cleared of adult heartworm and microfilariae to avoid reaction, then are given DEC daily PO throughout the mosquito season to prevent reinfection. Diethylcarbamazine (DEC) is also used to treat prepatent loose (lungworm infection) in cattle, although it is relatively ineffective against the mature form of *dictyocaulus viviparous*. It is routinely given IM at 22 mg/kg body weight for three consecutive days, although it is reported that one injection at 44 mg/kg gives better respiratory relief.

Praziquantel and epsiprantel are closely related analogs that have high efficacy against cestode parasites at relatively low dose rates but no effect on nematodes. In dogs, praziquantel is rapidly absorbed, and maximum blood levels are reached as early as 30-60 minutes after administration. After absorption, it is believed to be reexcreted back into the intestinal lumen via the mucosa, which may explain the extremely high efficacy even against
3-day-old *Echinococcus granulosus*. Epsiquentel at 5 mg/kg is highly effective against adult *Echinococcus granulosus*; but 10 mg/kg are required for high activity against 7-day-old *Echinococcus granulosus*. Cestodes buried in the crypts of lieberkühn surrounded by mucus and inflammatory exudates are usually rather inaccessible to anthelmintics in the lumen of the gut delivery to the sites of infection from the blood stream makes for more effective action. Resecretion into the gut is rapid; studies have identified the active drug in the ileum within 8 min of administration when the bulk of the administration dose still remained for higher up the gastrointestinal tract. Praziquantel exerts its antiparasitic effects by interfering with the regulation of intracellular Ca\(^{2+}\) concentration, impairing both motility and function of the suckers of the cestode. *In-vivo* studies have indicated that it induces paralysis of the parasites; thus, praziquantel acts, as many anthelmintics, primarily on neuromuscular co-ordination. Its margin of safety is wide. Clorsulon is a sulfonamide given PO as a suspension for liver fluke infection in cattle. A higher dose (7 mg/kg) is needed for immature flukes (eight week old) than for adult (3.5 mg/kg). In plasma clorsulon is bound to protein, which when ingested by liver flukes, inhibits enzymes of the glycolytic pathway. Although its safety margin is wide, clorsulon should not be used in dairy cows of breeding age, and the withholding period before slaughter is 8 days.
Bunamidine is another anticestodal compound. It is used in small animals and is most effective if given after fasting. It is observed and metabolized in the livers and leads to digestion of the tapeworms in the gut of the host. Vomiting and mild diarrhoea may be seen, and exercise or excitement should be avoided in dogs soon after dosage. Nitroscanate, like the substituted phenols, probably acts by uncoupling oxidative phosphorylation. It is one of newer broad-spectrum anthelmintics and is used in small animals against toxocara. Toxascaris, Taenia, Dipyldium, Ancylostoma, Uncinaria and Echinococcus species.

The benzimidazoles are the largest chemical family used to treat endoparasitic diseases in domestic animals. They are characterized by a broad spectrum of activity and a wide safety margin. Their high degree of efficacy is related both to their pharmacodynamic and pharmacokinetic properties. Those of current interest are thiabendazole, cambendazole, parbendazole, mebendazole, fenbendazole, oxfendazole, oxigendazole, albendazole, albendazole sulfoxide, thiophanate, febantel, netobimin, and triclabendazole.

Both albendazole and triclabendazole are active against liver flukes. However, unlike all the other benimidazole, triclabendazole has no activity against roundworms. The group includes thiabendazole analogues and benzimidazole carbamates; substitution of various side chains and radicals on the parents benzimidazole
carbamates; substitution of various side chains and radicals on the parent benzimidazole nucleus produces the individual members. Benzimidazole carbamates are characterized by novel substitutions on the benzimidazole nucleus and replacement of the thiazole ring by methylcarbamate. Such modifications have spawned a new benzimidazole generation with much slower rates of elimination, higher potencies, broader spectra, and complex metabolic pathways. A number of benzimidazoles (e.g. Febantel, thiophanate, and netobimin) exist in the form of pro-drugs, active because they are metabolized in the body to the biologically active benzimidazole carbamate nucleus. Synthesis of benzimidazoles carbamates depends on a cyclization process; metabolic or chemical modification generates an active anthelmintic in-vivo from an inactive prodrug precursor. Febantel is hydrolyzed to the active metabolite febendazole.

Netobimin undergoes processes of reduction, cyclisation, and oxidation to yield albendazole sulfoxide. Benzimidazole sulfoxides such as oxfendazole and albendazole sulfoxide bind poorly to parasite β-tubulin and probably act as prodrugs for fenbendazole and albendazole, respectively. The thio metabolites have high affinity for helminth tubulin. Oxidation/reduction between the thio and sulfoxide-substituted benzimidazole methyl carbamates is reversible. The benzimidazole methyl
carbamates is reversible. The benzimidazole anthelmintics have low toxicity in mammals; however, some of the benimidazole are teratogenic and depending on the dose rate, are indicated in early pregnancy, and require with holding periods.

Because most benzimidazoles are sparingly soluble in water, they are given PO as a suspension, paste, or powder, or by intraruminal injection. Differences in the rate and extent of absorption from the gastrointestinal tract depend on such factors as species, dosage, formulation, solubility, and operation of the oesophageal groove reflex.

Benzimidazoles bind to β-tubulin, a structural protein, and block polymerization of tubulin into microtubules. This damages the integrity and transport functions of cells within the parasite. The antiparasitic effect is a lethal, but relatively tardy, process. Binding of benzimidazole to β-tubulin is reversible and saturable. Antimitotic effects have been documented for a number of benzimidazole, and some experimental work has revealed effects such as chromosome doubling, due to malfunctioning of the mitotic spindle caused by depolymerization of microtubulus.

The wide safety margin of benzimidazoles is due to their greater selective toxicity is not absolute; some toxic effects based on antimitotic activity (tetratogenicity or embryotoxicity) can occur in target species. The most
Effective of the group are those with the longest half life in the body, such as oxfendazole, fenbendazole, albendazole and their prodrugs, because they are not rapidly metabolized to inactive products, effective concentrations are maintained for an extended period in the plasma and gut, which increases efficacy against immature and arrested larvae and adult nematodes. The following generalities apply to the benzimidazoles. They are more effective in ruminants and horses, in which their rate of passage is slowed by the rumen or caecum. Divided doses are more effective than a single dose because the nature of their antiparasitic action depends on prolongation of contact time. The most effective benzimidazoles are less readily metabolized to inactive soluble products that earlier compounds, i.e. the kinetics of elimination are slowed.

In the case of oxfendazole and probably other benzimidazoles absorbed drug is at least as important in achieving efficacy against nematodes in the abomasums and small intestine as the unabsorbed drug passing down the Gastrointestinal tract. Once in the blood stream, Oxfendazole recycles across the gut wall between the vascular system and the gastrointestinal tract. Worms in the mucosa of the abomasums and small intestine may be exposed to this recycling anthelmintic to a greater extent than to drug contained in the passing ingesta in the gastrointestinal tract. Albendazole and fenbandazole are reversibly
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oxidized to their sulfoxides, which are further irreversibly oxidized to the sulfones. Tubulin binding activity is associated with the thio-metabolites. However, the sulfoxides are metabolically converted to the thio compounds and thus share anthelmintic activity with them. The sulfones can not be converted to thio compounds and are inactive. Oxidation of the thio to the sulfoxides and the sulfoxide to the sulfone appears to occur mainly in the liver. Ruminal and intestinal fluids residue the sulfoxide to the thio. The relative rate of oxidation in liver and reduction in the gastrointestinal tract varies between cattle and sheep. The metabolism and excretion of thio-bendazolate is more extensive in cattle than in sheep. As a consequence, the systematic anthelmintic activity of most benzimidazoles is greater in sheep than in cattle, and dose rates in cattle are often higher than those in sheep.

Residues of albendazole, oxfendazole, fenbendazole, febantel, netobimin, and thiabendazole concentrate primarily in the liver in cattle and sheep, and persist longest in this tissue. Residues are more persistent for fenbendazole, oxfendazole, and febantel than for albendazole or thiabendazole. Although residues are detectable in milk for these drugs, their presence is relatively short lived. The persistence of high blood concentrations of such active benzimidazoles is important for activity against both tissue dwelling parasites, such as
arrested Ostertagia, ostertagi larvae, and parasites in the gut, such as Haemonchus and Trichostrongylus species.

In ruminants, oral dosing with the benzimidazoles removes most of the major adult gastrointestinal parasites and many of the larval stages. Albendazole, fenbendazole, oxfendazole, and febantel are active against inhibited fourth stage larvae of ostertagia species. The degree of efficacy of these compounds in the prevention of ostertagiasis type II in cattle may be related to the degree of hypobiosis of the larvae (i.e. those with love metabolic actively are less susceptible to disruption of microtubules by the benzimidazoles). Efficacy against Dictyocacilus viviporus has also been noted for these insoluble benzimidazoles.

Parbendazole\textsuperscript{18-21} is the primary benzimidazole for which teratogenic effects (Skeletal malformations) have been demonstrated in sheep. Cambendazole, oxfendazole, albendazole, and febantel are also teratogenic in sheep whereas fenbendazole, mebendazole, and oxibendazole are not. Teratogenic effect occurs at dosages much lower than those associated with acute toxicity in target species. Teratogenicity varies among animal species; dose rates. Specific benzimidazole, and stage of embryonic development are major influencing factors. Fenbendazole and oxfendazole are metabolically inter converted but show differential teratogenicity. Fenbendazole sulfoxide can occurs in two chiral forms, one of which predominates
when oxfendazole is administered and appears to be more embryo toxic than the other clinical form, which is more apparent after fenbendazole administration. Cattle seem to be unaffected by most benzimidazoles that are teratogenic in sheep. A similar relationship exists between rates and rabbits. Many benzimidazoles teratogenic in sheep are also teratogenic in rats.

Although benzimidazoles all have similar pharmacodynamics effects (anti-tubulin), species differences in sensitivity to embryo toxic effects are attributable to metabolic and pharmacokinetic disposition factors. Benzimidazoles can be classified as follows:

1. Those that are not teratogenic and have no teratogenic metabolite (e.g. Oxibendazole).

2. Those that are not teratogenic but give rise to a teratogenic metabolite (e.g. Albendazole).

3. Those that seem to be teratogenic and have no teratogenic metabolite (e.g. Oxefendazole).

4. Those that are possibly teratogenic and have additionally a metabolite that seems to be more toxic than is the present compound (e.g. Mebendazole).

In horses, the benzimidazoles are characterized by effective removal (90-100%) of almost all mature strongyles, but third and fourth stage larvae are more difficult to eliminate. High levels and repeated administration may be
necessary for extra intestinal migrating stages of large strongyles and for small strongyle larval embedded or encysted in the wall of the intestine. Repeated doses are thought to be advantageous because the lethal effect of benzimidazoles is a slow process; hence, their, incorporation into feed supplement. Ascarid removal in horses varies with various members of the benzimidazole group; frequently an increased dosage is required activity against strongyloides species varies also, but oxyurid is usually removed by any of the benzimidazoles at the recommended dose.

In horses and cats, membendazole is used for treatment of roundworms, hookworms and tapeworms. However, treatment must be given b.i.d. for 3 days. Fenbendazole has been used in a divided dose regimen in the bitch against tissue-dwelling larvac of Toxocara canis and Ancylostoma caninum; daily administration of 50mg/kg to bitches from day 40 of pregnancy through day 14 after parturition resulted in pups free of both parasites.

In cattle and sheep, triclabendazole at 10 mg/kg, P.O. is highly effective against immature Fasciola hepatica in the liver parenchyma and against the mature stage in the bile ducts. The maximum tolerated dose in the target species is 200mg/kg; thus, the safety margin is 20. Of the other benzimidazole and probenzimidazole used for nematode control, some have marginal efficacy at increased doses rates against liver flukes; albendazole and netobimin are active
against mature Fasciola hepatica. Because of the lack of efficacy against the immature stages, most benzimidazole are not indicated for treatment of acute fascioliasis and have limited value in control of the disease, cures after treatment use as a tartrate, embonate, or pamoate salt.

Aqueous solutions are subject to isomerisation on exposure to light, with a resultant loss in potency; therefore, suspensions should be kept out of direct sunlight. It is not recommended for use in severely debilitated animals because of its levamisole type pharmacologic action. Pyrantel tartrate is well absorbed by pigs and dogs, less well by ruminants. Metabolism is rapid and their metabolites are excreted rapidly in the urine (40% the dose in dogs); some unchanged drug is excreted in the feces (principally in ruminants). Blood levels usually peak 4-6 hr after administration P.O. The pamoate salt of pyrantel is poorly soluble in water, this offers the advantages of reduced from the gut and allows the drug to reach and be effective against parasites in the lower end of the large intestine, which makes it useful in horses and dogs. Pyrantel is used PO as a suspension, paste, drench, or tablets. It is effective against ascarids, large and small strongyles, and pinworms. Oxantel, a phenol analog of pyrantel, is combined with pyrantel is some anthelmintic preparation for dogs (and man) to increase activity against whipworms.
Morantel\textsuperscript{22-30} is the methyl ester analog of pyrantel and, in ruminants, it tends to be somewhat safer and more effective than pyrantel. It is absorbed rapidly from the abomasums and upper small intestine of sheep and metabolized rapidly in the liver; 17% of the initial dose is excreted in the urine as metabolites within 96 hr after dosing. Both pyrantel and morantel have a higher efficacy against adult gut worms and larval stages that well in the lumen or on the mucosal surface than against the stages found in the mucosa (such as arrested ostertagia larvae). A sustained released ruminal bolous for use in cattle, which releases the morantel over 90 days, has been developed. No withdrawal period (at least in some countries) is required for the biodegradable morantel bolous for cattle. Salicylamilides, substituted phenols, aromatic amide.

**SALICYLANILIDES**

Brotianide, dioxanide, closantel, niclosamide, oxyclozamide, rafoxanide.

**SUBSTITUTED PHENOLS**

Bithionol, disophonol, hexachlorophone, niclofolan (menichlopholan), nitrooxnil.

**AROMATIC AMIDE**

Diamfenetide (diamphenethide).

All members of these chemical grouping are active against liver flukes.
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Diamfenetide is unique in that it has exceptionally high activity against the youngest immature stages of the liver fluke in sheep, with a diminution of its activity as the flukes matures. Nitroxynil is normally administered SC; the rest of the group are given PO. The salicylanilides and substituted phenols act to uncouple or disconnect the mitochondrial reactions involved in electron-transport-associated events from ATP generation. This uncoupling is lethal to Fasciola hepatica and other blood-sucking helminthes including the nematode Haemonchus contortus. In-vivo mainly the adult flukes are affected, with variable activity against the immature flukes in the liver parenchyma.

The lowered efficacy of a number of the salicylanilides and substituted phenols against the immature flukes may be due to the high protein binding of these drugs in the blood. A number of these compounds however, appear to have activity against 6 weeks old fluke in cattle and sheep.

By affecting them either at the time of administration or, more probably, by persisting in blood until the flukes starts to ingest blood and become exposed to higher drug concentrations. Metabolism may affect the pharmacological activity of various fasciolicides, and some of this metabolism may occur in the gastrointestinal tract, for diamfenetide, which is given PO, this metabolism may be important for full efficacy. Nitroxynil is metabolized by ruminal bacteria, which destroy its activity and restricts administration in
injection. After absorption, diamfenetide is further metabolized in the liver to an amine metabolite that is active against flukes; it is not active against liver flukes *in-vitro* unless incubated in the presence of enzymatically fractional liver cells. Oxyclozanide is metabolized in the liver to the active glucuronide, which is excreted in the bile in high concentrations in the vicinity of the adult fluke. Most of the available fasciolicides are administered as oral suspensions or occasionally as solutions by SC injection. Niclosamide is poorly absorbed from the gastrointestinal tract; the bulk of the dose remains in the lumen of the gut where it exerts its taeniacidal effect by inhibiting oxidative phosphorylation in the parasite. It is used primarily in dogs and also in eastern Europe in ruminants infected with Moniezia species.

Increasing the dosage of these compounds frequently results in increased activity against the later parenchymal stages of *Fasciola* species, but when such increased dosages are used, the inherent safety margin of the particular drug must be recognised. The bile ducts are important in the excretion of many of these phenol-based compounds, as evidenced by the high proportion of these and their metabolites excreted in the feces rather than in the urine. The increased susceptibility of developing flukes is largely related to the long plasma half lives of the compounds, which requires lengthy withholding periods. The fasciolicidal activity of salicylanilides in sheep depends on the extent to
which the drug persists in plasma. For example, rafoxanide is fully absorbed and plasma levels of 29 μg/ml are found 24 hr after dosing. The plasma half life is 4 days, and rafoxanide binds to plasma proteins with a very high affinity. Up to 2 mg/ml can dissolve in plasma, but rafoxanide is virtually insoluble in water. Relatively high levels of residues are found in plasma even 42 days after dosing, but residues in other tissue are negligible. The plasma half life of disophenol is even longer, being 7-14 days in days in dogs and 30 days in sheep.

The high efficacy associated with long plasma half life has the disadvantage of long with holding periods.

The high efficacy of many salicylanilides and substituted phenols against blood sucking parasites, e.g. Halmonchus contortus and hookworms, may be related to their attachment to plasma proteins.

Presumably they are released to poison the parasites after it ingests blood. Pharmacokinetic data for many modern fasciolicides is sparse. Peak plasma levels, which may be an indicator of efficacy, are reached in 12 - 24 hr for salicylanilides and 3-4 days for bithionol sulfoxide. The absorption of fasciolicides given parenterally (nitroxynil) is rapid and complete plasma levels peak 30-60 minute after dosing. The relatively high residues of nitroxynil found in milk are due to the relatively high dose rate, parenteral
administration and the tendency to form stable complexes with serum and body proteins. Nitroxynil is retained in the liver and plasma of sheep at detectable levels (> 0.1 ppm) for 66 days after a single dose of 10 mg/kg. Although binding to serum albumin occurs, long-term exposure to nitroxynil is critical for antiparasitic activity. Plasma levels associated with activity against the mature fluke are 55 ppm. Closantel, rafoxanide, and oxyclozanide have long terminal half lives in sheep (14.5, 16.6 and 6.4 days, respectively), which are related to the high plasma protein of these three drugs (>99%), and residues in liver are detectable for weeks after administration.

Because these drugs, with the possible exception of diamfenetide, are general uncouplers of oxidative phosphorylation, their safety indexes are not as high as for many other anthelmintics, but nonetheless, are more than adequate if used as directed. Adverse effects are most commonly seen in animals severely stressed, in poor condition nutritionally or metabolically, or with severe parasite infections. Commonly, a slight loss of appetite and looseness of feces may be seen after treatment at recommended dosages. High dose rate may cause blindness and classic signs of uncoupled phosphorylation - hyperventilation, hyperthemia, convulsions, tachycardia, and ultimately health.
Consumer concerns arise because of narrow safety margins, pharmacodynamics effects, long half-lives, high binding to plasma protein, deposition in the liver, and excretion in detectable quantities in milk. However, because these fasciolicides are used no more than 2-3 times/year, residues are unlikely to occur in milk with any great frequency.

Nonetheless, safe levels could possibly be exceeded in infants or small children consuming \( \geq 1 \) lit of milk/day, even at the end of withholding periods.

**ANTHELMINTIC TESTING**

The ultimate test of anthelmintics activity is the ability of a chemical agent to eliminate worms from a specifically parasitized animal with a minimum of toxic effect to the host. Although at one time a suitable *in-vitro* test was considered a useful screening method, current thinking is directed toward *in-vivo* screening.

**IN-VITRO METHOD**

The *in-vitro* methods\(^{32}\) which has received much consideration as a quick means of determining parasiticidal effect, must take into account the availability of the worm material as well as the special physiological and anatomical characteristics of the worm preparation in relation to the test compound. As early as 1916, *in-vitro* test for ascaricides
were conducted substituting the softer earthworms and leach for the ascaris as testing medium.

This was done because of the resistance offered to chemical attack by the outer cuticle of round worms. Since the rather innocuous earthworm reacted to over 120 different drug a manner completely unrelated to the ascaris, this method was necessarily abandoned. Baldwin studied the feasibility of using certain anatomical segments of the round worm as test objects. Semi-isolated strips of muscle, obtained by denuding the body wall of the pig ascaris, were suspended in a buffered, synthetic medium and the neuromuscular effect of various drugs was measured. It was reported that both the anthelmintic properties and physiological responses elicited by the chemical investigated were reproducible and correlated well with in-vivo findings. In considering in-vitro testing methods, it should be born in mind that these preparations would be of little value if the activity of the paraciticide in question depended on: (i) alimentary tract absorption in the parasite, (ii) interference with the worm's supply of essential metabolites, or (iii) chemical alternation in the host's body before reacting the site of infection.
IN-VIVO SCREENING METHOD

The *in-vivo* screening methods\(^\text{33}\) enable the investigator to observe the potency of various drug on the parasite in its natural environmental, thereby presenting a truer picture of anthelmintic effect. Using this procedure, the number and condition of or a eliminated before and after drug administration can be recorded and compared with controls. After completion of dosage laboratory examination of a test animal may reveal migration of immature and mature worms of both sexes, the number of parasites remaining in the host dead or alive and any physiological changes in the host or helminth. This is also a possible means of ascertaining in a general way, the mode of drug action. In many instances laboratory animals with natural infections harbor more than one type of helminth. This is highly useful in determining the spectrum of an experimental drug. The possibility that drugs found active against animal helminthiases may retain their activity when used in human therapy should also be included in establishing a rationable for *in-vivo* screening. Even though exceptions to this rule are to be expected, it is as serviceable in the field of anthelmintics as it is in the field of pharmacodynamics. Disadvantages which arise from the use of this method are the expense of maintaining large colonies of test animals, the increased quantity of synthetic chemicals required for testing, and the time consumed in treating the animals and
evaluating results. Some of these difficulties can be offset by selecting relatively inexpensive experimental animals having multiple infections. The more promising drugs uncovered in initial screening can then be retested in a large animal.

The techniques used to parasitize laboratory animals have been well developed, as have the methods employed in determining anthelmintic activity. Most of the parasitic infections can be produced in small test animals by oral or intraperitoneal administration of the infective stage of the parasite. In many cases perasitization can be avoided by using naturally infected animals. The degree of parasiticidal or antiparasitic activity elicited by a chemical agent is usually determined as follows - (i) Ova-count reduction or disappearance of ova from the animal excrement; (ii) passage of norms, (iii) examination of blood sample and (iv) migration of the parasite within the host to an organ in which it is destroyed.

**DRUGS IN HELMINTHIASIS**

Since the anatomy of the pathogenic worm includes membranes, functional organs, nerve trunks and glandular system, it is not an easy task to determine the absorption and site of activity of most anthelmintics in the parasitic system. Indeed, the systemic effect of a drug on a worm can be as complicated as its effect on the host. Although it is difficult to define the action of most anthelmintics in an
exact statement, in general, their effectiveness is due to one of the following modes of action: (i) Narcosis, paralysis or death of the parasite causing as elimination; (ii) Irritation or burning of the worm tissue; (iii) Digestion of the helminth by a proteolytic agent; and (iv) Disturbance of the worm by a chemical agent causing it to migrate and subsequently be destroyed. The physiology of the parasitic worm is therefore an important consideration in developing effective anthelmintics.

The more common parasites may be divided into the following group according to their attachment: (i) Those which are unattachment in the intestinal tract, e.g. Ascaris and Enterobius; (ii) Those which are attached to the intestinal wall, e.g. Trichuris and Trichinella; and (iii) Parasites which are totally imbedded in mucosa such as strongyloides. The human blood flukes or schistosomes attach themselves to the intima of the portat or caval veins. The Loci of infection must therefore be considered in designing chemical agents to be used as anthelmintics. For example a drug which is rapidly absorbed in the higher intestine would be ineffective against parasites located in the colon or rectum. On the other hand, an active nucleus or active fragment which is slowly released because of chemical reaction or metabolic fate would show little activity against strongyloides. Stercoralis since these worms are located in the region of the duodenum and upper jejunum.
As in all forms of chemotherapy, an anthelmintic must have a wide margin between its toxicity to the worm and its toxic side effect on the host. A drug used in treating parasitic infections should be orally active and preferably effective in one dose (it is interesting to note that in some backward regions the natives consider an injection 'powerful' medicine).

From a practical stand point, the drug should also be inexpensive since parasitic infections are heaviest where the population is economically the poorest.

**CHLORINATED HYDRO CARBONS**

Tetra chloroethylene,\(^{35}\) Cl\(_2\)C = CCl\(_2\), the most important chlorinated hydrocarbon in the field of anthelmintics is active against intestinal flukes in human, hookworms infections in animal and man, and trichostrongylus in cattle. It has superseded carbon tetrachloride, CCl\(_4\), as the drug of choice in treating ancylostomiasis (hookworm disease) because it is effective, inexpensive, and has been well-tolerated in hundreds of thousands of doses. Although carbon tetrachloride is effective and inexpensive, its toxicity to liver cells makes it undesirable.

Tetrachloroethylene is manufactured as an industrial solvent by chlorination of ethylene chloride over pumice stone at 300 - 500°C. Hookworm flariform larvae resulting
from dog or cat hookworm infections can penetrate and migrate under the cutaneous layer of human skin causing "Creeping eruption" (Cutaneous larvae migrans). By using an ethyl chloride spray, which temporarily freezes the parasitized portion of the skin, the infective larvae are killed and later absorbed by phagocytosis.

Butyl chloride and hexachloroethane, Cl₃CC Cl₃, have also been active as antihookworm agents. It has been claimed that in cases where carbon tetrachloride, tetrachloroethane, and hexyresorcinol have failed to gain a response against hookworm infections, N-C₂, 4-dichlorobenzyl)-N-(β-hydroxyl ethyl) dichloroacetamide (Fig.1) an agent designed for the treatment of amebiasis, has been effective.

![Chemical structure](Fig. I)

**PHENOL**

Compounds possessing a high phenol coefficient and a favourable therapeutic index have been tested and used in treating many worm infections.

Tymol, 2-isopropyl-5-methylphenol (Fig.II)
which occurs in the essential oil of thymus vulgaris, and β-naphthol are effective in eliminating hookworm infections (ancylostomiasis). However, both of these agents have fallen into disfavour because of their small margin of safety at the recommended dose, α-Bromo-β-naphththal (Fig.III) is a potent antihookworm agent having a minimum of side effects.

The investigations of Lamson and his co-workers contributed a long, systematic list of phenolic anthelmintics. No distinct regularity could be elaborated from these studies, but the well known urinary antiseptic, 4-n-hexyl resorcinol was shown to possess excellent anthelmintic properties. Although it is not as potent as santonin, its low toxicity makes it a desirable nematoside and tuenocide. The
potency of hexyl resorcinols is attributed to its selectives "phenol burning" of the parasite tissue. Administered in dose up to 19, it also exerts a distinctive bactericidal effect, which allows sterilization of tissue damaged by the worms.

Hexyl resorcinol (Fig. IV) is synthesized by caproylation of resorcinol followed by reduction of the resulting amyl ketones.

\[
\begin{align*}
\text{OH} & \quad + \quad \text{CICO} \quad \text{C}_5\text{H}_{11} \\
\text{OH} & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \q
toxic. The carbamates of 2-hydroxybiphenyl and 4-benzylphenol are already in use as vermifugues, but their effectiveness is questionable. This increase of physiological effect by carbamylation has been demonstrated in other fields of medicinal chemistry, e.g. By ethinamate in sedation and maprobamate in ataractics. The finding that two separated benzene rings were more effective as taenicides than the related condensed (naphthol type) system, resulted in the synthesis of a variety of halogenated diphenylmethanes and diphenyl ethers. Two representative derivatives in this series which are very active against chicken tapeworm are 2, 2\text{\textsuperscript{1}}-dihydroxy 3,3\text{\textsuperscript{1}}, 5,5\text{\textsuperscript{1}}, 6,6\text{\textsuperscript{1}}-hexachlorodiphenylmethane (Fig.V) and 2,2\text{\textsuperscript{1}}-dihydroxy 3,3\text{\textsuperscript{1}}, 4, 4\text{\textsuperscript{1}}, 5, 5\text{\textsuperscript{1}}, 6, 6\text{\textsuperscript{1}}-octachlorodiphenyl ether (Fig. VI) etc.
Table 1.1: Some useful Anthelmintics

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Nonproprietary names</th>
<th>Proprietary Names</th>
<th>Principal helminthiosis treated with agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetra chloroethylene</td>
<td>-</td>
<td>-</td>
<td>Hookworm infection and ascariosis</td>
</tr>
<tr>
<td>4-hexylresorcinol</td>
<td></td>
<td>Crystoids anthelmintic</td>
<td>Ascariosis hook and whipworm infection</td>
</tr>
<tr>
<td>1-Diethyl-carbamyl-4-methyl piparizine</td>
<td>Diethyl carbamazine</td>
<td>Hetrazan</td>
<td>Filarisis and ascariosis</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td></td>
<td>Antepar citrate Multifuge citrate piparazine citrate</td>
<td>Ascariosis and pinworm infection</td>
</tr>
<tr>
<td>Hexamethyl pararosaniline chloride</td>
<td>Methylrosaniline chloride</td>
<td></td>
<td>Strong loidiasis</td>
</tr>
<tr>
<td>Sodium antimony III bis-pyro catechol-2,4-disulfonate</td>
<td>Stibophon</td>
<td>Fuadin</td>
<td>Schistosomiasis</td>
</tr>
</tbody>
</table>
NATURAL PRODUCT

The anthelmintic properties of many naturally occurring product have been known since the beginning of cultivation. In fact, a few heirloom of ancient medicine are still in use e.g. extract of male fern (Flix-mass) which was valued as a parasiticidal agent in ancient Greece; the area of nut and pomegranate bark used centuries ago in China, and Chenopodium (oil of worm seed) which was used as anthelmintic potion by the American, Indians.

PLANTS AS POTENTIAL ANTHELMINTIC AGENTS

Plants are the good source of well established traditional and modern drugs and phytochemicals. Ethnobotanical survey and documentation have been taken world wide. It is thought to be quite fruitful to study the world Ethnobotanical information about medicinal plants having anthelmintic activity. Some plants are common to are many regions where plates are unique. The potential importance of the medicinal plants is due to the presence of some specified chemical substances in the specific parts of the tissue of the plants. Since the time of Charaka and Sushruta many herbal medicines in different oral formations have been recommended for the treatment of helminthiasis. Extracts of drugs from plants sources such as Chenopodium ambrocioides, Embelia ribes Burm.
Trachyspermum ammi, Punica gratum, Artemisia maritima, Cucurbita maxima, Tridax procumbens Linn., and Gingiber officinale are some of the plants reported to possess anthelmintic activity.

A deep sweep in the available literature on the anthelmintic plants has revealed that a large number of compounds have been isolated from the anthelmintic plants, and therefore the following two anthelmintic plants were selected for the present investigation, in view of their significant medicinal values.

(i) *Ficus glomerata* Roxb and

(ii) *Embelia ribes* Burm

**FICUS GLOMERATA ROXB**

*Ficus glamerata*\textsuperscript{36-40} Roxb (Gular) belongs to natural order Moraceae. It is cultivated in throughout India. It is an evergreen tree found in throughout India.

Different parts of plants are used in the treatment of dysentery, vulnerary, kapha, biliousness, and diseases of vagina. The roots are useful in hydrophobia. The bark is useful in asthma, piles, and galactagogue.

The fruit is useful in blood diseases, biliousness, burning, sensations, fatigues, urinary discharges, thirst, menorrhagia, nose bleeding; causes "kapha" and
Photograph 1: Plant *Ficus glomerata* (Roxb.)
intestinal worms (Ayurveda). The leaves are astringent to the bowels and good for bronchitis. The fruit is useful in chronic bronchitis, dry cough, loss of voice, diseases of the kidney and spleen. The ashes are diuretic and useful in gleet (Yunani).

**EMBELIA RIBES BURM**

*Embelia ribes* Burm, which is commonly known as Baberang in hindi and belongs to natural order Myrsinaceae. It is an evergreen tree found in throughout India.

Its fruit is hot, dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alexiteric, laxative, alterative, cures tumours, ascites, bronchitis, mental diseases, dyspnaea, diseases of the heart, urinary discharges; used in snake-bite, jaundice, hemicrania, and worms in wounds (Ayurveda).
Photograph 2: Plant *Embelica ribes* (Burm.)
### Table - 1.2 : Plant - *Ficus glomerata* Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Part of the Plant</th>
<th>Compound Isolated</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Leaves</td>
<td>β-Sitosterol</td>
<td>I</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Leaves</td>
<td>β-Amyrin</td>
<td>II</td>
<td>51</td>
</tr>
<tr>
<td>3.</td>
<td>Stem-bark</td>
<td>Leucocyanidine-3-O-β-D-glucopyranoside</td>
<td>III</td>
<td>52</td>
</tr>
<tr>
<td>4.</td>
<td>Stem-bark</td>
<td>Leucopelargonidine-3-O-α-L-rhamno-pyranoside</td>
<td>IV</td>
<td>53</td>
</tr>
<tr>
<td>5.</td>
<td>Stem-bark</td>
<td>α-Amyrin</td>
<td>V</td>
<td>54</td>
</tr>
<tr>
<td>6.</td>
<td>Stem-bark</td>
<td>α-Amyrin acetate</td>
<td>VI</td>
<td>55</td>
</tr>
<tr>
<td>7.</td>
<td>Trunk bark</td>
<td>Stigmasterol</td>
<td>VII</td>
<td>56</td>
</tr>
<tr>
<td>8.</td>
<td>Leaves</td>
<td>Gluonolacetate</td>
<td>VIII</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Stem-bark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Fruit</td>
<td>Hentriacontane</td>
<td>IX</td>
<td>58</td>
</tr>
<tr>
<td>10.</td>
<td>Fruit</td>
<td>Tiglic acid</td>
<td>X</td>
<td>59</td>
</tr>
<tr>
<td>11.</td>
<td>Fruit</td>
<td>Lupeol</td>
<td>XI</td>
<td>60</td>
</tr>
<tr>
<td>12.</td>
<td>Fruit</td>
<td>Lupeol acetate</td>
<td>XII</td>
<td>61</td>
</tr>
</tbody>
</table>

### Table-1.3 : Plant II - *Embelia ribes* Burm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Part of the Plant</th>
<th>Isolated Compound</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Seeds</td>
<td>Embelic acid</td>
<td>XIII</td>
<td>62</td>
</tr>
<tr>
<td>2.</td>
<td>Seeds</td>
<td>Villangin</td>
<td>XIV</td>
<td>63</td>
</tr>
<tr>
<td>3.</td>
<td>Seeds</td>
<td>Tannin</td>
<td>XV</td>
<td>64</td>
</tr>
<tr>
<td>4.</td>
<td>Seeds</td>
<td>Quercetol</td>
<td>XVI</td>
<td>65</td>
</tr>
</tbody>
</table>
STRUCTURE OF ISOLATED COMPOUNDS

I. β-Sitosterol$^{50}$

II. β-Amyrin$^{51}$

III. Leuco Cyanidine- 3-O-b-D-glucopyranoside$^{52}$
IV. Leucopelargonidene-3-O-α-L-rhamno pyranoside\textsuperscript{53}

V. α - Amyrin\textsuperscript{54}

VI. α - Amyrin acetate\textsuperscript{55}
VII. Stigmasterol

VIII. Gluanol-acelate

IX. Henriciacontane $C_{31}H_{64}$
X. Tiglic acid\textsuperscript{59}

XI. Lupeol\textsuperscript{60}

XII. Lupeol acelate\textsuperscript{61}

XIII. Embelic acid\textsuperscript{62}
XIV. Villangin

XV. Tannin

XVI. Quercetol
PROBLEM TAKEN AND WORK DONE

Anthelmintics are therapeutic agents that are used to destroy parasitic worms or remove them from the infected host. The majority of helminth infections are acquired by contact with:

(a) Infected animals
(b) Ground contaminated by human or animals excrement.
(c) Water infected with cercariae
(d) Ingestion of infected meat.

Parasitic worms are dependent upon the host for permanent existence. They must therefore have some method of gaining access to the body of the host and their off springs (either eggs or larvae) must have a means of escaping from the host's body to perpetuate the species; helminth eggs or larvae are generally not able to produce an immediate infection in a new host.

A period of time, varying from a few hours in the case of oxyurids to months in the case of other parasites is necessary before the infective stage to again reached, before preventive measures against the spread of parasitic infections can be taken. An exact knowledge of this critical period in the life cycle of the helminth is essential.
Surveys have shown that one-third of the human race suffers from helminth diseases, of which a large number are multiple infection. Although helminth infections are usually associated with tropical regions more than 40 million Americans are also victims.

Fascinated by the important medicinal values of the two plants (i) Ficus glomerata Roxb. Plant and (ii) Embelia ribes Burm.; the author carried out their further phytochemical investigations$^{65-80}$ and the findings of the author is summarized below:

**Table 1.4 : A brief account on isolated compound**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Part of the plant</th>
<th>Isolated compounds</th>
<th>Molecular formula</th>
<th>Melting point (°C)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Ficus glomerata</em> Roxb.</td>
<td>Stembark</td>
<td>Lupeol-3-O-α-L-xylopyranosyl [1→4]-O-β-D-glucopyranoside</td>
<td>C$<em>{41}$H$</em>{68}$O$_{10}$</td>
<td>190-191</td>
<td>MS-I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>β-amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-rhamnopyranoside</td>
<td>C$<em>{42}$H$</em>{70}$O$_{10}$</td>
<td>200-202</td>
<td>MS-II</td>
</tr>
<tr>
<td>2</td>
<td><em>Embelia ribes</em> Burm.</td>
<td>Seed</td>
<td>Quercetol-3-O-β-D-arabinopyranoside</td>
<td>C$<em>{26}$H$</em>{18}$O$_{10}$</td>
<td>240-241</td>
<td>MS-III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seeds</td>
<td>Stigmasterol-3-O-β-D-arabinopyranosyl [1→4] O-β-D-glucopyranoside</td>
<td>C$<em>{40}$H$</em>{66}$O$_{10}$</td>
<td>190-192</td>
<td>MS-IV</td>
</tr>
</tbody>
</table>
II. ISOLATION AND STUDY OF THE SAPONIN, MS-I; LUPEOL-3-O-α-L-XYLOPYRANOSYL [1→4]-O-β-D-GLUCOPYRANOSIDE FROM THE STEM-BARK OF FICUS GLOMERATA ROXB.

This chapter deals with the isolation of the saponin MS-I (yield 0.07125%), molecular formula C$_{41}$H$_{68}$O$_{10}$, m.p. 190-191°C and (M$^+$) 720 (FABMS). It was obtained by column chromatography of the precipitate obtained by adding excess of solvent ether in the methanol soluble fraction of concentrated ethanolic extract of stem-bark of *Ficus glomerata* Roxb.$^{81-85}$

Compound MS-I showed significant bands, in the IR spectrum at $\nu^{KBr}_{max}$ cm$^{-1}$. 3906.1-3423.2 (-OH), 2817.4 (CH$_3$ stretching), 1596.2 (C=C stretching), 1461.2 (C–H bending vibration of methyl group), 1383.3 (CH$_3$ symmetric stretching), 1352.2 (CH$_3$ bending), 1112.7 (symmetric (C–O–C) and 769.2 (C–H out of plane bending). It's $^1$H-NMR signals were recorded at (300 MHz, DMSO): δ 0.68 (3H, s, Me-C$_{22}$), δ0.82 (3H, s, Me-C$_{23}$), δ 0.94 (3H, s, Me-C$_{24}$), δ0.80 (3H, s, Me-C$_{26}$), δ0.83 (3H, s, Me-C$_{27}$), δ0.84 (3H, s, Me-C$_{29}$) δ0.85 (2H,S,C$_{28}$–H), δ1.2-2.03 complex pattern, 26 H (polymethylene CH$_2$ and CH proton), δ4.40 (1H,m,C$_3$H), δ3.43 (5H,m, glucose proton), δ2.42 (6H,m, xylose proton), δ5.32 (1H,d, J-7.3 1’ anomeric proton), δ4.33, (1H,d, J-7.1, 1” anomeric proton), δ2.04 (3H,s,C’$_2$-OAc), δ2.05 (3H,s, C’$_3$-Oac), δ2.06, (3H,s,C’$_6$-Oac), δ2.08, (3H,s,C”$_2$-Oac), δ2.09, (3H,s,C”$_3$-OAc) and δ2.11 (3H,s,C”$_4$-OAc).
It's FABMS showed significant fragmentation peaks m/z = 720, 588, 426, 232, 206, 178 and 176.

On acid hydrolysis MS-I yielded sapogenin MS-I (A) which was identified as Lupeol (by superimposable spectral analysis). The sugars obtained from the acid hydrolysis of the saponin MS-I were identified as L-xylose (R_f-0.28) and D-glucose (R_f-0.18) by CoPC and CoTLC, whereas periodate oxidation, partial and enzymatic hydrolysis showed the bisdesmosidic nature of the glycoside and sugars were present in the ratio of 1 : 1. L-xylose was terminal sugar and D-glucose was linked to sapogenin via β-linkage and L-xylose was linked to D-glucose via α-linkage.

Thus the saponin MS-I was identified as; Lupeol-3-o-α-L-xylopyranosyl [1→4]-o-β-D-glucopyranoside.
III. ISOLATION AND STUDY OF THE SAPONIN (MS-II); β-AMYRIN-3-O-β-D-GALACTOPYRANOSYL [1→4]-O-α-L-RHAMNOPYRANOSIDE FROM THE ROOT OF FICUS GLOMERATA (ROXB).

This chapter deals with the isolation of the saponin MS-II (yield 0.0546%), molecular formula C_{42}H_{70}O_{10}, m.p. 200-202°C and (M') 734. It was obtained by column chromatography of the precipitate obtained by adding excess of solvent ether in the methanol soluble fraction of concentrated ehtanolic extract of root of Ficus glomerata\textsuperscript{86-89} Roxb. Compound MS-II showed significant absorption bands in the IR spectrum at $v_{max}^{KBr}$ cm$^{-1}$.

3906.1-3426.8 (free-OH), 2819.9 (CH$_2$ asymmetric stretching), 1595.2 (ring stretching, C=C stretching), 1351.9 (C−O stretching and O−H in plane bending vibration and CH$_3$ bending), 1162.4 (C−O−C stretching asymmetric), 1032.5 (ring breathing mode), 768.5 (C−H of plane bending) and 671.3 (C−O out of plane bending several bands). It's $^1$H-NMR signals were recorded at (300 MHz, DMSO): δ 0.79 (3H, s, Me-C$_{23}$), δ 0.86 (3H, s, Me-C$_{24}$), δ0.97 (3H, s, Me-C$_{25}$), δ0.73 (3H, s, Me-C$_{26}$), δ0.74 (3H, s, Me-C$_{27}$), δ0.76 (3H, s, Me-C$_{28}$), δ0.82 (3H, s, Me-C$_{29}$), δ0.84 (3H, s, Me-C$_{30}$), δ1.1-2.0, 21H, complex signal (polymethylene, CH$_2$ and CH proton), δ4.45 (1H,m,C$_3$-H), δ2.25 (1H,t,J=3.30,C$_{12}$-H), δ2.56 (2H,d,J=4.21,C$_{19}$-H), δ3.51 (5H,m, galactose proton), δ3.91 (4H,m, rhamnose proton), δ3.85 (3H, complex signal Me-rhamnose proton), δ4.47 (1H,d, J=3.2, 1’ anomic proton), δ4.49 (1H,d, J=3.3, 1” anomic proton), δ2.02 (3H,S,2'-OAc), δ2.04 (3H,s,3'-OAc), 2.06 (3H,s,2''-OAc), δ2.08 (3H,s,3''-OAc), δ2.12 (3H,s,4''-OAc) and δ2.16 (3H,s,6''-OAc).

It's FABMS showed significant fragmentation pattern m/z = 733, 572, 426, 218, 193, 191, 179 and 178.
On acid hydrolysis MS-II yielded the sapogenin MS-II (A) which was identified as β-Amyrin (by superimposable spectral analysis). The sugars obtained from the acid hydrolysis of the saponin MS-II were identified as L-rhamnose (Rf=0.36) and D-galactose (Rf=0.18) by CoPC and CoTLC, whereas periodate oxidation, partial and enzymatic hydrolysis showed the bisdesmosidic nature of the glycoside and sugars were present in the ratio of 1:1. D-galactose was terminal sugar and L-rhamnose was linked to sapogenin via α-linkage and D-galactose was linked to L-rhamnose via β-linkage.

Thus the saponin MS-II was identified as; β-amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside.
IV. ISOLATION AND STUDY OF THE FLAVONOIDAL GLYCOSIDE, (MS-III); QUERCETOL-3-O-β-D-ARABINOXYRANOSIDE. FROM THE SEEDS OF EMBELIA RIBES BURM.

This part describes the isolation and study of flavonoidal glycoside, MS-III (yield 0.08125%) which analysed for molecular formula C\textsubscript{20}H\textsubscript{18}O\textsubscript{10}, m.p. 240-241°C, (M\textsuperscript{+}) = 418 (FABMS) and was obtained by column chromatography of ethylacetate soluble part of the methanol soluble fraction of concentrated ethanolic extract of defatted seeds of *Embelia ribes*\textsuperscript{90-95} Burm. Compound MS-III showed significant bands in IR spectrum at $\nu_{\text{max}}^{\text{KBr}}$ cm\textsuperscript{-1}. 3906.4-3427.7 (free -OH), 2926.5 (C-H stretching vibration), 1612.1 (aromatic ring system), 1351.9 (C-O-C bending vibration), 1117.0 (>C=O), 1031.5 (C-O-C stretching vibration) and 830.2 (two adjacent - H atoms in ring system). It's $^1$H-NMR signals were recorded at (300 MHz, DMSO): δ6.31 (1H, d, J-2.3, C\textsubscript{6}-H), δ6.79 (1H, d, J-2.5, C\textsubscript{8}-H), δ5.15 (1H, d, J-2.42, C\textsubscript{3}-H), δ7.61 (1H, d, J-2.21, C'\textsubscript{2}-H), δ7.48 (1H, d, J-2.51, C'\textsubscript{6}-H), δ6.70 (1H, d, J-2.54, C'\textsubscript{5}-H), δ4.48 (1H, d, J-2.3, 1' anomiceric proton), δ2.02 (3H, s, C''\textsubscript{2}-OAc), δ2.04 (3H, s, C''\textsubscript{3}-OAc) and δ2.06 (3H, s, C''\textsubscript{4}-OAc).

Its FABMS showed significant fragmentation pattern m/z = 418, 286, 257, 153, 152, 136 and 124.
On acid hydrolysis, MS-III gave aglycone MS-III(A), which on 50% KOH degradation gave phloroglucinol m.f. C₆H₆O₃, m.p. 113-114°C and catechuic acid C₇H₅O₃, m.p. 200-201°C. The sugar obtained by acid hydrolysis of MS-III was identified as D-arabinose (Rₜ-0.23) by CoPC and CoTLC in different solvent systems.

Periodate oxidation and enzymatic hydrolysis showed that the aglycone MS-III(A) and sugar were in equimolar ratio and sugar was in pyranose form. It also showed that D-arabinose was linked to a aglycone via β-linkage.

Thus the flavonoidal glycoside (MS-III) was identified as; Quercetol-3-O-β-D-arabinopyranoside.
V. PART A : STUDY AND ISOLATION OF STEROIDAL SAPONIN (MS-IV) STIGMASTEROL-3-O-β-D-ARABINOPYRANOSYL [1→4]-O-β-D-GLUCOPYRANOSIDE FROM THE SEEDS OF EMBELIA RIBES BURM.

This chapter deals with the isolation of steroidal saponin MS-IV (yield 0.05306%), molecular formula C_{40}H_{66}O_{10}, m.p. 190-191°C and (M+) 706. It was obtained by column chromatography of the precipitate obtained by adding excess of solvent ether in the methanol soluble fraction of concentrated ethanolic extract of defatted seeds of Embelia ribes^{96-100} Burm. \[ T \cdot 18737 \]

Compound MS-IV showed significant bands, in the IR spectrum at $\nu_{\text{max}}^{\text{KBr}}$ cm$^{-1}$; 3754.3-3423.2 (-OH), 2817.4 (-CH$_3$ stretching), 1596.2 (C=C stretching), 1461.2 (C-H bending vibration of methyl group), 1383.3 (CH$_3$ symmetric stretching), 1352.2 (CH$_3$ bending) and 769.2 (C-H-out of plane bending). It’s $^1$H-NMR signals were recorded at (300 MHz, DMSO): $\delta$0.71 (3H, s, Me-C$_{18}$), $\delta$0.83 (3H, s, Me-C$_{19}$), $\delta$0.76 (3H, d, J-6.4, Me-C$_{25}$), $\delta$0.70 (3H, d, J-6.6, Me-C$_{26}$), $\delta$0.89 (3H, t, J-5.8, Me-C$_{28}$), $\delta$0.57 (3H, d, J-6.33, Me-C$_{29}$), $\delta$1.3-2.01 (25H, complex pattern, polymethylene CH$_2$ and CH proton), $\delta$5.56 (1H, dd, J-6.79, C$_{21}$-H), $\delta$5.51 (1H, dd, J-6.58, C$_{22}$-H), $\delta$4.48 (1H, m, C$_3$-H), $\delta$4.41 (6H, m, arabinose proton), $\delta$4.56 (1H, d, J-2.5, 1' anomic proton), $\delta$4.16 (5H, m, glucose proton), $\delta$4.58 (1H, d, J-2.3, 1” anomic proton), $\delta$2.03 (3H, s, C’$_2$-OAc), $\delta$2.06 (3H, s, C’$_3$-OAc), $\delta$2.08 (3H, s, C’$_6$-OAc), $\delta$2.14 (3H, s, C’$_2$-OAC), $\delta$2.16 (3H, s, C’$_3$-OAc) and $\delta$2.18 (3H, s, C’$_4$-OAc).
It's FABMS showed significant fragmentation pattern \( \text{m/z} = 706, 574, 412, 382, 290, 284, 274 \) and 256.

On acid hydrolysis MS-IV yielded sapogenin MS-IV (A) which was identified as Stigmasteral (by superimposable spectral analysis). The sugars obtained from the acid hydrolysis of the saponin MS-IV were identified as D-arabinose (\( \text{R}_f-0.23 \)) and D-glucose (\( \text{R}_f-0.18 \)) by CoPC and CoLTC, whereas periodate oxidation, partial and enzymatic hydrolysis showed the bisdesmosidic nature of the glycoside and sugars were present in the ratio of 1 : 1. D-arabinose was terminal sugar and D-glucose was linked to sapogenin via \( \beta \)-linkage and D-arabinose was linked to D-glucose via \( \beta \)-linkage.

Thus the steroidal saponin MS-IV was identified as; Stigmasterol-3-O-\( \beta \)-D-arabinopyranoside [1→4]-O-\( \beta \)-D-glucopyranoside.
PART B: STUDY OF ANTHelmINTIC ACTIVITY OF THE ISOLATED COMPOUNDS MS-I, MS-II, MS-III AND MS-IV.

This part describes the study of anthelmintic activity$^{101-109}$ of the compounds isolated from indigenous anthelmintic plants. Compounds MS-I and MS-II were isolated plant *Ficus glomerata* Roxb. (N.O. Moraceae) and compounds MS-III and MS-IV isolated from plant *Embelia ribes* Burm. (N.O. Myrsinaceae). All the isolated compounds MS-I, MS-II, MS-III and MS-IV were screened for their anthelmintic activity by Broom $et al.$,$^{110}$ method. Tween 80 was used as solvent whereas piperazine phosphate was used as standard anthelmintic agent.

The results revealed that the compound MS-II and MS-III show significant anthelmintic activity in comparison to compound MS-I and MS-IV and so may potentially be explored as commercial anthelmintic agents.
REFERENCES


