CHAPTER-2

SECTION - I
GENERAL EXPERIMENTAL PROCEDURE
Phenoxy acetic acid and their derivatives have been reported to possess a wide spectrum of biological activities such as herbicidal\textsuperscript{114-117}, bactericidal and diuretic\textsuperscript{118}. A few of them have also been employed as antitubercular agent\textsuperscript{119}, antipyretic\textsuperscript{120}, local anaesthetic\textsuperscript{121}, tranquilizer and sedatives\textsuperscript{122}. Some phenoxy substituted heterocyclic derivatives have been prepared and found to exhibit CNS depressant, anti-inflammatory, antiallergic, plant growth promoting hormones, anthelmintic, anticonvulsant and antimicrobial activities\textsuperscript{123-125}.

This idea has encouraged me to undertake the synthesis of such compounds containing phenoxy and substituted phenoxy derivatives with heterocyclic rings and screened them for various biological activity.

NATURE OF THE COMPOUNDS SYNTHESISED BY THE AUTHORESS

The authoress has been able to synthesise several phenoxy and substituted phenoxy acetic/proponic acids, and also naphthoxy acetic acid possessing phenothiazine, piperazine and morpholine moieties. The final synthesised compounds were screened for anthelmintic, antimicrobial and anti-inflammatory activities.

I have synthesised the following types of the compounds -
(I) \( N-[2-(\text{phenoxy, bromo, chloro, methyl and nitro phenoxy}) \text{ acetyl}] \) phenothiazines.

(II) \( N-[2-(\text{bromo, chloro and methyl phenoxy}) \text{ propionyl}] \) phenothiazines.

(III) \( N-[2-(1- \text{ and } 2-\text{naphthoxy}) \text{ acetyl}] \) phenothiazines.

(IV) \( N-[2-(\text{phenoxy, bromo, chloro, methyl and nitro phenoxy}) \text{ acetyl}] \) piperazines.

(V) \( N-[2-(\text{bromo, chloro and methyl phenoxy}) \text{ propionyl}] \) piperazines.

(VI) \( N-[2-(1- \text{ and } 2-\text{naphthoxy}) \text{ acetyl}] \) piperazines.

(VII) \( N-[2-(\text{methyl phenoxy}) \text{ acetyl}] \) morpholines.

(VIII) \( N-[2-(\text{bromo, chloro and methyl phenoxy}) \text{ propionyl}] \) morpholines.

(IX) \( N-[2-(1- \text{ and } 2-\text{naphthoxy}) \text{ acetyl}] \) morpholines.
The total synthesis as mentioned in the present thesis is comprised of two parts-I and II respectively.

PART - I

SYNTHESIS OF VARIOUS N-[2-(PHENOXY AND SUBSTITUTED PHENOXY) ACETYL/PROPIONYL] PHENOTHIAZINES, PIPERAZINES AND MORPHOLINES

This part consist of four steps.

STEP-FIRST

SYNTHESIS OF VARIOUS BROMO, CHLORO, METHYL AND NITRO PHENOLS

Various substituted bromo and nitrophenols were synthesised in the laboratory whereas chloro and methyl phenols were used commercially available. The synthesised phenols were confirmed by their sharp melting points and microanalyses.

STEP-SECOND

SYNTHESIS OF VARIOUS PHENOXY AND SUBSTITUTED PHENOXY ACETIC/PROPIONIC ACIDS

Equimolecular quantities (0.07 - 0.1 mole) of various phenols (dissolved in 20-30 ml of 33% aqueous sodium hydroxide solution) and monochloro acetic acid and 2-monochloro propionic acid were refluxed separately in a 250 ml round bottomed jointed flask fitted with a water condenser on a water bath for 30 minutes. The reaction mixture was then transferred into a...
250 ml beaker and was then evaporated almost to dryness. The residue was then dissolved in 100 ml of water. The solution was cooled and acidified with dilute hydrochloric acid to yield the free acid. The mixture was then extracted with ether. The ethereal solution was washed with water and evaporated to yield the various phenoxy and substituted phenoxy acetic/propionic acids.

\[
\begin{align*}
X \quad &\text{OH} \quad \xrightarrow{\text{NaOH}}\quad X \quad &\text{ONa} \\
&\quad \xrightarrow{\text{+Cl}(R)\text{COOH}} \\
&\quad \text{(excess alkali)}
\end{align*}
\]

\[
\begin{align*}
X \quad &\text{O-CH-COONa} \quad \xrightarrow{\text{dil. HCl}} \quad X \quad &\text{O-CH-COOH+NaCl}
\end{align*}
\]

The structures of the various phenoxy and substituted phenoxy acetic/propionic acids were confirmed by their sharp melting points and microanalyses (yield 58-78%).

**STEP-THIRD**

**SYNTHESIS OF VARIOUS PHENOXY AND SUBSTITUTED PHENOXY ACETYL/PROPIONYL CHLORIDES**

In the various phenoxy and substituted phenoxy acetic/propionic acids (0.05 - 0.06 mole dissolved in the appropriate organic solvents) was added excess of thionyl
chloride (0.06 - 0.07 mole) in a round bottomed jointed flask fitted with a water condenser and the mixture was refluxed on a water bath separately for about six to eight hours. Excess of thionyl chloride was distilled off under reduced pressure to give the corresponding phenoxy and substituted phenoxy acetyl/propionyl chlorides.

The reason for using excess of thionyl chloride is to avoid the anhydride formation.

The structures of all the phenoxy and substituted phenoxy acetyl/propionyl chlorides were confirmed by their sharp melting points/boiling points and microanalyses (yield 56-85%).
STEP-FOUR

(A) SYNTHESIS OF N-[2-(PHENOXY AND SUBSTITUTED PHENOXY) ACETYL/PROPIONYL] PHENOTHIAZINES

To an ice-cooled solution of phenothiazine (0.036 mole in 25 ml EtOAc/MeOH) in a 500 ml beaker was added 5 ml 4N sodium hydroxide solution. The phenoxy and substituted phenoxy acetyl/propionyl chlorides were added dropwise in the above solution separately with constant stirring for about 40-60 minutes to afford various phenoxy and substituted phenoxy acetyl/propionyl phenothiazines which were separated by ether. The ethereal solution was washed with sodium bicarbonate solution followed by distilled water (4×30 ml) and on concentration yielded solid product which were purified over the column of silica gel using the appropriate solvent(s) as eluant. Finally, the eluate was concentrated and the products were crystallized from appropriate solvent(s).

![Chemical Diagram]

Phenoxy/substituted phenoxy acetyl/propionyl Chlorides
(B) SYNTHESIS OF VARIOUS N-[2-(PHENOXY AND SUBSTITUTED PHENOXY) ACETYL/PROPIONYL]-1-PIPERAZINES

To an ice-cooled alkaline solution of piperazine (0.037 mole in MeOH/EtOAc) was reacted with phenoxy and substituted phenoxy acetyl/propionyl chlorides separately and worked up as usual (on page 33) to yield the final products.

SYNTHESIS OF VARIOUS N-[2-(PHENOXY AND SUBSTITUTED PHENOXY) ACETYL/PROPIONYL] MORPHOLINES

To an ice-cooled alkaline solution of morpholine (0.037 mole in EtOAc/MeOH) was reacted with phenoxy and substituted phenoxy acetyl/propionyl chlorides separately and worked up as usual (on page 33) to yield the final products.
The following Scheme-1 outlines this part of work -

Phenols

\[ \text{NaOH} \]

Na\textsubscript{1}O\textsubscript{H}

Sodium salt of phenols

\[ \text{Cl-CH-COOH} \]

monochloro acetic/propionic acids

\[ \text{NaOH} \]

Na\textsubscript{1}O\textsubscript{H}

Cl-CH-COO\textsubscript{Na}

Sodium salt of monochloro acetic/propionic acids

\[ \text{R} \]

\[ \text{O-CH-COONa} \]

Sodium salt of phenoxy acetic/propionic acids

dil. HCl

\[ \text{R} \]

\[ \text{O-CH-COOH} \]

Phenoxy acetic/propionic acids

\[ + \text{SOCl}\textsubscript{2} \]

\[ \text{R} \]

\[ \text{O-CH-C-Cl} \]

Phenoxy acetyl/propionyl chlorides
Phenothiazine + \( \text{amine} \) → Phenoxy/substituted phenoxy acetyl/propionyl phenothiazines

Piperazine + \( \text{amine} \) → Phenoxy/substituted phenoxy acetyl/propionyl-1-piperazines

Morpholine + \( \text{amine} \) → Phenoxy/substituted phenoxy acetyl/propionyl morpholines
PART - II

SYNTHESIS OF N-[2-(1- AND 2-NAPHTHOXY) ACETYL] PHENOTHIAZINES, PIPERAZINES AND MORPHOLINES

This part consists of four steps.

STEP-FIRST

Both 1- and 2-naphthols were used commercially available.

STEP-SECOND

SYNTHESIS OF 1- AND 2-NAPHTHOXY ACETIC ACIDS

Equimolecular quantities (0.08 mole) of 1- and 2-naphthols (dissolved in 20 ml of 33% aqueous sodium hydroxide solution) and monochloroacetic acid have been taken separately and worked up by the same procedure as described on page 30 to yield 1- and 2-naphthoxy acetic acids.
STEP-THIRD

SYNTHESIS OF 1- AND 2-NAPHTHOXY ACETYL CHLORIDES

A solution of 1- and 2-naphthoxy acetic acids (0.05 mole in EtOAc) was added excess of thionyl chloride (0.06 mole) and worked up as usual (on page 31) to yield 1- and 2-naphthoxy acetyl chlorides respectively. The use of excess thionyl chloride in this step was to avoid the anhydride formation. The mechanisms was same as described on page 32.


STEP-FOUR

(a) SYNTHESIS OF N-[2-(1- AND 2-NAPHTHOXY) ACETYL] PHENOTHIAZINES

To an ice-cooled alkaline solution of phenothiazine (0.036 mole in EtOAc) was treated with 1- and 2-naphthoxy acetyl chlorides separately and worked up by the same procedure as described on page 33 to afford the final products.
(b) SYNTHESIS OF N-[2-(1- AND 2-NAPHTHOXY)ACETYL]-1-PIPERAZINES

To an ice-cooled alkaline solution of piperazine (in EtOAc/MeOH) was treated with 1- and 2-naphthoxy acetyl chlorides separately and worked up by the same procedure as described on page 33 to yield the final products.
SYNTHESIS OF N-[2-(1- AND 2-NAPHTHOXY) ACETYL] MORPHOLINE

To an ice-cooled alkaline solution of morpholine in EtOAc/MeOH was added 1- and 2-naphthoxy acetyl chlorides separately and worked up by the same method as given on page 33 to afford the final products.

![Chemical diagram]

The purity of the compounds was monitored by TLC using silica gel 'G' plates. Melting points were taken on a Toshniwal melting point apparatus. All the compounds were analysed for their C, H, and N percentages. Their infra-red spectra were recorded on a Perkin Elmer 157 (in KBr, cm⁻¹) spectrophotometer.
The following SCHEME-2 outlines this part of work:

1- and 2-naphthol

\[ \text{NaOH} \]

Sodium salt of 1- and 2-naphthol

\[ \text{Cl-CH}_2\text{-COOH} \]

monochloro acetic acid

\[ \text{NaOH} \]

\[ \text{Cl-CH}_2\text{-COONa} \]

Sodium salt of monochloro acetic acid

\[ \text{dil. HCl} \]

Sodium salt of 1- and 2-naphthoxy acetic acids

\[ \text{1- and 2-naphthoxy acetic acids} \]

\[ + \text{SOCl}_2 \]

\[ \text{1- and 2-naphthoxy acetyl chlorides} \]
Phenothiazine

1- and 2-naphthoxy acetyl phenothiazines

Piperazine

1- and 2-naphthoxy acetyl piperazines

Morpholine

1- and 2-naphthoxy acetyl morpholines
SECTION - II
GENERAL DESCRIPTION OF THE ACTIVITY PERFORMED
TECHNIQUES OF THE EVALUATION OF ANTHELMINTIC,
ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES
GENERAL INTRODUCTION TO PHARMACOLOGY

Pharmacology is the science which includes all of the knowledge about drugs. The word Pharmacology is derived from the Greek words pharmacon means drug and logos means knowledge. A drug is any chemical agent which affects living protoplasm and is intended for use in the treatment, prevention or diagnosis of disease. The word "drug" is derived from the French word drogue means a dry herb. The main aim of pharmacology is to study the action of drug on the function of living organisms in health and to apply the knowledge derived from in the cure, prophylaxis or diagnosis of diseases. Pharmacology includes such allied fields as - pharmacognosy, pharmacodynamics, pharmaco therapeutics, toxicology, pharmacy, materia medica and chemotherapy.

In the development of organic therapeutic agents, pharmaceutical scientists have explored numerous approaches to finding and developing organic compounds that are now available to us in dosage forms suitable for the treatment of our ills and often for the maintenance of our health. Pure organic compounds, natural or synthetic, are the chief source of agents for the cure, the mitigation or the prevention of disease today. These remedial agents have had their origin in a number of ways - (i) from naturally occurring materials of both plants and animal origin, and (ii) from the synthesis of organic compounds whose structures are closely related to those
of naturally occurring compounds (e.g., morphine, atropine, steroids and cocaine) that have been shown to possess useful medicinal properties. The first two approaches have led to the development of our useful medicinal agents, a third approach (iii) that of pure synthesis, has provided significant discoveries of medicinal agents.

Only a half century ago, man relied almost exclusively on nature of produce the drugs he needed, and the contributions of pharmacy were confined largely to the preparation of extracts, tinctures and other dosage forms of the crude drugs and to isolation of active principles, especially alkaloids and glycosides. Synthetics began to appear at a noticeably accelerated rate in the 1920s and this is generally attributed to the very large expansion of the American chemical industry fostered by World War I. Many observers view the advent of the sulpha drugs in the early 1930s as marking the beginning of the modern era of synthetic drugs.

The great majority of today's new basic drugs are distinct organic chemical compounds. Most of these are products of synthetic organic chemistry, although some, such as reserpine, ACTH and most of the antibiotics, are products of natural origin. Even with drugs of the latter group, however, the chemist has played a very important role in devising processess to produce them economically not only in the large
quantities required but also in a sufficient state of purity. He has also succeeded in the deliberate chemical alteration of these naturally occurring compounds and produced derivatives which are either more potent or superior in other respect, for example, dehydrocholic acid, dihydroergotamine, fluorocorticosteroids, semisynthetic penicillins, methyltestosterone etc.

Drug that are in use today were obtained either as a result of planned studies or merely by chance observation. The exact mode and mechanism of action of many such substances have been worked out but in many cases the efforts have failed. It is the science of experimental pharmacology which is trying to rationalise the empirical therapy. The detailed pharmacological study of the compounds of all either of natural or synthetic sources with a view to investigate the possibility of their being included in the category of drugs and their possible clinical applications, come within the realm of experimental pharmacology with all drugs and new substances, therefore, a systematic study is concluded to elucidate their general pharmacological action, mechanism of action, absorption, fate excretion and toxic effects etc. Such studies, if carried out in human beings with new substances, are not free from risks and dangers and not always possible even with established drugs. Therefore, recourse a new moiety must be experimented on animals. Experiments are conducted either in vivo in healthy animals or diseased animals or
animals after producing conditions simulating disease in human beings or in vitro with isolated tissues, micro-organisms, parasites etc. The results of such experimental studies are, in the end re-evaluated in human beings (clinical pharmacology) before undertaking the final therapeutic trials.

It is, therefore, quite evident that experimental pharmacology forms one of the most important pillars of medical science, as we owe practically all our drugs today to this science. One of the main functions of the pharmaceutical industry is to create products. Products are defined as drugs, devices, or services that have a perceived import on the health care systems. Many important examples of the impact of drugs on health and longevity may be cited. Much of the credit can go to research, along with the development production and distribution facilities of the pharmaceutical industry. Chemist and other physical scientist have been predominantly responsible for synthesis, isolation and characterisation of medical agents. However, biological scientist have played and equally essential role and originating meaningful screening and testing models and in the overall evaluation of new agents. Organic chemists synthesize new drug as well as isolate and characterize natural products. In each case there is interest in the complex relationships between chemical structure and pharmacologic action.
The pharmacologic activity of a compound is an involved function of the structure, and very small changes may profoundly modify the pharmacologic effect. These structural modifications may involve replacing one group with another at a specific point in the molecule, shifting the same group from place to place in the parent molecule, saturating valence bonds or modifying the acidity or basicity.

The use of any drug in the treatment of disease may be discussed under two heads. The first of these include the drugs that are used in the treatment and cure of specific disease and the second category is the one which has the characteristic effect upon the animals and organisms but are not the specific remedies for a particular disease for example morphine, cocaine etc.

The development of new drugs depend upon clinical trials and its use in medicine. A successful drug would be one which is: (i) readily absorbed and slowly excreted and (ii) having low toxicity to invading organisms. The relationship is frequently expressed as the ratio of the maximum tolerated dose to the minimum curative dose which is termed as chemotherapeutic index (CI).

\[
CI = \frac{\text{Maximum tolerated dose}}{\text{Minimum curative dose}}
\]
This index has now been replaced by the term, **therapeutic index** (TI) which is used to designate a quantitative statement of the selectivity of a drug when a therapeutic and an untoward effect are being compared. TI is expressed as the ratio of the median lethal dose \( (LD_{50}) \) to the median effective dose \( (ED_{50}) \).

\[
TI = \frac{LD_{50}}{ED_{50}}
\]

The above factors depend upon mainly on the relationship between biological activity and chemical constitution of a particular drug. These relationship are identical to serve as a guiding factor in mapping the structural features of the compounds with analogous activities. The biological activity of the drug is not the sum of the activities of group or atoms present in it but due to the molecule as a whole. The individual activity of all the groups or atoms associated in the molecule is changed during the synthesis of the drug.

The idea about the structure activity relationship undergoes gradual changes with the advancement in the knowledge of chemical and physical properties of the molecule. Even the most advanced and carefully considered theories have not related regularities in the relation of chemical structures to physiological actions which could be used indiscriminately in one series of compounds after proving their value in the other.
This attractive hypothesis suggested a new approach in chemotherapeutic research with comprised of the trials of compounds closely related to an essential metabolite of a microorganisms. The slightest change in structure often does produce considerable change in biological properties. Therefore, in evaluation structure activity relationship, the total picture of steric factor, electron density, localization and the resultant physical and chemical properties of a given compound need to be considered. Chemotherapeutic value of a compound is usually determined in different stages. First the preliminary *in vitro* tests are performed and if the compounds are found active in such tests, these are subjected *in vivo* tests along with the tests to determine their toxicity in order to find their possible practical usefulness as a drug.
GENERAL INTRODUCTION TO ANTHELMINTICS

Helminthiasis or worm infestation, is one of the most widespread disease in the world. The prevalence of human helminthic infestations is existing generally throughout the globe and represents a major world health problem particularly in third world countries. The condition appears to be worsening with respect to the number of persons affected and the areas of the world involved. A number of worms are parasitic to man, who can carry on infestation through his entire life. The most serious common worm parasite is the Schistosoma or blood fluck. The principal species are S.mansoni, S.haematobium and S.japonicum. The nematodes (roundworms) and cestodes (tapeworms) are the important species present in man.

Many worms live in an intermediate host, such as the snail or other molluscs. Attention is being focused on the destruction of the host snail of the schistosoma as a means of reducing the threat of this worm to the human population. The flukes are among the more serious parasites because of their debilitating effect on the human system and the difficulty in effecting a cure, a problem compounded by the ever present possibility of reinfection in regions of poor sanitation.

The helminths are multicellular organisms possessing three germ layer and exhibiting a bilateral symmetry. The worm parasites of man belong to two phyla: Nemathelminthes
(roundworms) and **platyhelminthes** (flatworms). The roundworms (syn. Nematodes) include the hookworm, roundworm, whip worm, pinworm, threadworm, trichina and filariasis. The flatworms include cestodes and trematodes.

*Anthelmintic*[^128^] are drugs which have the capability of ridding the body of parasitic worms or helminths. The drugs which directly or indirectly kill the worms are called **vermicides** and they are only a few, as dichlorrophen, hexylresorcinol etc. Those which expel the worms from the body, usually paralysing them, are known as **vermifuges** e.g. piperazine, santonin, etc. and are mostly used against intestinal helminths.

Most of the anthelmintics have been used empirically and even today the exact mode of action of all of them is not known. The vermifuges usually exert a paralysing effect on the musculature of the worm acting either directly or indirectly through the nervous system. This causes relaxation of grip of the worms upon the intestinal mucosa and active persistalsis, often excited by purgatives, remove the paralysed or narcotised worms from the intestines. The vermicides possibly inhibit the essential enzyme systems of the worms and interfere with helminthic metabolism causing death of the parasite.

An ideal anthelmintic drug should meet the following requirements[^129^].
1. The drugs have a maximum toxic action on the invading helminths and minimum effect on the host i.e. a high chemotherapeutic index.

2. The drug should possess a wide range of activity on different kinds of worms.

3. The drug should not be absorbed from the intestines when used against intestinal helminths to produce better parasitotropic action.

4. The drug should be well absorbed when used against tissue helminths to kill both adult worms and larvae.

5. The drug should be nontoxic to the host, easy to administer and cheap.

A brief account of the different types of therapeutically anthelmintics are classified according to their action on different helminths as follows -

(A) NEMATODE INFESTATIONS

The nematodes constitute a large group of worms of which some species are important human parasites. It includes intestinal and somatic nematodes.

INTESTINAL NEMATODES : These are the following types.

(1) ANCYLOSTOMIASIS (HOOKWORMS DISEASE)

Ancylostomiasis is a general term used to describe a disease caused by parasites, *Ancylostoma duodenale,*
A. ceylanicum and Necator americanus in man\textsuperscript{130,131}. The disease is found to be in tropical countries. On contact with skin they penetrate lymphatic and small blood vessels and ultimately swallowed through the trachea and throat. They develop in small intestine as mature worms within four to seven weeks. Severe infection causes indigestion, eructation, anorexia, headache and vertigo. In advance cases of epigastric pain, the chronic fatigue, anaemia, and alternating diarrhoea has developed.

The drugs of choice are pyrantal\textsuperscript{132}, morantel\textsuperscript{133}, thibendazole\textsuperscript{134-136}, tetramisole\textsuperscript{137}, trichlorophenol piperazine\textsuperscript{138}, tetrachloroethylene\textsuperscript{139}, biphaniium hydroxide naphthoate, dimanthine\textsuperscript{140} etc.

(2) TRICHURIASIS (WHIPWORM DISEASE)

Trichuriasis is the disease caused by Trichuris trichiura or whipworm. It is very common in most part of the warm countries and is generally associated with ascaris and hookworms infection because of their similar geographical distribution.

The adult worms live in the large and small intestines of man, especially in the caecum and vermiform appendix. It is usually non-pathogenic, but heavy intestinal infestations may produce abdominal pain, diarrhoea and anaemia.
The drugs of choice are dithiazanine\textsuperscript{161}, dichlorvos\textsuperscript{142},
glycobiarsol\textsuperscript{143} etc.

(3) ASCARIASIA (ROUNDWORM DISEASE)

Ascariasis is caused by \textit{Ascaris lumbricoides}. The condition is highly epidemic where soil pollution with human faeces occurs. The worms gain access to the intestinal tract directly by ingestion of ova containing viable embryos. The larvae are liberated in small intestine, penetrate the intestinal wall and enter the circulating blood stream. The larvae deposit in the lungs, where many of them penetrate the alveoli. After growth period the larvae migrates up the respiratory passage and are swallowed. Abdominal pain, allergic manifestations and mechanical obstruction are observed in severe infestation.

The drugs of choice are piperazine\textsuperscript{144}, thiabendazole etc.

(4) ENTEROBIASIS (PINWORM OR THREADWORM DISEASE)

Enterobiasis is caused by \textit{Enterobius vermicularis} and are found all over the world. This infestation is more common in children. The infection is caused by direct ingestion of ova. In intestine eggs hatch and release larvae from which they mature in ileum. The mature eggs are deposited in the folds of anus, vulva and perennial skin. Heavy infestation may cause anorexia, abdominal pain, weight loss, insomnia and
nervousness.

The drugs of choice are mebendazole\textsuperscript{145}, piperazine, pyrvinium pamoate\textsuperscript{146} etc.

(5) STRONGYLODIASIS (STRONGYLOIDES)

The disease is most frequently caused by penetration in the skin by larve of \textit{Strongyloids stercoralis}, rarely by their ingestion. The larvae invade the blood stream and are carried to the lungs where they break the alveoli. The eggs are deposited in the mucous of the duodenum and upper jejunum. The eggs are hatched in the tissue of the host and the motile larvae penetrate the mucosa. Abdominal pain, anaemia, anorexia and diarrhoea are observed in severe infestation.

\[ T. 12.345 \]

The drugs of choice are cyanin oxes, dithiazanine iodide\textsuperscript{147} etc.

(6) TRICHINOSIS (TRICHINA DISEASE)

The disease is caused by the penetration in the muscle by larvae of \textit{Trichinella spiralis}. Severe infestation causes abdominal pain, nausea and lymphatic obstructions.

The drug of choice is thiabendazole.
(B) SOMATIC INFESTATIONS

FILARIASIS AND TROPICAL EOSINOPHILIA

Filarial worms are nematodes which infest tissues, and of the several members which infest man. Wuchereria bancrofti is the commonest in our country and the others are Loa loa and Onchocerca volvulus. The other diseases caused by these worms are loiasis and erysipelas by circulating freely in the blood and lymph which affect brain, subcutaneous tissue and eyes. The adult worms often produce lymphatic obstructions.

The drugs of choice are diethyl carbamazine and (+) suramin 129.

DRACONTIASIS (GUINEA WORM DISEASE)

Dracontiasis is the disease caused by Dracunculus medinensis. This infestation is transmitted by drinking of water containing infected cyclops (water flora). The adult female usually remains in subcutaneous tissue and many come out through a small ulcer, usually on the foot.

The drugs of choice are niridazole and metronidazole and thiabendazole.

(C) CEDESTES

TAENIASIS: Taeniasis is the disease caused by tapeworms. This infestation is transmitted by ingestion of infected beef or pork meat and can be prevented by avoiding the ingestion of
suspected meat or by its through cooking. Taenia saginata (beef tapeworm), Taenia solium (pork tapeworm) and Hymenolepis nana (dwarf tapeworm) etc. are common cestodes in our country which infest human intestine.

The drugs of choice are mepacrine hydrochloride, dichloroophen, chloroquine etc.

(D) TREMATODES

SCHISTOSOMIASIS AND CLONORCHIASIS (FLUKE DISEASES)

Schistosomiasis (blood flukes) and clonorchiasis (chinese lever fluke) are the disease caused by Schistosoma haematobium; S.mansone, S.faponicum and clonorchis sinensis worms. These infestations can be considered as systemic, as the parasites are localised in organs other than the gastrointestinal tract. The adult worms affect intestines, bladder, liver and lungs. Haemoaturia is to be observed under the severe infestation.

The drugs of choice are niridazole, stibophen, lucanthone, chloroquine, thioxamethanone etc. 129
EVALUATION TECHNIQUES

The authoress has used two different methods for the screening of anthelmintic activity of compounds.

1. SCREENING BY WATKIN'S METHOD

Evaluation of anthelmintic drugs possess a special problem. The wide variation of their species, their behaviour in host and susceptibility of the host to the anthelmintic drug are all difficult to simulate in experimental conditions.

Craig and Toye, reviewed in vitro and in vivo techniques in common use. In the former, the paralysant or lethal effect of the drug is tested on isolated worm, in vivo, efficacy is measured by a reduction of the worm infection in animals. Undoubtedly there are many symbiotic association between the parasite and the host hence in vitro testing gives only a crude approximation of the effectiveness of the drug.

Ascaris lumbricoids, Uncenara stencephala, Trichostranglus calcaratus and some other species of worms are generally employed for in vitro study.

In vivo screening may be carried out using roundworm, whipworm, hookworm, tapeworm and threadworm infection in mice, rats or dogs.

Tradelburg proved the various species of ascaris
which are the major helminths causing ascariasis and various other helminths infection in man and animals have remarkable anatomical similarities with common earthworm.

Furthermore, Watkins\textsuperscript{150} and various other co-wokers\textsuperscript{151} have used earthworm instead to various ascaris species for in vitro determination of the anthelmintic activity of various drugs and products.

Due to the availability of earthworm and their acceptances as substituents in place of various ascaris species, qualitative in vitro anthelmintic evaluation of synthesised compounds was done by Watkin method on earthworm using piperazine citrate as a standard by the authoress.

The suspension of the synthesised compounds was made in ethylene glycol (0.1% w/v). The piperazine citrate (standard drug) was made of the same concentration in ethylene glycol. Normal saline solution was also prepared in distilled water.

**PROCEDURE**

Thirteen petri-dishes of four inches diameter were taken. Normal saline (25 ml) solution was put in each petri dish. The different sample solution (2 ml) and standard solution (2 ml) were put in it. Finally, two living earthworms were put in each petridish having the above solution. The
earthworms were washed with normal saline solution prior to their putting in it. The time (in minutes) taken for complete paralysis and death of each worm was noted and mean paralysis time and mean lethal time for all samples, standard drug and blank were recorded. The death of worms was confirmed by external stimuli. The experiment was carried out in duplicate. In each petridish was kept (a) 2ml of 0.1% w/v drug suspension; (b) 25ml of normal saline solution and (c) two earthworms of approximately equal size.
SCREENING BY STEWARD'S METHOD

ANTIHOOKWORM ACTIVITY: Antihookworm activity (Steward method)\textsuperscript{152} of synthesised compounds was performed with young male rats weighing 25-40 g susceptible to Ancylostoma ceylanicum infection. Rats were infected with 500 infective larvae of \textit{A. ceylanicum} subcutaneously for each experiment.

Animals in different experimental group were administered the test compounds in varying doses with an infected untreated group as control. All experimental and controlled animals were sacrificed on 3rd day after last dose and their worms were counted and compared with control group. The percent efficacy was calculated by the following formula

\[
\text{Percent efficacy} = \frac{N-n}{N} \times 100
\]

where, \( N \) = Average number of worms recorded in control group.
\( n \) = Average number of worms recorded in treated group.

Atleast two replications of each experiment were done.

CESTOCIDAL ACTIVITY: Cestocidal activity of the compounds were carried out with young male rats weighing 25-40 g. The rats were infected with 200 viable eggs of \textit{Hymenolepis nana}. 18-20 days after infection, faeces of the individual rats were examined for the presence of eggs and those found infected, were used for screening. The compounds were administered initially at the single oral dose of 250 mg/kg. Insoluble
compounds were made into fine suspension with the help of tween 80. On the day 3 post treatment the animal did not receive any food for 5-6 hours before they were sacrificed for assessing their worm burden. The intestine from each animal was removed, washed well with normal saline solution and examined for worms and scolices under dissecting microscope. The criterion for assessing the activity was taken as the absolute clearance of parasite along with scolices from individual rats at the particular dose. Even, if a single scolex or worm remained in the intestine, the drug at that dose was considered to be ineffective. The percent efficacy of the compound was obtained from the number of the treated animals free from infection and those which did not respond to the drug treatment.

The standard drug used was mebendazole.
GENERAL INTRODUCTION TO ANTIMICROBIAL

The antimicrobial drugs occupy a unique niche in the history of medicine. The germ theory of disease was the vehicle of a dramatic revolution in medicine. During the entire preceding history of medicine, fewer than a handful of drugs had a known locus of action, and even fewer had been submitted to systematic laboratory investigation. The first synthetic antimicrobial drugs revolutionized in the treatment of certain created protozoal infections, especially syphilis, but the second major revolution in medicine in which the antimicrobial drugs played a major role awaited the appearance of sulphanil amide and penicillin; the exponential development in the antibiotic and systemic antibacterial field is the inevitable result of the momentum created by those two agents.

In microbial and parasitic infections curative drugs are essential either to destroy or to irradicate them from body of the host without producing any damage. The specific treatment of systemic infections caused by micro-organisms and parasites by drugs of known chemical structure, is known as chemotherapy and the drugs employed are called chemotherapeutic agents eg. sulphonamides as bacterial infections, isoniazid in tuberculosis, emetin in amoebiasis, quinine in malaria, organic arsenicals in syphilis etc.

During the early part of this century, tremendous studies were made in the systemic treatment of certain microzoal
infections. Nevertheless, these advances did not greatly affected directly the overall practice of medicine. The advent of sulphanilamide in 1935 marked the beginning of a major revolution in the practice of medicine. The subsequent profusion of antibacterial agent overwhelmed the physician with golden tools. The realization that certain microorganisms are successfully resisting the "Wonder Drug" not only impels a ceaseless search for new synthetic antibacterial agents but also forces a sober return to certain ancillary art of the medical and surgical management of infectious disease.

The sulphonamide sensitive organisms synthesize their own pteroyglutamic acid (PGA) but sulphonamide-resistant group either do not require PGA or can utilise performed PGA. The para-aminobenzoic (PABA) is an essential growth substance of many bacteria, as it is usually incorporated in the synthesis of PGA molecule, and an enzyme reaction is necessary for the utilization of PABA by the cell. Para aminobenzenesulphonamide (sulphanilamide) offers competition for a position in this reaction because of the similarity between the chemical structure of the two compounds, and if sufficient sulphanilamide is present to displace PABA from the enzymic reaction, the growth of the bacterial cell is inhibited by substrate composition. Although PGA synthesis by the bacteria is also immediately inhibited but bacteriostasis does not occur until the stored PGA is exhausted. Finally the bacteria are reduced
in number and weakened, and are readily destroyed by phagocytes and other antibodies present in the blood. Excess of PABA competitively and PGA noncompetitively counteract bacteriostasis induced by sulphonamides.

Antibacterial agents other than antibiotics and sulphonamides are frequently used in dental practice for the following purposes.

(1) To disinfect penetrating and nonpenetrating instruments.

(2) To control superficial infections of the skin, mucous membrane, or bone either for prophylactic purposes or a distinct therapeutic procedure, and

(3) To disinfect tooth cavities prior to the insertion of a filling material to routine tooth preparations, pulp capping, pulpeting, or endodontic procedures.

The examples are ethyl alcohol, boric acid and boric acid formulations, gentian violet, methylene blue, formaldehyde, weak solutions of sodium hypochlorite, iodine, hydrogen peroxide etc.

Fungi are plant like, nonphotosynthetic eukaryotes and grow either in colonies of single cell (yeast) or in filamentous multicellular aggregates (molds). Most fungi live
as saprophytes in soil or dead plant material and are very important in the mineralization of organic matter. The fungi comprise five widely different classes of primitive flora, including the bacteria. The variations in cell physiology and biochemistry are extreme among fungi. Thus, antifungal agents include a wide variety of chemical types of rather narrow antifungal spectrum. Broad spectrum antifungal agents in general are toxic and are irritants, as expected from their nonselectively; however, the many of these have limited absorption through the epidermis and so may be employed in dermatologic preparations. The wide spread use of broad spectrum antibiotics and corticosteroids in recent years has increased the frequency of secondary fungal infections, especially moniliasis, by altering normal bacterial flora and resistance to infections respectively. Not all antifungal agent are fungicidal; many are only fungistatic and certain of them may owe their efficacy to a keratolytic action. The antifungal agents are:

(1) Those employed locally - These include many synthetic drugs for e.g. polyene antibiotic, nystatin, hamycin etc.

(2) Those used systemically - These include the antibiotics, griseofulvin, and amphotericin B; imidazole derivatives, clotrimazole, ketoconazole and miconazole; and flucytosine.
Antifungal agents used for skin and mucous membrane infection include the antibiotic griseofulvin, nystatin and various other topically applied agent. The characteristic feature of griseofulvin is that, it is administered orally to achieve local effect.
EVALUATION TECHNIQUES

This part has been divided into two heads:

(a) Evaluation of antibacterial activity and
(b) Evaluation of antifungal activity.

(a) EVALUATION OF ANTIBACTERIAL ACTIVITY

Various method\textsuperscript{153-159} are available for the evaluations of the antibacterial activity. However, the most widely used method consists in determining the antibacterial activity of the drug by adding it in varying concentrations to the cultures of the test organisms. In the present work, the antibacterial activity of the synthesised compounds was evaluated by filter paper disc method\textsuperscript{160}. The main aim of these investigations was to study the changes in the activity with the variation in the structures of the molecule and thereby establishing a correlation between the structure of the compounds and their antibacterial property.

All the synthesised compounds (in dimethyl formamide, DMF) were screened for their antibacterial activity against the following bacteria using streptomycin as a standard.

(a) Escherichia coli, gram (-)
(b) Salmonella typhi, gram (-)
(c) Staphylococcus aureus, gram (+)
(d) Bacillus subtilis, gram (+)
This method consists of the following steps -

(i) Preparation of the medium, its sterilization and tubing;

(ii) Treatment of the glass apparatus and its sterilization;

(iii) Pouring of the needed medium into sterilised petridishes;

(iv) Preparation of the required concentration of the synthesised compounds and dipping the sterilized Whatman filter paper disc of size 6 mm into it;

(v) Incubation at particular temperature and

(vi) Measurement of the zones of inhibition.

Out of the different steps in the above method, the most important is the selection of the suitable medium and its preparation because it is the composition of the medium which exerts greatest influence upon the activity of a compound. The other factors which influence in vitro tests are:

(i) The kind and condition of the test organism.

(ii) The concentration of the drug solution and the dilution of the drug at the site of action (Incubation period).

(iii) Environment factors which may augment or counteract the interaction of the drug and the parasite.

(iv) Temperature of the incubation because for each bacteria there is an optimum temperature and for most of the pathogenic bacteria this temperature is 37°C.
(v) pH of the medium which is usually in the range of 7.2 to 7.6.

In the present work, the following composition of the nutrient agar medium is employed:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 litre</td>
</tr>
<tr>
<td>Agar agar</td>
<td>17.5 g</td>
</tr>
</tbody>
</table>

For the preparation of the medium, all the above ingredients except agar-agar were weighted and dissolved in (500 ml) water by the application of gentle heat. After the ingredients were dissolved completely, more distilled water (500 ml) was added. The pH of the medium was adjusted in the range of 7.5 ± 0.1 and then the weighted quantity of agar-agar was added to this solution and the mixture autoclaved for half an hour. The hot media was filtered through cotton to obtain a clear solution. The medium thus prepared was transferred in different culture tubes in 40 ml portions. The tubes plugged with cotton and were sterilized by steaming in an autoclave at 20 lbs/sq inch for an hour. All the glass apparatus were cleared with chromic acid and then sterilized by keeping in an oven.
PREPARATION OF THE NUTRIENT BROTH

As the beef extract is deficient in nitrogenous materials, the heat resistant protein derivatives in the form of peptone was added. Peptose is the principle source of nitrogen. It may also contain some vitamins and sometimes carbohydrates depending upon the kind of proteinous material digested. Sodium chloride is added to increase the salt content.

The ingredients, peptone (1 g), sodium chloride (5 mg) and beef extract (100 ml) were mixed and heated till they dissolved. It was then filtered through a filter paper by adjusting the pH at 7.5 ± 0.1 by using normal sodium hydroxide solution. The medium so obtained was poured into the sterile flask and plugged with sterile cotton plug which was then sterilize into an autoclave at 20 lbs/sq inch pressure for 15 minutes.

STOCK CULTURE AND INOCULUM

The bacteria was subcultured on the nutrient agar slants. The inoculum of the bacteria was prepared by transferring a loopfull of the bacteria from stock culture into sterile broth and incubated for 24 hours for the optimum growth in broth medium.

PREPARATION OF PLATES AND APPLICATION OF DISC

20 ml of sterilised agar base were transferred aseptically to each of the previously sterilized petriplates and
allowed to set uniformly. 0.1 ml of the 24 hours old broth (included bacteria) was then added uniformly to each petriplate.

The sterilized Whatman filter paper (No. 1) discs (6 mm diameter) were thoroughly moistened in the test samples (different concentrations) and placed on seeded agar plates alongwith the standard antibiotic, streptomycin. The seeded agar plates were then incubated at 37° for 36 hour.

READINGS AND INTERPRETATION

After 24 hours of incubation, the plates were examined and the diameter of the zones of complete inhibition was measured to the nearest whole milimeter with a sliding calipers.

(b) EVALUATION OF ANTIFUNGAL ACTIVITY

There are several methods available for recording the antifungal activity of the compounds. The one which is in common use\textsuperscript{161} in recent time has been adopted.

All the synthesised compounds (in ethylene glycol) were screened for their antifungal activity against the following fungi using griseofulvin as a standard.

A. Aspergillus niger
B. Penicillium crysogenum
C. Trichophyton mentagrophytes
D. Candida albicans
This method consists of the following steps -

(1) STERILIZATION OF THE APPARATUS

All the glass apparatus were cleaned with chromic acid followed by distilled water and then sterilized by heating at 200° in a hot air oven.

(2) PREPARATION OF THE MEDIUM

Sabouraud's glucose agar medium was used for antifungal screening which consist of -

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>40 gm</td>
</tr>
<tr>
<td>Peptone</td>
<td>10 gm</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>20 gm</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.2 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Streptomycin was used to check the growth of undesirable bacteria. The above mentioned ingredients were weighted and dissolved in 500 ml of distilled water. After the ingredients were dissolved completely, more distilled water (500 ml) was added to make the solution upto one litre and the pH of the medium was kept in the range of 7.6 ± 0.1. The medium was heated in an autoclave for half an hour and then transferred in 100 ml portions in previously sterilized conical flasks fitted with sterilized cotton plugs. The solution in conical flasks were again autoclaved at 15 lbs/sq inch for one hour and then used for the work.
Fairly uniform suspension of the spores of the selected fungi in sterilized distilled water was poured. The spore suspension of each test organism was kept in a broth at 35-40\(^\circ\). The petriplates were inoculated in the same manner as described on page 71 for antibacterial screening. These petriplates were incubated at 30\(^\circ\) for 48 hours. The zone of inhibition was considered as an indication for the antifungal activity by comparing with the standard.
GENERAL INTRODUCTION TO ANTI-INFLAMMATORY

Inflammation is a response of the tissue to an infection, irritation or foreign substance. It is a part of the host’s defense but when the response becomes too great, it may be for worse than the disease state which is counteracted, and in extreme cases, it may be fatal. The characteristics of inflammation are numerous: redness, swelling (edema), heat, pain, soreness and the corresponding histological changes. In other words, inflammation is a process by which body fights the energy of any living or non-living foreign body and characterized by certain changes in the body. These conditions may be attributed to vasodilation, leakage of plasma into tissues, increase of blood supply and stretching of the tissues respectively.

Rheumatic disease are inflammatory conditions and can be classified as connective tissue disease and belong to the complex group of autoimmune conditions. They include rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout, rheumatic fever, systemic lupus, erythematosus, psoriasis and poly artheritis nodosa. These are chronic, disabling, inflammatory conditions, which may affect single or multiple organ systems of the body.

A large number of substances the so-called mediators of inflammation are formed or released either concurrently or in successive time sequences at the site of injury from various
cell sources in response to an etiologic factor. A variety of cells contain a number of potent mediators and, in some instances inhibitors of the inflammatory response. These cell sources may include neutrophils (polymorphonuclear neutrophil leukocytes), basophils, mast cells, platelets, macrophages and lymphocytes. The numerous mediators of inflammation implicated in the inflammatory process and elaborated by the foregoing cells include histamine, serotonin, leukokinin, slowreacting substance of anaphylaxis (SRS-A), lysosomal enzymes, hynphokines, and prostaglandins. Medical effect of bark of willow (Salix vulgaris) and certain other plants in therapy of inflammation for many centuries. The presence of salicin in these plants gave an idea to use synthetic salicylates as anti-inflammatory agents such as acetyl salicylic acid and methyl salicylate. The first non-steroidal anti-inflammatory agent was paracetamol although it has been observed to have low degree of anti-inflammatory activity but a strong antipyretic activity. Deposition of crystalline monosodium urate hydrate in joints is known as gout. Commonly affect the joints of great toe. The drugs aspirin, salicylates, phenylbutasone, indomethacin, naproxen, mfenamic acid, gold compounds are the beneficial remedy for such disease. During the period between second world wars pyrazolone derivatives gained considerable popularity. Non steroidal anti-inflammatory drugs have a number of biochemical activities. They are said to inhibit formation of the inflammatory mediator (histamine,
serotonin, prostaglandins and kinins) and to moderate the activity of the inflammatory proteases. They are also believed to inhibit oxidative phosphorylation, which deprive inflammed tissue of needed metabolic energy in the form of adenosine triphosphate (ATP). They may displace anti-inflammatory peptides from albumins; or they may hyper polarize neuronal membranes, especially in the acidic environment of inflammed tissue. The above mentioned drugs are also believed to stabilize lysosomal membranes.

An obstacle to the discovery of new drugs to treat chronic conditions such as rheumatic disease is the difficulty in developing animal models that resemble the disease sufficiently for pharmacologic testing. The most widely used primary test to screen new non-steroidal anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by infection of the irritant carrageenan, which is a mucopolysaccharide derived from Irish sea moss, Chondrus crispus. Most clinically useful anti-inflammatory agents suppress this type of edema. The anti-inflammatory properties of indomethacin, a widely used nonsteroidal anti-inflammatory agent were initially detected by a carrageenan assay. Indomethacin is highly potent anti-inflammatory drug but exhibited a high degree of gastric toxicity. The advent of propionic acid derivatives in the horizon of anti-inflammatory therapy brought newer hopes in the minds of clinicians starting with ibuprofen, fenoprofen,
ketoprofen etc. which were found to be extremely safe drugs.

Anti-inflammatory agents act on various systems responsible for inflammation such as plasma clotting, arachidonic acid or complement systems. Inflammation can be reduced by inhibition of oxidative phosphorylation, inhibition of protein denaturation and acceleration of sulphhydril exchange. The agent may also reduce the inflammation by fabrinolysis that is by inhibition of complement. Interruption of arachidonic acid cascade is one of the mechanisms of anti-inflammatory actions. This can be achieved by inhibitory membrane phospholipids, blocking of cyclo-oxygenase pathway on lipoxygenase pathway and also act by inhibiting various enzyme systems like protease, 5 HT decarboxylase, histidin decarboxylase elastase etc.

Attempts have been made to differentiate between analgesic and combined analgesic plus anti-inflammatory activities by measuring the edema and pain produced by an irritant. Most compounds with analgesic and anti-inflammatory activity do not raise the threshold to pain in normal joints. The term antinociceptive is used to differentiate this combined from simple analgesic activity.

Anti-inflammatory agents are classified as follows:
(1) STEROIDAL ANTI-INFLAMMATORY AGENTS

They exert their action by inhibiting the release of phospholipids in lipoxygenase pathway which inhibited the release of arachidonic acid from membrane, e.g. dexamethasone etc.

(2) NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

They are said to inhibit biosynthesis of prosta glandin at cyclo-oxygenase pathway, e.g. indomethacin, aspirin, mefenamic acid etc.

SCREENING METHODS

The screening methods for anti-inflammatory activity have been classified as follows:

(1) NON-IMMUNOLOGICAL METHODS

Non-immunological methods have been further divided into the following headings -

For evaluation of acute inflammation: It is the following types - carrageenan induced hind paw oedema method$^{163}$; 5-hydroxytryptamine induced hind paw oedema method$^{164}$; formalin induced hind paw oedema method$^{165}$; hyaluronidase hind paw oedema method$^{166}$; histamine induced hind paw oedema method$^{166}$ and turpentine oil induced arthritis in knee joints method$^{167}$. 
For evaluation of subacute inflammation: It is of the following types — carrageenan granuloma pouch technique\textsuperscript{168} and cotton pellet granuloma technique\textsuperscript{179}.

For evaluation of chronic inflammation: It is only one type — formaldehyde induced arthritis method\textsuperscript{170}.

(2) NON-IMMUNOLOGICAL METHODS

It is the following types — adjuvant arthritis method\textsuperscript{171} and tuberculin sensitivity test method\textsuperscript{172}.

(3) MISCELLANEOUS METHODS

It is again of the following types — UV erythema method\textsuperscript{172} and urate crystal induced synovitis method\textsuperscript{173}.
EVALUATION TECHNIQUE

Anti-inflammatory activity of the synthesised compounds were carried out with albino rats weighing (80-120 g) of either sex. The test compounds and the standard drug were administered at a dose of 100 mg/kg body weight intraperitoneally. $\text{ALD}_{50}$ values were determined employing albino rats as test animals. The acute toxicity were determined in rats by intraperitoneally administration of the test compounds at graded doses and recording the mortality after 24 hours. All the compounds were found to be relatively less toxic as their $\text{ALD}_{50}$ values ranged from $>1000$ mg/kg intraperitoneally.

All the synthesised compounds were tested for anti-inflammatory activity by rat paw oedema test as described by Winter, Risley and Nuss utilizing carrageenan suspension as the phlogistic agent. Anti-inflammatory activity was determined by measuring the change in the volume of inflammed foot produced by injection of 0.05 ml of 1% freshly prepared carrageenan suspension.

The volume was measured by plethysmograph. Initial volume of right hind paw of albino rats were measured plethysmographically without administration of test compounds.

Albino rats (80-120 g) were divided into three groups each consisting of four rats. A group of rats was treated intraperitoneally with 100 mg/kg body weight of the aqueous
suspension (with few drops of tween 80) of the synthesised compounds. Another group was administered intraperitoneally 100 mg/kg body weight of aqueous suspension of phenylbutazone (standard drug) and the third group (control group) was fed with the same volume of distilled water. One hour after the drug administration the animals were injected with 0.05 ml suspension of carrageenan (1.0 percent in 0.9 percent saline) in the right hind paw planter apponeurosis. The measurement of the paw volume were taken using mercury displacement technique with the help of plethysmometer immediately before and 1, 2 and 3 hours after the carrageenan injection. The percent inhibition of inflammation after 3 hours was calculated by the method of Newbould using the following formula -

\[
\text{Percent Inhibition I} = 100 \left[1 - \frac{a - x}{b - y}\right]
\]

where, \(x\) = Mean foot volume of rats before the administration of carrageenan injection in the test and the standard group.

\(a\) = Mean foot volume of rats after the administration of carrageenan and test compounds injection in the test and standard group.

\(Y\) = Mean foot volume of rats before the administration of carrageenan injection in the control group.

\(b\) = Mean foot volume of rats after the administration of carrageenan injection in the control group.