CHAPTER 5

PHARMACOLOGICAL ACTIVITIES OF THE EXTRACT AND ISOLATED COMPOUNDS
Pharmacology is the 'SCIENCE OF DRUGS' as is evident from the name itself (Pharmakon - Drugs, and Logos - Discourse of Science). The drugs are the chemical substances to be used in minute amounts for diagnosis, cure, control or prophylaxis of disease. The main object of pharmacology is to provide a scientific foundation for medical treatment and also to add new and better drugs in the therapeutic armamentarium. The experimental pharmacology is rather a new branch of medical sciences and dates back to the latter half of the 19th centuries when Rudolf Buchheim founded an experimental pharmacology laboratory. Since that time this branch has made tremendous advancements specially because of the phenomenal growth of physiology, biochemistry, biophysics, pathology, microbiology, synthetic and analytical chemistry etc.

Drugs 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. Treatment carried on with the former group of drugs is rational therapy and with the latter is empirical therapy. It is the science of experimental pharmacology which is trying to
rationalise the empirical therapy. Besides this the detailed pharmacological study 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 actions, 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 in animals after producing conditions simulating disease in human beings, or in vitro with isolated tissues, microorganisms, 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 sciences, as we owe practically all our drugs today to this science. From a medical point of view this is all the more important because nothing is better than 'Seeing is believing'. This idea has encouraged me to take up the pharmacological activity.
The ethanolic extract of the whole plant of *Limonia crenulata* and the new isolated compound (anthraquinone glycoside): 8-hydroxy-6-methoxy-2-methyl-anthraquinone-3-O-β-D-glucopyranoside (structural study has already been described on page 37) from the same source have been found to exhibit anti-inflammatory activity which is discussed in section - I of this chapter.

Section - II of this chapter consists of the screening of analgesic activity of the new compound: 2-hydroxy-4-methoxy-phenyl-1-vinyl acetate which was isolated from the heartwood of *Aegle marmelos* (structural study has already been described on page 88).
SECTION - I

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Inflammation is a process by which body fights the energy of any living or non-living foreign body and characterized by redness, swelling, heat and pain. These conditions may be attributed to vasodilation, leakage of plasma into tissues, increase of blood supply and stretching of the tissues respectively.

The chief mediators of inflammation are kinin, histamin, serotonin and prostaglandin\textsuperscript{154}. Medicinal effect of bark of Willow (\textit{Salixable vulgaris}) and certain other plants in therapy of inflammation has been known 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. During the period between two world wars pyrazolone derivatives such as phenylbutazone, oxyphenbutazone etc. gained considerable popularity. However continued use showed a large number of highly noxious side effects. After second world war 'INDOMETHACIN' was discovered at Merck, Sharp and Dohma laboratories in U.S.A. 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 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 plasmin, clotting, arachidonic acid or complement systems. Inflammation can be reduced by inhibition of oxidative phosphorylation, inhibition of protein denaturation and acceleration of sulphhydryl exchange. The agent may also reduce the inflammation by fibrinolysis that is by inhibition of platelets or by mixed lymphocyte reaction or by inhibition of complement. Interruption of arachidonic acid cascade is one of the mechanisms of anti-inflammatory actions. This can be achieved by inhibiting membrane phospholipids, blocking of cyclo-oxygenase pathway or lipoxygenase pathway. The anti-inflammatory drugs are found to be regulate leukocyte function by inhibition of macrophages, phagocytes and release of lysosomal hydrolases. These drugs also act by inhibiting various enzyme systems like protease, 5 HT decarboxylase, histidin decarboxylase, elastase etc.

Anti-inflammatory agents are mainly used in 'rheumatism'. Rheumatisms are connective tissue diseases and belongs to the disorder of joints mainly the synovial joint and the para or articular joints. Disorder of joints are rheumatoid arthritis, osteo-arthritis, ankylosing spondylitis and gout. Rheumatism arthritis is a chronic disease primarily involving peripheral joints. Osteo-arthritis, is basically wear and tear degeneration of synovial joints. This results due to deteriora-
tion of articular cartilage and abnormal body formation in joints. Deposition of crystalline monosodium urate hydrate in joints is known as gout, commonly affect the joints of great toe.

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 prostaglandin 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;

(2) Immunological methods; and

(3) Miscellaneous methods.

(1) **NON-IMMUNOLOGICAL METHODS**

Non-immunological methods have been further divided into the following headings.
(a) **FOR EVALUATION OF ACUTE INFLAMMATION** - 
It is of six types.

(i) Carrageenan induced hind paw oedema method\textsuperscript{155}.

(ii) 5-Hydroxy tryptamine induced hind paw oedema method\textsuperscript{156}.

(iii) Formalin induced hind paw oedema method\textsuperscript{157}.

(iv) Hyaluronidase hind paw oedema method\textsuperscript{158}.

(v) Histamin induced hind paw oedema method\textsuperscript{159}.

(vi) Turpentine oil induced arthritis in knee joints method\textsuperscript{159}.

(b) **FOR EVALUATION OF SUBACUTE INFLAMMATION** - 
It is of two types.

(i) Carrageenan granuloma pouch technique\textsuperscript{160}.

(ii) Cotton pellet granuloma technique\textsuperscript{161}.

(c) **FOR EVALUATION OF CHRONIC INFLAMMATION** - 
It is of one type.

(i) Formaldehyde induced arthritis method\textsuperscript{162}.

(2) **IMMUNOLOGICAL METHODS**

Immunological methods are of two types.

(a) Adjuvent arthritis method\textsuperscript{163}.

(b) Tuberculin sensitivity test method\textsuperscript{164}.

(3) **MISCELLANEOUS METHODS**

It is of two types.

(a) UV erythema method\textsuperscript{164}.

(b) Urate crystal induced synovitis method\textsuperscript{165}.
EXPERIMENTAL

Anti-inflammatory activity was carried out with albino rats (weighing 180-150 g) of either sex. The ethanolic extract of *Limonia crenulata* and the new compound, 8-hydroxy-6-methoxy-2-methyl — anthraquinone-3-O-β-D-glucopyranoside (structural study described on page 37) were separately administered orally at a dose of 1000 mg/kg body weight i.p. in aqueous suspension and the standard drug (oxyphenbutazone) was administered at the dose of 100 mg/kg body weight i.p. in aqueous suspension. \( \text{ALD}_{50} \) values were determined employing albino rats as test animals. The acute toxicity was determined in rats by oral administration of the extract and compound at graded doses\(^{166}\) and recording the mortality after 24 hours. The extract and the compound were found to be relatively less toxic as their \( \text{ALD}_{50} \) values ranged from 2000-3000 mg/kg P.O. or above.

Both the extract and the compound were examined for anti-inflammatory activity by rat paw oedema test as described by Winter, Riseley and Nuss\(^{155}\) utilising carrageenan suspension as the phlogistic agent. Anti-inflammatory activity was determined by measuring the change in the volume of inflamed 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 was measured plethysmographically without the administration of extract and the compound.
Albino rats were divided into four groups each consisting of six rats. Two groups of rats were treated orally with 1000 mg/kg body weight of the aqueous suspension of ethanolic extract of *L. crenulata* and the new compound, 8-hydroxy-6-methoxy-2-methylantraquinone-3-O-β-D-glucopyranoside. Third group of rats was administered orally 100 mg/kg body weight of aqueous suspension of oxyphenbutazone (standard drug) and the fourth group (control group) was fed with the same volume of distilled water. One hour after the drug administration the animal were injected with 0.05 ml of freshly prepared 1% carrageenan suspension in normal saline in the right hind paw planter apponeurosis. The measurements of the paw volume were taken using Harris and Spencer mercury displacement technique with the help of plethysmometer, immediately before and 1, 2, 3, 4 and 5 hours after the carrageenan injection. The percent inhibition of inflammation after 5 hours was calculated by the method of Newbould\textsuperscript{167} 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 standard group. 
- \(a\) = Mean foot volume of rats after the administration of carrageenan injection in the test and the 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.

The results have been shown in the table - 20.
<table>
<thead>
<tr>
<th>Group of Rate</th>
<th>Dose (mg/kg per os)</th>
<th>Volume of paw (ml) after carrageenan administration (Mean ± S.E.) in different groups after hours</th>
<th>Total increase in paw vol. after 5 hours</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.61 ± 0.06, 0.70 ± 0.03, 0.83 ± 0.05, 0.92 ± 0.07, 0.99 ± 0.03, 1.05 ± 0.02</td>
<td>0.44 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>EtOH extract of L. crenulata</td>
<td>1000</td>
<td>0.69 ± 0.05, 0.71 ± 0.02, 0.75 ± 0.03, 0.78 ± 0.06, 0.81 ± 0.04, 0.83 ± 0.05</td>
<td>0.14 ± 0.03</td>
<td>68.19%</td>
</tr>
<tr>
<td>New compound</td>
<td>1000</td>
<td>0.67 ± 0.04, 0.69 ± 0.03, 0.70 ± 0.06, 0.72 ± 0.05, 0.78 ± 0.03, 0.82 ± 0.04</td>
<td>0.15 ± 0.03</td>
<td>65.99%</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>100</td>
<td>0.68 ± 0.05, 0.70 ± 0.02, 0.72 ± 0.03, 0.75 ± 0.04, 0.77 ± 0.02, 0.80 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>72.73%</td>
</tr>
</tbody>
</table>
The data shown in the table-20 indicated a significant inhibition in ethanolic extract of *L. crenulata* (68.19%) followed by new compound (65.91%) in comparison to standard drug (oxyphenbutazone) which showed 72.73% inhibition in the same conditions on the rats.
SECTION - II

EVALUATION OF ANALGESIC ACTIVITY

Analgesics\textsuperscript{168,169} are agents which relieve pain acting centrally to elevate pain threshold without disturbing consciousness or alternating other sensory modalities.

Pain is an universal experience of all mankind. Attempts to define this term have not proved satisfactory. However, pain\textsuperscript{170} is a highly individualized perception of stimulus as modified by a wide variety of personal attitudinal and emotional factors. There is also the difference between experimental and pathological pain. Experimental pain is usually brief and is accompanied by a sense of security and is relatively unresponsive to analgesics. Pathological pain may be prolonged. It is associated with anxiety and response to analgesic drugs. Pain receptor organs are distributed through the body. Clinically pain can be considered as:

(a) Superficial or cutaneous pain;
(b) Deep pain from muscles, joints, ligaments and bones;
(c) Visceral pain;
(d) Referred pain;
(e) Psychogenic or functional pain.

Pain from muscles, joints, ligaments and bones usually has a dull character and it may be accompanied by a sickening sensation due to an automatic response. Visceral pain is dull,
aching in character and is accompanied by sweating, nausea, fall in blood pressure and even shock in practice. Visceral pain may be due to spasm, ischaemia, myocardial infection inflammation (appendicitis) or stimulation of sensory nerve endings (poptic ulcer). Deep pain whether visceral or somatic in origin, may sometimes be misinterpreted as it is coming from some part of the body other than the actual site of stimulation. This is called referred pain. Psychogenic or functional pain is usually a vague pain which follows no definite anatomical pattern of distribution. Such pain is usually continuous from day to day and involves more than one part of body. It, however, does not disturb sleep. Pain is mediated by the nerve endings of the non-medulated sensory fibres which carry it to the spinal cord. The pain fibres are mainly carried to the thalamus. The chemicals may or may not be the reason for pain because it is uncertain. Analgesics\textsuperscript{171} are classified as:

1. **NARCOTIC ANALGESICS**

The analgesics under this class not only provide relief from pain but also produce depression of the central nervous system. They are:

(a) Naturally occurring opium alkaloids, for example morphine, codeine etc.

(b) Semi-synthetic derivatives of the natural opium alkaloids, for example heroin, dihydromorphinone, etc.
(c) Synthetic morphine substitutes, for example pethidine, methadone etc.

2. NON-NARCOTIC ANALGESICS

The analgesics, like salicylates produce relief of pain without hypnosis or marked impairment of mental activity. They are mainly useful in relieving dull aching pain of low intensity coming from integumental structures such as muscles and joints.

The mechanism of the analygesic effect of salicylates, is still controversial. Some assume that it is to be mainly supraspinal. The thalamus is responsible for the integration of pain sensation and also emotional reaction to pain. They are believed to act by a blockade of the pain centres in the thalamus.

METHODS USED FOR EVALUATION

The methods for characterising agents potentially useful for the relief of pain are numerous\(^ {172} \). There is no single approach that gives complete parallellism between the result in man and animals and clinical practice in man. Part of the difficulty is directly related to the fact that clinical pain is a pathological condition and differs from experimental pain. Recognizing that the characteristics of clinical pain can only be reproduced in part in the animal models. It is found that a battery of tests provide a firmer foundation for the evaluation of analgesic effects of the drugs as given below.
The rat tail flick procedure was adopted by the author. The albino rats was held in the holder so that their tail protrudes out. The tail was placed on the hot wire. The time of flicking of tail is recorded and compared with that of the standard. The time of interval is considered as the flick of the tail of the rat, the more potent would be the drug.
EXPERIMENTAL

In the present study of the analgesic activity of a new compound: 2-hydroxy-4-methoxyphenyl-1-vinyl acetate (structural study has already been described on page 88) from the heart wood of *Aegle marmelos* was determined by the rat tail flick procedure. A hot wire analgesiometer (Techno-corporation, Lucknow) was used for determining the pain threshold of rats. Cold water was circulated through the arrangement provided in the instrument to avoid the heating of area around the hot wire. Albino rats (weighing 120-150 g) were divided into five groups, each group having ten animals. The first group was given only carboxymethyl cellulose. Second, third and fourth groups of animals were given separately 1, 2 and 100 mg/100 g in carboxymethyl cellulose respectively. Fifth group of animals was given 1 mg/100 g of morphine-HCl in carboxymethyl cellulose.

Carboxymethyl cellulose showed no analgesic effect hence it was used for control group. The rats were placed in a rat holder through which the tail of animal protruded out. The normal reaction time of all the rats as observed by tail flick was measured. The current was adjusted so that more than 90% rats reacted with tail flick within 5 seconds and in no case it exceeded to 10 seconds. The compound was suspended using 0.5% carboxymethyl cellulose in distilled water. The suspension was given orally to the albino rats. The pain threshold was measured after the laps of 45 minutes. The reaction time was insignificant. The results are given in table-21.
# TABLE - 21

EVALUATION OF ANALGESIC EFFECT OF THE NEW COMPOUND : 4-METHOXY-2-HYDROXYPHENYL-1-VINYLCETATE OF THE HEART WOOD OF AEGLE MARMELOS.

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Batch Number of Rats</th>
<th>Dose (mg/100 g)</th>
<th>Latent period in second Before administration</th>
<th>Latent period in second After administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 minutes</td>
<td>60 minutes</td>
</tr>
<tr>
<td>1. Carboxymethyl cellulose</td>
<td>A₁</td>
<td>-</td>
<td>5 ± 0.03</td>
<td>6 ± 0.06</td>
</tr>
<tr>
<td>2. 4-methoxy-2-hydroxyphenyl-1-vinyl acetate</td>
<td>B₁</td>
<td>1</td>
<td>6 ± 0.04</td>
<td>15 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>2</td>
<td>5 ± 0.06</td>
<td>17 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>B₃</td>
<td>100</td>
<td>6 ± 0.05</td>
<td>20 ± 0.06</td>
</tr>
<tr>
<td>3. Morphine-HCl</td>
<td>C₁</td>
<td>1</td>
<td>5 ± 0.02</td>
<td>26 ± 0.03</td>
</tr>
</tbody>
</table>
The table - 21 of the results of the analgesic effect of the new compound: 2-hydroxy-4-methoxyphenyl-1-vinyl acetate, isolated from heart wood of *A. marmelos* reveals that there is no variation in the latent period before and after administration of carboxymethyl cellulose (CMC) alone has no analgesic activity. However when the results of CMC groups were compared with that of groups A₁ and B₁ it was noted that this new acetate in both doses 1 mg/100 g and 2 mg/100 g produced a marked analgesic effect over control group. Further increase in the dose 2 mg/100 g to 100 mg/100 g) however could bring about only a slight increase in the latent period indicating that increase in dose would not proportionally increase the analgesic effect. The overall study revealed that the new acetate has fairly good analgesic effect.