CHAPTER-V

BIOLOGICAL ACTIVITY
5.1. GENERAL INTRODUCTION OF PHARMACOLOGY

Biological activity of the drug is not the sum of the activities of groups or atoms present in it but due to the molecule as a whole. The idea about the structure–activity relationship underwent gradual changes with the advancement in the knowledge of chemical and physical properties of the molecules. It is assumed that the pharmacological activity is a function of the physical properties if it is a "structurally non-specific drug" or chemical properties if it is "structurally specific drug". The structure is then modified in the following manner to affect the properties:

(i) Shifts are made in the positions of functional groups.

(ii) Valence bonds are saturated.

(iii) Acidity or basicity is modified; and

(iv) Variation of configuration about asymmetric centres are made.

A correlation between the pharmacological action and the structure in a series of compounds, is its structure–activity relationship (SAR). A slight alteration in this structure might totally change a particular effect observed in the parent molecule. Even the most advanced and carefully considered theories have not revealed regularities in the relation of chemical structure to physiological actions which could be used indiscriminately in one series of compounds after proving their value in the other. According of W.A. Sexton, physical and chemical reactivity of a molecule after the structural variation may cause change in distribution in the cells and tissues and access the active sites of enzymes and receptors in reactions rates and in excretion patterns. Thus in evaluating structure activity relationship, the total picture of steric factor, electron density, localisation and the resultant physical and chemical reactivities of a given compound need 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 then subjected to in-vivo tests along with the tests to determine their toxicity in order to find their possible practical usefulness as a drug. Various physico-chemical parameters influence biological parameters of a drug and even above that structural variations may increase the therapeutic value of the compound by widening the gap between the therapeutic action and its side effects. In addition, certain structural modifications may enhance or uncover many dormant physiological and biochemical efficiencies of the drug.

It is well known that some of the metal ions such as Cu, Ni, Co, Fe, Cr, Zn etc. are very important for the efficient metabolism and growth of human and animal organisms. A thorough study and search of the literature in relation to the biological requirements and toxicity of Cu led to the following conclusions. Copper is an essential element and is required for normal metabolism in man. Since co-ordinated forms of Cu are always stable forms compared to ionized form, it exists in biological systems as a variety of complexes. The best known biological function of cobalt is its intimate involvement in the co-enzyme related to vitamin B₁₂ which is synthesized by bacteria. Nickel has some undisclosed function in living organism which can activate a number of metals in vitro bound to ribonucleic acids and is found in blood, having a special affinity for bone and skin and plays important role in pigmentation. The red brown protein, ferritin is widely distributed in plants and animals. It sequesters iron (III) in a soluble and non-toxic form and functions as a readily available storage of iron (III). Such an observation is of tremendous biological significance in the context of nature's selection of Fe(III) as the co-factor in enzymic processes.
Manganese often plays an important role in numerous biological processes associated with utilization of hydorgen peroxide or dioxygen. At least five functions of these types are known, viz., manganese superoxide dismutase, manganese catalase, manganese peroxidase, manganese ribonucleotide reductase and oxygen evolving complex in photosystem II. Recently the biochemistry of chromium has attracted a lot of interest due to its presence in the glucose tolerance factor (GTF) and its role in the enhancement of ribonucleic acid synthesis. It has been further reported that none of the N-coordinated complexes possess any glucose tolerance factor activity. Only O-coordinated complexes possess glucose tolerance factor activity. The importance of zinc to biochemistry, biology, pathology and clinical and veterinary medicine is now generally recognized. In the cells it is incorporated into more than 200 metalloenzymes involved in nucleic acid, protein, carbohydrate and lipid metabolism. Although zinc belongs to the essential metal ions, it becomes hazardous when present in excess. As a result there are metabolic disorders connected with both deficiencies and excess amounts of the metal ion. Cadmium and mercury have been found to be environmental pollutants. The toxicity of metals such as cadmium and mercury in a biological system may result from blocking the essential functional group of the biomolecules and inhibiting or enhancing their enzymatic activities or from displacing the essential metal ion in biomolecules. Cadmium appears to compete with zinc at the active sites of enzymes; isolation in the kidney and liver of a cadmium-containing metalloprotein, metallothionein, suggests that the protein is involved in detoxification processes.

The present work also aims at the study on change in respective activities of the drugs as a result of complex formation hence to recommend the proposed complexes in the place said drugs for medicinal use in suitable cases.
5.2. GENERAL INTRODUCTION OF ANTIINFLAMMATORY ACTIVITY

Inflammation is a response of the tissue to an infection, irritation of foreign substance. It is a part of the hosts defense but when the response becomes too great, it may be for worse than the disease state which is counteracted and in extreme case, it may be fatal. The characteristics of inflammation are numerous, redness, swelling (edema) heat, pain, soreness and the corresponding histological changes.

The inflammatory process involves a series of events that can be elicited by numerous stimuli, e.g. Antigen – Antibody interaction, infectious agent and thermal or other physical injury. Each type of stimulus provokes a characteristic pattern of response. The response is usually accompanied by the familiar clinical symptoms of erythema, edema, tenderness and pain. Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanism\(^{31}\).

(i) An acute transient phase characterized by local vasodilation and increased capillary permeability.

(ii) A delayed subacute phase, most prominently characterized by infiltration of leukocytes and phagocytic cells.

(iii) A chronic proliferative phase in which tissue degeneration and fibrosis occur.

Rheumatic diseases are inflammatory conditions and can be classified as connective tissue diseases and belong to the complex group of autoimmune conditions. It represents an autoimmune disease that involves both the humoral and cellular arms of immune response\(^{32,33}\). A complex interaction of genetic, immunological and local factors has been invoked along with the viral or other infections that may be involved in the initiation
of the disease or exacerbation of the disease. The process is thought to be initiated by a hypothetical joint-seeking (arthrotopic) antigen that is processed and presented by macrophages T-lymphocytes in conjunction with a major histocompatible antigen in the synovial membrane. The interaction of this complex with T cell receptors together with the action of macrophages derived cytokines, results in the activation, differentiation and clonal expansion of T cells. These elements of cellular immune response are accompanied by microvascular injury and an inflammatory reaction that includes development of an exudative synovial fluid that contains many neutrophils. Many cytokines have been found in the rheumatoid synovium, one of the most prominent of these is interleukin – 1 (IL – 1) responsible for some of the systemic manifestations of these diseases. They include rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout rheumatic fever, systemic lupus, erythematosus, psoriasis and poly arteritis nosoda. These are chronic, disabling, antiinflammatory condition, which may effect single or multiple organ systems of the body.

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 pharmacological testing. The most widely used primary test to screen new non-steroidal antiinflammatory 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 mass, chondrus crispus. Most clinically useful antiinflammatory agents suppress this type of edema. The antiinflammatory properties of indomethacin, a widely used non-steroidal antiinflammatory agent, initially detected by a carrageenan assay. Indomethacin is highly potent antiinflammatory drug but exhibited a high degree of gastric toxicity. The advent of propionic acid derivatives in the horizon of antiinflammatory therapy brought never hopes in the minds of
clinicians starting with ibuprofen, fenoprofen, ketoprofen etc. which were found to be safe drug.

Despite extensive research in the field of inflammation search for an ideal antiinflammatory agent, devoid of undesirable side effects, still continues. The commonest hazard of these agents is the occurrence of gastric irritation or ulceration, especially when in chronic use. Although parallelism seems to exist between the antiinflammatory and ulcerogenic activity of anti-rheumatic drugs; ibuprofen, fenoprofen and others claimed to have less of such toxicity. Diclofenac sodium is well tolerated compared with other non-steroidal antiinflammatory drugs and no other agent of this class appears to have a safety profile which is clearly superior to diclofenac. Diclofenac is rarely or never associated with some other serious side effects caused by other NSAIDS e.g. pancreatitis, aseptic meningitis, severe cutaneous or phototoxic reactions. It caused gastrointestinal tract disturbances, peptic ulcer and gastrointestinal tract bleeding. The other side effects are edema, skin rashes, dizziness, drowsiness, depression, headache, jaundice and bleeding tendency. The most common side effects of acidic non-steroidal antiinflammatory drugs in man, as well as in animals, are gastrointestinal symptoms, i.e. mucosal damage, bleeding and ulceration.

The metal complexes have been reported to play an important part in the biological activity of durg. The participation of the metalloproteins in respiratory, photosynthetic, nitrogen fixation, biosynthetic and metabolic processes is essential to the foundation of life. Several cobalt complexes are important as model to the metal-to-oxygen bonding that is involved in biological systems. Cobalt is the constituent of vitamin B₁₂. Proteins containing iron participate in oxygen transport. A number of copper proteins including enzymes have been reported.
A variety of recent observations indicated that copper complexes, when administered in conjunction with antiinflammatory drugs, exhibit synergistic activity.\textsuperscript{46} Copper has been shown to suppress inflammation and to possess antiulcer properties.\textsuperscript{47,48} Copper chelates of antiinflammatory agents are known to be less ulcerogenic than their parent acids.\textsuperscript{49} It has also been found that the Cu complexes of some antiarthritic drugs are themselves more active as antiinflammatory agents than their parent compounds.\textsuperscript{50,51} The molecular basis of action of the Cu complexes with well-established drugs such as diclofenac sodium, piroxicam etc. is not as yet clear. Formation of the uncharged Cu-piroxicam species is of particular interest, since it has been shown that such neutral Cu-drug complexes are essential for effective distribution of the pharmacoactive agents and maintaining the copper balance in blood plasma.

Antiinflammatory agents are classified as follows:

(A) Steroidal Antiinflammatory Agents

They exert their action by inhibiting the release of phospholipids in lipooxygenase pathway which inhibits the release of arachidonic acid from membrane.

(B) Non-steroidal Antiinflammatory Agents

They are said to inhibit biosynthesis of prostaglandin at cyclooxygenase pathway.

There has been substantial progress in elucidating the mechanism of action of NSAIDS, although a precise understanding of their therapeutic activities and side effects is still to be established. Inhibition of cyclooxygenase, the enzyme responsible for the biosynthesis of the prostaglandin and certain related autocoids is generally thought to be a major facet of the mechanism of non-steroidal antiinflammatory drugs.
Scheme I: Cyclo-oxygenase pathway of inflammation

Some evidences show that prostaglandin participate in pathogenesis of the inflammation and fever and this reinforces the hypothesis that inhibition of the biosynthesis of these autocoids could explain a number of clinical actions. Numerous subsequent observations have substantiated this point of view including the discoveries that prostaglandin are released whenever cells are damaged. They appear in inflammatory exudates and
non-steroidal antiinflammatory drug inhibits the biosynthesis and release of prostaglandin in all cells tested.

There is a good deal of evidence that therapeutic dose of aspirin-like compounds reduce prostaglandin biosynthesis in man. Such doses inhibit the production of prostaglandin by human platelets and reduce the prostaglandin content of human serum, urine and synovial fluid of arthritic knee joints.

The theory of mode of action of NSAIDS is that the activation of phospholipase A₂ induces release of arachidonic acid, which leads to the generation of some or all of the prostaglandin or thromboxanes. These compounds contribute in various ways to the genesis of inflammation, pain and fever. The NSAIDS inhibits cyclo-oxygenase step there by preventing the formation of prostaglandin endoperoxides (PGG₂ and PGH₂) and thromboxane A₂ and other prostaglandin and consequently reducing the signs and symptoms of inflammation.

SCREENING METHODS

The screening methods for antiinflammatory activity have been classified as follows:

(1) Non-immunological method
(2) Immunological method
(3) Miscellaneous method

(1) Non-immunological method:

This has been further divided in three types -

(a) For evaluation of acute inflammation: It is of following types -

(i) Carrageenan hind paw edema method

(ii) 5-hydroxy tryptamine induced hind paw edema method

(iii) Formalin induced hind paw edema method
(iv) Hyaluronidase hind paw edema method\textsuperscript{56}

(v) Histamine induced hind arthritis in knee joint method\textsuperscript{57}.

(b) For evaluation of substance inflammation: It is of the following types:

(i) Carrageenan granuloma pouch technique\textsuperscript{58}

(ii) Cotton pellet granuloma technique\textsuperscript{59}.

(c) For evaluation of chronic inflammation: It is only one type:

(i) Formaldehyde induced arthritis method\textsuperscript{60}.

(2) Immunological Method:

This method is of two types –

(i) Adjuvant arthritis method\textsuperscript{61} and

(ii) Tuberculin sensitivity test method\textsuperscript{62}.

(3) Miscellaneous Method:

It is of two types –

(i) UV erythema method\textsuperscript{62} and

(ii) Urate crystal induced synovitis method\textsuperscript{63}.

**EXPERIMENTAL**

Antiinflammatory activity of the complexes were performed using a plethysmometer to measure carrageenan induced rat paw volume following the method of Winter et al\textsuperscript{35}.

Adult male wister albino rats 90-125 g were fasted for 18 hrs but free access of water. Each treatment i.e. plain drug (diclofenac sodium, mefenamic acid, flufenamic acid and piroxicam) and its complexes were administered at a dose of 100 mg/kg body weight orally in 0.5% CMC (carboxy methyl cellulose) suspension. Half an hour following the treatment
0.1 ml. of 1% solution of a carrageenan, was injected in the right hind paw planter apponeurosis, the paw volume was measured immediately before carrageenan and again 3 hrs later by means of plethysmometer. Edema was measured in a precalibrated plethysmometer as the difference between the volume of the paw measured before and 3hrs after giving carrageenan. The percent inhibition (I) of inflammation after 3hrs was calculated by the method of Newbould\textsuperscript{64} using the following formula.

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

Where,

\[x = \text{Mean foot volume of rats before the administration of carrageenan injection in the test and the standard group.}\]

\[a = \text{Mean foot volume of rats after the administration of carrageenan injection in the test and the standard group.}\]

\[y = \text{Mean foot volume of rats before the administration of carrageenan injection in the control group.}\]

\[b = \text{Mean foot volume of rats after the administration of carrageenan injection in the control group.}\]

5.3. ANTIINFLAMMATORY ACTIVITY OF DICLOFENAC SODIUM COMPLEXES

Diclofenac sodium which is a phenyl acetic acid derivative, has analgesic, antipyretic and antiinflammatory action and is used mainly in the treatment of rheumatoid arthritis and rheumatic disorders. It is used in painful post-operative and post-traumatic inflammation, swelling, pain and dental surgery\textsuperscript{65,66}. The colour reaction of drug with Co\textsuperscript{2+} salts are studied\textsuperscript{67}. Earlier the Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Cu\textsuperscript{2+} complexes of diclofenac sodium were studied\textsuperscript{68} and results indicate that Cu and Co complexes of diclofenac show better activity than the Ni complex Cu coordination compounds which have not been generally recognized as possible active metabolites may be
responsible for the antiinflammatory activity of the clinically used antiinflammatory agents.

Diclofenac sodium is \((2-[(2, 6\text{-dichlorophenyl}) -\text{amino}] \text{ benzene acetic acid monosodium salt})\). Molecular formula of diclofenac sodium is \(\text{C}_{14}\text{H}_{10}\text{Cl}_{2}\text{N}\text{NaO}_{2}\) and molecular weight is 318.12. It is white crystalline solid which is stable in air, soluble in water and ethanol, insoluble in chloroform and ether and melting point at 283-285°C.

The results showing antiinflammatory activity of diclofenac sodium drug and its complexes with various metals are presented in Table 5A and Figure 5.1. As results indicate the activity of the drug increases when it is complexed with Cr(III) and Zn(II) while the drug becomes much less active when it is complexed with Fe(III). Activity of plain drug has been found to be almost similar to that of its complex with Mn(II). This result provides evidence for a unique metabolite, Cr-dependant fat and protein metabolism, Mn-dependant vital functions and Zn-dependant at the active site of enzymes process required for tissue maintenance. A metal coordination compound which may be responsible for the desired antiinflammatory activity of those agents which have clinical usefulness. That is to say, Cr, Mn and Zn coordination compounds which have not been generally recognized as possible active metabolites may be responsible for the antiinflammatory activity of clinically used antiinflammatory agents.

5.4. ANTIINFLAMMATORY ACTIVITY OF MEFENAMIC ACID COMPLEXES

Mefenamic acid is \((2-[(2,3\text{-dimethyl phenyl})-\text{amino}] \text{ benzoic acid})\) or \(\text{N-(2,3-xyllyl)-anthranilic acid}\). Molecular formula of mefenamic acid is \(\text{C}_{15}\text{H}_{15}\text{NO}_2\) and molecular weight is 241.28. It is a white to off-white, crystalline powder that is odourless and has very little initial taste, but it has a bitter after taste; darkens on prolonged exposure to light, is
nonhygroscopic and is stable at $25^0$, $37^0$ and $45^0$; decarboxylates at temperature above its melting point (at $300^0$, it is 100% decarboxylated in 3 min.); melts between $227^0$ and $232^0$C.

Mefenamic acid is anthranilic acid derivative. An analgesic drug indicated for the relief of mild to moderate pain when therapy will not exceed 1 week. Mefenamic acid is also indicated for the relief of pain resulting from dental extractions. It is contraindicated in patients with ulceration of the upper or lower intestinal tract, children under 14 year of age, women during preganancy and patinents known to be hypersensitive to the drug. Onwards effect include diarrhea which may be used to serve, autoimmune hemolytic anemia, thrombocytopenic purpura, leukopenia, pancytopenia, agranulocytosis and bone narrow hypoplasia. Minor reactions include drowsiness, gastrointestinal discomfort, dizziness, headache, vomiting, urticaria, rash, eosinophilia, bluered vision, insomnia and perspiration. Rarely palpitations, facial edema, dyspnea, eye pain, ear pain, dysuria, hematuria, reversible loss of colour vision and increased insulin need in diabetic patients. Mild renal and hepatic toxicity have also been reportedn. Since this drug is useful in mild to moderate pain, physicians would be well advised to consider its use only in case which either can not tolerate or do not respond to less toxic agents.

The results presented in Table 5B and Figure 5.2 show that complexes ‘C’ (Cr-mefenamic acid) and ‘I’ (Zn-mefenamic acid) are more active than the parent drugs.

As given in Table 5B and Figure 5.2 complexes ‘C’ and ‘I’ shows higher percent inhibition of edema as compared to the parent drug. These drug complexes show better antiinflammatory activity than the mefenamic acid. The chelation reduces considerably the polarity of the metal ions mainly because of partial sharing of its positive charges with the donors
groups and possibly by π-electron delocalization over the whole chelate ring. Such a chelation increases the lipophilic character of the metal chelate which probably leads to the break down of permeability barrier of cells resulting in interference with normal cells process.

In the present case Cr and Zn complexes of mefenamic acid show better activity than the Mn, Fe, Co, Ni and Cu complexes. This result provides evidence for a unique metabolite, Cr-dependant fat and protein metabolism and Zn-dependant at the active site of enzymes process required for tissue maintenance.

The inflammatory activity of M-mefenamic acid complexes are found follow the sequence :Cr>Zn>Ni>Co>Mn>Cu>Fe with respect to M (where M =Cr, Mn, Fe, Co, Ni, Cu, Zn). The molecular basis of action of the Cr, Mn, Fe, Co, Ni, Cu and Zn complexes with well established drug such as mefenamic acid is not as yet clear.

Mefenamic acid represents an effective anti-phlogistic analgesic, discovered after aminopyrine. It is evident from the investigation that combination of both effect, antioociceptive (analgesic) and antiinflammatory, is a rarity among these compounds. The mechanism of analgesic action is likely to be the inhibition of prostaglandin synthetase. The potency of mefenamic acid with respect to aspirin has been studied in relation to dose response including gastrointestinal bleeding. In both observations mefenamic acid is reproted to the better than aspirin.

The Zn-mefenamate had the most pronounced activity, where as Fe-mefenamate showed lowest activity. Prophylactic activities of the metal complexes varied during intoxication with other chloro compounds. The degree of hydrolysis of the metal complexes at physiological has been observed to pH-affect the activity of these complexes.
5.5. ANTIINFLAMMATORY ACTIVITY OF FLUFENAMIC ACID OF COMPLEXES

Flufenamic acid is anthranilic acid derivative, which shows analgesic and antiinflammatory action. Flufenamic acid is N-(α, α, α-trifluoro-m-tolyl)-anthranilic acid. Molecular formula of flufenamic acid is C_{14}H_{10}F_{3}NO_{2}. Molecular weight of flufenamic acid is 281.2. Flufenamic acid is white, crystalline powder, odourless. Practically insoluble in water, soluble in 4 parts of ethanol, in 7 parts of chloroform and in 3 parts of ether.

Flufenamic acid is also indicated for the relief of pain resulting from dental extractions. It is contraindicated in patients with ulceration of the upper or lower intestinal tract, children under 14 year of age, women during pregnancy and patients known to be hypersensitive to the durg. Since this drug is useful in mild to moderate pain, physicians would be well advised to consider its use only in cases which either can not tolerate or do not respond to less toxic agents.

The results presented in Table 5C and Figure 5.3 show that complex ‘C’ (Cr-flufenamic acid), ‘D’ (Mn-flufenamic acid) and ‘G’ (Ni-flufenamic acid) are more active than the parent durg.

As results indicate complexes ‘C’, ‘D’ and ‘G’ show higher percent (inhibition) of edema as compared to parent drug. These complexes show better antiinflammatory activity than the flufenamic acid. The antiinflammatory activity of M-flufenamic acid complexes are found to be in the sequence: Mn>Ni>Cr>Zn>Fe>Cu>Co with respect to M (where M = Cr, Mn, Fe, Co, Ni, Cu and Zn).

In the present case Cr, Mn and Ni complexes of flufenamic show better activity than the parent drug while the antiinflammatory activity of the parent durg is decreased when it is complexed with Fe (III), Co (II), Cu (II) and Zn (II). The activity of the complex with Cu (II) is lowest while that of
with Zn (II) is comparable to that of the parent drug. This result provide evidence for a unique metabolite Cr, Mn and Zn are also dependant metabolic process required for the tissue maintenance.

5.6. ANTIINFLAMMATORY ACTIVITY OF PIROXICAM COMPLEXES

Piroxicam is 4 – hydroxy – 2 – methyl – N – ( 2 - pyridyl) -2H, 1, 2-benzothiazine-3-carboxamide-1, 1-dioxide. Molecular formula of piroxicam is C_{15}H_{13}N_{3}O_{4}S and molecular weight is 331.33. An off-white to light tan or yellow crystalline and odourless compound. It forms monohydrate, that is yellow, very slightly soluble in pure water and organic compounds but highly soluble in acidic water, slightly soluble in alcohol and aqueous alkaline solutions.

Piroxicam is a non-steroidal antiinflammatory drug belonging to the oxicam group. It is noted for its potent and long lasting analgesic and antiinflammatory effects enabling once daily dose. This drug is structurally different from other groups. It has been used to treat rheumatoid arthritis, ankylosing, spondylitis and acute gout.

Piroxicam prevents the edema, erythema tissue proliferation, fever and pain by its powerful antiinflammatory action. Due to powerful antiinflammatory action, it is effective regardless of the etiology of the inflammation.

The results presented in Table 5D and Figure 5.4 show that all M-piroxicam (where M = Cr, Mn, Fe, Co, Ni, Cu and Zn) complexes are more active than the parent drug.

As given in Table 5D and Figure 5.4 all compounds show higher percent inhibition of edema as compared to parent drug. All these compounds show better antiinflammatory activity than the piroxicam. The antiinflammatory activity of M-piroxicam complexes are found to follow the
sequence Co>Mn>Cr>Cu>Fe>Ni>Zn with respect to M (where M = Cr, Mn, Fe, Co, Ni, Cu and Zn).

This result provides evidence for a unique metabolite Cr, Mn, Fe, Co, Ni, Cu and Zn dependant metabolic process required for tissue maintenance.

5.7. COMPARATIVE ACTIVITY OF NSAIDS AND THEIR COMPLEXES

The comparative studies the results presented in Table 5E and Figure 5.5 show that highest and lowest antiinflammatory activity of metal complexes with NSAIDS. In case of diclofenac sodium complexes of Cr are found to be highest antiinflammatory activity while the Fe-complexes are least antiinflammatory activity from the diclofenac sodium and as similar to the mefenamic acid complexes.

In the present case Mn-complex of flufenamic acid show better antiinflammatory activity while the least antiinflammatory activity of Co-complex of flufenamic acid from the flufenamic acid. As given Table 5D piroxicam complexes of Co is highest antiinflammatory activity while the least antiinflammatory activity of Zn-complex from the parent drug.

More active complexes possibly depressed the synthesis of the proinflammatory (vasodilator) prostaglandin, PGE$_2$ in the carrageenan pouch model of inflammation$^{75}$. This is in consistent with the work of Lee and Lands$^{76}$, and recently confirmed by Moddox$^{77}$, who found a depression in PGE$_2$ synthesis and a concomitant increase in the antiinflammatory (vasoconstrictor) prostaglandin, PGF$_{2\alpha}$, following the addition of copper sulphate or chloride to seminal vesicle homogenates. These results suggest that the mechanism action of effective complexes may be, at least in part, at the level of the prostaglandin mediation of inflammation. This is to say, these complexes may play a role in decreasing the synthesis of the proinflammatory PGE$_2$ and concomitantly, increasing the synthesis of the antiinflammatory PGF$_{2\alpha}$. 
**TABLE -5A : ANTIINFLAMMATORY ACTIVITY OF DICLOFENAC SODIUM AND ITS COMPLEXES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of animals used in each group</th>
<th>Dose (mg/kg) body wt.</th>
<th>Initial volume* 0.0 hrs</th>
<th>Final volume* after 3hrs</th>
<th>Vol. of edema* (Final-Initial)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘A’ Control</td>
<td>10</td>
<td>100</td>
<td>0.615</td>
<td>1.145</td>
<td>0.530</td>
<td>-</td>
</tr>
<tr>
<td>‘B’ Plain drug</td>
<td>10</td>
<td>100</td>
<td>0.683</td>
<td>0.996</td>
<td>0.313</td>
<td>40.84</td>
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<td>‘C’ Cr-drug complex</td>
<td>10</td>
<td>100</td>
<td>1.006</td>
<td>1.176</td>
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<td>67.92</td>
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<td>‘D’ Mn-drug complex</td>
<td>10</td>
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<td>0.830</td>
<td>1.140</td>
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<td>0.810</td>
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<td>‘F’ Zn-drug complex</td>
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<td>100</td>
<td>0.729</td>
<td>0.909</td>
<td>0.180</td>
<td>66.03</td>
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* Average of 5 readings
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<th>Compound</th>
<th>No. of animals used in each group</th>
<th>Dose (mg/kg) body wt.</th>
<th>Initial volume* 0.0 hrs</th>
<th>Final volume* after 3hrs</th>
<th>Vol. of edema* (Final-Initial)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>'A' Control</td>
<td>10</td>
<td>100</td>
<td>0.615</td>
<td>1.145</td>
<td>0.530</td>
<td>-</td>
</tr>
<tr>
<td>'B' Plain drug</td>
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<td>100</td>
<td>0.750</td>
<td>0.950</td>
<td>0.200</td>
<td>62.26</td>
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<tr>
<td>'C' Cr-drug complex</td>
<td>10</td>
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<td>0.843</td>
<td>0.177</td>
<td>66.60</td>
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<tr>
<td>'D' Mn-drug complex</td>
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<td>0.996</td>
<td>0.250</td>
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<td>'E' Fe-drug complex</td>
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<td>0.599</td>
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<td>'F' Co-drug complex</td>
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<td>'G' Ni-drug complex</td>
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<td>'H' Cu-drug complex</td>
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<td>0.192</td>
<td>63.77</td>
</tr>
</tbody>
</table>

* Average of 5 readings
### TABLE - 5C: ANTIINFLAMMATORY ACTIVITY OF FLUFENAMIC ACID AND ITS COMPLEXES

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of animals used in each group</th>
<th>Dose (mg/kg) body wt.</th>
<th>Initial volume* 0.0 hrs</th>
<th>Final volume* after 3hrs</th>
<th>Vol. of edema* (Final-Initial)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘A’ Control</td>
<td>10</td>
<td>100</td>
<td>0.615</td>
<td>1.145</td>
<td>0.530</td>
<td>-</td>
</tr>
<tr>
<td>‘B’ Plain drug</td>
<td>10</td>
<td>100</td>
<td>0.723</td>
<td>0.929</td>
<td>0.206</td>
<td>61.13</td>
</tr>
<tr>
<td>‘C’ Cr-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.706</td>
<td>0.886</td>
<td>0.180</td>
<td>66.03</td>
</tr>
<tr>
<td>‘D’ Mn-drug complex</td>
<td>10</td>
<td>100</td>
<td>1.003</td>
<td>1.130</td>
<td>0.127</td>
<td>76.03</td>
</tr>
<tr>
<td>‘E’ Fe-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.633</td>
<td>0.856</td>
<td>0.223</td>
<td>57.92</td>
</tr>
<tr>
<td>‘F’ Co-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.903</td>
<td>1.246</td>
<td>0.343</td>
<td>35.28</td>
</tr>
<tr>
<td>‘G’ Ni-drug complex</td>
<td>10</td>
<td>100</td>
<td>1.140</td>
<td>1.290</td>
<td>0.150</td>
<td>71.69</td>
</tr>
<tr>
<td>‘H’ Cu-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.565</td>
<td>0.870</td>
<td>0.305</td>
<td>42.45</td>
</tr>
<tr>
<td>‘I’ Zn-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.640</td>
<td>0.850</td>
<td>0.210</td>
<td>60.37</td>
</tr>
</tbody>
</table>

* Average of 5 readings
<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of animals used in each group</th>
<th>Dose (mg/kg) body wt.</th>
<th>Initial volume* 0.0 hrs</th>
<th>Final volume* after 3hrs</th>
<th>Vol. of edema* (Final-Initial)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘A’ Control</td>
<td>10</td>
<td>100</td>
<td>0.615</td>
<td>1.145</td>
<td>0.530</td>
<td>-</td>
</tr>
<tr>
<td>‘B’ Plain drug</td>
<td>10</td>
<td>100</td>
<td>0.673</td>
<td>0.946</td>
<td>0.273</td>
<td>48.49</td>
</tr>
<tr>
<td>‘C’ Cr-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.730</td>
<td>0.898</td>
<td>0.168</td>
<td>68.30</td>
</tr>
<tr>
<td>‘D’ Mn-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.694</td>
<td>0.811</td>
<td>0.117</td>
<td>77.92</td>
</tr>
<tr>
<td>‘E’ Fe-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.749</td>
<td>0.950</td>
<td>0.201</td>
<td>62.07</td>
</tr>
<tr>
<td>‘F’ Co-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.646</td>
<td>0.733</td>
<td>0.087</td>
<td>83.58</td>
</tr>
<tr>
<td>‘G’ Ni-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.610</td>
<td>0.830</td>
<td>0.220</td>
<td>58.49</td>
</tr>
<tr>
<td>‘H’ Cu-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.702</td>
<td>0.883</td>
<td>0.181</td>
<td>65.84</td>
</tr>
<tr>
<td>‘I’ Zn-drug complex</td>
<td>10</td>
<td>100</td>
<td>0.788</td>
<td>1.026</td>
<td>0.238</td>
<td>55.09</td>
</tr>
</tbody>
</table>

* Average of 5 readings
<table>
<thead>
<tr>
<th>NSAIDS</th>
<th>Antiinflammatory activity (%)</th>
<th>Complexing metals showing highest and lowest antiinflammatory activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Best activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metal</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>40.84</td>
<td>Cr</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>62.26</td>
<td>Cr</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>61.13</td>
<td>Mn</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>48.49</td>
<td>Co</td>
</tr>
</tbody>
</table>
**Fig. 5.1:** Antiinflammatory activity of diclofenac sodium and its complexes.
Fig. 5.2: Antiinflammatory activity of mefenamic acid and its complexes.
FIG. 5.3: ANTIINFLAMMATORY ACTIVITY OF FLUFENAMIC ACID AND ITS COMPLEXES.
Fig. 5.4: Antiinflammatory activity of piroxicam and its complexes.
FIG 5.5: PLAIN DRUG FORMING COMPLEXES WITH Cr, Mn, Fe, Co, Ni, Cu AND Zn.

A = DICLOFENAC SODIUM
B = MEFENAMIC ACID
C = FLUFENAMIC ACID
D = PIROXICAM

ANTINFLAMMATORY ACTIVITY (%)
5.8. REFERENCES


42. Rainsford, K.D.; Agents Actions, 1977, suppl. 1, 59.


75. Willis, A.L.; Personal Communication.
