REVIEW OF LITERATURE
Arthritis, the most distressing and disabling syndrome encountered in medical practice, has been aptly termed as "Great crippler" and the "King of human miseries".

Inflammation is a complex vascular, lymphatic and local tissue reaction elicited in higher animals by the presence of viable or non-viable irritants (Menkin, 1956). The cellular events in the injured tissue include fenestration of the micro-vascular, leakage of the elements of the blood into the interstitial spaces and migration of leucocytes into the inflammed tissue. On macroscopic level, this is characterised by erythema, edema, tenderness and pain (Goodman and Gilman, 1980). The term "Rheumatoid arthritis" was coined by Garrod in 1959 for an inflammatory affection of the joints. Rheumatoid arthritis, a chronic polyarthritis affecting the peripheral joints with exacerbations and remissions, probably involves the combination of an antigen (gamma globulin) with an antibody (rheumatoid factor) and a complement.

Pathophysiology of inflammation:

Inflammatory reaction has essentially three features - (1) dilatation of blood vessels and increased vascular permeability leading to erythema and oedema at the site of noxious stimulus (Transudative phase), (2) cellular infiltration (exudative phase) and (3) finally tissue repair (proliferative phase) (Bhargava and Gupta, 1977).
The classical signs of inflammation include redness, swelling, heat and pain (Conheim, 1932).

Inflammation may vary from the acute transient and highly localised response to simple mechanical injury or the complex persistent response involving the whole organism. It initiates a series of biochemical, immunological and cellular events which may follow the initial responses and ending with physical repair and restoration of the function of the injured tissue (Naik and Sheth, 1976). This complex response involves the liberation of chemical mediators like histamine, 5-hydroxytryptamine, S.R.S. - A, kinins and prostaglandins. Every pathological condition is associated with tissue injury hence inflammation may be the commonest change observed, though not necessarily in acute form. Remarkable resemblance has been observed in response to a wide variety of stimuli. The nature and consequences of the reaction may vary depending on the type of a stimulus and the defence capacity of the host. The inflammation can be grouped under various heads depending on the response of the tissue to injury.

a. **Acute inflammation** - When the tissue damage is triggered by mechanical trauma, thermal injury, chemical burn or acute allergy, it represents an early reaction followed by repair (Naik and Sheth, 1976).

b. **Chronic inflammation** - When an irritant of low grade intensity acts upon the tissues it results in chronic inflammation. It is characterised microscopically by the
presence of lymphocytes, plasma cells and macrophages. Chronic inflammation is usually a sequel to acute inflammation or may be chronic from the beginning and the resulting fibrosis is more marked than acute inflammation (Boyd, 1974).

**Chemical mediators of inflammation:**

a. **Histamine** - The concept of endogenous histamine was first postulated by Lewis (1927). Subsequently, it was reported that histamine itself is released by injury (Spector, 1958; Bhatt and Sanyal, 1963; Bhalla et al., 1970). However, Bhargava et al. (1976) observed that increased capillary permeability induced by histamine is mainly due to activation of H₁ receptors as it was completely blocked by mepyramine. However, H₂ receptor blocker (Burimamide) failed to inhibit histamine-induced increased capillary permeability. Therefore, H₁ - receptor blockers may be regarded as having anti-inflammatory activity.

b. **5-Hydroxytryptamine (5-HT)** - Rowley and Benditt (1956) observed oedema of rat paw on injection of serotonin (5-HT). Serotonin as well as histamine (Paratt and West, 1957; Bhatt and Sanyal, 1963) are liberated during early phase of inflammation and like histamine it might also be responsible for vascular reaction and the oedema formation after injury (Wilhelm, 1962). The permeability effects of 5-HT might be attributed to a direct effect on vascular epithelium or to the release of histamine (Spector, 1958).
c. **Prostaglandins** - Prostaglandins are considered to be chemical mediators of inflammatory reaction. Prostaglandins of E type are reported to suppress acute and chronic inflammation in normal and adrenalectomised rats (Zurier et al, 1973) and also are held responsible for initiation and maintenance of inflammatory process.

Crunkhorn and Willis (1969, 1971 a, 1971 b) observed vasodilatation, increased capillary permeability and migration of leucocytes by prostaglandins in rat and man. Synergistic effects on capillary permeability are seen between prostaglandins and bradykinin, histamine or 5-HT in experimental animals (Bekemeier et al, 1974). Willis (1969) and Di Rosa et al, (1971) showed the presence of prostaglandins in the inflammatory exudate and further reported that carrageenin induced - oedema in rats is mediated by histamine and 5-HT during first hour while increased vascular permeability is maintained by kinin release upto 3½ hours. However, between 2½ to 6 hours the mediator appears to be prostaglandin, the release of which is closely associated with migration of leucocytes. When the acute phase of inflammation is over, prostaglandins have been reported to promote granuloma formation, mediate collagen metabolism (Raisz and Koolemen Beynen, 1974) and activate mucopolysaccharide synthesis (Peters et al, 1974).

d. **Polypeptide** - The term "Bradykinia" was coined by Rochaesilva et al, (1949) and was reported to cause vasodilatation, increased capillary permeability and
of "chemical mediator of inflammation" as increased capillary permeability (Holdstock et al., 1957), pain (Armstrong, et al., 1957) and migration of leucocytes (Lewis, 1952).

e. **Proteases** - Two types of proteases - Kallikrein (Spector and Willoughby, 1968) and plasmin (Macfarlane and Pilling, 1948) have been identified during inflammatory process. Kallikrein has been reported to release kinins from their inactive precursor showing increased capillary permeability (Spector and Willoughby, 1968). Proteolytic enzyme (fibrinolysin or plasmin) might be an important link in inflammatory process (Macfarlane and Pilling, 1948).

f. **Other factors** - Factors like leucotoxin, leucocytosis promoting factors and lymphnode permeability factor (LNPF) have been shown to be responsible for causation of increased capillary permeability during inflammation (Hurley and Spector, 1961; Willoughby and Spector, 1964). Further, uncoupling of oxidative phosphorylation results in reduced biosynthesis of ATP which causes inhibition of mucopolysaccharide synthesis and inflammatory and tissue growth (Adams and Cobb, 1958). Steroidal and non-steroidal anti-inflammatory agents have been reported to uncouple oxidative phosphorylation (Whitehouse and Haslam, 1962).

**Probable mode of action of nonsteroidal anti-inflammatory drugs (NSAID):**

Inhibition of prostaglandin synthesis is the most
widely accepted mechanism of action proposed for NSAID (Vane et al., 1971). Ferriera and Vane (1974) demonstrated inhibition of prostaglandin synthesis by anti-inflammatory agents. The current opinion holds that during inflammatory hyperalgesia, fever and platelet aggregation, arachidonic acid is released from the phospholipid fraction of the cell membrane by the action of phospholipase A$_2$. Corticosteroids have been reported to inhibit the prostaglandin synthesis apparently by inhibiting the substrate or inhibiting the action of phospholipase (Flower, 1974), thus resulting in complete inhibition of all the proinflammatory mediators (Figure 1). NSAID are shown to decrease synthesis of cylic endoperoxide (PGG$_2$ and PGH$_2$) by inhibiting the enzyme cyclooxygenase. Kuehl and associates (1971) reported that PGG$_2$ plays significant role in inflammatory process and further showed that free radical liberated upon conversion of PGG$_2$ to PGH$_2$ may regulate the formation of pro-inflammatory mediators. PGH$_2$ is acted upon by isomerase and reductase contained within the prostaglandin synthetase complex producing PGE$_2$ and PGF$_2$ alpha. NSAID by inhibition of cyclooxygenase reduce the levels of PGI$_2$, PGE$_2$, PGF$_2$ alpha and TXA$_2$. Arrigony-Martelli (1977) and Shen and Winter (1977) have reported the other possible mechanisms of action of NSAID as follows:

1. Inhibition of chemotaxis of cells involved in inflammation.
Figure 1: Generation of potential mediators of inflammation by enzymatic conversion of arachidonic acid.
2. Inhibition of lysosomal membrane inhibition, antagonistic effects on mediators other than prostaglandins e.g. Histamine, 5-HT, SRS-A, kinins, lymph node permeability factor (LNPF), peptidases, complements and others.

3. Inhibition of the biosynthesis of mucopolysaccharides.

4. Uncoupling of oxidative phosphorylation.

History of anti-inflammatory drugs and discovery of Tromaril:

Rheumatoid arthritis being one of the most painful and crippling disease entities, affects a major part of the population of the world, yet a satisfactory treatment is not in sight. The study of inflammation and anti-inflammatory drugs occupied an important place in therapeutic armamentarium and the anti-rheumatic activity of cortisone was reported by Hench et al., (1949) and for this outstanding contribution, he was awarded the Nobel prize. The highly potent corticosteroids, are not devoid of adverse effects, require therapy for prolonged periods. Therefore, it necessitated the search for non-steroidal anti-inflammatory agents.

Various drugs like glucocorticoids, non-steroidal anti-inflammatory agents like salicylates, indole acetic acid derivative like indomethacin, anthranilic acid derivative like mefenamic acid, arylalkanoic acid derivative like ibuprofen, naproxen, ketoprofen, anti-malarials
like chloroquine, gold salts, chelating agents like D-penicillamine (Jaffe, 1965) and immunopromoters like B.C.G. vaccine (Rewald, 1974) and Levamisole (Husskisson et al., 1978) constitute the main study of the treatment of this disease. Moreover, inspite of the availability of large number of drugs, this disease still remains incurable. All the anti-inflammatory drugs produce undesirable effects like epigastric pain, gastrointestinal haemorrhage, and gastrointestinal ulceration, besides they very frequently interact with other drugs then affecting treatment. Therefore, search for safer non-steroidal anti-inflammatory agents is still on. In the intensive search of a new but safer non-steroidal anti-inflammatory agent synthetic analogues of anthranilic acid has been synthesized and subjected to pharmacological screening.

Fenamates belonging to anthranilic acid derivatives evoked considerable interest in view of their anti-inflammatory, analgesic and antipyretic activities. Amongst these, mefenamic acid, flufenamic acid and meclofenamic acid are available as an anti-inflammatory and analgesic-antipyretic agents. However, they are not free from undesirable effects shown by the other anti-inflammatory agents.

In view of the foregoing, modified analgues of these compounds were synthesized to obtain more potent drugs with minimal side effects. It resulted in the synthesis of new compound, N-B-phenylethyl anathranilic
acid, code named RH-8 (Tromaril), possessing potent anti-inflammatory, analgesic and antipyretic activity (Sisodia et al., 1966).

![Chemical structure of Tromaril](image)

**Chemical structure of Tromaril:**

It is a light buff-coloured solid, having a melting point of 117°C - 118°C, insoluble in water and soluble in common organic solvents. It has a pKa value of 5.25 ± 0.025. Its IR spectrum shows co-absorption at 1670 cm⁻¹ and NH at 3360 cm⁻¹. It is prepared by condensing 2-chlorobenzoic acid with p-phenylethylamine.

**Pharmacological actions of tromaril in experimental animals:**

Sisodia et al. (1980) reported dose-related anti-inflammatory activity of tromaril and found it to be equipotent with phenylbutazone in inhibiting carrageenin induced paw oedema, resin pellet granuloma, adjuvant arthritis and formalin peritonitis in rats. However, it was half as potent as oxyphenbutazone in delaying onset of ultraviolet erythema in guinea pigs and twice as potent as phenylbutazone in inhibiting mouse ear oedema. The inhibitory effects of tromaril on wound healing in rat and on granulation tissue formation on chorioallantoic membrane of chick embryo paralleled with phenylbutazone.
(Sisodia et al., 1980). Adrenalectomy failed to influence the inhibitory effect of tromaril.

Since the anti-inflammatory agents possess analgesic activity, it showed analgesic activity in rats similar to phenylbutazone (Sisodia et al., 1980) and was subjected to further studies involving analgesic activity. However, it was found to possess greater antinociceptive activity than aspirin and mefenamic acid as assessed by Haffner tail clip, Eddy’s hot plate and radiant heat method (Sisodia et al., 1980).

Anti-inflammatory agents are found to possess antipyretic activity. Therefore, tromaril was tested to assess this property (Sisodia et al., 1980). Tromaril has the same antipyretic activity as oxyphenbutazone on yeast-induced pyrexia in rats (Sisodia et al., 1980). However, in contrast with phenylbutazone, tromaril failed to show any ulcerogenic activity up to the dose of 400 mg/kg in rats (Sisodia et al., 1980). Tromaril was devoid of any enzyme induction activity in rats as compared to phenylbutazone. It inhibited degranulation of mast cells in rat following aseptic injury (Sisodia et al., 1980).

No significant effect was observed on cardiovascular system, central nervous system, respiratory system, and smooth muscle in experimental animals (Sisodia et al., 1980). Tromaril possesses a wide margin of safety as evidenced by i.p. LD50 values in mice (760 mg/kg IP rats), and oral LD50 value in mice, rats and dogs (72000 mg/kg).
Tromaril has been reported to inhibit prostaglandin synthetase responsible for its anti-inflammatory, antiplatelet aggregation activity like indomethacin (Kshirsagar et al., 1980). However, indomethacin decreases but tromaril increases urinary volume and electrolyte excretion (Kshirsagar et al., 1980). Further it has been postulated that indomethacin is one of the most potent inhibitors of prostaglandin synthesis (Goodman and Gilman, 1980).

Tromaril pretreatment inhibited significantly liver succinic dehydrogenase, glutamic oxaloacetic transaminase glutamic pyruvic transaminase and acid phosphatase content of oedematous or granulomatous tissue during carrageenin oedema and cotton pellet granuloma (Naik and Sheth, 1980). It also inhibited the alteration in the connective tissue components like hydroxyproline, hexosamine and sialic acid during carrageenin oedema and cotton pellet granuloma (Naik and Sheth, 1980). It is postulated that tromaril might inhibit the inflammatory process by inhibiting many biochemical processes at cellular level (Naik and Seth, 1980).

Valame et al., (1980) reported that tromaril failed to cause haemolysis of G6-PD deficient red cells in vitro.

**Pharmacokinetics of Tromaril**

Pharmacokinetic studies have been done in dogs and rats by Sisodia et al., (1980). Tromaril was found to be rapidly and well absorbed after oral administration and 50% of total amount of drug was absorbed within half an
hour (Sisodia et al., 1980). Thirty minutes after oral administration the peak plasma level of the drug was 3,2666 mg/ml in rat and 8 mg/ml in dog. Plasma half life was reported to be 18 minutes in rats and 21 minutes in dogs after intravenous administration (Sisodia et al., 1980).

Highest concentration of tromaril was found in liver. It crosses the blood brain barrier and significant portion is bound to plasma proteins (Sisodia et al., 1980). Tromaril was found to be excreted in urine for over 72 hours after a single oral dose, which is attributed to slow release of the drug from binding sites in tissues. The faecal excretion of the drug was continued for over a period of 72 hours after single oral dose. Therefore, the drug undergoes enterohepatic circulation as evidenced by the presence of drug in bile (Sisodia et al., 1980).

Bio-availability studies of Tromaril:

Rao et al., (1980) showed that peak serum concentration is achieved in about two hours after oral administration which was found to be dose-related. The blood level declined in 4 hours (Rao et al., 1980). Presence of food has been reported to increase the absorption of tromaril (Kashirsagar et al., 1980).

The acute, subacute and chronic toxicity of tromaril (RH-8) has been studied in mice, rats and dogs. The intraperitoneal LD₅₀ value in mice was 760 mg/kg. However, oral LD₅₀ value in mice, rats and dogs was found to be
greater than 2000 mg/kg. The subacute and chronic toxicity study of tromaril showed that it was well tolerated by rats and dogs. Further, no significant pathological changes, morphological or functional, was observed at the oral low dose level. In long term study, no significant haematological, chemical or pathological changes were observed at the low dosage level on oral administration (Sisodia et al., 1980).

Teratogenic effects of tromaril were investigated in rats and rabbits. On oral administration of tromaril to pregnant rats (20-640 mg/kg/day) and rabbits (20-200 mg/kg/day) during the organogenesis period, no teratogenic effect was reported on foetuses. Moreover, tromaril was devoid of any effect on post-natal development of their pups (Sisodia et al., 1980). In the mutagenic studies, the incidence of micronuclei and P/N ratio in bone marrow erythrocytes in tromaril and ibufen-treated mice was reported to be similar to the untreated controls indicating lack of induction of any genetic damage by these drugs (Shobha Devi and Polasa, 1980). However, phenylbutazone and indomethacin showed a significant increase in the frequency of micronuclei in the polychromatic erythrocytes and altered the P/N ratio.

Clinical studies of tromaril:

Gastro-intestinal haemorrhage has been reported after the administration of corticosteroids (Kowalenski 1959), salicylates (Marchetti et al., 1974) mefenamic acid
(Wolfe et al., 1976), indole and phenylpropionic acid derivatives (Bhargava, 1973). Significant increase in blood loss was reported after administration of aspirin, flurbiprofen and tromaril (Dahanukar et al., 1980) but only the difference between tromaril and flurbiprofen group is significant. Tromaril has been found to possess significant antithrombotic activity (Manikeri et al., 1980). Tromaril and flurbiprofen were reported to inhibit platelet aggregation without affecting coagulation tests. On the contrary, aspirin inhibits platelet aggregation in some tests of coagulation (Manikeri et al., 1980; Gupta et al., 1980). Tromaril has been reported to be effective and safe in arthritis, arthralgia, osteoarthritis and gout as anti-inflammatory drug in view of their good gastric tolerance and devoid of side effects (Swamy et al., 1980; Mathur et al., 1980; Sattur et al., 1980; Rao et al., 1980). Rao (1980) observed significant anti-inflammatory analgesic effect in rheumatoid arthritis. No untoward effect was observed on haemopoetic, renal or hepatic systems after the use of tromaril (Sisodia et al., 1980). Rao et al., (1980) and Lokabai et al., (1980) reported tromaril and oxyphenbutazone to be equally effective.

Tromaril showed antipyretic activity equal to that obtainable with aspirin (Gupta et al., 1980). Raotg et al., (1980) failed to detect the presence of tromaril in breast milk of lactating mothers.
In contrast with indomethacin, tromaril was reported to increase the urinary volume, sodium, potassium and protein output significantly in patients with nephrotic syndrome (Kishirsagar et al., 1980).

Properties of other anti-inflammatory drugs:

After the discovery of aspirin in 1899 by Dresser, various anti-inflammatory drugs were introduced such as aminophenols like phenylbutazone, indole acetic acid derivatives like indomethacin, anthranilic acid derivatives like mefenamic acid and arylalkanoic acid derivatives like ibuprofen, naproxen, ketoprofen. However, amongst these drugs aspirin remains the drug of choice for rheumatic and arthritis conditions (Goodman and Gilman, 1980). Indomethacin, synthesized by Shae et al. (1963), was considered to be more effective than any other anti-inflammatory drug for the long term management of chronic arthritis with minimal toxicity to the patient (Norcross, 1965). In studies conducted by Winter et al. (1963), higher efficacy of indomethacin over aspirin for its anti-inflammatory action was established as it was found to be 35 times as potent as aspirin in hind paw oedema and cotton pallet tests. Moreover, indomethacin was reported to be more potent anti-pyretic agent by these workers. Indomethacin has been reported to be better than placebo in rheumatoid arthritis in clinical studies (Deodhar et al., 1973). Aspirin and ibuprofen have been reported to be more effective than placebo and equally effective in analgesic activity.
(Giansiracusa et al., 1975). Objective assessments of joint measurement were found to be similar for ibuprofen and indomethacin (de Blecourt, 1975).

Tolmetin has been reported to possess a marked anti-inflammatory activity as it inhibited carrageenin-induced paw oedema (Carson et al., 1971; Wong, 1975). It was more active than aspirin but less active than indomethacin (Awouters et al., 1975; Wong, 1975). Most of the anti-inflammatory drugs possess analgesic activity. However, unlike established analgesic action of aspirin (Winder, 1959; Paalzow, 1969; Dubas and Parker, 1971), the analgesic activity of indomethacin, distinct from its anti-inflammatory effect remains uncertain. Collier et al., (1968) reported it to be an effective analgesic in abdominal construction response. Tolmetin failed to respond in Haffner tail clip assay, however, it significantly inhibited acetic acid-induced writhing (Sangal, 1982). Salicylates increased cardiac output and right ventricular and systemic pressures (Tehney and Miller, 1955). Aspirin and indomethacin, which inhibit prostaglandin synthesis, enhance the coronary dilatation induced by increased cardiac activity, hence might be useful in preventing coronary insufficiency in conditions of cardiac stress (Talesnik and Sunahara, 1973). Aspirin is most vulnerable in producing initial respiratory stimulation followed by depression, which leads to respiratory alklosis followed by metabolic acidosis (Bosenstein and Borison, 1963).
While observing the central nervous system effects of anti-inflammatory agents, headache drowsiness and depression, were observed with indomethacin (Healey, 1967) and Naproxen (Katona, 1973) whereas salicylates are likely to cause significant neurological effects like convulsions, or coma, tinnitus and hearing loss (Waltner, 1955).

Most of anti-inflammatory agents possess antipyretic activities. Tolmetin showed antipyretic action in Brewer's yeast-induced pyrexia (Sangal, 1982), tolmetin produced lesions in gastric mucosa in fasted rats in single (Shriver, et al., 1975) or multiple doses (Mann, 1977; Wong et al., 1973).

In clinical trials, superiority of tolmetin in comparison to aspirin, has been found to be controversial because multicentre studies (April et al., 1975; Bain et al., 1975) observed no significant difference between two drugs.

Indomethacin was found to be more potent in inhibiting platelets as it decreased the release of platelet bound C-serotonin (Zucker and Peterson, 1970). Tolmetin stored only little effect on platelet adhesiveness unlike aspirin (Sangal, 1982). However, aspirin was found to possess fibrinolytic activity and slightly prolonged coagulation and prothrombin times (Rishi et al., 1976). The haemopoietic effects of indomethacin included neutropenia, thrombocytopenia and rarely aplastic anemia, while ibuprofen also failed to modify platelet function and to
prolong bleeding time (Goodman and Gilman, 1980)

Aspirin induced hyperglycemia and hypoglycemia in certain diabetic patients (Reid et al., 1957; Whitehouse, 1965). Indomethacin instead of any direct action, just inhibited the glucagon-induced hepatic glucose production (Ganguli et al., 1979) and angiotensin-induced hyperglycemia (Singh et al., 1978). However, tolmetin showed a significant hypoglycemia (Sangal, 1982). The effect of aspirin and phenylbutazone on uric acid excretion was found to be dose dependent (Yu and Gutman, 1959; Goodman and Gilman, 1975 and Wallace et al., 1967) while tolmetin was found to decrease the serum uric acid level (Sangal, 1982).

Aspirin was shown to enhance fibrinolytic activity (Rishi et al., 1976). However, Done (1960) reported inhibition of fibrinolysis with anti-inflammatory agents in vitro.