Review of Literature
REVIEW OF LITERATURE

The present study is concerned with exploring the potential pharmacological actions of a few flavone derivatives. The main focus is on their role in pain and inflammation and mechanisms involved therein. Hence a review on the following topics has been considered appropriate.

a) The current concepts on pain, pain pathways, various types of analgesic drugs, their mechanisms of action and adverse effects have been reviewed in the first stage.

b) In a similar fashion the recent concepts on inflammation, mediators of inflammation, the mechanism of action, therapeutic uses and adverse effects of steroidal and nonsteroidal anti inflammatory drugs have been reviewed subsequently.

c) The review of literature also includes a brief description on the chemistry of flavonoids and their biological effects with special emphasis on antinociceptive, anti inflammatory actions and possible mechanisms mediating these effects.
PAIN

The International Association for the study of pain (IASP) has defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”. Each patient's experience and expression of pain are different (Oxenham et al. 2006).

The above definition implies that, perception of noxious stimuli is not the same thing as pain which is a subjective experience and includes an emotional component as well. The sensation of pain and the distress response it evokes are greatly influenced by the individual’s previous experiences.

Types of Pain

There are different types of pain, such as acute pain, chronic pain and neuropathic pain (Fields et al., 1994b).

Acute Pain

Acute pain is experienced immediately when the body is injured or damaged. This type of pain alerts the individual to injury and helps in identifying the location of the damage. It is uncomfortable to experience, but easy to treat, is distinguished by having a specific cause and purpose and generally produces no persistent psychological reaction. Examples of acute pain are wounds, fractures, burns etc.
Acute pain automatically results in a stress reaction with an increase in blood pressure, heart rate, respiration rate, enhanced alertness and greater motor strength for a faster response. When acute pain is precipitated by trauma or acute medical conditions, diagnosis and treatment of the underlying causes are always the first priority. There is a chance that untreated or under treated acute pain will increase the risk of evolving into a chronic pain condition.

**Chronic pain**

There are several factors that can cause, perpetuate or exacerbate chronic pain. The patient may simply have a disease that is characteristically painful for which there is presently no cure such as arthritis, cancer, migraine, headaches, fibromyalgia or diabetic neuropathy (Fields *et al.*, 1994b).

There may be neural and somatic perpetuating factors that are initiated by bodily disease and may persist after that disease has resolved. Examples include damaged sensory nerves, sympathetic afferent activity and painful reflex muscle contraction. A variety of psychological conditions can exacerbate or even cause pain.

There have been some theories that inadequate treatment of acute pain can lead to chronic pain. Sometimes chronic pain can have a psychosomatic or psychogenic cause (Oxenham *et al.* 2006).
Some common pain situations

Cutaneous pain

Cutaneous pain is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and due to the high concentration of nerve endings produce a well defined, localized pain of shorter duration. Examples of injuries that produce cutaneous pain include minor cuts, minor burns and lacerations.

Visceral pain

This type of pain originates from body’s visceral organs. Visceral nociceptors are located within body organs and internal cavities. Visceral pain is extremely difficult to localize and several injuries to visceral tissue exhibit “referred pain” where sensation is localized to an area completely unrelated to the site of injury. Myocardial ischemia is the best example of referred pain.

Somatic pain

Somatic pain originates from ligaments, tendons, bones, blood vessels and even nerves themselves. It is detected with somatic nociceptors; examples include sprains and broken bones.

Phantom limb pain

A type of referred pain from a limb that has been lost or from which a person no longer receives physical signals. It is an experience almost universally reported by amputees and quadriplegics.
Neuropathic pain

This can occur as a result of injury or disease to the nerve tissue itself (Fields et al., 1994a). For example, damage to peripheral nerves, as occurs in diabetic neuropathy or to primary afferents as in herpes zoster can result in pain that is referred to the body region innervated by damaged nerves.

Pain may also be produced by damage to central nervous system, particularly the spinothalamic pathway. Neuropathic pains typically have an unusual burning, tingling or electric shock like quality and may be even triggered by very light touch. A variety of mechanisms contribute to neuropathic pain. Damaged primary afferents including nociceptors become highly sensitive to mechanical stimulation and begin to generate impulses in the absence of stimulation. Thus both central and peripheral nervous system changes may contribute to neuropathic pain.

Pain pathways

The small diameter fibres of peripheral nerves are the primary afferent nerves of pain sensation. In the peripheral tissue these nerves have sensory endings which are activated by a variety of stimuli (mechanical, thermal and chemical). These sensory nerve endings are normally activated mainly by stimuli of noxious intensity that can cause some degree of tissue damage. These receptors are thus different from mechanical and thermal receptors by their response mainly to higher threshold stimuli. The non myelinated C fibres with low conduction velocity and Aδ myelinated fibres with rapid conduction
are the primary nociceptive afferents. Local release of a variety of chemical agents resulting from tissue injury excites these nerve terminals. The pain receptors are maximally present in the skin, periosteum, arterial walls and joint surfaces whereas in deep viscera they are not wide spread (Rang et al 2003).

Hence localization of cutaneous pain is easier than visceral pain. From physiological view, pain conducted by two different sensory afferents may be considered as fast and slow pain. Fast pain is generally conducted by Aδ fibres and is well localised, is sharp or pricking in nature. Slow pain is conducted by unmyelinated C fibres, is poorly localised, burning, dull or aching in nature.

The cell bodies of the spinal nociceptive afferent fibres lie in dorsal root ganglia and end in the gray matter of the dorsal horn (fig -1). Most of the nociceptive afferents terminate in the superficial regions of the dorsal horn. The C fibres and some Aδ fibres innervate cell bodies of lamina I and II whereas other Aδ fibres terminate in lamina V in the interior part of the dorsal horn.

The main projections pathways of pain from the dorsal horn to the thalamus originate from the cells in lamina I and V. Spinothalamic tract fibres synapse with cells present in the ventral and medial parts of thalamus. The axons arising from these cells project to the somato sensory cortex. The sensory cortex plays an important role in processing the intensity and location of the pain and helps in cognitive evaluation of the pain.
Figure 1
Pain Pathway

Spinothalamic tracts also are linked with frontal cortex and the limbic system. This pathway may be responsible for the affective or unpleasant emotional dimension of pain. The fibres mediating slow pain also have projections to reticular areas of the brain stem and terminate in the intralaminar nuclei of thalamus. These two areas constitute part of the principal “arousal system” of the brain. This may be the reason why a subject remains sleepless while experiencing severe pain.

Modulation in the pain pathway

Facilitation and hyper excitability

The nociceptive pathway is subjected to various types of positive and negative modulation at different levels. The primary afferent neurons contain several peptides like substance - P and calcitonin gene related peptide (CGRP) which are released as mediators at peripheral and central terminals in pain pathway. The facilitation and thus the hyper excitability happens both peripherally and from central influences. The transmission at the first synaptic relay in the dorsal horn is facilitated by several neuro peptides and inflammatory mediators.

Substance - P and CGRP released from primary afferent neurons also act in periphery to sustain and amplify the inflammatory reaction and hence in further activation of nociceptive afferent fibres. N-methyl d-aspartate (NMDA) and nitric oxide (NO) also facilitate nociceptive transmission and this may be responsible for pathological hyperalgesia usually associated with
inflammatory responses. Many central mechanisms also contribute to facilitate this hyperalgesia of which nerve growth factor (NGF) is considered to play an important role. Increased NGF production (particularly in inflammation) is considered to augment the electrical excitability, chemosensitivity and peptide content of nociceptive afferent neurons. The increased levels of NGF may be an important mechanism by which nociceptive transmission becomes facilitated by tissue damage leading to hyperalgesia (Rang et al., 2003).

**Inhibitory control**

Regulation of nociceptive impulses is mediated at the spinal cord level as well as from descending inhibitory control. These impulses attenuate and dampen the excitation of primary afferent neurons or the activity of spinothalamic tract.

**Gate control theory**

The substantia gelatinosa cells of lamina II of the dorsal horn send short inhibitory interneuron projecting to lamina I and V where the primary afferent neurons terminate. These inter neurons regulate the transmission at the first synapse of the nociceptive pathway between the primary afferent fibres and the spinothalamic tract. The regulation of nociceptive pathway at the primary synapse is popularly known as “Gate control theory” proposed by Wall and Melzack in 1965 (Fig-3). It has been proposed that non-nociceptive afferent input (from mechano receptors through Aβ fibres) and descending
Figure 3
Gate Control theory

inhibitory neurons activate the inhibitory interneuron of substantia gelatinosa cells. These interneurons originating from substantia gelatinosa cells act to inhibit the nociceptive transmission at the synapse between primary afferent neuron and spinothalamic tract.

**Descending inhibitory control (Fig-2)**

An important constituent of the gating mechanisms that control impulse transmission in the dorsal horn arise from higher centres. The periaqueductal gray (PAG) area of the midbrain forms a key part of this descending regulatory system. The PAG receives inputs from hypothalamus, cortex and thalamus and it may be the main mechanism by which cortical and other inputs act to control the nociceptive gate in the dorsal horn (Rang *et al.*, 2003). The neuronal pathway activated by stimulation of PAG passes through nucleus raphe magnus (NRM) and from there fibres run via dorsolateral funiculus of the spinal cord and synapse with dorsal horn inter neurons. 5-Hydroxy tryptamine (5-HT) is believed to be the major neurotransmitter in this synapse. Endogenous opioid peptides, (enkephalins) are also considered to play a role in the descending inhibitory pathway. It is well known that dorsal horn inter neurons regulate the activity of synapses in the spino thalamic tract. In addition to the connections from PAG, input from spino thalamic neurons also activate NRM. The dorsal horn transmission is also inhibited by a noradrenergic pathway from locus ceruleus (Rang *et al.*, 2003).
Figure 2

Descending Pathway

Chemical mediators in the nociceptive pathway (Rang et al., 2003)

Sensitivity of the nociceptive nerve endings

The polymodal nociceptors are the main type of peripheral sensory neurons that respond to any noxious stimulus. Majority of them are non myelinated C fibres. The peripheral nerve endings of these fibres respond to thermal, mechanical and electrical stimuli. However in most cases the peripheral nociceptive endings are mainly stimulated by a variety of chemicals.

Endogenous substances

1. Kinins

Brady kinin and kallidin are mainly vasodilator peptides formed from a protein substrate kininogen which is plasma α – globulin. These are formed during tissue injury by the proteolytic cleavage of kininogen. Bradykinin is a potent pain producing chemical and acts by combining with specific G-protein coupled receptors. It is believed that bradykinin B₁ receptors play a significant role in inflammation and hyper algesia. Bradykinin may also release prostaglandins which in turn enhance the direct actions of bradykinin.

2. Prostaglandins

Prostaglandins of the E and F series are released during inflammation and tissue ischaemia. It has been proposed that prostaglandins per se may not cause pain but they may strongly enhance the pain producing effect of
bradykinin or 5-HT. Prostaglandins may sensitize the nerve terminals to other agents partly by inhibiting potassium channels and partly by facilitating cellular depolarisations (Ca$^{2+}$ and Na$^{+}$) caused by noxious agents (Rang et al., 2003).

**Exogenous substances**

**Capsaicin**

It is an active chemical present in chilly peppers. Similar substances are also present in ginger and black pepper. Capsaicin binds to vanniloid receptor present on nociceptive afferent neurons and open ligand gated cation channels. Entry of sodium and calcium into the primary afferent neuron causes depolarization and initiation of action potentials. The vanniloid receptor is also activated by other capsaicin like agonists and by other stimuli including temperature in excess of about 45°Celsius (the threshold for pain) and protons. Anadamide has been identified as endogenous ligand for vanniloid receptor. Many other pain producing substances like bradykinin also act by sensitizing vanniloid receptor (VR$_1$).

**Other mediators**

Many Chemical substances and metabolites are released from injured or inflamed tissues that may affect nociceptive nerve terminals. 5HT, histamine, adenosine triphosphate (ATP) and protons are considered to have some role in nociceptive phenomena.
Modulation of the pain pathway

The origin of pain impulse from peripheral nerve endings and transmission via spinal cord to various higher centres involves the release of several transmitters at different junctions. The excitation of nociceptors and the release of transmitters at different synapses are modulated by a wide range of local and central influences.

Tachykinins

Tachykinins like substance - P and neurokinin- A are widely distributed especially in nociceptive primary afferent neuron and the dorsal horn. When the nociceptive neurons are activated, these neuro peptides are released both at the central and the peripheral terminals. When nociceptors are activated, the release of substance - P in the periphery may cause neurogenic inflammation. In the spinal cord dorsal horn, substance-P released is responsible for nociceptive signal transmission. Tissue inflammation through the action of nerve growth factor (NGF) increases the substance-P content of nociceptive neurons and thus potentiating the excitatory responses in the spinal cord. It has been suggested that substance- P in addition to its primary role in nociceptive pathway also is involved in inflammatory conditions like arthritis, asthma, and inflammatory bowel disease. It has been postulated that many actions of bradykinin and neurokinin A are mediated by their actions on Enkephalin₁ (Enk₁) and Enk₂ receptors (Rang et al., 2003).
Endogenous opioid peptides

Several peptides with morphine like pharmacological actions have been identified as endogenous opioid peptides. These peptides are formed from large molecular weight precursors like preproopiomelanocortin, prepro enkephalin and prepro dynorphin. From these precursors are formed the opioid peptides, β-endorphin, met- enkephalin, leu-enkephalin and dynorphin. These peptides are widely distributed in various parts of CNS and periphery. Pro-enkephalin peptides are present in areas of the CNS that are considered to be related to pain perception, lamina I and II of spinal cord, spinal trigeminal nucleus, and PAG. The presence of enkephalin in the above areas may suggest a possible role for them in the descending pathways from the midbrain to the dorsal horn. Enkephalins have been also identified in brain areas that modulate the affective behaviour like amygdala, hippocampus, locus ceruleus and frontal cerebral cortex (Gutstein and Akil.., 2006).

These evidences strongly indicate the essential role played by endogenous opioid peptides in the sensory perception of pain as well as in the consequent behavioral (emotional) responses exhibited by an individual.

Other central mediators

The nociceptive impulse originating from the nociceptors is amplified by chemicals released from the primary afferent neuron itself (substance P, and neurokinin A) which perpetuate the inflammatory reaction. The release of NGF like substances in the periphery also increases inflammation and in fact
produces hyper algesia. Several other mediators have been identified in the nociceptive pathway and in the descending inhibitory pathway which are considered to play a prominent role in the transmission and modulation of pain.

**Glutamate**

This excitatory amino acid released from the primary afferent neuron acts on AMPA receptors and is responsible for fast synaptic transmission at the first synapse in the dorsal horn. Glutamate is also believed to initiate a slow, NMDA receptor mediated response, which plays a crucial role in facilitation of pain transmission.

**Gama amino butyric acid (GABA)**

Many spinal cord interneurons release GABA and inhibit the transmitter release by primary afferent terminals in the dorsal horn.

**5-Hydroxy tryptamine (5HT)**

The inhibitory neurons running from NRMC to the dorsal horn release 5HT.

**Nor adrenaline**

This catecholamine is the transmitter of the inhibitory pathway from locus ceruleus to the dorsal horn and possibly also in other anti nociceptive pathways.
Adenosine

The descending inhibitory purinergic pathways are believed to act on pain transmission through adenosine1 (A1) receptors and produce analgesia. On the contrary activation of A2 receptors may induce pain.

Treatment of Pain

The ideal treatment for any pain is to remove the cause. However, symptomatic treatment of pain with a suitable analgesic offers relief of both physical symptoms and psychological benefit to the sufferer.

A wide variety of analgesic drugs are available to treat pain of different origin. Mild pain of musculoskeletal origin is managed with non steroidal anti-inflammatory (NSAID) analgesics. Visceral pain and post operative pain are mainly treated with opioid analgesics. Neuropathic pain constitutes a special category where the drugs of the above groups remain ineffective. It is managed by different groups of drugs like anticonvulsants and antidepressants.

Non Steroidal Antiinflammatory Drugs (NSAID)

NSAID are effective in the treatment of mild to moderate pain and are also useful adjuncts in the treatment of severe pain. All these compounds inhibit cyclooxygenase and except for acetaminophen all have antiinflammatory action, especially at higher doses. They are particularly effective for mild to moderate head ache and for pain of musculoskeletal
origin. The following non steroidal anti inflammatory drugs (NSAID) are currently employed in therapy (Burke et al., 2006).

- **Salicylates:** Aspirin and Diflunisal
- **Para aminophenol derivative:** Acetaminophen
- **Acetic acid derivatives:** Indomethacin, Sulindac and Etodolac
- **Heteroaryl acetic acid derivatives:** Tolmetin and Ketorolac
- **Phenylacetic acid derivative:** Diclofenac
- **Fenamates:** Mefenamic acid, Meclofenamate and Flufenamic acid
- **Propionic acid derivatives:** Ibuprofen, Naproxen, Fenoproven, Oxaprozin
- **Pyrazolone derivatives:** Phenylbutazone, Oxyphenbutazone, Antipyrine, Aminopyrine and Dipyrene.
- **Enolic acid derivatives:** Piroxicam, Meloxicam and Nabumetone
- **COX-2 Selective inhibitors:** Celecoxib, Valdecoxib, Rofecoxib, and Etoricoxib.

**Analgesic effect of NSAID**

NSAID are mainly effective against pain associated with inflammation, because they decrease production of the prostaglandins that sensitize nociceptors to inflammatory mediators such as bradykinin. Therefore, they are effective in arthritis, bursitis, and pain of muscular and vascular origin, toothache, and dysmenorrhoea, the pain of postpartum states, the pain of cancer metastases in bone and all conditions that are related with increased
prostaglandins. There is some evidence that they have a central effect by an action mainly on spinal cord.

Mechanism of action

The main action of NSAID is inhibition of arachidonic acid metabolizing activity of cyclooxygenase (COX) (Vane et al. 1971). The principal therapeutic effects of NSAID derive from their ability to inhibit prostaglandin production. The first enzyme in the prostaglandin synthetic pathway is prostaglandin G/H synthase, also known as cyclooxygenase or COX. This enzyme converts arachidonic acid to the unstable intermediates PGG$_2$ and PGH$_2$ and leads to the production of thromboxane A$_2$ (TXA$_2$) and a variety of prostaglandins. There are two isoforms of cyclooxygenase, Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2). COX-1 is a primarily constitutive isoform found in most normal cells and tissues, while cytokines and inflammatory mediators that accompany inflammation induce COX-2 (Siebert et al., 1997).

Opioid analgesics

Opioids are the most potent pain relieving drugs currently available. Furthermore, of all analgesics they have broader range of efficacy, providing the most reliable method for rapidly relieving pain. Opioids activate pain inhibitory neurons and directly inhibit pain transmission neurons.

The following Opioid analgesics are employed in therapy (Gutstein and Akil , 2006).
**Morphine and related opioid agonists**

There are many natural and synthetic agents, Morphine, Heroin, Hydromorphone, Oxymorphone, Levorphanol, Levallorphan, codeine, hydrocodone and oxycodone.

- **Pipridine and phenyl piperidine derivatives:** Meperidine, Fentanyl, Sufentannil, Alfantanyl, Remi fentanil.
- **Methadone and congeners:** Methadone hydrochloride and propoxyphene.
- **Opioid agonists / antagonists, partial agonists:** Pentazocine, Nalbuphine, Butarphanol, Buprenorphine.

**Opioid Receptor**

Three types of opioid receptors (µ, δ, κ) have been studied in detail (Waldhoer et al., 2004). The µ receptors are believed to be responsible for most of the analgesic effects of opioids, respiratory depression, euphoria, sedation and dependence. Most of the analgesic opioids are µ - receptor agonists. The δ – receptors may also contribute to analgesia at the spinal level and more importantly in the periphery. The κ receptors contribute to analgesia at the spinal level and may elicit sedation and dysphoria. They produce few side effects and do not contribute to dependence. Some analgesics act through κ -receptors. A fourth subtype of σ receptors was reported by Walker et al (1990).
There is pharmacological evidence for further subdivisions of each of these subtypes. Akil and Watson (1994) have suggested the presence of one additional sub type of κ receptor, with high affinity for the benzomorphan class of opiate alkaloids. Existence of δ -opioid receptor subtypes (δ₁ and δ₂) was proposed from behavioral studies by Jiang et al., (1991). Occurrence of µ -opioid receptor subtypes (µ₁ and µ₂) were proposed after behavioral and pharmacological studies (Pasternack., 1986).

**Mechanism of action**

All opioid receptors are linked to G-protein coupled receptors. Opioids inhibit adenylyl cyclase leading to decrease in intracellular C- AMP with consequent decrease in cell excitability. Opioids activate K⁺ channels and subsequent increase in K⁺ conductance to produce hyper polarization of neurons and a decrease in their excitability. Opioids inhibit Ca²⁺ conductance by suppressing voltage gated N type Ca²⁺ channels. Since Ca²⁺ influx is needed for the stimulus induced neurotransmitter release, opioids decrease the release of neurotransmitter like substance P and glutamate from nociceptive terminals.

Opioids are believed to act at several sites in the pain pathway. Eventhough the primary site of action of opioids is considered to be the higher centres in brain; peripheral sites of opioid action are also suggested. Opioids may act on the peripheral terminals of nociceptive afferent neuron and also act directly on the dorsal horn exerting an inhibitory influence on pain
transmission. The release of substance P from dorsal horn neurons is inhibited by opioids. Several studies have proved that, the opioids activate neurons in PAG and projections to NRM. The above two structures constitute the important descending controls of the pain pathway. Projections from Nucleus Raphe Magnus run to the substantia gelatinosa of the dorsal horn and inhibit pain transmission. Enkephalins and 5-HT are considered to be important mediators in the descending pathways.

**Other analgesic drugs**

Besides opioids and NSAID, several other drugs are used as analgesics particularly to treat neuropathic pain states, which respond poorly to conventional analgesic drugs and cause a major clinical problem.

**Tramadol**

This is a metabolite of the anti depressant trazolone and is widely used for post operative pain. It is a weak agonist at \( \mu \) - opioid receptors and also an inhibitor of nor adrenaline reuptake.

Tricyclic anti depressants, imipramine and amitriptyline act centrally by inhibiting noradrenaline reuptake and are highly effective in relieving neuropathic pain.

Antiepileptic drugs such as carbamazepine, gabapentin and occasionally phenytoin are sometime effective in neuropathic pain.
Ketamine, a dissociative anesthetic which works by blocking NMDA receptor channels, has analgesic properties.

Intravenous lidocaine, a local anaesthetic drug, can give long-lasting relief in Neuropathic pain states. It probably acts by blocking spontaneous discharge from damaged sensory nerve terminals.

**Treatment of chronic pain**

**Antidepressant medications**

The tricyclic antidepressants are extremely useful for the management of patients with chronic pain (Mcquay *et al*., 1996). The analgesic effect of tricyclics has a more rapid onset of action and occurs at a lower dose than is typically required for treatment of depression. Furthermore, patients with chronic pain, who are not depressed, obtain pain relief with antidepressants. There is evidence that tricyclic drugs potentiate opioid analgesia and are considered as useful adjuncts for the treatment of severe persistent pain such as occurs with malignant tumors (Luissier *et al*., 2004). Tricyclics are of particular value in the management of Neuropathic pains such as painful diabetic neuropathy and post herpetic neuralgia.

**Anticonvulsants and antiarrhythmics**

These drugs are useful primarily for patients with Neuropathic pain. Phenytoin and carbamazepine were first shown to relieve the pain of
trigeminal neuralgia. This pain has a characteristic brief shooting, electric shock like quality (Mcquay et al., 1995).

Anti arrhythmic drugs such as low dose lidocaine and mexiletine seem to be effective for pains that respond to anticonvulsants, as well as other conditions, including post operative and burn pain. These drugs block the spontaneous activity of primary afferent nociceptors when they are damaged. They are considered for use in patients with pain associated with damage to peripheral nerves (Oskarsson et al., 1997).

**Adverse effects to analgesic drugs**

The availability of a wide range of analgesic drugs enables the physician to choose an effective drug to suit the requirements of the patient. However no analgesic drug is free from adverse effects whether employed for acute pain or chronic pain syndromes. A brief consideration of the adverse effects to different classes of analgesics is presented here.

**Adverse effects of NSAID therapy**

**Effects on gastrointestinal tract**

The most common symptoms associated with these drugs are gastrointestinal, including, anorexia, nausea, dyspepsia, abdominal pain and diarrhea (Hawkey., 2001). These symptoms may be related to the induction of gastric or intestinal ulcers. 15-30% of NSAID users suffer from these symptoms. Ulceration may range from small superficial erosions to full
thickness perforation of the muscularis mucosa. There may be single or multiple ulcers and ulceration may be accompanied by gradual blood loss leading to anemia or life threatening hemorrhage (Burke et al., 2006). The concurrent use of corticosteroids, heavy alcohol consumption or *H. pylori* infection further aggravates the above risk.

The selective COX -2 inhibitors have been shown to be less prone than fully efficacious doses of other NSAID to induce endoscopically visualized gastric ulcers (Deeks et al., 2002).

Gastric damage by NSAID can be brought about by two distinct mechanisms. Inhibition of COX-1 in gastric epithelial cells depresses mucosal cytoprotective prostaglandins, especially PGI\(_2\) and PGE\(_2\). These eicosanoids inhibit acid secretion by the stomach, enhance mucosal blood flow and promote the secretion of cytoprotective mucus.

Another mechanism by which NSAID may cause ulceration is by local irritation from contact of orally administered drug with the gastric mucosa. Local irritation allows back diffusion of acid into the gastric mucosa and induces tissue damage. It is also possible that enhanced generation of lipooxygenase products (e.g. Leucotrienes) contributes to ulcerogenecity in patients treated with NSAID.
Renovascular complications

NSAID use may result in salt and water retention, edema and worsening of renal function in renal/cardiac and cirrhotic patients. Regular NSAID use may also decrease the effectiveness of antihypertensive medications and diuretic drugs. Many NSAID especially aspirin may reduce uric acid excretion and may also produce hyperkalemia.

CNS effects

Headache, vertigo, dizziness, confusion and lowering of seizure threshold are some of the CNS adverse effects of NSAID.

Cardiovascular effects

The inhibitory effect of NSAID on platelet function increases the risk of hemorrhage. Even though COX-2 inhibitors do not share this effect, the cardiovascular side effect of coxibs acquires another dimension. The selective inhibitors of COX-2 depress PGI\textsubscript{2} formation by endothelial cells without inhibiting platelet thromboxane. Selective inhibition of PGI\textsubscript{2} may increases the risk of thrombosis. There is increased incidence of myocardial infarction and stroke in patients treated with rofecoxib (Bresalier et al., 2005), valdecoxib (Nussmeier et al., 2005) and celecoxib (Solomon et al., 2005).

Patients with increased risk of cardiovascular disease or thrombosis are particularly prone to cardiovascular adverse events of COX-2 inhibitors (Burke et al., 2006).
Pregnancy and lactation

The use of NSAID to terminate pre-term labour has been associated with closure of ductus arteriosus and impaired fetal circulation. NSAID may delay labour and increase the risk of postpartum hemorrhage.

Hypersensitivity reactions

Many hypersensitivity reactions may be exhibited by individuals treated with NSAID. Vasomotor rhinitis with profuse watery secretions, angioedema, urticaria, asthma, flushing and hypotension and shock may be encountered with NSAID use. The hypersensitivity reactions may occur in 10-25% of patients with asthma, nasal polyps or chronic urticaria.

Adverse effects of opioid analgesics

Morphine and related opioids produce a wide spectrum of unwanted effects including respiratory depression, nausea, vomiting, dizziness, mental clouding, dysphoria, pruritis, constipation, increased pressure in the biliary tract, urinary retention and hypotension.

Tolerance and dependence

Tolerance to opioids develops rapidly and is readily demonstrated. Dependence comprises of physical and psychological dependence. Morphine produces tolerance and dependence (Ballantyne and Laforge., 2007). Tolerance develops rapidly accompanied by physical withdrawal syndrome.
Tolerance extends to most of the pharmacological effects of morphine, including analgesia, emesis, euphoria, and respiratory depression.

Physical dependence is characterized by a clear cut abstinence syndrome, fever, sweating, piloerection, nausea, diarrhoea and isomnia. Extreme restlessness and distress are accompanied by a strong craving for the drug.

**Adverse effects of antiarrythmic drugs**

The adverse effects of lidocaine are mainly manifestations of actions on the central nervous system and include drowsiness, disorientation and convulsions (Roden et al., 1994).

**Adverse effects of tricyclic antidepressants**

Important side effects are sedation, (H1 block) postural hypotension, (α- adrenoceptor block) dry mouth, blurred vision, constipation (muscarinic block), occasionally mania and convulsions, impotence, risk of ventricular dysrhythmias and interaction with other CNS depressants.

**Adverse effects of Anti convulsants**

Side effects of phenytoin are ataxia, vertigo, gum hypertrophy, hirsutism, megaloblastic anaemia, fetal malformation and hypersensitivity reactions. The important side effects of carbamazepine are sedation, blurred vision, water retention and hypersensitive reactions.
INFLAMMATION

Inflammation may be defined as the local tissue response due to any injurious stimulus. It is a kind of defense mechanism of the body to eliminate or limit the spread of injurious substance and may also proceed to remove the consequent necrosed cells or tissue (Harsh Mohan., 2006). A wide variety of noxious agents may be responsible for inflammation, which includes

- Physical noxious stimuli like heat, cold, radiation or mechanical trauma.
- Chemical agents such as acid or alkali or any other micro organism.
- Infective agents and toxins released from bacteria, virus or any other micro organism.
- Endogenously released immunological agents due to antigen antibody reaction or specific cell types.

The inflammatory response is essential for survival to overcome environmental pathogens and injury. However in certain situations and diseases the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse complications (Burke et al., 2006).

Phases of inflammatory reaction

Irrespective of the nature of initial stimulus, the classic inflammatory response includes calor (warmth), rubor (redness), tumor (swelling) and dolor
Different mechanisms are likely to mediate three distinct temporal phases of the inflammatory response.

- The acute phase is characterized by transient local vasodilatation and increased capillary permeability.
- A delayed sub acute phase in which there is infiltration of leucocytes and phagocytes cells.
- A chronic proliferative phase, in which there may be tissue degeneration and subsequent fibrosis.

For an easy understanding, inflammatory response may be considered as acute and chronic based on certain distinct features.

**Acute inflammation**

Acute inflammation is generally of short duration representing the early body reaction which is usually followed by a quick tissue repair (Gaybay and Kushner., 1999).

**Chronic inflammation**

The inflammatory process is not characterised by the classical signs of acute inflammation. Instead chronically inflamed tissue is characterised by infiltration of mononuclear immune cells, tissue destruction and attempts at healing which include angiogenesis and fibrosis.
Many mechanisms are involved in the promotion and resolution of the inflammatory response. The process of vasodilatation, chemotaxis, cell adhesion, migration and other proliferative reactions involve the role of several substances collectively known as the mediators of inflammation (Sehran and Chirang., 2004; Kyriakis and Avuruch., 2001).

**Chemical mediators of inflammation**

Many endogenous chemical mediators play a significant role in mediating various reactions of the inflammatory process like vascular permeability, vasodilation, chemotaxis, cellular migration and tissue damage (Dale., 1994). These mediators may originate from different types of cells, plasma, or from the damaged tissue itself. They may be considered in two groups: a) Mediators released by cells and b) Mediators originating from plasma.

**Cell derived chemical mediators: (Dale., 1994)**

(i) Vaso active amines (Histamine, 5-Hydroxy tryptamine).

(ii) Arachidonic acid metabolites through cyclooxygenase pathway (prostaglandins, thromboxane A2 and prostacyclin) and metabolites through lipoxygenase pathway (HETE, Leukotrienes).

(iii) Lysosomal components.

(iv) Platelet activating factor.

(v) Cytokines (Interleukins, Tumor necrosis factor)

(vi) Nitric oxide and many types of free radicals.
Plasma derived mediators

These are products of the kinin system, the coagulation system, the fibrinolytic system and compliment system. The components of these cascades are proteases that are inactive in their native form; are active by proteolytic cleavage, each activated component then activating the next (Gaybay and Kushner., 1999)

Biological effects of various mediators of inflammation

Histamine

Histamine is a basic amine present in many tissues of the body particularly in lungs, skin and G.I.T. Mast cells and basophils are rich source of histamine, where it is stored in granules as a complex with an acidic protein and heparin. Histamine is released from mast cells by exocytosis during inflammatory or allergic reaction. Histamine produces many effects by acting on H₁, H₂, or H₃ receptors on target cells. The triple response after an intradermal injection of histamine reveals as a reddening of the skin, a wheal and a flare in surrounding area. The reddening results from vasodilatation of small arterioles and pre capillary sphincters and the wheal is caused by the increased permeability of post capillary venules. These effects are mainly mediated through activation of H₁ receptors .The flare is an axon reflex that involves activation of sensory nerves which in turn may release a peptide vasodialator (Rang et al ..,2003 ).
5 - Hydroxy tryptamine (5-HT)

5- HT is present in high concentration in platelets and is released when platelets aggregate at sites of tissue damage. 5-HT is considered to play a role in vasodilatation, sensitization of nociceptors and as an inflammatory mediator.

Arachidonic acid metabolites

The eicosanoids are not preformed and stored in tissues, but are generated \textit{de novo} from phospholipids. The eicosanoids are important mediators of various reactions in the inflammatory process. The arachidonic acid released from tissue phospholipids may be acted upon by two forms of cyclooxygenase (COX); COX-1, a constitutive enzyme and COX-2, which is induced in inflammatory cells by inflammatory stimuli. Arachidonic acid may be alternatively metabolised by 5-lipoxygenase that can generate, several types of Leukotrienes. PGE$_2$, PGD$_2$ and prostacyclin (PGI$_2$) are powerful vasodilators. In areas of acute inflammation, the vasodilator eicosanoids PGE$_2$ and PGI$_2$ are generated by the local tissue and blood vessels and PGD$_2$ is released from mast cells. They act in combination with other mediators to dilate the precapillary arterioles and increase blood flow in areas of acute inflammation. Prostaglandins also sensitize afferent C fibres to the action of bradykinin for initiating the pain. Prostaglandins of E series are also implicated in the production of fever.
Leukotrienes are the arachidonic acid metabolites derived by lipoxygenase pathway. Leukotriene $\text{B}_4$ (LTB$_4$) is an important mediator in all types of inflammation. LTB$_4$ may cause adherence chemotaxis and activation of polymorphs and monocytes. LTB$_4$ also stimulates proliferation and cytokine production from macrophages and lymphocytes. Leukotrienes are present in the tissues in many inflammatory diseases, like rheumatoid arthritis, psoriasis, ulcerative colitis and also in bronchial asthma.

**Platelet activating factor (PAF)**

PAF is believed to be an important mediator in many allergic and inflammatory reactions. PAF is generated and released from most inflammatory cells and also from platelets. PAF is released by the action of the enzyme phospholipase A$_2$ on tissue phospholipids. PAF causes vasodilation, increases vascular permeability, chemotaxis, activation of leucocytes and activation and aggregation of platelets. It is spasmogenic for smooth muscles and mainly implicated in bronchial asthma (Rang *et al.*, 2003).

**Cytokines**

Cytokines are mainly peptide mediators released by cells of the immune system. Important cytokines belong to the family of interleukins, chemokines, interferons, colony stimulating factors, growth factors and tumor necrosis factors. Cytokines have both pro inflammatory and anti inflammatory properties (Janeway *et al.*, 2004).
Pro inflammatory cytokines

The important pro inflammatory cytokines are Tumor necrosis factor alpha (TNF- \( \alpha \)) and interleukin -1 (IL-1) and IL-6. These are released from macrophages and many other types of cells and evoke the synthesis of various chemokines. Cytokine growth factors like platelet derived growthfactor, fibroblast growthfactor are important in tissue repair process.

Anti inflammatory Cytokines

Cytokines like tissue growth factor (TGF), IL-4, IL-10 and IL-13 can inhibit the production of chemokines and thus exert an inhibitory effect on inflammation.

Chemokines

These peptides control the migration of leukocytes in immune and inflammatory reactions.

Bradykinin

Bradykinin and kallidin are vaso active peptides derived from kininogens. Bradykinin causes vasodilatation and increased vascular permeability. It is also very effective in stimulating nociceptors.
Nitric oxide (NO)

The actions of nitric oxide on inflammation are complex in nature. Many cytokines stimulate the expression of inducible form of nitric oxide synthase (iNOS) by all inflammatory cells. NOS is also present in the bronchial epithelium (asthma subjects), in colon (ulcerative colitis) and in synovium (arthritis). Nitric oxide has pro inflammatory actions. It is a potent vasodilator, increases vascular permeability and increases the synthesis of pro inflammatory prostaglandins. Some actions of nitric oxide are anti inflammatory in nature. The nitric oxide released from endothelial cells inhibits adhesion of neutrophils and platelets and platelet aggregation.

Free radicals

Biologically derived free radicals also play a role in tissue injury and inflammatory response. These include superoxide anion, singlet oxygen, hydroxyl radical, hydrogen peroxide, peroxynitrite and hypochlorous acid. These oxidants are generated by phagocytic cells like neutrophils and macrophages and cause severe tissue damage (Woodfork and Dyke., 2004).

Steroidal and Nonsteroidal antiinflammatory drugs

Inappropriate and aberrant inflammatory or immune reactions are the main causes of several diseases and the scientific community is constantly exploring new avenues for the discovery of safe and effective anti inflammatory agents. Glucocorticoids and the Nonsteroidal anti inflammatory
agents are the major classes of drugs used for this purpose. A brief sketch about these agents is felt appropriate here.

**Corticosteroids**

The corticosteroids secreted by adrenal cortex are glucocorticoids with primary effect on carbohydrate metabolism and mineralocorticoids with a primary role in regulating electrolyte balance. The physiological functions of corticosteroids involve almost all systems of the body and hence the effect of corticosteroids use in pharmacological doses may also have repercussions almost in all body functions. Among the various properties of glucocorticoids, their important action on inflammation and immune mechanism signifies them as ideal candidates in the treatment of chronic inflammatory diseases and for immunosuppression. The immunosuppressive and anti-inflammatory actions of glucocorticoids are linked inseparably because both the above actions involve inhibition of leukocyte function (Chrouses., 1995).

Even though glucocorticoids do not treat the underlying cause of the disease, the anti-inflammatory activity of these drugs has enormous utility in many clinical situations. Glucocorticoids can prevent or suppress inflammation in response to a variety of provoking factors including radiant, mechanical, chemical, infectious and immunological stimulants. Multiple mechanisms are involved in the suppression of inflammation by glucocorticoids (Schimmer and Parker., 2006).
Almost all stages of inflammation are inhibited by corticosteroids. They decrease the release of vasoactive and chemo attractive factors, diminish the secretion of lipolytic and proteolytic enzymes, decrease extravasations of leucocytes to areas of injury and finally decrease fibrosis.

- The release of arachidonic acid and its metabolites is prevented by an inhibitory effect of glucocorticoids on phospholipaseA$_2$. These drugs are believed to stimulate the production of lipocortin, an inhibitor of phospholipase A$_2$.
- The production and release of cytokines like, IL-1, IL-6, and TNF-α from macrophages and monocytes is inhibited by these compounds.
- The release of endothelial leukocyte adhesion molecule-1 and intracellular adhesion molecule-1, cytokines and acute phase reactants from endothelial cells is prevented by glucocorticoids.
- The IgE dependent release of histamine and leukotrienes from basophils is blocked by glucocorticoids.
- Glucocorticoids not only prevent the release of arachidonic acid metabolites from fibroblasts but also suppress growth factor induced DNA synthesis and fibroblast proliferation.
- Similar to their action on macrophages and monocytes, glucocorticoids inhibit also the synthesis and release of cytokinins from lymphocytes.
(IL-1, IL-2, IL-3, IL-6, TNF-α, granulocyte/monocyte colony stimulating factor (GM-CSF1) and interferon gamma)

Cortisol (hydrocortisone), dexamethasone, prednisone, prednisolone, methyl prednisolone and triamcinolone are the important corticosteroids available in different dosage forms. Beclomethasone, budesonide, flunesolidone and triamcinolone are available as inhalation to be used in bronchial asthma.

Therapeutic uses

A. Substitution therapy:

1. **Acute adrenal insufficiency**: This life threatening emergency requires immediate management. Therapy includes large amounts of parenteral (I.V) Cortisol, correction of fluid and electrolyte imbalance and treatment of precipitating factors.

2. **Chronic adrenal insufficiency**: Congenital adrenal hyperplasia

3. **Therapeutic uses in non endocrine diseases**

Rheumatoid Arthritis

Glucocorticoids are used in rheumatoid arthritis mainly in patients with progressive diseases who fail to respond to NSAID or who are unable to tolerate the side effects of other disease modifying drugs. In patients with major symptomatology confined to one or a few joints intra articular steroid
injections is considered. 5 to 20 mg of triamcinolone injected can control acute inflammation of a specific joint without causing systemic side effects.

**Renal diseases**

Patients with nephrotic syndrome due to systemic lupus erythmatosus or primary renal disease may be benefited with corticosteroid therapy.

**Allergic diseases**

Glucocorticoids suppress the allergic manifestations in hay fever, serum sickness, urticaria, contact dermatitis, drug reactions, bee stings and angioneurotic edema etc which are of limited duration. In life threatening situations dexamethasone sodium phosphate is administered intravenously.

**Bronchial disease**

Corticosteroids are often used in bronchial asthma and in chronic obstructive pulmonary disease (COPD). Parenteral glucocorticoids are given during severe asthma attacks. Inhaled steroids can decrease the need for oral corticosteroids or replace them entirely. In the treatment of children with mild asthma, glucocorticoids can be administered as inhalation than oral corticosteroids.
**Infectious diseases**

Glucocorticoids in combination with an antibiotic is used to treat AIDS patients with *Pneumocystis carinii pneumonia*. Glucocorticoids increase oxygenation and lower the incidence of respiratory failure. Glucocorticoids decrease the incidence of long-term neurological impairment associated with H. influenza type B meningitis in infants.

**Ocular diseases**

Corticosteroids find their frequent use in a number of inflammatory and allergic conditions of eye. For the disease of the outer eye and anterior chamber they are instilled locally as eye drops, applied as ointments and sometimes administered as injections locally. However diseases of the posterior segment require their systemic administration.

**Skin diseases**

Corticosteroids as topical preparations are highly effective in the treatment of a wide variety of skin diseases. Atopic dermatitis (eczematous skin disease), dermatoses, lichen simple chronicus, seborrheic dermatitis, mycosis fungoides and pemphigus. Topical preparations vary in their concentration. They are also used systemically for severe episodes of acute skin disorders.
**Gastro intestinal diseases**

Corticosteroids are helpful in the treatment of chronic inflammatory bowel disease, ulcerative colitis, and Crohn’s diseases. Hydrocortisone (100mg) can be administered as a retention enema for treating mild ulcerative colitis. In severe acute ulcer conditions oral prednisone (10-30mg/kg/day) can be administered as a retention enema. For severely suffering patients with fever, anorexia, anemia and impaired nutritional status large doses should be used (40 to 60 mg prednisone per day).

**Malignancies**

Because of the anti lymphocytic effects, glucocorticoids are used in the chemotherapy of acute lymphatic leukemia, Hodgkin’s and other lymphomas. Prednisone is the commonly used corticosteroid.

**Cerebral edema**

Corticosteroids have been found to be useful in the reduction or prevention of cerebral edema associated with neoplasm, especially the metastatic ones.

**Miscellaneous diseases and conditions**

**Sarcoidosis**

Sarcoidosis is treated with corticosteroids (1mg/day of prednisolone) to induce remission.
Thrombocytopenia

In thrombocytopenia prednisolone (0.5mg/kg) is used to decrease the bleeding tendency. For severe cases daily doses of prednisone (1 to 1.5 mg/kg) are employed.

Auto immune destruction of erythrocytes

Patients with auto immune destruction of erythrocytes are treated with prednisone (1mg/kg per day).

For treating severe hemolysis higher doses may be used. Small maintenance doses may be required for several months in patients who respond.

Organ transplantation

During organ transplantation higher doses of prednisone (50 to 100mg) are given along with other immunosuppressive agents.

Adverse effects of corticosteroids

Adverse effects resulting from withdrawal therapy

Sudden withdrawal of corticosteroids after prolonged therapy may result in acute adrenal insufficiency through suppression of the patient’s capacity to synthesize corticosteroids. Glucocorticoids withdrawal syndrome consists of fever, myalgia, arthralgia and malaise.
Adverse effects resulting from continued use of glucocorticoids

Effect on fluid and electrolytes

Continuous use of corticosteroids can cause hyperkalemia, alkalosis, edema and hypertension, particularly in patients with primary hyperaldosteronism.

Effect on metabolic actions

Glucocorticoids may cause hyperglycemia.

Immune responses

Prolonged administration of glucocorticoids inhibits immune system and the inflammatory response and increase the susceptibility to infection.

Effect on G.I.T

Peptic ulceration risk will be increased. Bleeding and silent perforation of ulcers may occur. Dyspeptic symptoms may be frequent with high dose corticosteroid therapy either alone or when given along with NSAID.

Myopathy

Myopathy is seen in patients receiving long term steroid therapy. Myopathy is characterised by weakness of proximal muscles of arms, legs, shoulders and pelvis.
Behavioral changes

Mild euphoria frequently accompanies high dose steroid treatment. Nervousness, decreased sleep and mood changes are noted by few. Rarely a depressive illness may be precipitated.

Eye

Glaucoma may develop in susceptible individuals after prolonged topical therapy. Ocular complications include cataract, particularly in children with bronchial asthma who are receiving long term steroid therapy. Increased intraocular pressure leading to inducement of glaucoma is also seen.

Osteoporosis

Compression fractures of vertebrae and spontaneous fractures of long bones can occur, especially in elders. Osteoporosis occurs in patients of all ages who receive long term glucocorticoids therapy. It affects trabecular bone and cortical rim of the vertebral bodies and ribs.

Osteonecrosis

The necrosis of bone of femoral head is seen with long time therapy. In children, the metabolic effects may result in inhibition of growth, even with low doses. When the drugs are used in anti inflammatory and immuno suppressive therapy, the metabolic actions and the effects on water and
electrolytes balance and organ systems are side effects and Cushing syndrome may occur (Saag., 2003)

**Nonsteroidal antiinflammatory drugs (NSAID)**

Of all the therapeutic agents in the world, NSAID are the most widely used compounds. They are primarily used for their anti inflammatory, analgesic and antipyretic actions. All these effects are observed due to the primary effect of these compounds in inhibiting prostaglandin synthesis. The first enzyme in the prostaglandin synthetic pathway is known as cyclooxygenase or COX. There is good correlation between the potency of these drugs as COX inhibitors and their anti-inflammatory activity. There are two forms of cyclooxygenase enzyme, COX-1 and COX-2. COX-1 is primarily a constitutive isoform found in most normal cells and tissues and has a primary effect in maintaining tissue homeostasis. COX-2 is an inducible form of cyclooxygenase, which is released from inflammatory cells for activation by cytokines and other inflammatory mediators. Thus COX-2 may be considered responsible for the synthesis of prostanoid mediators of inflammation (Vane and Botting., 1998)

Most NSAID inhibit both COX-1 and COX-2 with little selectivity. It is believed, that the antiinflammatory effect of NSAID is mainly related to their inhibition of COX-2 while their unwanted effects in particular gastro intestinal irritation are due to the inhibitory effect on COX-1. Based on this concept selective COX-2 inhibitors have been developed with markedly less gastric irritation potential. Aspirin and other NSAID inhibit the COX enzymes and
prostaglandin production. These compounds do not inhibit the lipoxygenase pathways of arachidonic acid metabolism and hence do not suppress leukotrienes production.

Even though all NSAID inhibit prostaglandin synthesis, there are minor but important differences among them in the above action. Aspirin covalently modifies COX-1 and COX-2, irreversibly inhibiting cyclooxygenase (COX) activity. This irreversible inhibition has a major implication in inhibiting thromboxane A2 production in platelets which lasts for the lifetime of the platelet (8-12 days). Platelets being devoid of nucleus may not generate COX-1 after exposure to aspirin. Majority of NSAID are organic acids which act as reversible competitive inhibitors of cyclooxygenase activity.

Apart from the inhibition of cyclooxygenase some NSAID possess other properties that may also contribute to their anti inflammatory effect. At higher concentrations NSAID are known to reduce production of super oxide radicals, decrease nitric oxide synthase, decrease pro inflammatory cytokines (TNF-α and IL-1), modify lymphocyte activity, alter cellular membrane compounds and inhibit the expression of adhesion molecules (Burke et al., 2006).

**Therapeutic uses of NSAID**

The inhibition of cyclooxygenase in inflammatory cells and at other sites has endowed them with significant antiinflammatory analgesic and anti pyretic effects. NSAID are mainly used against pain of low to moderate
intensity. Their maximal efficacy is generally much less than the opioids. Pain
due to inflammation and post operative pain are very well controlled by NSAID
while the pain arising from the hollow viscera (except menstrual pain) is not
usually amenable to NSAID treatment. Paracetamol remains the main choice
among the NSAID to control fever of any origin. The important clinical
application of NSAID is their use in the treatment of musculoskeletal
disorders such as rheumatoid arthritis and osteo arthritis. NSAID provide only
symptomatic relief from pain and inflammation in the above conditions and
may not arrest the progression of pathological injury to tissues. Many types of
mild arthropathies, ankylosis  spondylosis and gout are treated by any one of
the NSAID.

The property of COX inhibition by NSAID has made them useful in
other condition like closure of patent ductus in neonates, systemic
mastocytosis and Barter’s syndrome. Recent reports indicate the potential
use of aspirin or other NSAID in cancer prevention (Burke et al., 2006)
**FLAVONOIDS**

Flavonoids, the derivatives of benzopyrone are widespread in photosynthesizing plants. More than 4000 flavonoids have been reported since 1980 (Harborne *et al.*, 1988).

**Chemistry of Flavonoids**

The flavonoids are an important group of polyphenolic compounds derived from nature. The basic skeleton of flavonoid is the γ–pyrone ring occurring as phenyl benzopyrone (Flavone). Various substitutions, mainly phenolic OH groups, take place in 3, 5, 7, 3‘ and 4‘ positions of the flavone nucleus. Flavonoids are generally found in nature as glycosides with sugar moiety attached to the 3rd or 7th position. Depending upon the position of OH groups, saturation of OH groups and of the pyrone nucleus, flavonoids are classified into various sub groups, viz; isoflavones, flavonol, flavone, flavonones, flavans and chalcones (Fig - 4) (Harborne *et al.*, 1975).

**Isoflavones**

The isoflavones have their phenyl group (B ring) attached to the 3rd position of the benzopyrone nucleus. Isoflavones have been shown to possess potential estrogenic actions.
Figure 4

Basic Structure of various flavonoids

Flavone

Isoflavone

Flavonol

Flavanone

Flavan

Chalcone
**Flavonol**

Flavonols have a OH group in the 3rd position of the ring. Gossypin and rutin are important members of this group.

**Flavanones**

Flavanones result from the saturation of the double bond in the 2-3 position of the benzopyrone nucleus.

**Flavans**

Reduction of the carbonyl group of the pyrone ring and subsequent saturation of this ring gives flavans; catechin and epicatechin are standing examples of this group.

**Chalcones**

Higher pH results in the opening of the pyrone ring in the 1-2 position of flavanone. Hesperidin methyl chalcone is the best known example of this class.

**Natural occurrence of Flavonoids**

Flavonoids are present in vascular plants (Harborne and Simmonds., 1964), but more rare in bryophytes. Some flavonoid classes have a restricted distribution e.g. Isoflavonoids occur predominantly in Fabaceae family. Flavonoids are present in different plant organs, such as leaves,
stems, flowers, fruits, and within a plant, the flavonoid glycosides are found in the cell vacuole, while aglycones are located in the leaf outer wall in leaf waxes and plant exudates.

**Natural occurrence of Flavonoids**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>Plant source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-hydroxy flavone</td>
<td>2-OH</td>
<td><em>Primula and Diosmysia</em> spp</td>
</tr>
<tr>
<td>5-hydroxy flavone</td>
<td>5-OH</td>
<td><em>Primula imperialis</em> and other spp.</td>
</tr>
<tr>
<td>5,7–dihydroxy flavone</td>
<td>5, 7-(OH)$_2$</td>
<td><em>Populus</em> spp (Chrysin)</td>
</tr>
<tr>
<td>5, 8–dihydroxy flavone</td>
<td>5, 8-(OH)$_2$</td>
<td><em>Primula modestia</em> and other spp (Primetin)</td>
</tr>
<tr>
<td>Apigenin</td>
<td>5, 7, 4′-(OH)$_3$</td>
<td><em>Antirhinum</em> spp, <em>Scrophularaceae</em> and other spp</td>
</tr>
<tr>
<td>Bicalein</td>
<td>5, 6,7-(OH)$_3$</td>
<td><em>Scutellaria</em> spp, <em>Oroxyxylum indicum</em>, <em>Plantago major</em></td>
</tr>
<tr>
<td>Galangin</td>
<td>3, 5, 7-(OH)$_3$</td>
<td><em>Alpinia officinarum</em></td>
</tr>
<tr>
<td>Luteolin</td>
<td>5, 3, 3′, 4′-(OH)$_4$</td>
<td><em>Roseda luteola</em></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>3, 5, 7, 4′ - (OH)$_4$</td>
<td>Widespread</td>
</tr>
<tr>
<td>Gossypetin</td>
<td>3, 5, 7, 8, 3′, 4′-(OH)$_6$</td>
<td>Widespread</td>
</tr>
</tbody>
</table>
Biological significance of flavonoids

The polyphenolic flavonoids and anthocyanins are present in various parts of the plant. These secondary metabolites have been ascribed with many important functions in plants. The different hues and colours resulting from a combination of several polyphenolics are considered important for pollination of flowers. Many flavonoids possess antiviral activity and also protect plants from predators. Regular consumption of these natural compounds by humans obviously has many nutritional and possibly therapeutic implications. The biological effects of flavonoids are appreciated in recent times and many amazing facts are reported.

Capillary integrity

Rusznyak and Szent Gyorgi (1936) carried out the pioneering work in guinea pigs and reported that, crude preparations of vitamin-C containing flavonoids could improve the capillary resistance in scurvy animals more effectively than pure vitamin C. This observation was later confirmed by Rusznyak and Benko (1941). Further studies conducted in rats revealed an unequivocal effect of flavonoids in maintaining capillary integrity in these species (Benko et al., 1970, Varkonyi et al., 1971). Hesperidine methyl chalcone increased the capillary resistance of small intestine, large intestine and kidney of guinea pigs kept on a scurvy diet (Gabor et al., 1968)
Anti inflammatory effect

Among the various actions of flavonoids, the anti inflammatory action has been extensively studied by several workers (Rinehart., 1955; Gupta et al., 1971; Gabor, 1986 and Parmar and Ghosh., 1978). Flavonoids have been found to exert a beneficial effect in rheumatoid arthritis (Rinehart, 1955) and also in gingival inflammatory conditions (Carvel and Halperin, 1961). Experimental evidence for the anti inflammatory effect of taxifolin (Gupta et al., 1971) and gossypin (Parmar and Ghosh., 1978) have been provided. Gossypin significantly reduced the paw edema and was also effective against adjuvant and formalin induced arthritis in rats (Parmar and Ghosh., 1978)

Flavone derivatives such as apigenin and chrysin showed higher activity only in acute inflammation whereas luteolin, bicalein, spinosin, santin, ermanin, nepitrin and wogonin exhibited higher level of activity in acute as well as chronic inflammatory models (Lee et al., 2004, Martinez et al.,1997 and Agarwal.,1982).

Muthiah et al., (1993) reported the anti inflammatory effect of several methoxy flavones. In a study by Arivudai Nambi et al., (1996) on hydroxy flavones, they suggested that, hydroxylation favoured anti inflammatory activity more than methoxylation.

Flavonol glycosides showed similar activity as exhibited by their aglycones when given orally. Flavonone glycosides like naringin and
hesperidins when administered intraperitoneally showed good response in both acute and chronic inflammatory models (Perriera et al., 2007).

A flavone titonine and its methoxy and acetyl derivatives (7,3′4,-trimethoxy flavone and 7, 3 – dimethyl 4 – acetyl flavone) possess different degree of anti inflammatory potential (Carvalho et al., 1999). They suggested that the relative potencies of flavone / flavonol and isoflavone are dependent on the pattern and number of hydroxylation / methoxylation on A/B ring, similar to a report on the structure-activity study by Thirugnanasambantham et al., (1990, 1993) on the antinociceptive effect of flavonoids.

Significant anti inflammatory and anti arthritic activities of silymarin, a mixture of flavano lignans, was also reported in the papaya latex induced model of inflammation and mycobacterial adjuvant arthritis in rats. Gupta et al., (2000) suggested a role for inhibition of 5- Lipoxygenase in the anti inflammatory and anti arthritic activities of silymarin.

A number of other flavonoids also are reported to possess anti inflammatory activity. Hesperidin, apigenin, luteolin and quercetin have been reported to exhibit significant anti inflammatory activity (Alcaraz et al., 1987).

**Mechanisms involved in the anti inflammatory action of flavonoids.**

Flavonoids may produce their anti inflammatory effect by a multitude of ways to inhibit the inflammatory process. Formation and release of various mediators of inflammation like histamine and prostaglandin are affected by
flavonoids (Lorenze et al., 1973; Fewtrell and Gomperts., 1977a; Baumann and Bruchhausen., 1979)

Flavonoids inhibit the increased capillary permeability during inflammation (Parmar and Ghosh. 1978). The adhesion of leucocytes to endothelial surface and subsequent migration is influenced by flavonoids like HR (Pearson et al., 1979).

Inhibition of cyclooxygenase

Phospholipid metabolism is catalyzed by enzymes such as phospholipase A\(_2\) (PLA\(_2\)), cyclo oxygenase (COX), and lipoxygenase (LO) that lead to the production of inflammatory mediators such as prostaglandins (PGs) and Leukotrienes (LTs). Inhibition of these enzymes therefore remains an attractive target for development of anti inflammatory agents (Payan et al., 1995). Inhibition of prostaglandin E\(_2\) and leukotriene C\(_4\) in mouse peritoneal macrophages and thromboxane B\(_2\) production in human platelets by flavonoids from Stachys chrysanta and Stachys Candida was studied by Kaltsa et al., (2002).

5, 7 - di hydroxy 7 - methoxy flavone was reported to have moderate inhibitory activity of prostaglandins (Daott et al., 2003).

Harris et al., 2006 reported that luteolin and chrysin suppressed PGE\(_2\) formation equally well, despite differential effects on COX-2 protein expression and on superoxide and hydroxyl radical scavenging. These data
indicate that flavones may display similar anti inflammatory activity via different mechanisms.

Cheng et al., 2004 have evaluated 2',4',7'-trimethoxy flavone for its inhibitory activity of PGE$_2$ production from LPS treated mouse macrophage RAW 264.7 cell line.

Wogonin (5,7-dihydroxy 8-methoxy flavone) was found to suppress pro inflammatory enzymes including COX-2 (Park et al., 2001 and Chi et al., 2005).

Among flavonols, kaempferol, quercetin, myricitrin and 6-c-methyl quercetin 3, 7, 3' trimethyl ether were reported to inhibit COX-1 catalyzed PG biosynthesis in vitro.

Prenylated flavonoids such as brousso chalcones A, and cycloheterophylline were reported to inhibit COX. Promising COX-2 inhibitors from prenylated flavonoids have also been identified (Kim et al., 2005, Jang et al., 2002).

**Inhibition of lipoxygenase (LO)**

Several flavonoids were investigated on various in vitro assays for LO inhibitory activity. Among them, flavonol, (3-hydroxy flavone), fisetin, quercetin and kaempferol possessed 5-LO inhibitory activity whereas oroxylin - A and wogonin showed 12 - LO inhibition in vitro assay (Laughton et al., 1991, You et al., 1999). Luteolin, baicalein and cirsilol showed both 5-LO and 12- LO
inhibition (Yoshimoto et al., 1983, Yamamoto et al., 1998). From the above reports it is clear that 5, 6, 7 or 5, 7, 8 tri substitution of A ring of flavone favors 12 - LO inhibition and hydroxyl substituent at 4’ favours 5 - LO inhibitory effects. Gupta et al., (2000) suggested a role for inhibition of 5- lipoxygenase in the anti inflammatory and antiarthritic activities of silymarin.

Crisilol, a flavone when derivatized by introducing alkyl groups of 5-10 carbons at 5 or 6 position of A ring markedly decreased IC$_{50}$ values for 5-lipoxygenase inhibition. From the recent Structure activity relationship studies of flavonoids inhibiting rabbit reticulocyte 15-LO-1, it is evident that in parallel to the free radical scavenging properties, presence of a catechol arrangement in the B or A ring and a carbonyl group with C-2,3 double bond in C ring favours LO inhibition (Sadik et al., 2003).

Flavonoids such as hesperidin, naringin and quercitin when pre-incubated with H$_2$O$_2$ treated endothelial cells, demonstrated strong inhibition of acetyl transferase (AT) activity (Balestrier et al., 2003). AT is known to participate in platelet activating factor (PAF) biosynthesis, a potent inflammatory lipid.

**Inhibition of cytokines**

Flavonoids inhibit cytokine release from RAW 264.7 Cells (Xagorari et al., 2002) and may modulate the increasing number of cellular processes involving redox titration including the regulation of tyrosine phosphatase activity (Gamet -payarastre et al., 1999).
Inhibition of free radicals

Several types or reactive species are generated in the body as a result of metabolic reactions in the form of free radicals or non radicals. These species may be either oxygen derived or nitrogen derived and called pro oxidants. They attack macromolecules including proteins, DNA and lipid etc, causing cellular/tissue damage.

Flavonoids have been found to inhibit a wide range of enzymes involved in oxidation systems such as 5-lipoxygenase, cyclooxygenase, mono oxygenase and xanthine oxidase (Laughton et al., 1991; Siess et al., 1995; Cotelle et al., 1996; Cushman et al., 1991). These biological activities are related to their anti oxidative effects (Bors et al., 1990: Rice-Evans et al., 1995;1996).

Flavonoids can exert their antioxidant activity by various mechanisms, for example, by scavenging radicals, which initiate lipid peroxidation and lipid peroxide radicals, by binding metal ions, and by inhibiting enzymatic systems responsible for free radical generation.

Modulation of Nitric oxide (NO) production

The relevance of NO as a mediator of inflammation is more recently known (Lidia et al., 2000). NO is biosynthesized in mammalian cells by nitric oxide synthase (NOS), which has, neuronal (NOS I) , inducible (NOS II / iNOS), and endothelial (NO III) isozymes. Both NOS III and NOS I are
constitutively expressed whereas NOS II is expressed on induction by lipopolysaccharide (LPS) and number of cytokines in different cell types. Once expressed, this isoform generates nanomolar amounts of NO for hours and days. NO has a role in both acute and chronic inflammation (Kulkarni et al., 2000).

Recent studies indicates that NO stimulates the synthesis of inflammatory PGs by activating COX-2. Thus inhibition of nitric oxide pathway might have beneficial effects on inflammatory diseases, including joint disease. Myricitrin reduces the over expression of nitric oxide synthase and nuclear factor kappa b (NF kb) activation induced by lipo polysaccharide on RAW 264.7 cells (Chen et al., 2004).

Wogonin, baicalin and baicaleine were examined for their effect on LPS induced nitric oxide production and i NOS and COX -2 expression and they inhibited LPS induced nitric oxide production (Kim et al., 2005).

5, 4′ – Dihydroxy 6, 7, 8, 3’, 5’ - pentamethoxy flavone demonstrated dose dependent suppression of NO production (Liang et al., 2001). Wogonin, quercetin and luteolin inhibit NO production from LPS induced cells (Kim et al., 2001). Thus the above evidences indicate various mechanisms by which flavonoids could exert their anti-inflammatory action.
A novel antinociceptive property was reported for hydroxy ethyl rutoside by Ramaswamy et al., (1980). Subsequent work by Viswanathan et al., (1984) revealed potent antinociceptive action for gossypin in different experimental models. The effect was found to be mediated through opioid pathways and the possible development of tolerance to its antinociceptive action and dependence were investigated. Results revealed that, though gossypin utilized opioid pathways there was no tolerance development to gossypin analgesia either in acute or chronic experiments and no withdrawal signs were identified (Viswanathan et al., 1985). Further studies also indicated that gossypin pretreatment could significantly attenuate the acute tolerance development to morphine analgesia (Ramaswamy and Viswanathan et al., 1997) and gossypin treatment suppressed the withdrawal signs in acutely as well as chronically morphine dependent mice (Viswanathan et al., 1985). Moreover gossypin antinociception was found to involve the cholinergic and GABAergic systems which are important neurotransmitters in central nervous system (Viswanathan et al., 1993).

Thirugnanasambantham et al., (1985) reported that chrysin, morin and rutin exhibited antinociceptive action in mice. Later, a detailed structure activity study was undertaken with flavone and many of its methoxy and hydroxy derivatives. The flavone nucleus itself was found to exert a significant antinociceptive action which was modified to varying degree by different substitutions (Thirugnanasambantham et al., 1990 and 1993). A series of
dihydroxy flavone compounds were screened by Girija et al., 2002 for exploring potential antinociceptive action of these compounds.

A study by Anjaneyulu et al., (2003) investigating quercetin on thermal hyperalgesia in mouse model of diabetic neuropathic pain suggested that, the antinociceptive activity of quercetin may probably involve modulation of opioidergic mechanism and its potential use in attenuating diabetic neuropathic pain. The antinociceptive action of quercetin against several chemical and mechanical models of pain was further confirmed by Gadotti et al., (2005). The study by Kaur et al., (2005) revealed that quercetin induced antinociceptive effect by involving the modulation of adrenergic pathways.

Naidu et al., (2003b) attempted to explore the possible involvement of nitric oxide (NO) System in quercetin reversal of morphine tolerance and dependence in mice. Co administration of L- nitro arginine methyl ester (L-NAME) or quercetin with morphine during the induction phase delayed the development of tolerance to the antinociceptive action of morphine and also reversed naloxone precipitated withdrawal jumps. The results of the study suggested that quercetin reversal of morphine tolerance and dependence may involve its ability to suppress nitric oxide synthase (NOS) activity (Gadotti et al., 2005). Kaempferol-3, 7-O- alpha-di rhamnoside and quercetin 3,7-O-alpha di rhamnoside isolated from the leaves of Tilia argentea were shown to possess potent antinociceptive and anti inflammatory activity (Toker et al., 2004).
Meotti et al., (2006a) investigated the antinociceptive effect of myricitrin. They provided evidence for a role of L-arginine-nitric oxide and protein kinase C pathways in the above effect.

Five flavonoid derivatives of 5, 7-dihydroxy naringenin isolated from *Viscum album* were investigated and reported to have anti inflammatory and antinociceptive activity without inducing gastric damage. (Orahan et al., 2006).

**Flavonoids and anxiolytic activity**

In addition to the role in analgesia as described above, flavonoid compounds have also been found to exhibit another novel effect on CNS functions. Wolfman et al., 1994 have reported that 5, 7–dihydroxy flavone (chrysin) possessed anxiolytic actions without inducing sedation and muscle relaxation. They also postulated that this flavonoid is a partial agonist of the central benzodiazepine receptors.

Flavonoids from *Leptospermum scoparium* were found to have affinity for the benzodiazepine receptor. Dose related sedation and anxiolytic effect were exhibited in rats (Haberien et al., 1994).

Anxioselective properties of 6,3′–dinitro flavone, was tested in mice using elevated plus maze by Wolfman et al., (1996) and it was found that 6,3′–dinitro flavone has a benzodiazepine partial agonist profile, with low selectivity for central benzodiazepine receptor type I and receptor type II.
Paladini et al., (1999) studied the effect of halogen and nitro substituted flavones on affinity of ligands for the benzodiazepine receptor and reported that chemical modification of the flavone nucleus increases their anxiolytic potency.

Two flavonoids from *Artemisia herba alba* Asso were reported for in vitro GABA-benzodiazepine receptor activity (Salah et al., 2005).

Xu et al., (2006) have demonstrated the anxiolytic like effect of bicalein and compared its activity with other anxiolytics.

**Neuroprotective actions**

Ingae yoon et al (2004) investigated the effects of gossypin on the toxicity induced by oxidative stress or β-amyloid (Aβ) in primary cultured rat cortical cells. The results indicated that gossypin exerted neuroprotective effects in the cultured cortical cells by inhibiting oxidative stress and Aβ induced toxicity and that the antioxidant properties of gossypin may contribute to its neuroprotective action,

**Anti ulcer effect of Flavonoids**

Generally compounds exhibiting analgesic and anti inflammatory effects like NSAID induce gastric mucosal damage. However such an effect has not been reported for any flavonoid. But, in fact, many flavonoids have been documented to possess a protective effect against ulceration.
Vogin Rosi (1961) showed that a combination of orange bioflavonoid complex with vitamin C could protect the animals against histamine and reserpine induced ulcer. Gastric anti ulcer effect of catechin, naringin and gossypin in various experimental models has been extensively studied by Parmar (1977a). These flavonoids protected the animals from ulcers induced by pyloric ligation, restraint and drugs. They also reduced the gastric acid secretion. The effect of flavonoids on histaminergic system, viz; antihistaminic effect (Ramaswamy et al., 1979), histidine decarboxylase inhibition (Reimann et al., 1977) and mast cell stabilizing effects (Fewtrell and Gomperts, 1977a) may play an important role in the anti ulcer and anti secretory property of these compounds. In addition, flavonoids may protect gastric mucosa against free H^+ ions by a radical scavenging action (Slater and Eakins, 1975) and by a direct action on mucosal capillaries (Parmar and Ghosh., 1980).

β – Hydroxy ethyl rutosides, gosyypin, naringin and (+) cyanidanol-3 were shown to exhibit anti ulcer activity (Parmar et al.,1978). Quercetin , kaempferol, morin, myricetin and rutin when tested were found to inhibit the mucosal content of PAF in a dose dependent manner suggesting the protective role of these substances may be mediated by endogenous PAF (Izzo et al.,1994).

Preventive effect of the flavonoid wogonin against ethanol –induced gastric mucosal damage in rats was tested by Park et al., (2004).

They concluded that the flavonoid wogonin could be used as a preventive agent of alcohol induced gastropathy.
Effect on experimental cataract and retinopathy

The cataract observed in diabetic and galactosemic conditions was found to involve the enzyme aldose reductase in the lens. Flavonoids have been shown to inhibit cataract formation in diabetic and galactosemic cataracts (Varma et al., 1977; Parmar and Ghosh, 1979). The inhibition of the enzyme aldose reductase in the lens was found to be responsible for the above beneficial effect.

Cardiovascular actions

A coronary vasodilator action of flavonoids was reported by Setniker et al., (1961). Brkic and Laszt (1972) showed that HR and catechin could develop collateral circulation after left coronary occlusion. Perflavone has been suggested to be useful in the treatment of angina, atherosclerosis and myocardial infarction (Wagner., 1977).

Parmar and Ghosh (1978, 1980) suggested that flavonoids reduce capillary permeability by decreasing the general susceptibility of the capillaries to various permeability factors. They also have a direct constrictor action on the capillary bed. HR has been reported to increase the affinity of endothelial cells to each other and make the inter-endothelial junctions tighter (Pearson and Gordon.,1979). Based on this action, flavonoids have been successfully used in dysfunctional uterine bleeding, periodontal disease, hemorrhoids and in Reynaud’s syndrome (Parmar and Ghosh, 1980).
Flavonoid and anti tumor activity

Yusukava et al., (1990) have reported that the ability of myricitrin to inhibit tumor promotion is due to the activation of immune responses against tumors. Myricitrin has been reported as having an anti mutagenic effect which is attributed to a free radical scavenger action (Ednnharder and Grunhage., 2003).

The effect of a few methoxy flavones, 3’, 4’ - dimethoxy flavones, 5, 7, 4’- trimethoxy flavone and in particular 7,3’ - dimethoxy flavone on cytochrome p450 1B1 in Scc-9(squamous cell carcinoma) cell was measured with quantitative DNA method. The flavones inhibited the CYP1B1-mRNA expression. Curcumin and quercetin were also found to inhibit CYP 1B1 mRNA expression ( Kristina Walle and Thomas Walle., 2007).

Need for the present study

From the foregoing review, it is clear that flavonoid compounds exhibit multivarious biological actions and have enormous therapeutic utility. A significant anti nociceptive and anti inflammatory action of flavonoids merit closer investigation. Eventhough a wide variety of drugs are currently available to treat pain and inflammation their undesirable side effects necessitate the search for safe and effective compounds. Novel molecules that inhibit several mediators of inflammation and pain are being developed in different parts of the world. The polyphenolic flavonoids in addition to possessing significant antinociceptive and anti inflammatory effects are
reported to be devoid of gastric mucosal irritation. In fact this is the most common side effect shared by all NSAID. Previous investigations have analyzed the antinociceptive efficacy of many flavone derivatives and have reported compounds with moderate efficacy. However flavonoid compounds with high efficacy have not been reported so far.

It is well known that structural modifications may alter the efficiency of any active ingredient. In this regard the hypothesis proposed by earlier investigators (Thirugnanasambantham et al., 1990; 1993) deserves active consideration. The postulations by Thirugnana sambantham et al., (1990,1993) included that screening of polyhydroxylated flavones may yield high efficacy antinociceptive agents.

Hence in the present study, it has been envisaged to screen few more dihydroxy flavone derivatives for their potential effect on nociception and inflammation.