CHAPTER - II

REVIEW OF LITERATURE
2.3.2.2 Cells involved in chronic inflammation

2.3.2.2.1 Macrophages in chronic inflammation

Macrophages arise from common precursor in bone marrow. Monocytes are circulated in the blood and migrated into tissues and differentiated into macrophages. Two different types of macrophages exist in haematopoietic system. Wandering macrophages, have less half-life of about one day and tissue macrophages have several months to years. During chronic inflammation, by the process of extravasation, the macrophages accumulate and persist in particular site of inflammation (Allison AC et al., 1978).

2.3.2.3 Lymphocytes in chronic inflammation

Both B cells and T cells are involved in chronic inflammation. The inflammatory stimulated macrophages secrete proinflammatory cytokines and chemokines that promote recruitment of leukocytes. Macrophages display the antigen to lymphocytes and induce co-stimulatory molecules like B7.1, B7.2 (Priya Rajavelu et al., 2008), that stimulate the T cell response. Activated T lymphocytes recruit the monocytes from the circulation with IFN-γ a powerful activator of macrophages. Lymphocyte accumulation leads to morphological changes of lymphoid organs especially lymph nodes (Takeshi Yamanaka et al., 2003)
2.3.2.4 Mediators of chronic inflammation

Table 2.2: Mediators of chronic inflammation

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2.4 ENZYMES INVOLVED IN INFLAMMATION

2.4.1 Phospholipase A2 (PLA2)

PhospholipaseA2 (PLA2) hydrolyse membrane phospholipids and lipoproteins (Jesus Balsinde et al., 2002). The hydrolysis of membrane phospholipids releases prostaglandins and leukotrienes. These proinflammatory mediators involved in inflammatory pathways. These bioactive members include arachidonic acid and lysophospholipids can be converted to eicosanoids. These bioactive eicosanoids work synergistically in different inflammatory cells (Smith WL et al., 1989). PLA2 hydrolysis phospholipid at the sn-2 position releasing a fatty acid and lysophospholipid which are further enzymatically metabolized to various lipid mediators. PLA2 enzymes were first discovered in rattle snake and cobra venom at the end of the 19th century and later in human pancreatic extracts (Dennis EA et al., 2011). Till today more than 30 enzymes possessing PLA2 activity are identified in mammals (Schaloske RH et al., 2006).
2.4.1.1 Types of PLA2 enzymes.

2.4.1.1.1 Secretory phospholipase A2 (sPLA2)

Secretory phospholipase A2 are low molecular weight, Ca\(^{2+}\) requiring enzymes first discovered in bee and snake venom (Davidson FF et al., 1990). The human genome contains nine sPLA2 genes encoding catalytically active enzymes comprising PLA2 groups 1, 2, 5, 10 and 12. Several isoforms of sPLA2 are expressed and released by human immune cells. Increased levels of various forms are detected at site of inflammation.(Seilhamer JJ et al., 1989).

Secretory phospholipase A2 is considered as primary PLA2 enzyme catalysing arachidonic acid release (Balboa MA et al., 1996) from membrane lipids. Apart from arachidonic acid they can release omega-3 fatty acids from cell membrane. sPLA2 found in inflammatory cells and exudates is called the nonpancreatic secreted phospholipase A2 since its amino acid sequence is more closely related to those of sPLA2 in the venom from snakes rather than to those from pancreas. Secreted PLA2 enzymes commonly exhibit a property of increase in enzyme activity called interfacial activation when substrate is in aggregated form rather than in a monomeric form (Mounier CM et al., 2004). Concentration of calcium depends on the phospholipids’ structure (Akiba S et al., 1999). Central function of sPLA2 is providing host defence against bacterial infection due to their ability to kill Gram positive and Gram negative bacteria. It is found that sPLA2 together with cPLA2 synergistically influence the inflammatory pathways.

2.4.1.1.2 Cytosolic phospholipase A2 (cPLA2)

Cytosolic PLA2 are large cytosolic proteins of molecular weight 61-114 daltons containing a C2 domain which is associated to the membrane involving Ca\(^{2+}\) mediated mechanism (Kita Y et al., 2006). Cytosolic PLA2 was first discovered by Christina Leslie and Ruthkramer in 1986 in neutrophils and platelets (Alonso F et al., 1986). The cPLA2 enzyme was purified sequenced and cloned by James Clark and Ruthkramer in 1991. Later six cPLA2 subtypes are identified (Dennis EA et al., 2011), among the cPLA2 subtypes cPLA2α enzyme is well studied isoform. cPLA2α is encoded by the PLA2G4A gene (Leslie CC et al., 1997) and found expressed in different splice variants
in cells. cPLA2α is controlled by phosphorylation and intracellular Ca\textsuperscript{2+} levels. The cPLA2α enzymes contain three phosphorylation sites viz., Ser505, Ser727 and Ser515, which are phosphorylated by mitogen activated protein kinase (MAPKS), Calcium calmodulin kinase 2 and MAPK interacting kinase (MKK-1) (Ghosh M et al., 2006). Ca\textsuperscript{2+} binding induces translocation of cPLA2α to intracellular membranes, while cPLA2α plays a vital role in regulating catalytic activity releasing arachidonic acid from phospholipids. Possessing acyl chain specificity for arachidonic acid is prominent feature of cPLA2α (Clark JD et al., 1991 & Murakami M et al., 1998). It exhibits both transacylase as well as lysophospholipase activity.

2.4.1.1.3 Calcium independent phospholipase A2 (iPLA2)

The calcium independent PLA2 has common structural features with cPLA2. The iPLA2 enzyme is characterized in macrophages for the first time in 1994 (Ackermann EJ et al., 1994), from then six human iPLA2 enzymes were identified which were diverse in structure and function.

iPLA2 activity is associated with processes such as proliferation, bone formation, apoptosis and monocyte recruitment. iPLA2 maintains lipid and membrane homeostasis within cells (Tang J et al., 1997). Lack of substrate specificity is characteristic feature of iPLA2, thus iPLA2 can release a variety of fatty acids including arachidonic acid. iPLA2 enzymes are associated with pathological conditions such as diabetes and neurodegenerative disorders. iPLA2 plays a key role in phospholipid remodelling, signal transduction, Fas receptor mediated apoptosis and transmembrane ion flux.

2.4.1.1.4 PAF Acetyl Hydrolase / Oxidised Lipid Lipoprotein PLA2

This enzyme is also capable of cleaving oxidized lipids at sn-2 position up to nine carbons long and accessing substrate in the aqueous phase (Six DA et al., 2000). Its active site is triad which is formed by the combination of serine, histidine and aspartic acid. This enzyme was shown that it is anti-inflammatory enzyme, and also as a marker for coronary heart disease (Samanta, U et al., 2008). Research on heart disease is focused on the inhibitors, which are capable of inhibiting this enzyme thereby
decreasing cardiovascular diseases. Lp-PLA2 modifies LDL to promote endothelial cell binding of monocytes thus contributing to inflammation and foam cell formation (Stafforini, DM et al., 2009).

2.4.1.5 Adipocyte phospholipase A2 (AdPLA2)

AdPLA2 is an enzyme that is abundantly expressed in white adipose tissue. It is also known as group XVI phospholipase A2. It is encoded by the PLA2G16 gene. Adipocyte PLA2 belongs to the lecithin retinol acyl transferase family and it is 18 kDa proteins. Amino acids His23 and Cys113 are important for AdPLA2 activity (Yuan, C et al., 2006). This enzyme is capable of liberating both sn-1 and sn-2 fatty acids through G-protein coupled mechanism involving prostaglandins and EP3 (Reginato, M. J et al., 1998).

2.5 PLA2 INHIBITORS

Many natural and synthetic PLA2 inhibitors have been identified and reported. These PLA2 inhibitors are being used as anti-inflammatory drugs. The extensive research on PLA2 inhibitors has gained prominence since the release of arachidonic acid from membrane phospholipids by PLA2 is one of the rate limiting factors for eicosanoid production and platelet activating factor (PAF). Thus inhibition of PLA2 activity is more effective anti-inflammatory approach (Dennis, EA et al., 1994). The natural PLA2 inhibitors include eugenol and anisic acid, while synthetic PLA2 inhibitors include diclofenac and oxyphenbutazone.

2.5.1 Eugenol

Eugenol is a pale yellow oily liquid extracted from clove oil. It belongs to phenyl prophanoid class of chemical compound. Its medicinal properties make it as a good antiseptic, analgesic and anti-inflammatory agent. Eugenol forms a highly stable complex with PLA2 active site with three hydrogen bonds of amino acids H48, D49 and C45 and vanderwaals interactions with F5, Y22, Y28, G30, C45 and H48. This explains
the use of eugenol as a traditional medicine for curing inflammation (Kumar et al., 2007).

2.5.2 Anisic acid

Anisic acid is defined as a crystalline volatile carboxylic acid obtained from aniseed. Anisic acid is also known as 4-methoxy benzoic acid. Anisic acid is widely used as antiseptic and in treating snakebites. The analysis of X-ray crystal structure showed that anisic acid was located in the hydrophobic channel and fitted well in the binding site of enzyme, forming a number of hydrophobic interactions with the amino acids in the channel, such as L2, F5, 19, A18, 119 and Y22. These hydrophobic interactions of anisic acid results in the inhibition of phospholipase A2 (Singh et al., 2006).

2.5.3 Diclofenac

Diclofenac is chemically known as 2-(2, 6-dichloraniline) phenyl acetic acid, it was first developed by Novartis formerly known as Ciba-seigy in 1973 and introduced in 1979. It is used as standard drug for inflammatory conditions. Diclofenac was located in the hydrophobic channel and fitted well in the binding site of enzyme. The binding was stabilized by a hydrogen bond established between H48 and an oxygen atom of the carboxylic group of diclofenac. Diclofenac molecule undergoes changes in conformation upon binding with the enzyme. The observable changes occur in calcium binding loop-terminal region and surface loops (Singh et al., 2004).

2.5.4 Oxyphenbutazone

Oxyphenbutazone is a nonsteroidal anti-inflammatory drug used to cure rheumatoid disorders. Oxyphenbutazone is a metabolite of phenyl butazone. The structure of PLA2 and oxyphenbutazone complex was analysed and observed that oxyphenbutazone fits well in the binding site of the enzyme with the help of hydrophobic interactions. Y52 and K69 residues were interacting with ligand. Along with these residues other residues in the hydrophobic channel such as L3, F5, A18 and M8, 19 also interact with
Flexibility and strong hydrophobic nature are distinguished properties of oxyphenbutazone for interaction with enzyme. The anti-inflammatory property of oxyphenbutazone is due to its PLA2 inhibitory activity (Singh et al., 2004).

2.6. CYCLOOXGENASES

Cyclooxygenases are defined as prostaglandin hormone synthases which produce prostaglandins. The formed prostaglandins based on their nature can take part in inflammation process or safeguards and maintain the integrity of various cells, tissues and organs (Carol A. Rouzer et al., 2009). Cyclooxygenase was first discovered and purified in 1976 and cloned in 1988. Cyclooxygenase-1 and cyclooxygenase-2 are very similar in structure around 60-65% of sequence is conserved. These two enzymes convert arachidonic acid into prostaglandins which are considered as local hormones exerting a wide variety of beneficial and harmful effects. COX-1 is denoted as prostaglandin endoperoxide H synthase-1 and COX-2 is denoted to as prostaglandin endoperoxide H synthase-2. These isoforms of cyclooxygenases have structural identity of 60% showing that the structures of these two enzymes are highly conserved. These two isoforms slightly differ from each other as COX-1 has eight residues in N-terminal whereas COX-2 has eighteen residues in C-terminal. These two isoforms differ in two lone substitutions as Val523 and 434 in COX-2 are substituted from the original ILeu523 and 434 residues found in COX-1 (Smith W. L. et al., 2000).

2.6.1 Types of cyclooxygenases

2.6.1.1 Cyclooxygenase-1

COX-1 was enzymatically active prostaglandin endoperoxide synthase (PGHS) was isolated in 1976 from rat seminal vesicles (Hemler et al., 1985). COX-1 control normal cell activities such as gastric mucous production and renal blood flow maintenance (Cashman and McAnulty et al., 1995).
2.6.1.1 Expression of COX-1

COX-1 is membrane bound hemo and glycoprotein having 600 amino acids and its molecular weight is 71kDa mainly found in the endoplasmic reticulum of prostanoid producing cell (Miyamoto et al., 1976). It is expressed in all tissues preferably smooth muscle cells, endothelial cells, platelets, glomerulus, collecting duct, renal microvasculature and gastrointestinal tract. COX-1 involves in regulation of cellular and metabolic activities such as regulating blood flow in kidneys, safeguarding stomach lining integrity, platelet function and homeostasis in many organs (Crofford LJ et al., 1997).

2.6.1.2 Cyclooxygenase 2

Cyclooxygenase 2 is a membrane bound inducible dimeric enzyme of prostaglandin G/H synthase family that allow cells to respond to inflammatory cytokines, tumour promoters and growth factors. COX-2 catalyses the crucial step in the prostaglandin synthesis pathway. It has been described as a mediator of inflammation as well as modifier of prostanoid signaling. It can be considered as marker in studies of immune response and inflammatory disease drug target (Kulmacz R. J et al., 2003).

2.6.1.2.1 Expression of COX-2

Cells of the human body contain very low amount of COX-2, however it seems to be expressed more frequently in central nervous system and urogenital tract. COX-2 expression can be increased by exposure to growth factors, endothelial cells, fibroblasts, lipopolysaccharides, monocytes or macrophages and cytokines. It is normally found in reproductive organs, osteoblasts and endothelial cells (Fu et al., 1990 and Neilson et al., 1992).

The physiological significance of COX-2 has been shown in reproduction as it plays an important role in parturition and induction of ovulation (Wimsaif et al., 1993). In central nervous system, COX-2 plays a major role in processing of visceral and spinal sensory inputs (Breder et al., 1995). In gastrointestinal tract COX-2 is present in normal gastric
mucosa. It is also reported that COX-2 has been a key factor in embryogenesis (Zhang et al., 1999) and tumorigenesis (Eberhart et al., 1994).

**2.6.1.2.3 Mechanism of COX-2 action**

The cyclooxygenase activity of COX-2 oxygenates arachidonic acid with two molecules of oxygen to produce the cyclopentane hydroperoxy endoperoxide, PGG2. The enzyme anchors to the lipid bilayer by membrane binding domain and forms the catalytic site of COX. This structural alignment enables arachidonic acid to access into the COX active site, which is a long channel of hydrophobic nature that extends from membrane binding domain. Arg120 is mainly responsible for interaction of arachidonic acid with COX enzyme. Upon proper binding of substrate, arachidonic acid to the enzyme, Tyr385 removes the hydrogen from carbon 13. Then molecular oxygen forms an endoperoxide bridge joining carbon 9 and 12. Ring closure between carbon 8 and 12 occurs resulting in formation of bicyclic cyclopentyl dioxygen prostaglandin. (Smith WL et al., 1991).

**2.7 COX-2 IN HEALTH AND DISEASES**

Activation of prostanoid receptors initiates an amazing array of biological effects. The most important targets for prostaglandins include central and peripheral nervous system, cardiovascular system, gastrointestinal tract, reproductive system and renal system.

**2.7.1 COX-2 and brain**

Prostaglandins have been considered as mediators of fever. PGE2 produce fever by acting on neurons sensing temperature in the preoptic area (J. Engelberts D et al., 1993). COX-2 induction by interleukin-1 or lipopolysaccharide in endothelial cells of the cerebral blood vessels results in the production of PGE2 (Yamagata K et al., 1993). Prostaglandins are also recognized as inflammatory mediators in neural tissue and brain. In neurons of forebrain, COX-2 mRNA is constitutively expressed and immunoreactivity can be seen. COX-2 expression in the brain is more in new born
resulting in the higher levels of cerebral prostaglandins. These cerebral prostaglandins regulate blood flow in the new born (Jones SA et al., 1993). Acute stress increase COX-2 levels in cerebral cortex (C.D. Munhoz et al., 2008). Current research shows that COX-2 enhances amyloid plaque formation in brain parenchyma that leads to Alzheimers disease. The plaque formation by COX-2 made COX-2 as a therapeutic target in neurological disease (Joan Clària et al., 2003).

2.7.2 COX-2 and cardiovascular system

Prostaglandins produced from COX-2 such as PGI2 and TXA2 mainly affects smooth muscle cells and platelets. Both PGI2 and TXA2 have opposite actions. PGI2 enhances vasorelaxation, while TXA2 enhance vasoconstriction (Hecker M et al., 1995). In platelets, TXA2 inducts platelet aggregation while PGI2 inhibit platelet aggregation (Catella-Lawson F et al., 1999).

2.7.3 COX-2 and gastrointestinal tract

2.7.3.1 Gastric secretion

PGE2 and PGF2α are most abundant COX-2 derivatives present in gastric mucosa. Earlier, it is showed that PGE2 is antisecretory and antiulcerogenic in function (Robert A et al., 1967). Later research revealed that PGE1 or PGE2 inhibit acid and pepsin secretion and increase bicarbonate secretion upon intravenous administration, which results in reduced acidity of gastric juice (Eberhart CE et al., 1995). The production of PGE2 is increased in patients suffering from gastritis and Helicobacter pylori infection. PGE2 increase the gastrin levels (Sawaoka H et al., 1998). Acid secretion and gastrin levels become normal after administration of COX-2 inhibitors.

2.7.3.2 Gastrointestinal motility

Vascular reactivity studies in isolated intestinal muscle reveal that PGE2 causes relaxation of circular muscle and contraction of longitudinal muscle while PGF2α causes contraction of both muscles (Moore PK et al., 1998). Further, longitudinal and
circular muscle layers isolated from colon have shown the same response as that of intestine (Milton-Thompson GJ et al., 1995).

2.7.3.3 Gastrointestinal inflammation

Prostaglandins along with cytokines, bacterial products, reactive oxygen species, and lipid mediators play a major role in gastrointestinal inflammation (Shahbazian A et al., 2001). Products from COX-2 such as PGE2 and TXB2 and from lipoxygenases such as LTB4 and HETES are present in huge amounts in inflammed tissue (Wallace JL et al., 1992). An increase in evidence associated with inflammatory bowel disease (IBD) proved that prostaglandins counteract the proinflammatory activity of LTB4 (Masferrer JL et al., 1994). Preliminary results reveal that COX-2 specific inhibitors are safe and beneficial to the patients suffering from IBD.

2.7.4 COX-2 and reproductive system

Prostaglandins from COX-2 are essential for inducing uterine contraction during labour (Zuckerman H et al., 1974). Prostaglandins derived from COX-2 play a prominent role during delivery because COX-2 mRNA in the amnion and placenta significantly increases before and after the initiation of labour (Gibb W et al., 1996). COX-2 produce prostaglandins, which play a role in ovulation, successful rupture of follicle and implantation of embryo in the uterus endometrium (Dubois RN et al., 1998). Experimental mice lacking COX-2 show multiple failures in reproductive function such as ovulation, fertilisation, implantation and decidualisation.

2.7.5 COX-2 and renal system

Prostaglandins play a wide variety of roles in adult kidney. PG12 and PGE2 are highly influential renal vasodilators that modulate intrarenal vascular tone and glomerular thermodynamics and renal perfusion (Schlondorff D et al., 1986). PGE2 modulates renal water homeostasis by antagonizing the hydro-osmotic effect of anti-diuretic hormone in the collecting duct. PGE2 cause direct inhibition of sodium reabsorption in
tubular epithelial cells, thereby controlling renal sodium concentration. However, it is of interest to know which isoform of COX is involved in the maintenance of renal function.

2.8. CYCLOOXYGENASE INHIBITORS

Cyclooxygenase inhibitors inhibit cyclooxygenase enzymes, either COX-1 or COX-2 or both, which involved in the synthesis of inflammatory mediators derived from fatty acid known as prostaglandins. Cyclooxygenase inhibitors are commonly known as non-steroidal anti-inflammatory drugs (NSAIDS), which inhibit prostaglandin synthesis. Cyclooxygenase inhibitors are of two types they are selective inhibitors and nonselective inhibitors. Selective inhibitors inhibit either COX-1 or COX-2, whereas nonselective inhibitors inhibit both COX-1 and COX-2 enzymes.

2.8.1 Nonselective COX inhibitors

2.8.1.1 Indomethacin

Indomethacin is a anti-inflammatory drug used to cure inflammation. It reduces inflammation by inhibiting the synthesis of prostaglandins. Indomethacin was discovered in 1963 and approved for use in 1965. Indomethacin inhibition of COX involves the substitution of the 2-methyl group on the indole ring into a hydrophobic core in the COX-2 active site. (Harman et al., 2007).

2.8.1.2 Ibuprofen

Ibuprofen is a non-steroidal anti-inflammatory drugs used to cure inflammatory disorders. Ibuprofen is chemically known as 2-(D-isobutyl phenyl) propionic acid. Ibuprofen have melting point of 74-77°C. Ibuprofen is the first member of propionic acid derivatives. Ibuprofen is introduced in 1969 as a better alternative to aspirin. Ibuprofen competitively inhibits arachidonic acid binding and move rapidly from the COX active site. Ibuprofen lacks carboxylic group which helps in interacting with Arg120 without charged interactions. These larger methyl sulfonyl phenyl derivative
block the COX active site, thus preventing the interaction of COX active site with arachidonic acid (Rimon et al., 2009).

2.8.2 Selective COX inhibitors

2.8.2.1 Celecoxib

Celecoxib, the first COX-2 selective drug approved for treatment for signs and symptoms of osteo arthritis and rheumatoid arthritis. The COX-2 is the enzyme upregulated in several cancers. Celecoxib exhibits anti-inflammatory activity with reduced or minimal gastrointestinal and renal side effects. Monsanto, USA has tested the chemotherapeutic activity of COX-2 inhibitors in various animal models and in clinical trials on patient with familial adenomatous polyposis (Robert S. Bresalier et al., 2000). Celecoxib inhibits the growth of primary tumours by suppressing tumour growth factors and metastatic pathways. Celecoxib is effective at inhibiting angiogenesis. (Koki and Masferrer et al., 2002)

2.8.2.2 Rofecoxib

Rofecoxib is chemically known as 4-(4-(methyl sulfonyl) phenyl)-3-phenyl-2(5H)-furanone. It belongs to stibenes family. Stibenes are organic compounds containing a 1, 2-diphenyl ethylene moiety. Mechanism of rofecoxib includes the inhibition of the COX-2 isoenzymes at the site of inflammation with subsequent reduction in the synthesis of prostaglandins from arachidonic acid (Bombardier C et al., 2000).

2.9 NATURAL COX-2 INHIBITORS

2.9.1 Quercetin

Quercetin 3O-rhamnoside and Quercetin 3O-xylosyl (1→ 2) rhamnoside isolated from organic extracts of medicinal plants. These compounds reduce acute inflammation in in vivo and in vitro methods such as RAW 264.7 cell culture assay and TPA induced edema in mouse. The activity range of quercetin is compared with standard drug, indomethacinc. Quercetin inhibits the inflammatory induced NF-κB pathway (Bacon et al., 2003). It also inactivates the AKT pathway in chronic inflammatory cancer cells
(Conforti and Menichini et al., 2011). It stops the induction of nitric oxide synthesis and downregulates the TNF-α induced PGE2 and COX pathways. It inhibits the proinflammatory cytokines IL-1β, IL-6 synthesis (Gomes and Pan et al., 2008).

2.9.2 Celastrol

Celastrol is quinine methide pentacyclic triterpenoid extracted from root extracts of *Tripterygium wilfordii* and *Celastrus regelii*. It acts as potent analgesic, anti-inflammatory and antiarthritic agent. It controls the dextran-induced paw edema in rats. It acts through the suppression of NF-κB signaling in inflammatory and tumour cells and inhibits the action of IKKα and NF-κB induced pathways (Takkada and agarwal et al., 2003). It also inhibits the AKT dependent pathways in cancer cells (Xiaonan Wang et al., 2011).

2.10 LIPOXYGENASE (LOX)

Human 5-lipoxygenase is located on chromosome 10. 5-LOX gene is 90 kb long DNA. 5-LOX has been purified from human, rat, guinea pigs leucocytes, all the 5-LOX shown 90% homology. 5-LOX activity needed several cofactors and substrates that include ATP, Ca$^{2+}$, phosphatidyl choline, fatty acid hydroperoxides, arachidonic acid and molecular oxygen. 5-LOX catalyse the initial oxidation of arachidonic acid to yield 5-HPETE then converting 5-HPETE to LTA4. Calcium is required for activity of 5-LOX. Role of calcium seems to increase lipophilicity of 5-LOX in order to promote membrane associations. Lipoxygenase belongs to a group of fatty acid dioxygenases which catalyze the insertion of O$_2$ molecule stereospecifically into polyunsaturated fatty acid such as arachidonic acid. In 1974, 12-hydroxy eicosa-5, 8, 10, 14-tetraenoic acid (12-HETE) was identified in human platelets suggesting that expression of LOX enzymes in human platelets (Yeung J et al., 2014). After that some of the LOX enzymes were detected in rabbit reticulocytes. The first X-ray structure of lipoxygenase and its catalytic site were reported in 1993 by X-ray analysis and homology modelling (Boington JC et al., 1993). Crystalline form of lipoxygenase was isolated and purified from soybean by using ammonium sulfate solutions. The main domain of lipoxygenase consists of large alpha helix surrounding a central long 43 amino acid helix. Theorell et
al found that the molecular weight of LOX is 102 kDa by sedimentation method. All LOX isoforms possess single polypeptide chain, folded into two major domains

2.10.1 Structure of 5-LOX

The 5-LOX enzyme looks like an elliptic cylinder with large diameter of the ground square of 6.1 nm and short diameter of 4.5 nm and height 10nm. The substrate binding site is boot shaped cavity that is directly accessible. Its entrance is lined by arginine403, glycine407 and leucine597 and the bottom is lined by side chains of phenylalanine353, isoleucine418 and isoleucine593. (Hartmut Kuhn et al., 2005).

2.10.2 Regulation of 5-LOX

Regulation of 5-LOX was highly controlled process. Amount of free arachidonic acid available as substrate and its accessibility for 5-LOX are determinants for LT biosynthesis. LT production is regulated by both 5-LOX and cPLA2 (cytosolic phospholipase A2). Both these enzymes interact with membrane. Upon stimulation 5-LOX and cPLA2 migrates the membrane, where cPLA2 liberates AA from phospholipids. FLAP enhances transfer of AA to 5-LOX. Free AA supplied from both exogenous and endogenous sources are efficiently metabolized by 5-LOX. In normal conditions 5-LOX is present in cytosol or nuclear compartment associated with chromatin 5-LOX can be phosphorylated on three residues such as Ser271, Ser523, serine663 by different protein kinases.

2.10.3 Enzymes in Leukotriene biosynthesis

2.10.3.1 5-Lipoxgenase activating protein (FLAP)

FLAP is a novel protein essential for synthesis of leukotrienes, based on this function the discovered protein is named as five lipoxygenase activating protein (FLAP). FLAP is a unique 161 amino acid containing protein (18,157Da), stimulates the 5-LOX (William L.Smith 2000).
2.11 PROSTAGLANDINS

Prostaglandins are a group of biologically active lipid substances derived from plasma membrane. They were first extracted from human seminal plasma in 1933 independently by Goldblatt and Von Euler in England, later they found to be widely distributed in animal tissues. Prostaglandins are inflammatory mediators produced from arachidonic acid by phospholipase A2, further metabolized by cyclooxygenase-2 and 5-Lipoxygenases. Prostaglandins modulate the immune system by promoting and inhibiting the inflammation.

2.11.1 Types

Prostaglandins are of following types they are 1. PGE\(_1\), PGE\(_2\), PGE\(_3\), 2. PGI\(_2\) 3. PGD\(_2\) 4. PGF\(_{1\alpha}\), PGF\(_{2\alpha}\), PGF\(_{3\alpha}\)

2.11.2 Receptors

Prostaglandins perform their function through 7-transmembrane G Protein coupled receptors. The prostanoid receptor family composed of E prostanoid receptor EP1-4, PGD, PGF, PGI, thromboxane receptors.

2.11.3 Prostaglandins and inflammation

2.11.3.1 PGE\(_2\) and inflammation

PGE\(_2\) is prominent prostaglandin produced in the body exhibiting versatile biological activities, such as regulation of blood pressure, maintaining gastrointestinal integrity, regulation of immune responses and fertility. Abnormal levels of PGE2 results in a wide range of inflammatory conditions (Legler DF et al., 2010). PGE2 induce redness and edema in inflammed tissues due to arterial dilation and increased micro vascular permeability where as pain is due to the action of PGE2 on peripheral sensory neurons. PGE2 then acts through binding to one of the four receptors termed EP1, 2, 3, 4. (Trebino CE et al., 2003). Among the four receptors EP3 and EP4 receptors exhibit high affinity towards PGE2. Receptors EP2, EP3 and EP4 mediate edema associated with collagen and carrageenan induced arthritis and paw edema. PGE2 as a pro inflammatory mediator contributes to the regulation of the cytokines produced by dendritic cells and
differentiation of T cell into T helper1 (Th1) and T helper 2 (Th2) cells (Egan KM., 2004).

2.11.3.2 PGI₂ and inflammation

PGI₂ is vital prostanoid which have the capability of regulating cardiovascular homeostasis. Major sources of PGI₂ include endothelial progenitor cells, endothelial cells and VSMCS. PGI₂ is generated from PGH₂ by the action of COX-2 and PGIS a member of the cytochrome p450 super family that specifically converts PGH₂ to PGI₂. In vivo studies in experimental animals and humans showed that COX-2 was the dominant source of PGI₂, increased antigen induced leukocyte accumulation in bronchoalveolar fluid and peribronchiolar and perivascular inflammatory infiltration.

2.11.3.3 PGD₂ and inflammation

PGD₂ is synthesised in peripheral tissues and central nervous systems. (Jowsey IR et al., 2001). PGD₂ is involved in the regulation of pain perception (Urade Y et al., 1999, Eguchi N et al., 1999). PGD₂ synthesizing enzymes are of two types they are hematopoietic PGD synthase (H-PGDS) and lipocalin type PGD syntheses (L- PGDS). H-PGDS is generally localised in the cytosol of immune and inflammatory cells where as L- PGDS is more restrained to tissue based expression (Herlong JL et al., 2006). PGD₂ has strong association with inflammation and atopic conditions. PGD₂ is the predominant prostanoid produced by activated mast cells which initiate IGE mediated type 1 acute allergic responses (Roberts II LJ et al., 1980). PGD₂ causes allergic asthma such as bronchoconstriction and airway eosinophil infiltration (Hardy CC et al., 1984).The proinflammatory effects of PGD₂ appear to be mediated by both DP1 and DP2 receptors. PGD₂ synthesised by activated immune cells would be capable of activating multiple inflammatory signalling pathways.

2.11.3.4 PGF₂α and inflammation

PGF₂α is synthesised from PGH₂ by the enzyme PGF synthase. It acts via the FP receptor associated with Gq protein increases the intracellular calcium levels. COX-1 induced PGF₂α plays important role in female reproductive system, maintainence of internal organ functions. Elevated levels of PGF₂α act as a risk factor in inflammatory
mediated diseases such as arthritis, autoimmune diseases and cardiac diseases (Sugimoto Y et al., 1997, Saito O et al., 2003).

2.11.3.5 Thromboxanes

Thromboxanes are one of the inflammatory mediators belongs to family of lipids known as eicosanoids. Thromboxanes had got their name due to their function in clot formation. Thromboxanes are produced by the action of Thromboxane A synthase. Thromboxane A synthase was found in platelets converting PGH₂ to Thromboxane. Thromboxanes perform their function by binding to receptors such as Thromboxane receptors, G-protein coupled receptors coupled to the G protein G₉.

2.11.3.6 Thromboxanes and inflammation

TXA₂ an unstable Thromboxane with a half life of about 30 sec. Thromboxane synthase synthesize TXA₂ from PGH₂. TXA₂ is primarily mediated through the TP receptor coupled with G proteins and regulate phospholipaseC, adenylcyclase and Rho protein. TP receptor activation mediates several physiological functions such as smooth muscle contraction and relaxation, platelet adhesion and activation of endothelial inflammatory responses (Nakahata N et al., 2008).

2.12 CYTOKINES AND INFLAMMATION

Cytokines are defined as the group of secreted polypeptides which mediates inflammation. Based on their effect on inflammation, cytokines are broadly classified into two groups they are proinflammatory cytokines and anti-inflammatory cytokines. Proinflammatory cytokines initiates inflammation whereas anti-inflammatory cytokines reduce inflammation. The effect of cytokines depends on timing of cytokine release, receptor density, presence of competing or synergistic elements and tissue responsiveness to each cytokine (Dinarello CA et al., 1998).
2.12.1 Proinflammatory cytokines

Proinflammatory cytokines are defined as those cytokines which mediates inflammation. TNF-α, IL-1β, IL-6, and other chemokines such as G-CSF are involved in both acute and chronic inflammation.

2.12.2 Role of Interleukin-1β in inflammation

Interleukin-1β is a polypeptide produced predominantly after antigenic challenge. Mononuclear phagocytes, keratinocytes, fibroblasts, T and B lymphocytes are main source for interleukin-1β. IL-1β is a highly inflammatory molecule, which stimulate the production of arachidonic metabolites. IL-1 exists in two forms IL-1α and IL-1β, both these forms trigger fever by enhancing prostaglandin E2 synthase by vascular endothelial cells of hypothalamus. IL-1 enhances the release of histamines from mast cells at the site of inflammation. IL-1β acts as growth factor for T lymphocytes and it induces the formation of IFN-γ, IL-3 and other lymphokines synthesis. IL-1β acts as growth factor for B cells. It helps in synthesis of PGE2 thus enhancing inflammation (Mosmann TR et al., 1986 & Kelso A et al., 1995).

2.12.3 Role of Interleukin-6 in inflammation

Interleukin-6 is a unique cytokine takes part in resolution of immune responses. IL-6 produced by a variety of cells including T cells, mononuclear phagocytes and fibroblasts (T. Hirano et al., 1992) upon stimulation by infections and antigenic challenges. Upregualation of IL-6 production is seen in chronic and autoimmune diseases such as thyroiditis, type-1 diabetes and rheumatoid arthritis (P.L.J. Tan et al., 1990). IL-6 is used as a acute and chronic inflammatory marker. (T. Hirano et al., 1990).

2.12.4 Role of Tumor necrosis factor-α in inflammation

Tumor necrosis factor α and β are cytokines produced by the activated macrophages, fibroblasts, T cells and natural killer cells and mast cells (Hart PH et al., 1989). TNF-α induce fever by stimulating PGE2 synthesis by endothelium of hypothalamus. TNF-α stimulates the production of collagenase which is a key factor contributing to joint damages in arthritis (J. Vilcek et al., 1991). TNF-α is also known as lymphotoxin.
produced by activated T and B lymphocytes. TNF-α and β along with other pro-inflammatory cytokines IL-1 and IL-6 produced in huge amounts in acute and chronic infections. (B.B. Aggarwal et al., 1992).

2.13 CELL SIGNALLING PATHWAYS IN INFLAMMATION

Inflammation can be defined as a localized protective event elicited by pathogens or tissue injuries. During inflammatory response appropriate cells are immediately recruited at the site of infection to remove the pathogen. The inflammatory response is well orchestrated event caused by the interaction of several inflammatory mediators. The process of inflammation is initiated in response to infections, autoimmune and toxic stimulus. Bioactive peptides released by neuron and heat shock proteins or mitochondrial proteins released by dying cells trigger cytokine production by tissue cells. Many derangements in cell signaling occur during process of inflammation. LPS of gram negative bacteria has been reported to activate macrophages to produce inflammatory mediators such as NO, TNF-α and COX-2 mimicking the inflammatory response. LPS induces a series of signal production leads to activation of NF-kB, mitogen activated protein kinases. PI3/Akt plays key role in cell proliferation, differentiation and extra cellular signal transduction to the nucleus. TLR4 activates the MAPKInase, which further phosphorylates IKBα leads to translocation of NF-kβ 65 into nucleus and binds to promoter region of DNA induce the expression of proinflammatory mediators. Inflammatory cytokines TNF-α and IL-β activate the PI3K, it catalyses the phosphorylation of phosphotidyl inositol 4 phosphate and phosphotidyl inositol 4.5 phosphate yielding PIP2 and PIP3.

2.13.1 NF-kB SIGNALING

NF-kB is a transcription factor that plays a vital role in activation of immune system, inflammation, as well as in regulation of proliferation and cell survival. Members of this transcription factor family are activated in response to a variety of stimuli, including cytokines, growth factors, bacterial and viral infections. NF-kB is activated by many proliferative cytokines (TNF-α and IL-1β) and of the toll like receptors which recognize a variety of molecules associated with pathogenic bacteria and viruses. NF-kB protein is a heterodimer composed of a P65/Rel A and a P50 sub unit. Rel domain is common to all NF-kB protein for binding with DNA domain. Canonical NF-kB heterodimer reside
in the cytosol bound to an inhibitor of NF-kB called IκBα. NF-kB heterodimers are activated by a number of signals such as infections, ROS, Inflammatory cytokines, lipopolysaccharide, PGE2 and leukotrienes (Gustin et al., 2004) that drives the chronic pancreatitis, gastritis, inflammatory bowel disease and cancer (Greten et al., 2004). Stimulation of a cell by an activator such as TNF-α, IL-1β and TLR results in a cascade events activating IκK complex composed of IκKa, β, γ subunits, this activated IκK complex phosphorylate IκB then the phosphorylate IκB undergoes programmed death by ubiquitination at specific lysine residue. Ubiquitinated IκB target to the 26s proteosome for self-degradation. (Baldwin Jr., 1996, Ethridge et al., 2002).

2.13.1.1 NF-kB inhibitors
Research on tumor cells has identified NF-kB as a novel target for cancer prevention. Animal studies shown that inhibition or removal of NF-kB enhances the sensitivity of tumor cells to cell death (Schwartz et al., 1999, Luo et al., 2004), thus making NF-kB an attractive target for chemotherapeutic agents. Several classes of therapeutic agents that are capable of inhibiting of NF-kB are in clinical use. Glucocorticoids are used as anti-inflammatory and immunosuppressive agents who are capable of inhibiting NF-kB pathway. NF-kB inhibitors are used as promising therapeutic agents along with routinely used chemotherapeutic agents.

2.14 OXIDATIVE STRESS AND INFLAMMATION
Oxidative stress is a most important phenomenon with clinical significance in a wide variety of disease conditions such as cancer, muscle hypertrophy, Parkinson disease, diabetes, Alzheimer’s disease, cardiovascular disease (Benavonte-Garcia et al., 1997, Vaughan et al., 1997). Oxidative stress is featured by the production of oxygen derived free radicals like hydrogen peroxide, superoxide, nitric oxide, hydroxyl radicals. These free radicals are collectively termed as reactive oxygen species (ROS), apart from oxygenated species, nitric species such as nitric oxide (NO⁻) and peroxynitrite (ONOO⁻) play an important role in causing oxidative stress. These nitric species are collectively termed as reactive nitrogen species (RNS). ROS and RNS exert oxidative stress making each human cell to undergo 10,000 oxidative hits per second (Mahindra Singh et.al
which results in alteration of redox potential leading to un-repairable damage to biomolecules such as lipids, nucleic acid, proteins leading to cellular dysfunction and death.

2.14.1 Free radicals and antioxidants

In 1954, Geshman and Gilbert discovered that the lethal effects of ionizing radiation seen in Japanese people may be due to the formation of reactive oxygen species (ROS), this discovery gave rise to prominent research on reactive oxygen and nitrogen species (Gilbert et al., 1981). Denham Harman, trailblazer of free radical research first discovered the link between ageing and free radical chemistry in early 1950. In his theory on free radicals, he clearly highlighted that cellular ageing is associated with oxidative stress. Herman contribution gained worldwide recognition with the discovery of superoxide dismutase by McCord and Fridovich, a natural anti-oxidant enzyme which is capable of destroying the free radicals formed in the body. Forty years later Diplock et al., 1998 published a far reaching review strongly supported the fact that oxidative damage is an important causative factor in the development of diseases and antioxidants are capable of preventing and curing the diseases. Diplock et al., 1998 concluded that maintenance of well-being and health depends on the intake of antioxidants through diet which can modulate free radical process resulting in the removal of free radicals formed in the body. Current research is focused on the collaborative role of free radicals and antioxidants in diseases such as Alzheimer’s disease and diabetes.

2.14.2 Generation of free radicals

Free radical reactions occur continuously in cells and tissues as a consequence of various enzymatic and non-enzymatic reactions. Enzymatic reactions which are sources of free radicals are involved in phagocytosis, prostaglandin biosynthesis and in cytochrome P450 system. Non-enzymatic reactions mainly involve ionizing radiations. Apart from biological source non-biological sources such as tobacco smoke, automobile exhaust, ionizing radiation, hydrogenated oils, toxic metals are considered as richest source of free radicals (Prior et al, 1995 & Diplock et al., 1998).
Nitric oxide (NO\(^-\)) is another well-known free radical found in various living systems. It is generated by the vascular endothelium and other cells (Mencadd and Higgs et al., 1991). Nitric oxide reacts with superoxide anion to produce a highly reactive free radical peroxo nitrite intermediate (ONOO\(^-\)) which is capable of destroying biomolecules and causing them to malfunction (Beckman et al., 1990 & Saran et al., 1990).

### 2.14.3 Types of free radicals

A wide variety of free radicals cause oxidative destruction in the biological system they include reactive oxygen species such as superoxide anion (O\(_2^\cdot\)), peroxyl (RO\(_2^\cdot\)), hydroxyl (OH\(^\cdot\)). Reactive nitrogen species include nitric oxide (NO\(^-\)), peroxy nitrite (ONOO\(^-\)), peroxy nitrous acid (ONOOH), nitrogen dioxide (NO\(_2\)) and super oxide anion radical (O\(_2^\cdot\)). The reduction product of oxygen (Halliwell et al., 1994) is most often involved in the initiation of oxidation stress. Phagocytic cells like macrophages extensively produce superoxide anion radical to destroy the invading microbes such as bacteria, viruses (Babier et al., 1933 & Haliwell et al., 1994). It can be produced in the electron rich environment of the mitochondria membrane in the respiratory chain. In addition to this it can be produced endogenously by enzymes such as xanthine oxidase and NADPH oxidase pathways. NADPH oxidase pathway facilitates superoxide production to destroy pathogens. Superoxide radicals are highly capable of damaging red blood cells, lungs, joints (Lin et al., 1993 & Haliwell et al., 1994).

### 2.14.4 Free radical associated inflammatory diseases

Oxidative damage to various cells, tissues and organs by free radical results in various chronic diseases affecting various organs and systems. Free radicals and their formation are highly associated with chronic inflammatory diseases. Free radicals form one of the defence mechanism implied by immune system to expel the invaders by recruiting inflammatory cells to the site of injury (Allen et al., 2003). Free radicals activate NFkB, a nuclear transcription factor resulting in increased synthesis and release of IL-1\(\beta\), IL-6 and TNF-\(\alpha\) (Tumour necrosis factor), all these proinflammatory mediators provoke tissue damage.
2.14.4.1 Cardiovascular disease

Degenerative blood vessels and heart disease is one of the prominent and serious effects of free radicals. Free radical attack results in build-up of plaques in the walls of coronary arteries. The plaques will rupture and superimposed with thrombosis. This is the actual basis for the acute syndrome of myocardial infarction and unstable angina. Calcifying of plaques increase the risk of cerebral infarction (stroke) leads to severe disability or death (Brown et al., 1997).

2.14.4.1.1 Role of lipid peroxidation

Major progress in research regarding cardiovascular disease found that oxidation reactions play a vital role in atherogenesis. Lipids in the arterial wall yield peroxides and other free radicals (Herman et al., 1992 & Ames et al., 1993). Oxidised LDLs are cytotoxic and help in sustaining an inflammatory reaction in the arterial vessel wall (Estubauer et al., 1991, Basu et al., 1999, Cherubini et al., 2005). Oxidized LDLs are readily taken by macrophages to form foam cells which play a vital role in forming the atheromatous plaque. Medial smooth muscle also acts as source for foam cells, these foam cells migrate to the intima of arterial wall and facilitates the formation of plaque along with products of oxidation (Mason et al., 1997, Diplock et al., 1998 & Cherubini et al., 2005). The plaque continues to proliferate in accordance with connective tissue damage involving the deposition of collagen and elastin. Lipids such as cholesterol, LDL and the lipids accumulated in the injured area become oxidized further boosting free radical activity results in adherence of platelets to plaques (Kritchergky et al., 1999). With the increase in the size of plaque, blood flow becomes abnormally altered initiating thrombosis resulting in the occurrence of acute syndrome of unstable angina and myocardial infarction.

2.14.4.2 Cancer and inflammation

Cancer is the uncontrolled autonomous growth of abnormal cells that can rise in any organ or tissue of the body. Cancer cell is once normal cell which continue to grow and multiply without any limit. This may be due to various endogenous and exogenous
factors including oxidative stress, which brings a pathological change in the cell’s DNA resulting in tumour. Transformed cells evading immune system defence will only become tumours. Free radicals can deteriorate the immune system which can allow the abnormal cells to grow continuously (Ames et al., 1993). Harman suggested that risk of cancer increases with age this is due to increased level of endogenous free radical reactions and decreasing antioxidant defences.

DNA is a major target of free radical damage. Free radicals target and attack the nucleic acid bases resulting in various forms of base damage yielding products such as thymine glycol or abasic sites and 8-hydroxy guanosine. Apart from these, free radicals can attack and damage deoxyribose sugar as well as DNA protein cross-links. These damages to DNA result in mutations that yield cancer in germ cells and somatic cells. Free radical reactions with inadequate antioxidant defences results in increased rate of mutations in proto-oncogenes which are involved in normal cell growth and development, tumour suppression genes which suppress cell proliferation (Helliwell and Aruoma et al., 1993).

Cancer causing agents either initiating or promoting the carcinogenesis. Initiating agents include UV rays, radiation, chemical pollutants, tobacco consumption, and viruses. Promoting agents include hormones such as androgens for prostate cancer, oestrogens for breast cancer and ovarian cancer. Inflammation induces iNOS (inducible nitric oxide synthase) as well as COX and LOX which are capable of initiating carcinogenesis.

2.14.4.2.1 Role of ROS in carcinogenesis

Oxidative damage to DNA proved in vivo and in vitro confirming that ROS damage to cells contributes to carcinogenesis in many ways (Diplock et al., 1998). They may cause translocations and base pair mutations (Collins et al., 1996), abnormal cell to cell communication that favours unrestricted cell proliferation (Halliwell et al., 1996), structural changes in DNA such as gene sequence amplification (Halliwell et al., 1996), interference with genes that modulate cell growth preventing programmed cell death by necrosis and apoptosis (Robbin et al., 1995) and damage to DNA repair enzymes results in existence and survival of mutations. White cells of immune system are highly capable of removing altered cells which have potential to cause cancer. ROS decrease
the membrane fluidity of white blood cells. Loss of membrane fluidity decreases the ability of lymphocytes to eliminate the altered cells and invading agents (Bendich et al., 1999). Free radicals also damage the DNA of immune cells resulting in mutations and reduced cell function (Fabiani et al., 2001).

2.14.4.3 Alzheimers disease

Alzheimers disease (AD) is a neurodegenerative disorder which is characterized by the decline in the ability to remember, learn and think. Alzheimers disease more often occurs in old age.

2.14.4.3.1 Role of ROS in Alzheimers disease

In the histopathology of Alzheimers disease, oxidative reactions including lipid peroxidation found leading to oxidative stress (Zhos et al., 1995). The dementia of Alzheimers disease is associated with neurofibrillary tangles, senile plaques and loss of nerve cells from the cerebral cortex. Senile plaques are spherical forms with a dense concentration of protein called β-amyloid surrounded by decaying nerve cell terminals (Murphy et al., 1992). β-amyloid is neurotoxic and its toxicity is mediated by lipid peroxidation of membrane lipids leading to generation of an oxidative microenvironment causing lysis therefore loss of neurons. The generation of free radicals by β-amyloid has been proved, supporting the oxidative stress involvement. β-amyloid precursor protein (APP) is considered as precursor of β-amyloid, which has tendency to aggregate into insoluble fibrils which in turn form the basis of plaques (Murphy et al., 1992 & Glenn et al., 1994).

2.14.4.4 Osteoarthritis

Osteoarthritis, a joint disease is one of the common disorder affecting humans. At present osteoarthritis is considered as dynamic repair process of synovial joints triggered by a various mechanical and metabolic factors (Doherty et al., 2001). All the tissues of the joint such as bone, synovium, cartilage, ligament and muscle depends on
each other for health and function. Damage to any one affects other resulting in osteoarthritis affecting the whole joint (Doherty et al., 2001).

2.14.4.1 Role of ROS in osteoarthritis

Reactive oxygen species and the products of their reaction shown to decrease the fluidity of synovial fluid thus affecting its function (Merry et al., 1989). Free radicals in excess destroy the synovium, loss of joint fluid and support between bones. High levels of superoxide radicals in the exudates of patients suffering with active synovitis supported the fact that free radical damage may be the main cause of osteoarthritis (Zheu et al., 1998, Kucera et al., 1998). Tilak et al., 2006 propose that chondrocyte lipid peroxidation both physiological and pathological plays a key role in cartilage destruction by oxidizing cartilage collagen resulting in brittleness of cartilage, thereby initiating osteoarthritis.

2.15 PLANT DERIVED ANTIOXIDANTS

A number of plant derived substances collectively termed phytonutrients or phytochemicals gained prominence for their antioxidant activity. Polyphenol compounds such as flavonoids serve protection against a variety of free radicals formed as a result of oxidative stress (Cotelle N et al., 2001). Antioxidant property of flavonoids is largely contributed to broad therapeutic effects of flavonoids.
**Table 2.3: Categories of different Anti-oxidant systems**

<table>
<thead>
<tr>
<th>Types of Antioxidants</th>
<th>Components</th>
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<tbody>
<tr>
<td><strong>Endogenous Antioxidants</strong></td>
<td>Bilirubin</td>
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<td></td>
<td>Antioxidant thiols</td>
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<tr>
<td></td>
<td>i) glutathione</td>
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<tr>
<td></td>
<td>ii) lipoic acid</td>
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<tr>
<td></td>
<td>iii) N-acetyl cysteine</td>
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<tr>
<td></td>
<td>NADPH and NADH</td>
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<tr>
<td></td>
<td>Ubiquinone (coenzyme Q10)</td>
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<td></td>
<td>Uric acid</td>
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<tr>
<td></td>
<td>Antioxidant Enzymes</td>
</tr>
<tr>
<td></td>
<td>i) copper/zinc and manganese-dependent superoxide dismutase (SOD)</td>
</tr>
<tr>
<td></td>
<td>ii) iron-dependent catalase</td>
</tr>
<tr>
<td></td>
<td>iii) selenium-dependent glutathione peroxidise</td>
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<tr>
<td><strong>Dietary Antioxidants</strong></td>
<td>Vitamin C</td>
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<td></td>
<td>Vitamin E</td>
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<tr>
<td></td>
<td>Beta carotene and other carotenoids and oxycarotenoids</td>
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<tr>
<td></td>
<td>i) lycopene</td>
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<tr>
<td></td>
<td>ii) lutein</td>
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<td></td>
<td>Antioxidant polyphenols</td>
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<tr>
<td></td>
<td>i) flavonoids</td>
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<td></td>
<td>ii) flavones</td>
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<td></td>
<td>iii) flavonols</td>
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<tr>
<td></td>
<td>iv) proanthocyanidins</td>
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</tbody>
</table>
OBJECTIVES

1. Preliminary studies on in vitro and in vivo anti-inflammatory activities of *Mesua ferrea* bark and phytochemical analysis

2. Silica gel column chromatographic fractionation analysis of MFBEE and studies on anti-inflammatory effects of different fractions on production of nitric oxide and TNF-α in RAW 264.7 cells

3. Studies on structural elucidation and analysis of bio-active fraction, MFCE-F5 by HPLC, LC-MS, FT-IR, NMR techniques

4. Studies on anti-inflammatory activities of isolated compound Mesuaferrin A on proinflammatory enzymes, signal transducing molecules and docking studies

5. Studies on antioxidant activities of *Mesua ferrea* bark and isolated Mesuaferrin A