CHAPTER – I

INTRODUCTION
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1.1 INFLAMMATION

Inflammation is an important biological response of living organisms against infectious and noninfectious agents and tissue injuries which is clinically characterized by fundamental signs of redness, swelling, warmth, pain and loss of function (Choi et al., 2011 & Issa AY et al., 2006). Inflammation leads to renovation of tissue structure and function (Lawrence T, Willoughby DA et al., 2002). The mechanism of inflammatory cascades can be studied under arachidonic acid dependent and arachidonic acid independent pathways (Yoon and Baek et al., 2005). Arachidonic acid dependent pathways are mediated by cyclooxygenase (COX-2), lipoxygenase (5-LOX) and phospholipase A2 (PLA2) inflammatory enzymatic system as shown in figure 1.1. In contrast the second mechanism of inflammation involves a network of inflammatory cytokines responsible for activation of many inflammatory mediator genes. Nuclear factor kappa B (NF-κB) is considered as important transcriptional factor that can modify the inflammatory response. NF-κB has a role in promoting several proinflammatory intermediaries such as induced nitric oxide, TNF-α, IL-1β and PGE₂ (Ghosh S et al., 1998 and Hiscott J et al., 1993).

In arachidonic acid dependent mechanism, prostaglandins (PGE2) play a crucial role in induction of inflammatory response, their biosynthesis is significantly heightened in inflamed tissues, (Williams & Morley et al., 1973 and William & peck et al., 1977). Prostaglandins (PGE-2) or thromboxanes (TXA2) are collectively termed as eicosanoids, these are the bioactive inflammatory mediators formed from 20 carbon polyunsaturated fatty acids stored in plasma membrane by the sequential action of PGG/H synthase or cyclooxygenases (Nathan C et al., 2002). The arachidonate 5-lipoxygenase catalyzes oxidation of arachidonate at the 5-position to yield the 5-hydroperoxy eicosa tetraenoic acid (5-HPETE), then HPETE converted to leukotriene A4 (LTA4).
Figure 1.1: Arachidonic acid dependent and independent pathways of inflammation (Modified from Issa, Volate & Wargovich et al., 2006)

Cyclooxygenase is a key enzyme involved in synthesis of inflammatory mediators. COX exists in two major isoforms COX-1 and COX-2. All these isoforms distributed in different tissue and express in physiological and pathological conditions (W.L. Smith and D.L. Dewitt et al., 1996). Lipoxygenases are heterogeneous family of lipid peroxidating enzymes exists in different isoforms such as 5-LOX, 12-LOX and 15-LOX produce their respective inflammatory mediators 5-HETE, 12-HETE and 15-HETE. COX/LOX dual pathways are believed to be promising targets for development of anti-inflammatory agents without side effects (J. Morkel-Pelletier et al., 2003). In arachidonic independent pathway, transcription factors NF-κB coregulates the transcription of COX-2 and iNOS (Posadas et al., 2000). NO synthesized by iNOS activates soluble guanylate cyclase (SGC) and the resulting increase in cGMP induced upregulation of COX-2 expression in inflammatory cells (Park et al., 2002). Nitric oxide stimulates COX-2 expression through MAPKinase pathway (Yang et al., 2006).
TNF-α and IL-1β are most important proinflammatory cytokines produced by tissue macrophages, Mast cells, endothelial and epithelial cells which mediates acute and chronic inflammation (Cotran et al., 1999). TNF-α and IL-1β provoke generation of cell bonding molecules, and nitric oxide are capable of NF-κB and PI3K pathway activation (Balkwill and Mantovani et al., 2001).

Improvement of new anti-inflammatory drugs specially modified for treating inflammatory diseases such as rheumatism, asthma, inflammatory bowel diseases is attempted to associate their actions with the inflection of cytokines and linked signal pathways (Sacca R et al., 1997 & Barnes P.J et al., 2002).

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) have multiple roles with in the circulatory system. At lower concentrations ROS and RNS are important signaling molecules, where as in higher concentrations both participate in the alternation of molecular and cellular mechanism from a basal state to an activated state resulting in increased inflammatory signaling pathways (Patel et al., 2000, Cooper et al., 2002 & Strokes et al., 2002).

Oxidative stress is described as a discrepancy linking antioxidant and pro oxidant species. Oxidative stress is associated with cardiovascular diseases, atherosclerosis, hypertension and diabetes mellitus (Giugliano et al., 1995). Reactive oxygen species (ROS) including hydrogen peroxide (H₂O₂), hydroxyl radical, superoxide anion and RNS, Peroxy nitrate (ONOO⁻) (Griendling et al., 2001) contribute pro-oxidant/antioxidant imbalance. O₂⁻, H₂O₂, and ONOO⁻ are three of the key pro-oxidants contribute to oxidative stress (Wassmann et al., 2004). In inflammatory conditions, both ROS and RNS are considered to be toxic in their upregulated status due to lowest activity of antioxidant system (Griendling et al., 1994). Multiple sources of reactive oxygen species (ROS) have been identified in vascular cells. These include pro-oxidases like NADPH oxidase, (Meyer et al., 1999), xanthine oxidase (Miyachi et al., 1986), uncoupled NOS are thought to be main contributors to intracellular O₂⁻. Both enzymatic and non-enzymatic systems contribute free radical scavenging within the cell (Wassman et al., 2004) thus protecting from radical induced inflammation and cancer. Many man-made antioxidant agents have been developed to cure oxidative stress related diseases. However, the aspects including side effects, high cost, and lack of availability associated with synthetic antioxidants made scientists to focus on natural antioxidants.
Natural antioxidants got prominence as they are free from side effects. (Cai Y, Luo Q et al., 2004). Plant derived antioxidants such as ascorbic acid; rutin and quercetin play a protective role by scavenging the generated free radicals hence they are highly beneficial to cure the diseases caused by oxidative stress.

Anti-inflammatory drugs are generally prescribed for the healing of chronic inflammatory diseases, neurological diseases and cancer (Cheng & Harrish et al., 2005 & Wolfe, Lichtenstin and Singh et al., 1999). Anti-inflammatory drugs are classified into steroidal and nonsteroidal anti-inflammatory drugs (NSAIDS). Cortisole and hydrocortisole are a group of glucocorticoids used as most potent anti-inflammatory drugs to treat inflammatory diseases (Khan, Park & Lee et al., 2005). Glucocorticoids directly repress the NF-κB and AP-1 transcription factors responsible for genes encoding proinflammatory enzymes and cytokine mediators. The clinical use of glucocorticoids is associated with several deleterious side effects i.e. hypertension, hyperglycemia, water and electrolyte imbalance, osteoporosis and exhibit nonspecific reactions in their mechanism (Mangelsdorf et al., 1995).

Nonsteroidal anti-inflammatory drugs (NSAIDS) are a group of anti-inflammatory drugs used to treat inflammatory symptoms such as swelling, pain and fever. NSAIDs inhibit cyclooxygenases which catalyze the oxygenation of arachidonic acid to form prostaglandinG2 during the formation of prostaglandins and thrombaxanes (Volate and Wargovich et al., 2006). Cyclooxygenase enzymes are present in mammalian cells as isoforms COX-1 and COX-2 (Xie et al., 1991). COX-1 is a constitutive enzyme which maintains homeostasis of renal system and gastrointestinal tract, whereas COX-2 is an inducible enzyme induced by inflammatory conditions. Nonspecific inhibition of both COX-1 and COX-2 by NSAIDS results in adverse side effects like gastrointestinal toxicity and myocardial infarctions. Hence great effort has been devoted in developing natural COX-2 selective NSAIDS as effective anti-inflammatory drugs from medicinal plants that get rid of side effects associated with usage of NSAIDS.
1.2 NEED FOR THE DEVELOPMENT OF NOVEL ANTI-INFLAMMATORY AND ANTIOXIDANT AGENTS

Adverse side effects coupled with both steroidal and nonsteroidal anti-inflammatory drugs focused on the progress of novel anti-inflammatory and antioxidant agents which are free from side effects. A number of phytocompounds with specific activity have been identified, screened and developed to treat the inflammatory and oxidative stress associated diseases by targeting both oxidative and proinflammatory pathways. The phytoconstituents having potential anti-inflammatory and antioxidant activities include curcumin from turmeric (Khanna D et al., 2007), genistein from soyabeans (Pandey MK et al., 2007), withanolides from withania somnifera and silymarin from artichoke (Khanna D et al., 2007). The novel anti-inflammatory compounds are developed based on inhibition studies of NF-κB activation, proinflammatory enzymes such as COX-2, PLA2, 5-LOX, proinflammatory cytokines and mediators which include TNF-α, IL-1β, IL-6 and nitric oxide (Karin et al., 2004)

1.3 PLANT-DERIVED ANTI-INFLAMMATORY AGENTS

Plants occupy premier position in human history as an ultimate source of phytocompounds having high therapeutic value. It is found that 40% of all medicines derived from natural sources and out of them 25% are from plant sources. Plant-derived compounds are significant therapeutic agents which are used to cure both acute and chronic inflammatory diseases.

1.3.1 Plant-derived anti-inflammatory compounds that acts on arachidonic acid-induced nitric oxide and nuclear factor kappa B (NF-κB) mediated pathways

Cellular and molecular mechanisms involved in inflammatory response are promising targets for discovery and development of anti-inflammatory drugs to cure inflammatory diseases. Key inflammatory mediators such as PGE2, LTA4, TNF-α and iNOS whose production pathways have been the selective targets for improvement of novel anti-inflammatory drugs. (Levine JD et al., 1999)
1.3.2 Plant-derived inhibitors affecting prostanoid formation

Boswellic acid is a potent phytoconstituent of *Boswellia serrata*; the secondary metabolites from resin of boswellic acid are acetyl beta boswellic acid, it is proved as potent anti-inflammatory compound by inhibition of COX-2/LOX dual pathways (Takada Y et al., 2006). Curcumin is a naturally occurring anti-inflammatory agent, isolated from *turmeric* used for controlling various inflammatory disorders and wound healing (Song EK et al., 2001). Based on *in vivo* and *in vitro* studies, curcumin found to inhibit the inflammatory macrophage activation, inhibition of cyclooxygenase and lipoxygenase metabolic pathways (Kim DS et al., 2010 & Zhang F et al., 1999). Resveratrol is a polyphenolic compound isolated from roots of white hellebore (*Veratrum grandiflorum*). It inhibits both COX-2/5-LOX dual pathways and proinflammatory cytokine TNF-α. Quercetin is isolated from *Allium cepha* acts as most potent anti-inflammatory agent by targeting arachidonic acid dependent pathways (COX and LOX) and independent pathways such as TNF-α, IL-1β and IL-6.

1.3.3 Plant-derived inhibitors affecting nitric oxide production

Baicalin and wogonin are present in *Scutellaria baicalensis* Georgi belongs to the family lamiaceae. The anti-inflammatory potential of baicalin is due to its antioxidant property and ability to restrain LPS induced NO production, iNOS gene expression and increase in TNF-α levels in RAW 264.7 cells (Chen YC et al., 2001 & Chiu JH et al., 2002).

1.3.4 Plant-derived inhibitors affecting NF-κB signaling pathway

Avicins are family of triterpenoid saponins separated from *Acacia victoria* Bentham belongs to the family leguminosae. They inhibit the COX-2 expression by inhibiting NF-κB pathway (Haridas et al., 2001). Parthenolide is a sesquiterpene lactone isolated from *Tanacetum parthenium* which is commonly used in folk medicine as a remedy for treating inflammatory diseases. The anti-inflammatory potential of parthenolide is due to its ability to inhibit NF-κB pathway (Sheehan M et al., 2002). Silymarin is a bioactive flavonoid isolated from *Silybum marianum* belongs to the family asteraceae,
whose potent anti-inflammatory activity is due to the ability of inhibiting NF-κB pathway (Manna SK et al., 1999).

1.3.5 Plant-derived inhibitors affecting proinflammatory cytokine formation

Nobiletin is a polymethoxy flavonoid isolated from citrus fruits. Nobiletin is considered as potent inhibitor of PGE2 and proinflammatory mediators (Ishiwa J et al., 2000 & Lin N et al., 2003). Pycnogenol is a phenolic compound purified from the bark of *Pinus maritime Mill* belongs to the family pinaceae. Pycnogenol is an effective anti-inflammatory compound which is highly capable of reducing the synthesis of IL-1β and appearance of IL-1β mRNA in LPS stimulated RAW 264.7 cells (Cho KJ et al., 2001). Ginsenosides are terpenes isolated from *Panax ginseng* C. A. Meyer belong to the family araliaceae. (Cho JY et al., 2001).

1.4 PLANT-DERIVED ANTIOXIDANT AGENTS

Antioxidant secondary metabolites of medicinal plants can minimize the generation of reactive oxygen species (Cai Y et al., 2008) and alleviate the inflammatory diseases caused by oxidative stress (Akin et al., 2006). The phenolic and flavonoid constituents of metabolites contribute to antioxidant activities of medicinal plants (Zhang et al., 2006). Antioxidants like flavonoids, polyphenolic compounds and phenolics derived from plant sources significantly prevent oxidative stress conditions caused by free radicals such as reactive nitrogen and oxygen species. Reactive oxygen species are originated from oxidases of neutrophils. (Masoumeh et al., 2004). Some of the antioxidants from medicinal plants induce antioxidant enzymes which deplete oxidants in inflammatory cells.

Glycyrrhizin is triterpenoid saponin isolated from bark of *Glycyrrhiza glabra* (Fabaceae), is a potent antioxidant (Sam SKG et al., 2001). Silymarin is a flavonoid isolated from flower buds of *Cynara cardunculus var. scolymus* (Asteraceae), is a potent antioxidant (Gupta et al., 2006). Eugenol is a constituent of the phenyl propanoids group of chemical compounds extracted from the leaf of *Ocimum sanctum* Linn (Lamiaceae), shows potent antioxidant activity (Devi PU et al., 1999). Bakuchiol
is a meroterpene isolated from the seed of *Psoralea corylifolia* Linn (Fabaceae), used as an antioxidant (Haraguchi et al., 2000). Santalol is an organic compound comes under sesquiterpene category isolated from bark of *Santalum album* Linn (Santalaceae), shows antioxidant activity (Gupta et al., 2006). *Withania somnifera* (Solonaceae) roots contain oxygenated ergostane type steroids known as withanolides are potent antioxidant compounds (Vande velde V et al., 1982). Ascorbic acid commonly known as vitamin C is obtained from fruit of *Emblica officinalis* (Euphorbiaceae) is a well-known potent antioxidant (Bhattacharya A et al., 1999). Curcumin is a diaryl heptanoid obtained from the leaf of *Curcuma domestica* Valeton (Zingiberaceae), is a potent antioxidant (Shree Jayan et al., 1994). Carotenoids are organic compounds present in chloroplasts and chromoplasts obtained from roots of *Daucus carota* (Apiaceae) are potent antioxidants (Bishayee A et al., 1995). Fenchone is a monoterpene obtained from fruit oil of *Foeniculum vulgare* Mill (Apiaceae) shows antioxidant activity (Ruberto G et al., 2000).

1.5 DESCRIPTION OF *MESUA FERREA*

1.5.1 Introduction

*Mesua ferrea* belongs to the family clusiaceae. It is usually well-known as nagakesar in India and has been used as traditional medicine. Dried flower powder is used as remedy for gastrointestinal tract disorders, allergy and asthma. Seed oil is used in treating skin infections such as scabies, sores and wounds. Seed oil is generally prescribed for treating rheumatism. These are used in treating gastric ulcers. Bark decoction is used in treating gastritis and bronchitis. It is inhabitant of Srilanka and also widely distributed in north east region of India.

1.5.2 Classification

Kingdom-plantae

Class-Magnoliopsida
Order-Malpighiales
Family-Clusiaceae
Genus-Mesua
Species-Ferrea
Botanical name-\textit{Mesua ferrea}

\textbf{Figure 1.2: Mesua ferrea}

\subsection*{1.5.3 Botanical description}

It is medium sized evergreen tree grows up to 30-40 meters tall with 90cm diameter. Leaves are 5 to 6 inches long, with narrow and egg shaped. Flowers are bisexual, terminal and solitary. Fruit is a capsule with oval shape. They are 3-5 cm long with 1-4 seeds. Bark is hard and resistant with bright brown color. Phytochemical analysis of \textit{Mesua ferrea} exposed that occurrence of secondary metabolites like xanthones, coumarins, biflavonoids and terpenoids (Walia.S et al., 1976). Phytochemical and structural elucidating studies on the extracts of \textit{Mesua ferrea} bark shows the presence of Mesua ferrin-A, Mesua ferrin-B, anthraquinone, β-Sitosterol and Friedelin.

\subsection*{1.5.4 Pharmacological review on Mesua ferrea}

The whole plant of \textit{Mesua ferrea} has been extensively used in traditional medicine for curing of various diseases. Many researchers focused on evaluation of therapeutic potential of \textit{Mesua ferrea} based on biochemical and cell-based \textit{in vitro} and \textit{in vivo} methods. Sumitra chanda et al., 2013 described \textit{Mesua ferrea} seed extracts in all solvents ranging from petroleum ether to ethanol have shown significant broad spectrum anti-bacterial activities. Chloroform, dimethyl formamide and ethanol extract exhibit antifungal activities against \textit{Aspergillus candidus} and \textit{Mucor hiemalis} (Sumitra chanda et al., 2013). From \textit{in vitro} antioxidant studies of \textit{Mesua ferrea} leaf extracts on DPPH, NBT Riboflavin assays have shown that the ethanol extract showed potent antioxidant activity than other extracts (Narendra Prasad et al., 2012). Ethanolic extract of \textit{Mesua ferrea} dried flowers shown significant wound healing activity (GP Choudhary et al., 2012). The total phenolic content of \textit{Mesua ferrea} seed oil is compliant with
antioxidant and anti-inflammatory activities (Manoj Kumar Chahar et al., 2012). *In vivo* carrageenan-induced rat paw edema is appreciably repressed by ethanolic extracts of *Mesua ferrea* flowers at 200 and 400 mg/kg b.w. (Pinkesh Tiwari et al., 2013). Isolated compounds of *Mesua ferrea* bark extract are screened by *in silico* studies for targeting various inflammatory enzymes and mediators (Abhilash et al., 2012). *Mesua ferrea* bark extracts exhibit dose-dependent HRBC membrane stabilization assay (Manjunatha BK et al., 2013).

Though the *Mesua ferrea* has been studied *in vitro* and *in vivo* parameters pertaining to different biological activities, no attempts were made so far to evaluate further to study the effects on proinflammatory enzymes, proinflammatory cytokines and for isolating the active compounds.

Hence, the current study has been focussed to evaluate anti-inflammatory effects of *Mesua ferrea* bark extract and its fractions on *in vivo* and *in vitro* anti-inflammatory parameters and to carry out bioactivity-guided fractionation, isolation and characterization of anti-inflammatory compound(s) of therapeutic importance.