CHAPTER II

Review of Literature

Inflammation is fundamentally a protective response, the goal of which is to get rid the organism of both the initial cause of cell injury (e.g., microbes, toxins) and the consequences of such injury (e.g. necrotic cells and tissues). Cell injury induces releases of pro-inflammatory cytokines including tumor necrosis factor alpha(TNF-α), interleukin-1 (IL-1) from leukocytes, monocytes, and macrophages. These cytokines further trigger other pro-inflammatory cytokines and increase the expression of many cellular adhesion molecules (CAMs), selectins, integrins, and immunoglobulins. On the other hand, phagocytosis of bacteria or foreign particles is occurred. During this phase, high amounts of reactive oxygen species (ROS) such as superoxide anion ($\cdot O_2$ –), hydroxyl radical(HO·), and hydrogen peroxide (H_2O_2) are produced and an increase in the expression of phospholipase A_2, 5-lipoxygenase (5-LOX), and cyclooxygenase -2 (COX-2), inducible nitric oxide synthase (iNOS) (Huang *et al.*, 2004; Kumar *et al.* 2007).

Function of leukocytes (neutrophils and macrophages) are to deliver to the site of injury and to activate the leukocytes to perform their normal functions in inflammatory in host defense. Leukocytes ingest offending agents, kill bacteria and other microbes, and get rid of necrotic tissue and foreign substances. A pitfall of the defensive potency of leukocytes is that they may induce tissue damage and prolong inflammation, since leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

The process that host to elimination microbes that is functional responses of phagocytes in host defense consist of sequential steps-active recruitment of the cells to the sites of infection, recognition of microbes, phagocytosis, and destruction of ingested microbes.

Recruitment of the cells to the sites of infection

The recruitment of leukocytes to sites of injury and infection is a multistep process involving attachment of circulating leukocytes to endothelial cells and migration through the endothelium (Figure 1). The first events are the induction of adhesion molecules on endothelial cells, by a number of mechanisms. Mediators such as histamine, thrombin, and platelet activating factor (PAF) stimulate the redistribution of P-selectin from its normal intracellular stores in granules to the cell surface. Resident tissue macrophages, mast cells, and endothelial cells respond to injurious agents by secreting the cytokines TNF, IL-1, and chemokines (chemoattractant cytokines). Within 1 to 2 hours, the endothelial cells which are activated by TNF and IL-1 begin to express E-selectin. Leukocytes express at carbohydrate ligands for the selectins, and bind to the endothelial selectins. Then the bound leukocytes detach and bind again, and begin to roll along the endothelial surface. TNF and IL-1 also induce endothelial expression of ligands for integrins, mainly VCAM-1 (the ligand for the VLA-4 integrin) and ICAM-1 (the ligand for the LFA-1 and Mac-1 integrins). Meanwhile, chemokines that are produced at the site of injury enter the blood vessel, bind to endothelial cell heparan sulfate glycosaminoglycans and are displayed at high concentrations on the endothelial surface. These chemokines act on the rolling leukocytes and activate the leukocytes. The combination of induced expression of integrin ligands on the endothelium and activation of integrins on the leukocytes causes the firm integrin-mediated binding of the leukocytes to the endothelium at the site of infection. The leukocytes stop rolling, their cytoskeleton is reorganized, and they spread out on the endothelial surface. The next step in the process is migration of the leukocytes through the endothelium, called transmigration or diapedesis. Chemokines act on the adherent leukocytes and stimulate the cells to migrate through inter endothelial to the site of injury or infection. The net result of this process is that leukocytes, neutrophils and monocytes, rapidly accumulate around the infectious microbes. This reaction is typically elicited by microbes, but it may be seen in response to a variety of noninfectious stimuli as well. Figure 1

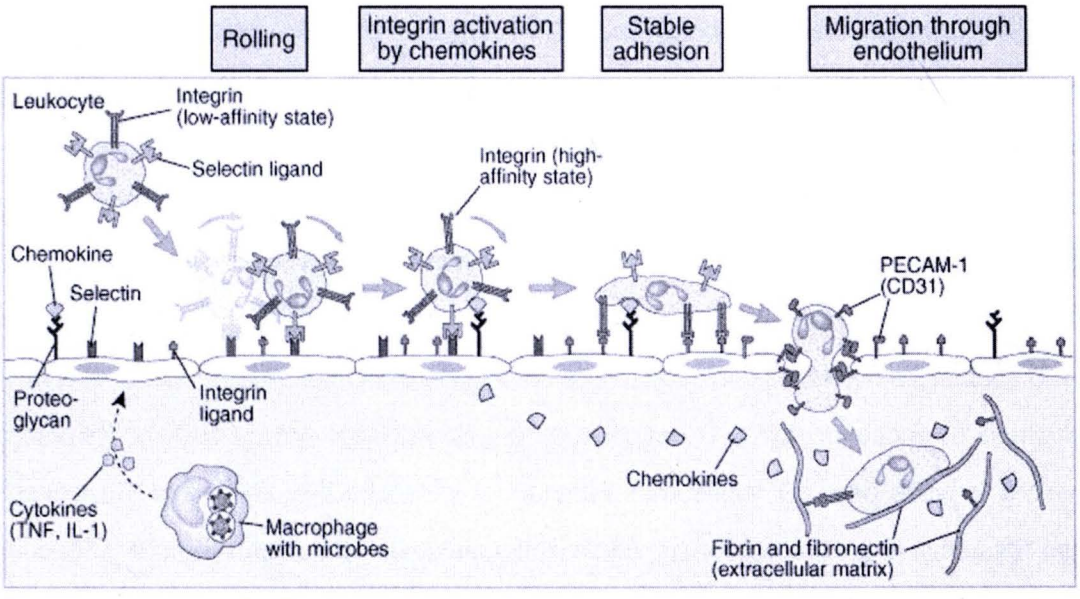

Figure 1: At sites of infection, macrophages that have encountered microbes produce cytokines (such as TNF and IL-1) that activate the endothelial cells of nearby venules to produce selectins, ligands for integrins, and chemokines. Selectins mediate weak tethering and rolling of blood leukocytes, such as neutrophils, on the endothelium; integrins mediate firm adhesion of neutrophils; and chemokines increase the affinity of neutrophil integrins and stimulate the migration of the cells through the endothelium to the site of infection (Abul *et al.*, 2005).

Recognition of microbes

In the blood and tissues neutrophils and macrophages express surface receptors that recognize microbes and stimulate the phagocytosis and killing of the microbes. These receptors include the following:

1. Different seven-transmembrane G-protein-coupled receptors recognize microbes and some mediators that are produced in response to infections and tissue injury. These receptors are found on neutrophils, macrophages, and most other types of leukocytes; and are specific for diverse ligands. Receptors of this class recognize short peptides containing *N*-formylmethionyl residues, as well as chemokines, chemotactic breakdown products of complement such as C5a, and lipid mediators of inflammation, including platelet-activating factor, prostaglandin E, and leukotriene B_4 (LTB_4). Since all

bacterial proteins are initiated by *N*-formylmethionine, this receptor allows neutrophils to detect and respond to bacterial proteins. Binding of ligands, such as microbial products and chemokines, to the G-protein-coupled receptors induces migration of the cells from the blood through the endothelium and production of microbicidal substances by activation of the respiratory burst. In a resting cell, the receptor-associated G-proteins form a stable inactive complex containing guanosine diphosphate (GDP) bound to Gα subunits. Occupancy of the receptor by ligand results in an exchange of GTP for GDP. The GTP-bound form of the G-protein activates numerous cellular enzymes, including an isoform of phosphatidylinositol-specific phospholipase C which functions to degrade inositol phospholipids and ultimately to increase intracellular $Ca^{2+}$and activate protein kinase C. The G-proteins also stimulate cytoskeletal changes, resulting in increased cell motility (Abul *et al.*, 2005; Kumar *et al,*. 2007).

2. Mannose receptors and scavenger receptors function are to bind and ingest microbes. The mannose receptor is a macrophage lectin that binds terminal mannose and fucose residues of glycoproteins and glycolipids. Macrophage scavenger receptors bind a variety of microbes as well as modified LDL particles. Macrophage integrins, notably Mac-1 (CD11bCD18), may also bind microbes for phagocytosis (Linehan *et al.*,2000; Kumar *et al.*, 2004).

3. Phagocytes express receptors for cytokines that are produced during immune responses. One of the most important of these cytokines is IFN-γ, which is secreted by natural killer (NK) cells during innate immune responses and by antigen-activated T lymphocytes during adaptive immune responses. IFN-γ is the major macrophage-activating cytokine.

4. Receptors for opsonins promote phagocytosis of microbes coated with various proteins and deliver signals that activate the phagocytes. The process of coating a particle, such as a microbe, to target it for phagocytosis is called opsonization, and substances that do this are opsonins. These substances are antibodies, complement proteins, and lectins. One of the most efficient systems for opsonizing particles is coating the particles with IgG antibodies, which are termed specific opsonins and are

recognized by the high-affinity Fcγ receptor of phagocytes, called FcγRI. Components of the complement system, especially fragments of the complement protein C3, are also potent opsonins, because these fragments bind to microbes and phagocytes express a receptor, named the type 1 complement receptor (CR1), which recognizes breakdown products of C3. These complement fragments are produced when complement is activated by either the classical (antibody-dependent) or the alternative (antibody-independent) pathway. A number of plasma proteins, including mannose-binding lectin (MBL), fibronectin, fibrinogen, and C-reactive protein, can coat microbes and are recognized by receptors on phagocytes. For example, a macrophage cell surface receptor called the C1q receptor binds microbes opsonized with plasma MBL, and integrins bind fibrinogen-coated particles (Abul *et al.*, 2005; Kumar *et al.*, 2007).

5. Toll-like receptors (TLRs), which are homologous to a *Drosophila* protein called Toll, are function to activate leukocytes in response to different types and components of microbes. There are 10 mammalian TLRs have been identified. Different TLRs play essential roles in cellular responses to bacterial lipopolysaccharide (LPS, or endotoxin), other bacterial proteoglycans, and unmethylated CpG nucleotides, all of which are found only in bacteria, as well as double-stranded RNA, which is produced only by some viruses. These receptors function are mediated through receptor-associated kinases to stimulate the production of microbicidal substances and cytokines in the leukocytes (Han *et al.*, 2005; Lee *et al.*, 2008).

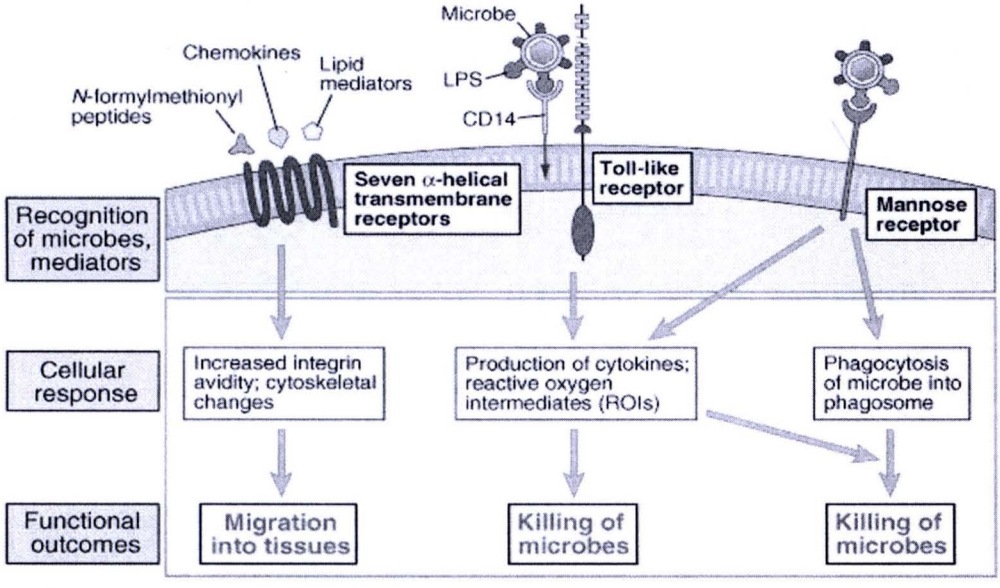

Figure 2: Different classes of cell surface receptors of neutrophils and macrophages recognize different stimuli. The receptors initiate responses that mediate the functions of neutrophils and macrophages (Kumar *et al.*, 2007).

Phagocytosis and destruction of ingested microbes

Phagocytosis is a cytoskeleton-dependent process of engulfment of large particles (>0.5 μm in diameter) and the release of enzymes by neutrophils and macrophages are responsible for eliminating the injurious agents. Phagocytosis involves three interrelated steps (Figure 4): (1) recognition and attachment of the particle to be ingested by the leukocyte; (2) its engulfment, with subsequent formation of a phagocytic vacuole; and (3) killing or degradation of the ingested material. Bacterial lipopolysaccharide (LPS or endotoxin) is a gram-negative bacteria product that is a mixture of fragments of the outer cell walls of gram-negative bacteria and contains both lipid components and polysaccharide moieties. LPS is a potent stimulator of innate immune responses that enhance killing of the bacteria, but it may also cause significant pathologic changes in the host. In innate immunity LPS is a potent activator of macrophages which lead to release cytokines such as IL-1 and TNF (called endogenous pyrogens) result in increase the enzymes, especially inducible nitric oxide synthase, cyclooxygenase-2 and initiate inflammation (Abul *et al.*, 2005; Kumar *et al.*, 2007).

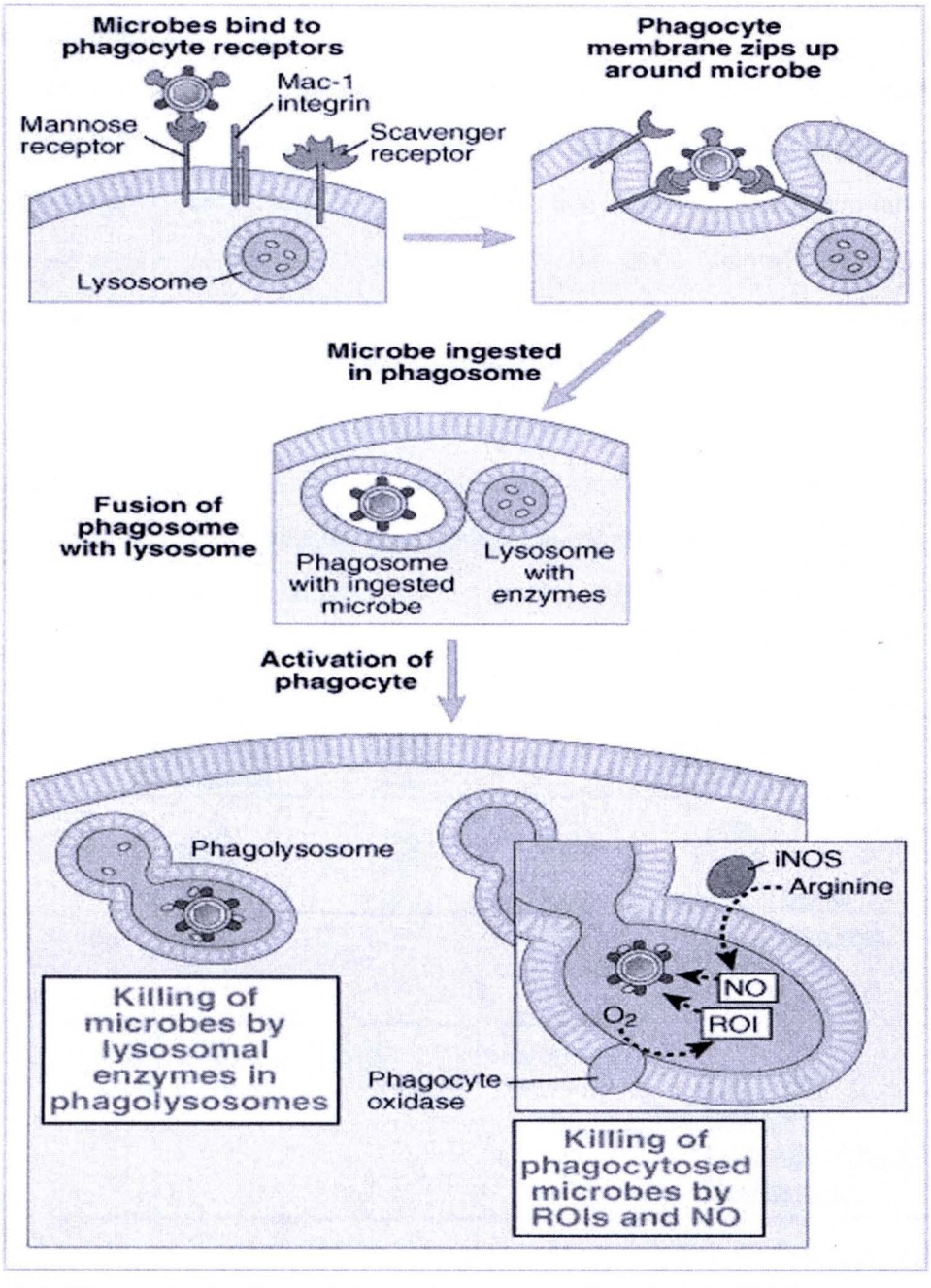

Figure 3: Phagocytosis of a particle by macrophages (Abul *et al.*, 2005)

Macrophages

Macrophages are mononuclear phagocytes originate in the bone marrow from a common haematopoietic stem cells (HSC). In response to macrophage colony-stimulating factor, they divide and differentiate into monoblasts and pro-monocytes before becoming monocytes, which exit from the bone marrow then enter the bloodstream. Monocytes undergo a series of change to become a macrophage in the body tissue. Macrophage is classified into two major groups: free macrophages and fixed macrophages. Fixed macrophages are found in organs and connective tissues. They have special names to designate specific location for instance in pulmonary airways, they are called alveolar macrophage, in connective tissue are histiocytes, in neural tissue are microglia, in liver are kupffer cells, in granulomas are epithelioid cells, in bone are osteclasts, in spleen called sinusoidal cells

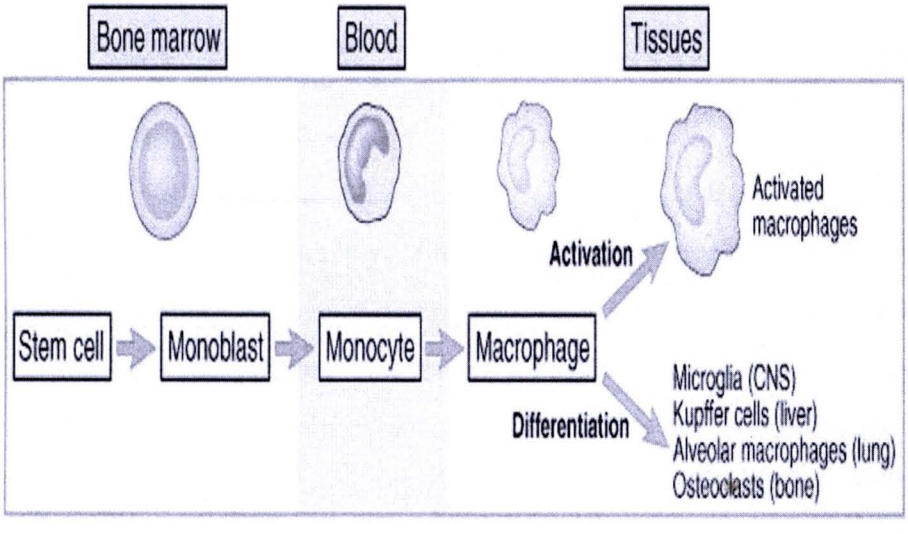

Figure4 : Mononuclear phagocytes develop in the bone marrow, circulate in the blood as monocytes, and are resident in all tissues of the body as macrophages. They may differentiate into specialized forms in particular tissues. (Abul *et al.*, 2005)

Macrophages play central role in innate and adaptive immunity and play a key role in host defence against parasitic bateria, pathogenic protozoa, fungi and helminthes as well as against tumers. In innate immunity, macrophages response to microbes by secreting cytokines that activate phagocyte and stimulate cellular reaction of innate immunity leading to inflammation (Ma *et al.*, 2003; Mosser 2003; Gorden *et al.*, 2005; Zhang *et al.*, 2008).

Cytokines

Cytokines are protein produced and secreted by cells of the immunity system (activated macrophage and lymphocytes). They are produced in response to antigens and microbes which stimulate diverse responses of cells involved in immunity and inflammation. Some cytokines promote inflammation are called pro-inflammatory cytokines. They are produced by activated macrophages and are involved in the up-regulation of inflammatory reactions. These cytokines are tumor necrosis factor (TNF-α), interleukin-1β (IL-1 β) and interleukin-6 (IL-6) (Dinarello CA. 2000; Stow *et al.*, 2009).

Tumor necrosis factor-α (TNF-α)

TNF-α is a major cytokine that mediates inflammation. It is produced mainly by activated macrophages. The secretion of TNF-α can be stimulated by endotoxin and other microbial products, immune complexes, physical injury, and a variety of inflammatory stimuli. The principal physiologic function of TNF-α is to stimulate the recruitment of neutrophils and monocytes to sites of infection and to activate these cells to eradicate microbes. These effects are mediated through several actions on vascular endothelial cells and leukocytes. It induces the expression of adhesion molecules (selectins and ligands for leukocyte integrins) that make the endothelial surface adhesive for leukocytes, initially for neutrophils and subsequently for monocytes and lymphocytes which is the most important event in the recruitment of leukocytes to sites of infection. It also stimulates endothelial cells and macrophages to secrete chemokines, cytokines (IL-6. IL-1β), eicosanoids, and nitric oxide (NO).

In infections, TNF-α is produced in large amounts and causes systemic and pathologic abnormalities as show in Fig. 5.

- TNF-α induces fever by increase synthesis of prostaglandins (PGE_2) then it acts on the hypothalamus, which generates a systemic response back to the rest of the body, causing heat-creating effects to match a new temperature level.

- TNF-α increases synthesis of certain serum proteins, such as serum amyloid A protein and fibrinogen that effect on hepatocytes and induce systemic acute-phase reactions.

- When serum concentration of TNF-α reachs 10^{-7} M or more, it results in a marked fall in blood pressure, or shock caused by decrease myocardial contractility and vascular smooth muscle tone. Furthermore TNF-α causes intravascular thrombosis, mainly as a result of loss of the normal anticoagulant properties of the endothelium and causes severe metabolic disturbances, such as a fall in blood glucose (Abul *et al.*, 2005; Kumar *et al.*, 2007).

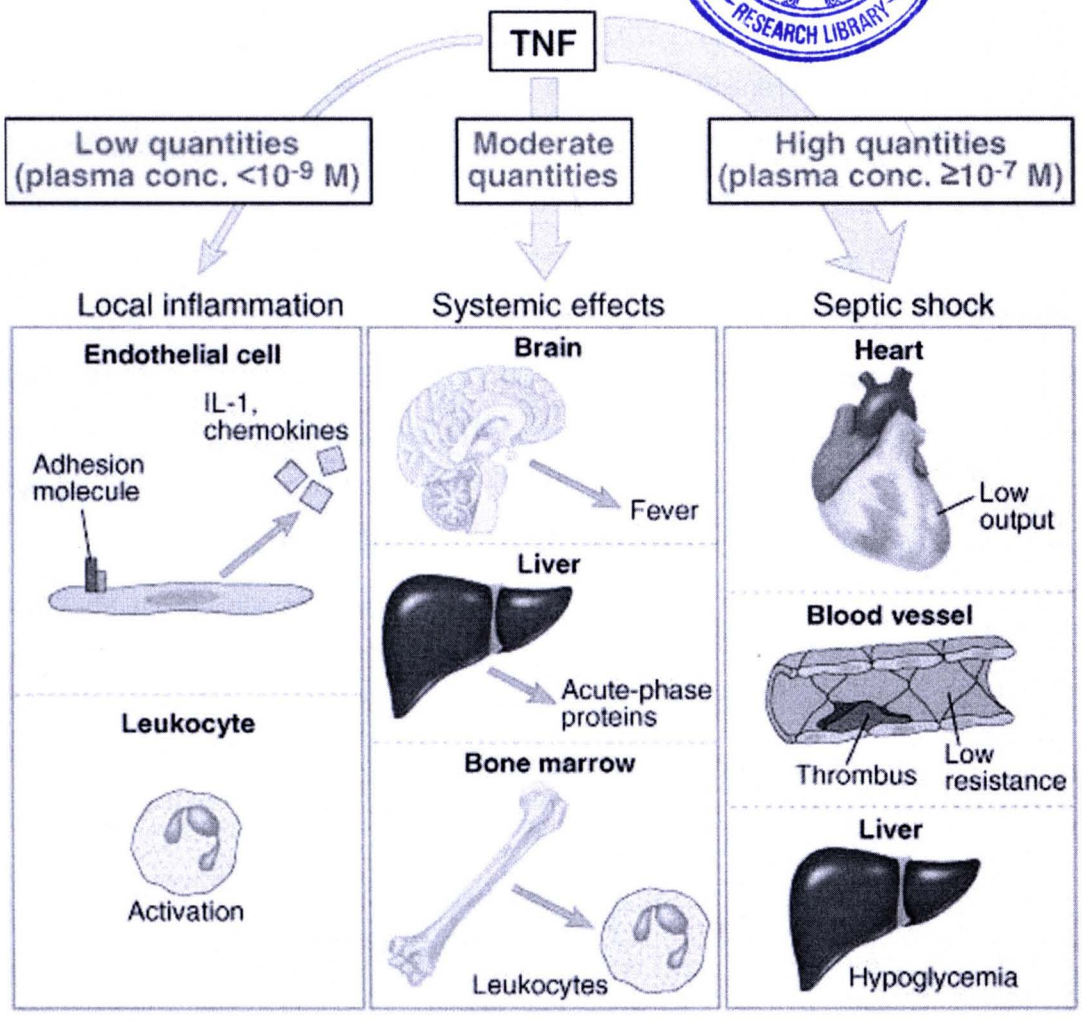

Figure 5: Biologic actions of TNF (Kumar *et al.*, 2007)

Interleukin-1 (IL-1)

IL-1 is produced by macrophages, neutrophils, epithelial cells and endothelial cells which are induced by bacterial products (such as LPS) or some cytokines (such as TNF-α). There are two forms of IL-1: IL-1α and IL-1β. IL-1β is most found in circulation. IL-1 possesses biologic effects quite similar to TNF as shown in Figure 6.

- It increases the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes, that the cells fight to pathogens in sites of infection and reset the hypothalamus leading to an increased body temperature which expresses as fever.

- It stimulates endothelial cells and macrophages to secrete IL-6 and nitric oxide (NO) and causes inflammation (Dinarellot 1997).

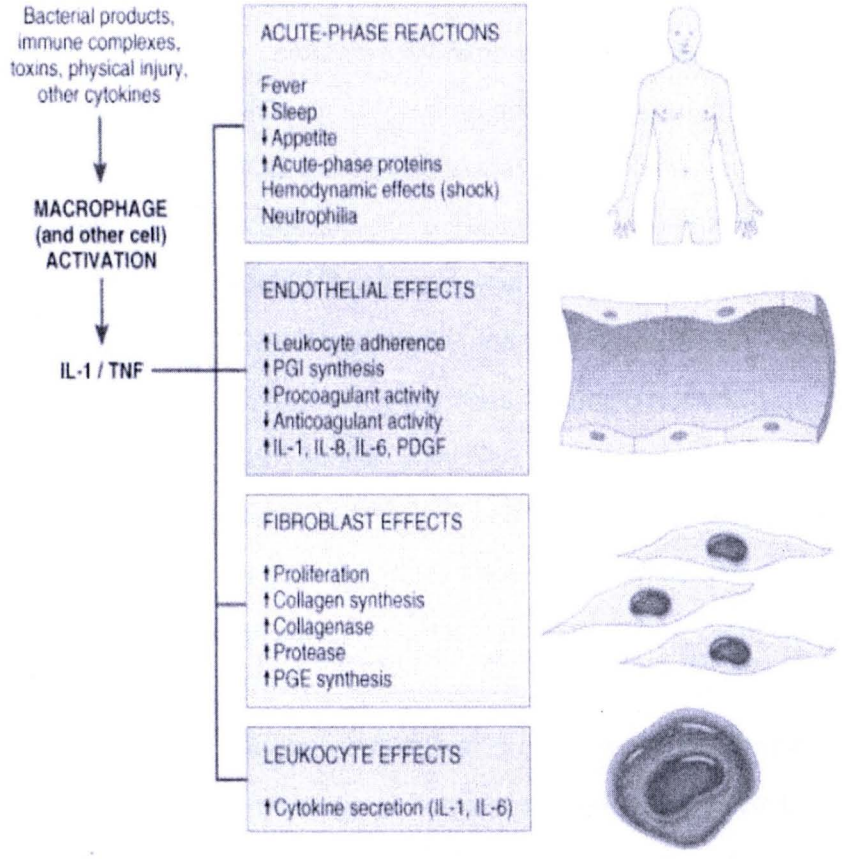

Figure 6: Major effect of interleukin-1 (IL-1) and tumor necrosis factor (TNF) in inflammation (Kumar *et al.*, 2007).

Interleukin-6 (IL-6)

IL-6 is produce by a variety of cell in immune system the most important sources are macrophages and monocytes at inflammatory site. IL-6 stimulates production of acute phase response This response consists of increased production of leukocytes; fever, which increases resistance to infection and changes in the levels of several plasma proteins, complement proteins, fibrinogen, C-reactive protein, and serum amyloid A protein. All these proteins play direct role in host defense. In extreme cases of severe infection, leads to shock, disseminated coagulation with multiorgan failure, and even death. IL-6 has biologic effects similar to TNF and IL-1. (Abul *et al.,* 2005; Kumar *et al.,* 2007)

Nitric oxide (NO)

NO is a soluble gas released from the endothelial cells and macrophages. It is synthesized from L-arginine catalysed by nitric oxide synthase enzyme (NOS). In mammals, three isoforms of NOS are discovered and named according to the activity or tissue types : endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS), eNOS and nNOS are constitutively expressed, Ca^{2+} - dependent and low output. In contrast, inducible NOS (iNOS) is induced when macrophages and other cells are activated by cytokines (e.g. TNF, IFN-γ) or LPS and the enzyme is Ca^{2+} - independent.

NO plays important roles in body functions, including host defense, nonspecific immune response to infection (innate immunity), cytotoxicity and tissue damage, vasodilatation of smooth muscle cells (Figure 7 and tabel 1) (Kumar *et al.,* 2007).

In the inflammatory reaction, NO is an important inflammatory mediator working together with other pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) lead to expression of iNOS in monocytes, macrophages and neutrophil. Over production Of NO by iNOS has been implicated in various pathophysiology of human diseases such as multiple sclerosis, septic shock, tumor development, asthma and neurodegenerative diseases (Clancy *et al.*, 1998; Bogdan 2001; Kleinert *et al.*, 2004; Sharma *et al.*, 2007; Toda *et al.,* 2009).

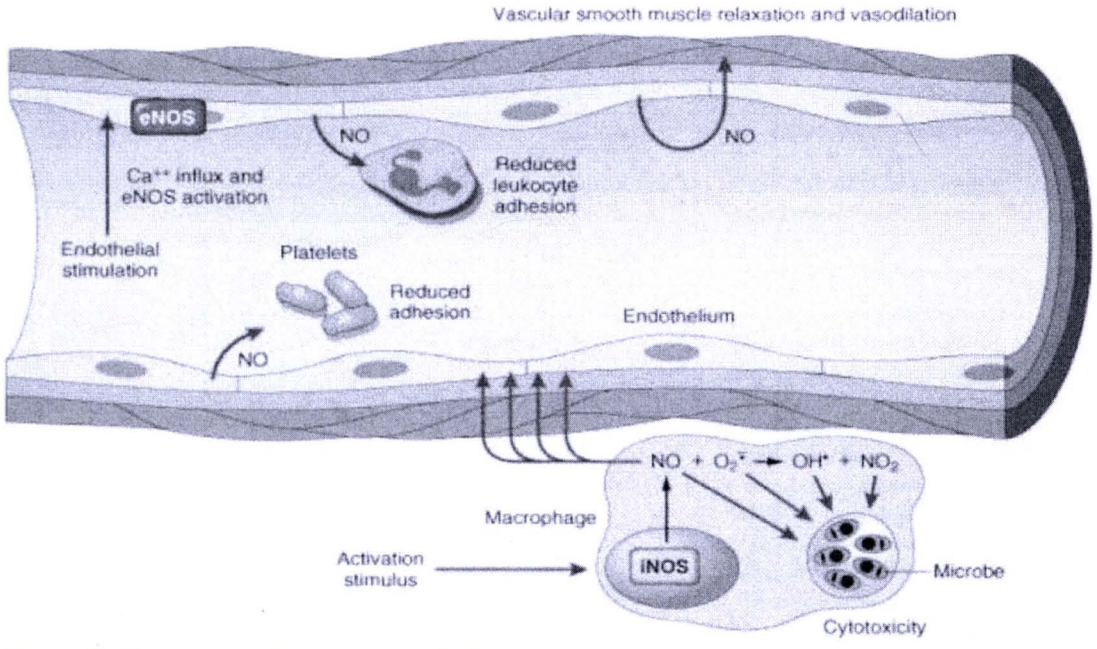

Figure 7: Functions of nitric oxide (NO) in blood vessels and macrophages, produced by two nitric oxide synthase enzymes (eNOS and iNOS). NO causes vasodilation, and NO free radicals are toxic to microbial and mammalian cells (Kumar *et al.*, 2007).

Table 1: Function of NO in immune system (Kumar *et al.*, 2007)

Category	Producers of NO (examples)	Phenotypic effect of NO	Examples of underlying molecular mechanisms
		Effector functions	
Antimicrobial activity	Macrophages, microglia, neutrophils, eosinophils, fibroblasts, endothelial cells, epithelial cells, astroglia	Killing or reduced replication of infectious agents (viruses, bacteria, protozoa, fungi, helminths)	•Direct effect of NO on the pathogen •Indirect effects of the NOS pathway (e.g., reaction of NO with other effector molecules, arginine depletion; see text)
Anti-tumor activity	Macrophages, eosinophils	Killing or growth inhibition of tumor cells	•Inhibition of enzymes essential for tumor growth (e.g., enzymes of the respiratory chain, *cis*-aconitase, ribonucleotide reductase arginase, ornithine decarboxylase) •Growth inhibition *via* iNOS-dependent depletion of arginine •Cell-cycle arrest (downregulation of cyclin D1) •Induction of apoptosis (by activation of caspases and accumulation of p53) •Sensitization of tumor cells for TNF-induced cytotoxicity
Tissue-damaging effect (immunopathology)	Macrophages, microglia, astroglia, keratinocytes, mesangial cells	Necrosis or fibrosis of the parenchyma	•Apoptosis of parenchymal cells •Degradation of extracellular matrix •Deposition of matrix, proliferation of mesenchymal cells •Influx of inflammatory cells via chemokine regulation
		Immunoregulatory functions	
Anti-inflammatory–immunosuppressive effect	Macrophages ('suppressor phenotype')	Inhibition of: •T cell proliferation •B cell proliferation •Antibody production by $CD5^+$ B cells •Autoreactive T and B cell diversification Inhibition of leukocyte recruitment (adhesion, extravasation, chemotaxis)	•Apoptosis of T cells or APCs •Downregulation of MHC class II, costimulatory molecules or cytokines •Disruption of signaling cascades and transcription factors •Inhibition of DNA synthesis •Downregulation of adhesion molecules or chemokines
Modulation of the production and function of cytokines, chemokines, and growth factors (pro- or anti-inflammatory effects)	Macrophages T cells endothelial cells fibroblasts	Up- and downregulation, e.g., of: •IL-1, IL-6, IL-8, IL-10, IL-12, IL-18, IFN-γ, TNF •TGF-β, G-CSF, M-CSF, VEGF, •MIP-1α, MIP-2, MCP-1	Modulation of •Signaling cascades (e.g. G-proteins, Jak, MAP kinases, caspases, protein phosphatases) •Transcription factors (e.g. NF-κB, Sp1, AP-1) •Proteins regulating mRNA stability or mRNA translation •Latent cytokine precursor complexes •Enzymes that process cytokine precursors
T helper cell deviation	e.g., macrophages	•Induction and differentiation of T_H1 cells •Suppression of T_H1 (and T_H2) cell responses •Suppression of tolerogenic T cell responses	1. Possible stimulation of IL-12-mediated signaling 2. Suppression of IL-12 production

Prostaglandins

Prostaglandins are potent bioactive lipid messengers derived from arachidonic acid (AA). Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid (5, 8, 11, 14-eicosatetraenoic acid) which is derived from dietary sources or by conversion from the essential fatty acid linoleic acid. It is released from membrane phospholipids through the action of cellular phospholipases (e.g. phospholipase A_2) AA metabolites, also called eicosanoids, are synthesized by two major classes of enzymes: cyclooxygenases or COX results in production of prostaglandins and thromboxanes and lipoxygenases or LOX which results in production of leukotrienes. COX have two isoforms ; COX-1 and COX-2. COX-1 is constitutively expressed in all cell types and is involved in normal kidney, gastrointestinal and reproductive functions whereas COX-2 is inducible by a wide variety of mitogens, hormones, cytokines and other stimuli and is thus associated with inflammation and diseases.

Prostaglandins are divided into series based on their structural features as PGD, PGE, PGF, PGG, and PGH. The most important ones in inflammation are PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin), and TXA_2 (thromboxaneA_2) (table 2). Prostaglandins are also involved in the pathogenesis of pain and fever. In inflammation, PGE_2 is hyperalgesic that makes the skin hypersensitive to painful stimuli. PGD_2 is the major metabolite of the cyclooxygenase pathway in mast cells; along with PGE_2 and $PGF_{2\alpha}$, they cause vasodilation and increase the permeability of postcapillary venules, potentiating edema formation. Ferver is induced by pyrogens which subsequently stimulate the production of PGE_2 and increase body temperature through heat regulating center in hypothalamus. Bacterial LPS from infecting organisms, or circulating IL-1, stimulate the expression of COX-2 and of PGE synthase in endothelial cells or macrophages that constitute the blood-brain PGE_2 which is generated by PGE synthase diffuses out of the endothelial cells or macrophages into the organum vasculosum lamina terminalis (OVLT) region of the hypothalamus which is responsible for controlling fever.(Vane *et al.*, 1998; Hinz and Brune 2002;Turini and Dubois. 2002; Marco *et al.*, 2002)

Table2: Inflammatory actions of eicosanoids (Kumar *et al.*, 2007)

Action	Metabolite
Vasoconstriction	Thromboxane A_2 , leukotrienes C_4, D_4, E_4
Vasodilation	PGI_2, PGE_1, PGE_2, PGD_2
Increased vascular permeability	Leukotrienes C_4, D_4, E_4
Chemotaxis, leukocyte adhesion	Leukotriene B_4, HETE, lipoxins

In state of over stimulation of the macrophags lead to over expression of mRNA of cytokines including TNF-α, IL-1β , IL-6 and over expression iNOS, COX-2. All these cytokines and enzymes are involved in the inflammatory diseases such as rheumatoid arthritis, atherosclerosis, lung fibrosis, septic shock and tissues damage. At present there are two general classes of drugs commonly used in the treatment of inflammatory diseases. They are steroid and nonsteroidal anti-inflammatory agents (NSAIDs)

Steroid

Steroidal compound structure and efficacy are similar to glucocorticoid hormone from adrenal cortex. Their effects are on entire body systems including carbohydrate, protein and lipid metabolism, electrolyte and water balance, cardiovascular system, skeletal muscle, central nervous system, forming elements of blood, anti-inflammatory and immunosuppressive action. Example of drugs in this group are dexamethasone, betamethasone, fludrocortisone, triamcinolone and prednisolone.

Mechanism of action of steroid for anti-inflammatory effect

Steroid is present in the blood in bound form on the corticosteroid-binding globulin (CBG). The intracellular receptor is bound to stabilizing proteins, including two molecules of heat shock protein 90 (Hsp90) and others. When the complex binds a molecule of cortisol, an unstable complex is created and the Hsp90 and associated molecules are released. The steroid-receptor complex is dimerize, enter the nucleus, bind to a glucocorticoid response element (GRE) on the regulatory region of the gene,

and regulate transcription by RNA polymerase II and associated transcription factors. The resulting mRNA is edited and exported to the cytoplasm for the production of protein that brings about the corresponding hormone response. The steroid-receptor complex interaction with a GRE is an interaction with and altering the function of other transcription factors, such as NF-ΚB in the nucleus of cells which result to reduce expression of pro-inflammatory cytokines, COX-2 and iNOS.

In the nucleus Glucocorticoid receptors (GR) can bind as a dimer onto the glucocorticoid response element (GRE), and regulate steroid-responsive genes regulating metabolic homeostasis. Recognized important function of activated GR is the inhibition of transcription of several cytokines and chemokines that are in inflammatory diseases. Another way steroid binding to glucocorticoid receptor (GR) activate to produce lipocortin which inhibits the activity of phospholipase A_2 that results in reducing the prostaglandins and reduction the number of lymphocytes, monocytes, eosinophils, and basophils at the site of inflammation.

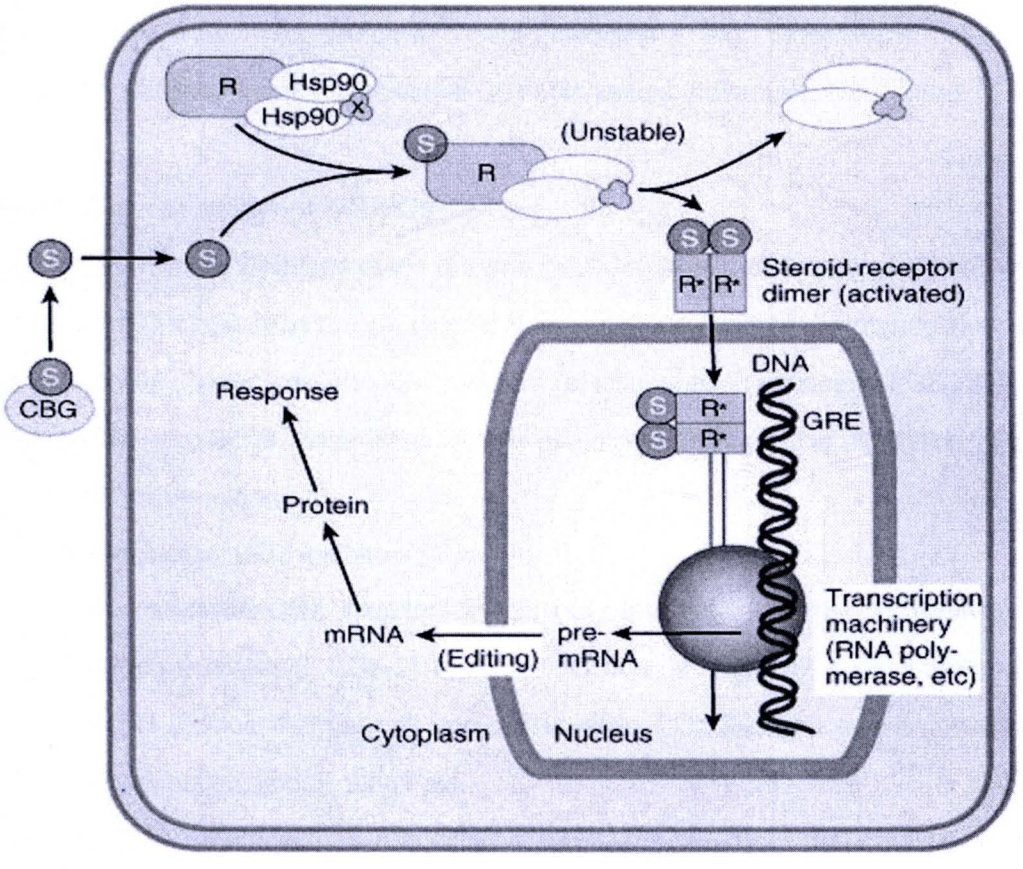

Figure 8: Mechanism of action of steroid (Schimmer *et al.*, 2006)

Adverse effects of steroid

Steroid is valued for its therapeutic application but it also causes many systemic side effects in long term administration. Since it suppresses the hypothalamic - pituitary –adrenal (HPA) axis and bring about iatrogenic Cushing's Syndrome. Fat tends to be redistributed from the extremities to the trunk, the back of the neck, and the supraclavicular fossae. There is an increase growth of fine hair over the face, thighs and trunk. Steroid-induced punctate acne may appear. Furthermore it causes insomnia, increased appetite, bone loss, peptic ulcers, myopathy, psychoses and glaucoma, increased susceptibility to infection and a risk for reactivation of latent tuberculosis (Chrousos *et al.*, 2007; Schimmer *et al.*, 2006).

Nonsteroidal anti-inflammation drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs are drugs with anti-inflammatory effects. Two major groups of NSAIDs are classified on the basic of their selectivity on COX enzyme. There are non-selective COX inhibitors (e.g. indometacin, ibuprofen, naproxen, piroxicam) and the selective COX-2 inhibitors (celecoxib, etoricoxib,).

Mechanism of action of NSAIDs

NSAIDs are inhibitors of the enzyme cyclooxygenase (COX-1 and COX-2) which catalyze arachidonic acid to form prostaglandins and thromboxanes. Prostaglandins act as messenger molecules in the process of inflammation especially PGE_2, PGI_2 and $PGF_{2\alpha}$. Suppression of these inflammatory prostaglandins result in alleviation of pain, fever, and inflammation.

- **Non-selective COX inhibitors**

  Non-selective COX inhibitors inhibit both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 COX-2) enzymes. COX-1 is primarily constitutive isoform found in most normal cell and tissue while COX-2 production is induced by inflammation, shock, tumor etc.

- **Selective COX-2 inhibitors**

Selective COX-2 inhibitors selectively inhibit COX-2 enzyme and prostaglandins production. While COX-1 but not COX-2 is expressed predominantly in gastric epithelial cells and is the major source of cytoprotective prostaglandins formation. Inhibition of COX-1 at this site is account for gastric adverse events. NSAIDs are also known to reduce production of superoxide radicals, inhibit the expression of adhesion molecules, decrease nitric oxide synthase and decrease pro-inflammatory cytokines.

Adverse effects of NSAIDs

1. **Gastrointestinal system**: GI side effect is the most common symptoms associated with these drugs. The adverse effect is account for inhibition of cytoprotective prostaglandins (PGE_2 and PGI_2) which result in abdominal pain, nausea, diarrhea, anorexia, gastric erosions/ulcers, anemia, GI hemorrhage and perforation.
2. **Central nervous system**: Headaches, tinnitus, dizziness, confusion, depression, lowering of seizure threshold, hyperventilation (salicylates).
3. **Renal system**: Salt and water retention, edema, worsening of renal function in renal/cardiac and cirrhotic patients, decreased effectiveness of antihypertensive medications, decreased effectiveness of diuretic medications, decreased urate excretion (especially with aspirin), hyperkalemia.
4. **Platelets**: Inhibited platelet activation.
5. **Uterus** : Prolongation of gestation, inhibit labo.
6. **Cardiovascular system**: Fluid retention, hypertension, congestive heart failure, risk of myocardial infarction are associated with COX-2 inhibitors more frequently than non selective COX inhibitors.

7. **Hematologic system:** Rare thrombocytopenia, neutropenia, or even aplastic anemia (Furst *et al.*, 2006; Barke *et al.*, 2007).

Although a variety of nonsteroidal anti-inflammatory drugs (NSAIDs) are employed for the treatment of inflammatory diseases, the adverse effects of these drugs limited their therapeutic use both for non selective COX inhibitor and selective COX-2 inhibitors.

Research and development of the new drugs in these classes are still in need. The goal for development of new anti-inflammatory drugs are to lessen the adverse effect and to maximize the therapeutic effect. New drugs from plants are interesting source for the scientists.

Glycosmis parva, a plant in Thailand, are composed of different kinds of alkaloids and steroidal compounds . At present there is no report up on its pharmacological actions. However there are several reports are found in the compounds related to constituents found *G. parva* especially β-sitosterol. Thus it is our interest to investigate the chemical constituents of this plant for its effect on the macrophage, which are targeted cells responsible for inflammatory process.

Glycosmis parva Craib

G. parva Craib are plant belonged to the family Rutaceae. It is commonly know as Som-chuen, Prayon-kluean for its local name in Thailand. This plants is evergreen shrubs or undertrees. Leaves alternate, 1-5 foliate. Flowers usually small, axillary panicles, calyx 4 or 5 partial imbricate. Petals 4 or 5 imbricate, stamen 8 to 10 free, filaments dilated below. Ovary 2 to 5 celled, the style very short, not jointed ovule 1 in each cell. Fruits globose, freshy, berry. Seed 1 to 3 oblong, testa membranous.

At least two major groups of compounds are identified from the hexane extraction of the branches and leaves of this plant. They are acridone alkaloids (N-methylataphilline and 5-hydroxy-N-methylseverifoline) and steroidal compounds (β-sitosterol and stigmasterol) (Kongsubsopa 2000). Several new compounds are identified from *G. parva* extracts as demonstrated in Appendix B-31 (Reungrungsi N. and Chansriniyom C.)

Pharmacological effect of compounds related to the chemical constituents found in *G. parva*

1. Antimalarial effect

5-hydroxy-N-methylseverifoline isolated from Citrus, Glycosmis, or Severinia plants (members of the family Rutaceae) have shown antimalarial activities in vitro and in vivo. 5-hydroxy-N-methylseverifoline suppressed 92% of *Plasmodium yoelii* at a concentration of 10 ug/ml in vitro(Fujioka *et al.*, 1989).

2. Antiproliferative effect

5-hydroxy-N-methylseverifoline possesses antiproliferative activity toward monolayers and suspension of several types of cancer cell lines including :human lung carcinoma (A-549), melanin pigment producing mouse melanoma (B-16 melanoma 4A5), T-cell leukemia (CCRF-HSB-2); human gastric cancer cell, and lymph-node metastasized (TGBC11TKB) (Kawaii *et al.*, 1999).

3. Anti-inflammatory effect

β-sitosterol isolated from n-hexane extract of *Euphorbia hirta* reduced ear edema when test in TPA-induced ear model in mice (Vazquez *et al.*, 1999).

β-sitosterol isolated from the root of *Dystaenia takeshimona* showed inhibitory activity of COX-2 by 98.2%and 5-LOX by 77.3% and reduced production of protaglandinD_2(PGD_2) , leukotrienC_4 (LTC_4) in mouse bone marrow- derived mast cells (Kim *et al.*, 2006).

β-sitosterol has been shown to inhibit the oedema in oxazolone-induced contact-delayed-type hypersensitivity model (Prieto *et al.*,2006).

β-sitosterol isolated from *Radix Adenophorae* reduced airway inflammatory and airway hyperresponsiveness (AHR) in murine model of asthma through suppression of IL-13, IL-5, IgE, eosinophils, CCR3 expression (Roh *et al.*, 2008).

β-sitosterol isolated from *Rhus sylvestris* has been reduced secretion of IL-6 and TNF-α in RAW 264.7 macrophage cell line stimulated with LPS (Ding *et al.*, 2009).

Fractionation of the acetone extract of *Sideritis foetens* composed of campesterol 7.6%, stigmasterol 28.4%, β-sitosterol 61.1% decreased carrageenan paw

oedema, inhibition of mouse ear oedema induced by TPA and decreased neutrophil infiltration into inflamed tissue (Navarro *et al.*, 2001).

A mixture of β-sitosterol and stigmasterol were isolated from *Buddlleja globosa* reduced TPA – induced inflammatory in mice by 78.2% at the concentration of 1 mg/20ul/ear (Backhouse *et al.*, 2008).