Chapter 1
INTRODUCTION
1.1. INFLAMMATION

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair (Nathan, 2002). Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury (Vodovotz et al., 2008). The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection (Frank and Fries, 1991; El-Gamal et al., 2010). However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease (Franklin et al., 2008). Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer’s and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever (Lucas et al., 2006; Kassuya et al., 2009).

Inflammation is a complex pathophysiological process mediated by a variety of signalling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α (Kinne et al., 2000; Yu et al., 2010), that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site (White, 1999). In addition, it is broadly accepted that cytokines, produced by either immune or central nervous system cells, might directly sensitize the peripheral nociceptors (Obreja et al., 2002).

Inflammation is an important cellular response triggered by various mechanical, chemical or immunological stress factors and it is regulated by a delicate balance
between local factors that finally determine the outcome of the disease process: progression or resolution. The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger (Huwiler and Pfeilschifter, 2009). This is followed by an anti-inflammatory phase, in which resident tissue cells may acquire the potential for protecting themselves from further activation and injury.

More recently, inflammation was described as “the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality” or “the reaction to injury of the living microcirculation and related tissues” (Spector and Willoughby, 1963). Although, in ancient times inflammation was recognised as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host.

Based on visual observation, the ancients characterised inflammation by five cardinal signs, namely redness (rubor), swelling (tumour), heat (calor; only applicable to the body extremities), pain (dolor) and loss of function (functio laesa). The first four of these signs named by Celsus in ancient Rome (30-38 B.C.) and the last by Galen (A.D. 130-200) (Hurley, 1972).

The classical description of inflammation accounts for the visual changes seen. The sensation of heat is caused by the increased movement of blood through dilated vessels into the environmentally cooled extremities. Redness is due to the additional number of erythrocytes passing through the area. Swelling (edema) is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting of sensory nerves due to oedema. Loss of function refers to either simple loss of mobility in a joint, due to the oedema and pain, or to the replacement of functional cells with scar tissue.
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Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti-inflammatory agents exert their effect through a spectrum of different modes of action (Samuelsson et al., 1978).

Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time (Posadas et al., 2004).

Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Chronic inflammation may also occur due to the persistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti-inflammatory mechanisms.

Chronic (or acute) inflammation is a multiple process mediated by activating inflammatory or immune cells (Lundberg, 2000), among which macrophages play a central role in managing many different immunopathological phenomena including the overproduction of proinflammatory cytokines and inflammatory mediators, generated by activated iNOS and COX-2 (Walsh, 2003). Under inflammatory conditions, immune cells are also stimulated by adhesion molecule activation signals in order to enhance the migration capacity to inflamed tissue and finally to form heterotypic cell clustering between the immune cells, endothelial cells and inflamed cells (Tao et al., 2009).

Macrophages in the inflammatory reaction initially requires an interaction between surface receptors such as Toll-like receptors (TLR) and stimuli (Takeda and Akira, 2001), and subsequent up-regulation of intracellular signalling events mediated by enzymes such as phosphoinositide 3-kinases (PI3K) and mitogen activated protein kinases (MAPKs) as well as transcription factors (e.g., nuclear factor [NF]-κB and
activator protein [AP]-1 (Sekine et al., 2006). Overall, these events lead macrophages to express pro-inflammatory genes such as inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 (Burmester et al., 1997; Bresnihan, 1999). Because large amounts of macrophage-derived inflammatory mediators can cause collateral or severe damage such as septic shock, rheumatoid arthritis and arteriosclerosis (Michaelsson et al., 1995; Stuhlmuller et al., 2000), the effective blockade of these inflammatory responses is an important therapeutic target.

Inflammatory diseases are a major cause of morbidity of the work force throughout the world. These have been called the “King of Human Miseries” (Chatterjee and Pal, 1984). Pain is an objectionable sensory and emotional incident associated with actual or potential tissue inflammation. Pyrexia or fever is caused as a secondary impact of inflammation (Khan et al., 2007). Inflammation, pain and fever are all associated with enhanced production of prostaglandins (Rang et al., 2003). Thus, most anti-inflammatory agents are expected to possess analgesic and antipyretic activity (Tripathi, 2001; Dewanjee et al., 2009).

1.1.1. Analgesia

Pain is an unpleasant subjective experience that is the net effect of a complex interaction of the ascending and descending nervous systems involving biochemical, physiological, psychological, and neocortical processes (Chisholm-Burns et al., 2008). Pain can affect all areas of a person’s life including sleep, thought, emotion, and daily activities. There are several ways to classify pain, but the first distinction usually made is that between acute and chronic pain. Pain is a subjective sensation which cannot be measured objectively, and its intensity is not always a direct reflection of the nociceptive inputs provoking it. Nociceptive inputs which are easily ignored by an individual in one situation may be unbearable in another (Buschmann, 2002).

The management and treatment of pain is probably one of the most common and yet difficult aspects of medicinal practice. Analgesic therapy is domain by two major classes of analgesic drugs; viz. opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as
gastrointestinal disturbance, renal damage (with NSAIDs drugs), etc. (Dahl and Reader, 2000; Bures et al., 2002).

1.1.2. Inflammatory diseases

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions (Palladino et al., 2003; Kang et al., 2008). As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defence mechanisms (Kupper and Fuhlbrigge, 2004) and regulation of these mechanisms is crucial, as inappropriate or misdirected immune activity is implicated in the pathogenesis of a large variety of inflammatory skin disorders. While some of these conditions are easily remedied, treatments for chronic inflammatory diseases such as psoriasis and atopic dermatitis are not 100% successful (Chi et al., 2003). High levels of inflammatory cytokines and reactive oxygen species are proposed to contribute to the pathophysiological mechanisms associated with various inflammatory skin disorders (Trouba et al., 2002).

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, and inflammatory bowel disease are often associated with inflammatory processes (Polya, 2003; Iwalewa et al., 2007). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer’s disease (AD) (Howes and Houghton, 2003).

Rheumatoid arthritis (RA) and osteoarthritis (OA) are frequent and important diseases with complex pathophysiology. There is convincing evidence that cytokines (e.g., IL-1 and TNF), prostaglandins (PG), and nitric oxide (NO) play critical roles in the development and perpetuation of inflammation and cartilage and meniscus damage in rheumatoid arthritis and osteoarthritis.
Obese individuals have high circulating levels of a range of inflammatory markers produced by adipose tissue, including TNF-α, interleukin-1 (IL-1), and IL-6 (Bullo-Bonet et al., 1999; Yudkin et al., 2000). These factors, whose levels can be reduced by weight loss, are likely to contribute to vascular damage in obese individuals.

Since its discovery in the early 1990s, COX-2 has emerged as a major factor in inflammatory reactions in peripheral tissues (Hinz and Brune, 2002). By extension, COX-2 expression in brain has been associated with pro-inflammatory activities, which are thought to be instrumental in the neurodegenerative processes occurring in acute and chronic diseases.

Many malignancies arise in the areas of infection and inflammation (Ebert et al., 2002; Martinez-Maza and Breen, 2002). There is a growing body of evidences that chronic inflammation is strongly associated with incidence of cancer. For example, colon cancer can arise from inflammatory bowel disease such as chronic ulcerative colitis and Crohn’s disease persistent more than 10 years.

1.1.3. Standard drugs for inflammation and side effects

Many steroids, specifically glucocorticoids and Mineralocorticoids reduce inflammation or swelling by binding to corticoid receptors. These drugs are often referred to as corticosteroids. Long-term corticosteroids use has several severe side effects eg. hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, anxiety effects etc. (Donihi et al., 2006).

There are over 50 different NSAIDs available (Chiroli et al., 2003) and they can be divided into different groups based on their chemical structure, pharmacokinetics and selectivity towards Cox-1 or Cox-2 (FitzGerald and Patrono, 2001; Bancos et al., 2009). NSAIDs can be classified (Paul, 2004) broadly into two types based on their chemical structure. Most NSAIDs are carboxylic acids; but a few, most noticeably phenylbutazones, are enolic acids. Carboxylic acid containing drugs include salicylate derivatives (eg. aspirin), carbocyclic and hetrocyclic acid derivatives (eg. indomethacin), fenamic acid derivatives (eg. Ibuprofen, ketoprofen, fenbufen, flurbiprofen, suprofen and naproxen) and phenyl acetic acid derivatives (eg.
diclofenac, aceclofenac, etc.). Enolic acid containing drugs include oxicam
derivatives (eg. piroxicam, tenoxicam and meloxicam) and pyrazoles (eg.
phenylbutazone and oxyphenbutazone). Non acidic group compounds include
nabumenton (Derle, 2006).

Most of the NSAIDs have three major types of action (Vane, 1998):
1) Anti-inflammatory action for treating several conditions including rheumatoid
arthritis, osteoarthritis, musculoskeletal disorders and pericarditis.
2) Analgesia for treating pain of mild to moderate intensity. Their maximum
therapeutic efficiency is much lower than that of the opioids, but they do not cause
dependence.
3) Antipyretic action, which mediates by the release of endogenous pyrogen from
monocytes and macrophages in the presence of infection or inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) typically relieve inflammation and
associated pain by inhibiting cyclooxygenase enzymes involved in the production of
prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by
distinct genes on different chromosomes (Polya, 2003). NSAIDs can cause liver
damage (Purcell et al., 1991), renal failure (Fored et al., 2001), aseptic meningitis
(Nguyen and Juurlink, 2004) and can interfere with bone fracture healing (Wheeler
and Batt, 2005). NSAID use is associated with a high risk of upper gastrointestinal
symptoms and lesions such as oesophagitis, gastritis, peptic ulcers, and their severe
complications including bleeding and perforation (Cryer and Kimmey, 1998) and
results mostly from inhibition of Cox-1 in the gastric mucosa.

Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting
prostaglandins synthesis and/or production (Todd and Sorkin, 1988; Skoutakis et al.,
1988). The drug also affects polymorphonuclear leukocytes function in vitro, thereby
reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical
generation, and neutral protease production (Mahgoub, 2002). Diclofenac has also
been reported to suppress inflammation induced by various phlogistic agents in
experimental animal models (Al-Tuwaijri and Mustafa, 1992). However, it may cause
side effects including gastrointestinal disorders when administered by oral route and
cutaneous lesions by intramuscular injection (Lopes et al., 2006; Suwalsky et al.,
There are several published reports of cases of diclofenac-associated hepatotoxicity (Purcell et al., 1991; Aydin et al., 2003).

Indomethacin is used in the treatment of disorders such as rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. Indomethacin produce its therapeutic and toxic effect by inhibiting prostaglandin synthesis in various tissues (Lione and Scialli, 1995). However long time use of indomethacin causes gastrointestinal complications, including intestinal perforation (Cassady et al., 1989); bronchopulmonary dysplasia and respiratory distress syndrome (Eronen et al., 1994).

Aspirin is the most widely used drug in the world today, because of its ability to act as anti-inflammatory medicine (Serhan et al., 2004; Schwab et al., 2007). However, patients with a history of peptic ulcer or other gastrointestinal disorders, are prone to gastroduodenal lesions on prolonged use of aspirin. The toxicity of aspirin is both dose- and disease-dependent.

Ibuprofen is also a commonly and successful used NSAIDs. However, long term use of ibuprofen, sulindac, phenylbutazone, and piroxicam has been associated with hepatotoxicity (Manoukian and Carson, 1996).

The coxibs like rofecoxib, lumiracoxib, celecoxib and etoricoxib were reported to be associated with reduced gastrointestinal toxicity from the upper gastrointestinal tract when compared to non-selective NSAIDs; however there are also reports that coxibs are associated with serious cardiovascular events (Pilotto et al., 2009) and hepatotoxicity (Alegria et al., 2002).

Based on all these findings, the US Food and Drug Administration (FDA) in 2005 mandated that all NSAIDs should include a warning to highlight the potential increase in the risk of serious cardiovascular events, along with the warning about potential severe life-threatening gastrointestinal events. The same has been delivered by the European Medicines Agency (EMEA) as well as by a large number of national drug agencies all over the world. Thus, a careful evaluation of the risk profiles for adverse events before prescribing non-selective NSAIDs and coxibs is strongly recommended (Layton et al., 2008).
1.1.4. Inflammatory mediators

The inflammatory response is a complex and highly regulated sequence of events that start with an initial production of pro-inflammatory mediators that recruit professional inflammatory cells to the site of injury to clear the offending trigger. Macrophages play major roles in the immune and inflammatory responses involved in host defence. Activated macrophages secrete a number of different inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), reactive oxygen species (ROS), prostaglandin E2 (PGE2), nitric oxide (NO), etc (Kaplanski et al., 2003; Bosca et al., 2005).

1.1.4.1. Cyclooxygenase (COX)

COX is the key enzyme that catalyses the first two steps in the biosynthesis of the prostaglandins (PGs). The COX pathway leads to the generation of prostaglandins and thromboxanes, which mediate the pain and edema associated with inflammation. There are two isoforms of COX: COX-1 and COX-2. COX-1 is detectable, but COX-2 is not detectable in most normal tissues, however, COX-2 can be induced by many factors such as pro-inflammatory cytokines, phlogistic factors, etc. Studies indicated that COX-2 plays an important role in inflammation (Oshima et al., 1996; Shu et al., 2006). Thus, those agents that could suppress the activity or protein expression of COX-2 are likely to be valuable medicine for anti-inflammatory and pain ease. Thus, decreasing of synthesis and activity of COX-2 can result in anti-inflammatory action both in localized and systemic conditions (Salvemini et al., 1993).

1.1.4.2. Prostaglandins

Prostaglandins (PGs) are generated by a variety of cell types, including activated macrophages (Harris et al., 2002). The rate-limiting enzyme in PG synthesis is cyclooxygenase (COX). Prostaglandins are the end products of the metabolism of arachidonic acid by cyclooxygenases (COX) and prostaglandin synthases (PGS), and comprise a series of classical pro-inflammatory mediators like PGD₂, PGE₂, PGF₂α, and PGI₂.
1.1.4.3. Arachidonic acid

The lipoxygenase pathway utilizes arachidonic acid by 5-lipoxygenase to produce the lipoxygenase products e.g. leucotrienes (LTs) which are also involved in inflammatory reactions as pro-inflammatory mediators. Leukotrienes, i.e. LTC4 and LTD4 cause edema together with increased microvascular permeability.

1.1.4.4. Thromboxane

Thromboxane A2 (TXA2) is an arachidonic acid metabolite produced during the catalysis of arachidonic acid by the sequential action of COX and thromboxane synthase (TXS), and is well established as a potent vasoconstrictor. This metabolite participates in various physiological and pathological processes ranging from synaptic transmission to inflammation (Turini and DuBois, 2002). Platelets represent the best known cell type to produce TXA2 in response to various stimuli. However, many other cells and tissues are also able to synthesize TXA2 (Nakahata, 2008).

1.1.4.5. Leukotrienes

Leukotrienes (LT) are end products of the metabolism of arachidonic acid by 5-lipoxygenase. Leukotrienes have physiological roles in innate immune responses and pathological roles in a variety of inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, allergic rhinitis, but most prominently in bronchial asthma (Werz and Steinhilber, 2005).

1.1.4.6. Polyunsaturated fatty acids (PUFA)

Linoleic acid (LA) and α-linolenic acid (ALA) belong to the n−6 (ω−6) and n−3 (ω−3) series of polyunsaturated fatty acids (PUFA). LA and ALA are precursors for the synthesis of higher unsaturated species: arachidonic acid deriving from LA, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) deriving from ALA. One possible mechanistic explanation for these anti-inflammatory and anti-tumorigenic effects may be that an increased consumption of EPA and DHA results in an increased incorporation of these fatty acids into phospholipids at the expense of
arachidonic acid. Consequently, they also replace arachidonic acid as a substrate for COX and LO that results in a reduced formation of PGE2, TXA2, LTB4 and LTE4 (Huwiler and Pfeilschifter, 2009).

1.1.4.7. Histamine

Histamine (HA) is a biogenic amine that affects a variety of functions in the human body. It has been known to play a role in inflammation, gastric acid secretion, and neurotransmission (Passani et al., 2007; Huang and Thurmond, 2008). Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H1R, H2R, H3R, and H4R), all of which are G-protein coupled receptors (GPCRs) (Schneider et al., 2002). Histamine appears to play a complex role in pain modulation. Histamine released from mast cells is an established mediator of acute allergic reactions and chronic inflammation. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 h (Okoli et al., 2007).

1.1.4.8. Nitric oxide (NO)

It is widely known that nitric oxide (NO), synthesized from L-arginine by nitric oxide synthase (NOS), is involved in diverse physiological processes. An excess in NO production is largely thought of as causing a variety of inflammatory diseases, such as sepsis, psoriasis, arthritis, multiple sclerosis, and systemic lupus erythematosus (Clancy et al., 1998).

1.2. INTRODUCTION TO LIVER

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles (Shanmugasundaram and Venkataraman, 2006). Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby
providing protection against foreign substances by detoxifying and eliminating them (Saleem et al., 2010).

Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death (Kaplowitz and Tsukamoto, 1996).

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic (Ajith et al., 2007). Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (Timbrell, 2001). During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress (Kohen and Nyska, 2002) which leads to the hepatic damage.

1.2.1. Liver diseases

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease, drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year (Gupta and Misra, 2006). According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually (Negi et al., 2008).
Depending on the duration of the disease the liver diseases are classified as acute or chronic. If the disease does not exceed six months it is considered as acute liver disorder while diseases of longer duration are classified as chronic.

Acute viral hepatitis and drug reactions account for the majority of cases of acute liver disease. Hepatitis A and B are the commonest causes of viral hepatitis in Europe and hepatitis E is common in India. Hepatitis C is not usually recognised as an acute infection because it rarely causes jaundice at this stage.

Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer (Tessitore and Bollito, 2006; Kohle et al., 2008). Chronic hepatitis or long term intoxication can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis and necrosis, and eventually may progress to hepatoma.

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, schistosomiasis, etc; or chemical factors such as CCl$_4$ administration, alcohol intake, etc. were known to contribute to liver fibrosis. Hepatic fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholestatic and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes and causes apoptosis and/or necrosis (Miyoshi et al., 1999).

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C (Seki et al., 2005; Kitase et al., 2005). In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality (Albano et al., 2005).

Drug/chemical-mediated hepatic injury is the common sign of drug toxicity (Lee, 2003) and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for
withdrawal of drugs from the market (Cullen and Miller, 2006). Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related). Unpredictable reactions, also referred to as idiosyncratic, can be viewed as either immune-mediated hypersensitivity or nonimmune reactions. Most potent predictable hepatotoxins are recognized in the animal testing or clinical phase of drug development.

1.2.2. Drugs for liver diseases

The liver is quantitatively the most important site of drug metabolism. However, many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects.

Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically. Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications (Seeff et al., 2001). Developing pharmacologically effective agents from natural products has become a new trend by virtue of their little toxicity or few side effects. There are few plant derived drugs in the market which are used for the liver disorders.

1.2.2.1. Silymarin

Silymarin, derived from the seeds of *Silybum marianum* L. (Family: Asteraceae or Compositae), commonly known as milk thistle, has been used for centuries as a natural remedy for liver and biliary tract diseases (Saller et al., 2001). Milk thistle protects and regenerates the liver in most liver diseases such as cirrhosis, jaundice, and hepatitis (Flora et al., 1998). Silymarin offers good protection in various models of experimental liver disease. It acts by antioxidative, antilipid peroxidative (Pascual et al., 1993), antifibrotic (Mourelle et al., 1989), membrane stabilizing, immunomodulatory and liver regenerating mechanisms (Pradhan and Girish, 2006).
Limitations

Silymarin is insoluble in water and typically administered as a sugar coated tablet (Thakur, 2002) or as an encapsulated standardized extract. The absorption by oral route is as low as 2-3 percent of the silybin recovered from rat bile in 24 h. About 20-40 percent of the administered dose of silymarin is excreted in bile as sulphates and glucuronide conjugates in human beings (Saller et al., 2001).

Side Effects

Silymarin has low level of toxicity. Although, silymarin has a good safety record, there are few reports of gastrointestinal disturbances and allergic skin rashes (Negi et al., 2008).

1.2.2.2. Liv-52

Liv-52 was introduced in 1954 as a specially formulated Ayurvedic herbal remedy for the treatment of viral hepatitis and has been widely prescribed for infective hepatitis since then (Mukerjee and Dasgupta, 1970). Experimentally, Liv-52 prevented injurious effects of carbon tetrachloride and other toxic substances on the liver.

Liv.52 is available as tablets and syrup containing the following herbs: Capparis spinosa; Cichorium intybus; Solanum nigrum; Terminalia arjuna; Cassia occidentalis, Achillea millefolium; Tamarix galica and Phyllanthus amarus. These herbs are processed and formulated according to the principles of Ayurveda, which are aimed at enhancing efficacy and avoiding toxicity (Charak and vimanasthan, 1981).

1.2.3. Hepatotoxicant

Liver toxicity mainly occurs due to alcohol, viral and induced by drugs. The third factor for the cause of acute liver disease is the use of drugs like paracetamol, pain killers and antibiotics.
1.2.3.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are the centrepiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. Non-steroidal anti-inflammatory drugs (NSAIDs), which are often used for the relief of non-specific fever (Radwan, 2000), continue to be important for the palliation of pain. They are the most frequently used medications for the treatment of a variety of common chronic and acute inflammatory conditions (Manoukian and Carson, 1996), and continue to be important for the palliation of pain and in decreasing inflammation and fever (Skoutakis et al., 1988; McGettigan and Henry, 2000).

Nearly all of the NSAIDs have been implicated in causing liver injury (Rabinovitz and Van Thiel, 1992). Diclofenac, and particularly sulindac, are reported to be more commonly associated with hepatotoxicity (Bjorkman, 1998). Several NSAIDs have been withdrawn from clinical use because of associated hepatotoxicity (Rabkin et al., 1999). The new more selective COX-2 inhibitors (e.g. celecoxib, rofecoxib, nimesulide) are also associated with hepatotoxicity (Merlani et al., 2001). Hepatotoxicity from NSAIDs can occur at any time after drug administration, but like most adverse drug reactions, most commonly occurs within 6–12 weeks of initiation of therapy (Aithal and Day, 1999).

There are two main clinical patterns of hepatotoxicity due to NSAIDs (Rabinovitz and Van Thiel, 1992; Aithal and Day, 1999). The first is an acute hepatitis with jaundice, fever, nausea, greatly elevated transaminases and sometimes eosinophilia. The alternative pattern is with serological and histological (periportal inflammation with plasma and lymphocyte infiltration and fibrosis extending into the lobule) features of chronic active hepatitis. Some of the NSAIDs which causes liver damage are listed below.

1.2.3.1.1. Diclofenac

Diclofenac sodium has antipyretic, analgesic and anti-inflammatory effects, is an inhibitor of cyclooxygenase enzyme. Like other nonsteroidal anti-inflammatory drugs,
clinical use of diclofenac has been associated with a small but significant incidence of hepatotoxicity, ranging from mild, asymptomatic, reversible increases in liver function tests to jaundice and hepatitis, including several reports of fatal hepatitis (Purcell et al., 1991; Banks et al., 1995).

In many cases, the clinical and biochemical features of diclofenac hepatotoxicity suggest the involvement of reactive or toxic metabolites. These products presumably were formed via the hepatic cytochrome P$_{450}$ (CYP)-catalyzed oxidation of diclofenac to reactive benzoquinone imines that are trapped by GSH (glutathione) conjugation. It is, therefore, possible that reactive benzoquinone imines may be formed and contribute to diclofenac mediated hepatic injury (Tang et al., 1999).

1.2.3.1.2. Sulindac

Liver injury from sulindac appears within a few days to six weeks after therapy is initiated. Fever, rash, eosinophilia, and edema are frequently found in association with evidence of liver injury. It is considered as one of the most likely NSAIDs to produce hepatic injury (Garcia Rodriguez et al., 1992).

1.2.3.1.3. Nimesulide

The anti-inflammatory drug, nimesulide is a selective COX-2 inhibitor, with only residual activity against COX-1 (Giuliano et al., 2001). It is almost exclusively metabolized and cleared by the liver (Chatterjee and Sil, 2007). The drug can cause several types of liver damage, ranging from mild abnormal function such as increase in serum amino transferase activity to severe organ injuries such as hepatocellular necrosis or intrahepatic cholestasis (Lucena et al., 2001).

1.2.3.1.4. Bromfenac

This acetic acid derivative was introduced in 1997 as a non-narcotic analgesic of the phenyl acetate class for short-term pain relief, but was removed from the market in 1998 owing to several instances of fulminant hepatic failure (FHF) leading to death or transplant that occurred after prolonged administration (Goldkind and Laine, 2006).
1.2.3.1.5. Indomethacin

Indomethacin has produced hepatocellular necrosis, sometimes accompanied by microvesicular steatosis and striking cholestasis (Fenech et al., 1967); children are more vulnerable and the drug is not recommended in the pediatric age group based on several deaths involving hepatocellular necrosis (Boardman and Hart, 1967).

1.2.3.1.6. Ibuprofen

Ibuprofen was withdrawn from use in the 1960s because of fatal hepatocellular injury.

1.2.3.2. Alcohol

Alcohol administration causes accumulation of reactive oxygen species, which in turn causes lipid peroxidation of cellular membranes and proteins and DNA oxidation resulting in hepatocyte injury (Zhou et al., 2002). Alcohol treatment of rats is known to cause the translocation of fat from the peripheral adipose tissue to liver, kidney and brain for accumulation (Nadro et al., 2006).

1.2.3.3. Carbon tetrachloride (CCl₄)

Many compounds including clinically useful drugs can cause cellular damage through metabolic activation of the compound to highly reactive substances such as free radicals. One such toxicant is carbon tetrachloride (CCl₄); the hepatotoxicity of CCl₄ is attributed to the formation of trichloromethyl and trichloromethyl peroxyl radicals, initiating lipid peroxidation and resulting in fibrosis and cell necrosis (Kadiiska et al., 2000). Long-term administration of CCl₄ causes chronic liver injury (Hernandez-Munoz et al., 1990).

1.2.3.4. Acetaminophen

Acetaminophen (APAP) has been widely used as a medicine for pain and fever relief (Whitcomb, 1994). It is commonly considered as a “safe drug” when taken within the suggested therapeutic dose. However, APAP can be hepatotoxic when an
overdose is administered. Clinically, APAP has been demonstrated to be nephrotoxic and hepatotoxic (Bonkovsky et al. 1994).

1.2.3.5. Galactosamine

Galactosamine (GalN) has been proposed to be hepatotoxic due to its ability to destruct liver cells (Anandan et al., 1999). Its toxicity is of clinical importance because there is a close resemblance between the multifocal necrosis produced by d-GalN and the lesion of viral hepatitis in humans. This amino sugar is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis within 8 h after administration (Ravikumar et al., 2005).

“The burden of disease” is an all encompassing term that captures not only the frequency (such as the incidence and prevalence) of the disease but also reflects how the disease impacts other aspects of the health of a population. These include negative impact of disease on longevity (such as pre-mature death and years of lost life), morbidity (pain and impaired health related quality of life), and economic consequences of the disease (such as direct health care expenditures in caring for the disease and indirect costs related to lost income from premature death or disability). Therefore, one needs to take all these aspects into account to understand the true magnitude of a disease’s burden. Such understanding is also essential in formulating health care policies to prioritize health interventions and to allocate scarce resources across a range of medical diseases. For example expensive interventions (e.g., screening to detect early disease, treatment) will add cost and therefore may increase the overall disease burden, however these interventions may actually reduce the overall disease burden by prolonging life, and improving quality of life (Kanwal and El-Serag, 2009).

1.3. HERBAL MEDICINE

India is a rich source of medicinal plants and a number of plant derived extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China (Xia dynasty) and India (Vedic period). The first written reports date back to
600 B.C. with the Charaka Samhita of India and the early notes of the Eastern Zhou dynasty of China that became systematized around 400 B.C. The recipes, once formulated, were usually expanded rather than abandoned during subsequent centuries. Expansion was stimulated by a growing understanding of the natural evolution of frequently encountered diseases and by emerging hypotheses regarding their causes (Schuppan et al., 1999).

The use of medicinal plants in curing diseases is as old as man (Aibinu et al., 2007). The World Health organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. These plants which are found in our environment enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine (Akah and Nwabie., 1994). Since ancient times of civilization, people have been relying on plants as either prophylactic or therapeutically arsenal to restore and maintain health, and plants are well known as an important source of many biologically active compounds.

Plant derived natural products such as flavonoids, terpenes and alkaloids (Witherup et al., 1990; Shukla et al., 2010) have received considerable attention due to their diverse pharmacological properties including inflammatory, antipyretic and analgesic activities. Consumption of natural products reduce the risk of developing pathological conditions, including cancer, nervous system disorders, cardiovascular, genetic, and inflammatory diseases (Jurenka, 2009; Newman and Cragg, 2007). Plants contain numerous bioactive molecules that can improve the body’s resistance to cellular stress and prevent the cytotoxicity of various agents.

Natural products and their derivatives have traditionally been the most common sources of drugs, and still represent a fairly large percentage of the pharmaceutical market (Kirkpatrick, 2002). It has long been recognised that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead structures for drug discovery (Okoye and Osadebe, 2010).
Plants are a rich source of active ingredients for health care products, with many blockbuster drugs being directly or indirectly derived from plants (Newman et al., 2000). Examples of some drugs are shown in table 1. However, many high value plant-derived natural products remain undiscovered or unexplored for their pharmacological activity (Raskin et al., 2002).

India has been identified as one of the top twelve mega bio-diversity center of the world. This is because India has a vast area with wide variation in climate, soil, altitude and latitude (Tiwari, 2008). India is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Parekh et al., 2005). India with its biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc (Tiwari, 2008). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Manna and Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive (Parekh and Chanda, 2007a).

Due to the known side effects of approved pharmaceuticals, patients often turn to alternative medicine which is considered “natural” and “healthy”. Herbal medicine is thus gaining popularity, but lack of knowledge of the mechanisms and side effects of these preparations as well of safety regulations for their preparation may have serious consequences (Boullata and Nace, 2000).
Table 1: Plant derived drugs, with their clinical uses and sources.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Action or clinical use</th>
<th>Plant source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromelain</td>
<td>Anti-inflammatory; proteolytic agent</td>
<td>Ananas comosus (L.) Merrill</td>
</tr>
<tr>
<td>Codeine</td>
<td>Analgesic</td>
<td>Papaver somniferum L.</td>
</tr>
<tr>
<td>Colchicine</td>
<td>Antitumor agent; antigout</td>
<td>Colchicum autumnale L.</td>
</tr>
<tr>
<td>Danthron</td>
<td>Laxative</td>
<td>Cassia spp.</td>
</tr>
<tr>
<td>Digitalin</td>
<td>Cardiotonic</td>
<td>Digitalis purpurea L.</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Cardiotonic</td>
<td>Digitalis lanata Ehrh.</td>
</tr>
<tr>
<td>Quinine</td>
<td>Antimalarial</td>
<td>Cinchona ledgeriana Moens ex. Trimen</td>
</tr>
<tr>
<td>Salicin</td>
<td>Analgesic</td>
<td>Salix alba L.</td>
</tr>
<tr>
<td>Silymarin</td>
<td>Antihepatotoxic</td>
<td>Silybum marianum (L.) Gaertn.</td>
</tr>
<tr>
<td>Taxol</td>
<td>Antitumor</td>
<td>Taxus brevifolia Nutt.</td>
</tr>
</tbody>
</table>

1.3.1. Standardization of herbal medicine

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. According to WHO, 80% of the rural population in developing countries depend on traditional medicines to meet their primary health care needs (Bannerman et al., 1993). Authentication and standardization are prerequisite steps while considering source materials for herbal formulation in any system of medicine (Ahmad et al., 2009).

In traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. (Kamboj 2000). It is very important that a system of standardization is established for every plant medicine in the market because the
scope for variation in different batches of medicine is enormous. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (Raina, 2003). Some of the standardization test for herbal medicines are listed below (Ritch, 2000).

**Macro and microscopic examination:** For identification of right variety and search of adulterants.

**Foreign organic matter:** Remove of matter other than source plant to get the drug in pure form.

**Ash values:** It is criteria to judge the identity and purity of crude drug – Total ash, sulfated ash, water soluble ash and acid insoluble ash etc.

**Moisture content:** To check moisture content is helps in prevent degradation of product.

**Extractive values:** These are indicating the approximate measure of chemical constituents of crude drug.

**Crude fiber:** To determine excessive woody material criteria for judging purity.

**Qualitative chemical evaluation:** It covers identification and characterization of crude drug with respect to phytochemicals constituent.

**Quantitative chemical evaluation:** To estimate amount the major class of constituents.

**Toxicological studies:** Pesticide residue, potentially toxic elements, and microbial count approach to minimize their effect in final product.

### 1.3.2. Toxicological aspects of herbal medicine

Phytotherapy has never stopped gaining in popularity. In low and middle income countries, it often represents the main, if not, only therapeutic system to which majority of people are referred to for their primary health care (WHO, 2007; Mukinda and Eagles, 2010). Its widespread use is further substantiated by the affordability, knowledge of medicinal plants and the belief that they are harmless (Springfield et al., 2005), since these treatments are “natural” and commonly used for self-medication
without supervision. Although medicinal plants may cause several biological activities in humans, very little is known regarding the potential toxicity for many of these bioactive substances. The increase in number of users as oppose to the scarcity of scientific evidences on the safety of the medicinal plants have raised concerns regarding toxicity and detrimental effects of these remedies (Saad et al., 2006). Because they are considered natural and are available without a prescription, many users ignore the potential for toxicity (Larrey, 1997). Many herbal remedies have not been submitted to rigorous scientific testing and are largely justified by prescribers via trial-and-error experience. Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD$_{50}$/ED$_{50}$) of drugs and xenobiotics (Rang et al., 2001; Maikai et al., 2008).

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in large numbers as analgesics and antipyretics. But they are having many side effects in the body organs. Diclofenac, is one of the non-steroidal anti-inflammatory drugs, and widely used in treatment of several rheumatic diseases, and as an analgesic and anti-inflammatory agent. There are many reports that long term use of diclofenac has toxic effects to the liver as well as other organs (Sallustio and Holbrook, 2001). Natural products/plant derived extracts has great capacity to overcome this problem. Therefore, the prime objective of the present study is to select a medicinal plant which can be used as anti-inflammatory agent as well as in hepatic disorders/damage; and may not have side effects to the other organs of the body.

1.4. SELECTION OF THE PLANT FOR PRESENT STUDY

When selecting a plant for pharmacological activities, four basics methods are usually followed (Suffness and Douros, 1979):

a) Random choice of plant species
b) Choice based on ethnomedical use
c) Follow up of existing literature on the use of the species
d) Chemotaxonomic approaches

Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Based on the second and third
approach, selection of the plant has been made in the present work. In light of the above context, *Woodfordia fruticosa* Kurz. flowers were selected for the study.

1.4.1. *Woodfordia fruticosa*

*Woodfordia fruticosa* Kurz. (syn. *Woodfordia floribunda* Salisb.) belongs to the family Lythraceae, is a much branched beautiful shrub, 1-3 m high. It is the plant of tropical and subtropical regions with a long history of medicinal use. English names of the plant are Fire Flame Bush and Shiranjitea. The plant is abundantly present throughout India, ascending up to an altitude of about 1500 m, and also in a majority of the countries of South East and Far East Asia like Malaysia, Indonesia, Sri Lanka, China, Japan and Pakistan as well as Tropical Africa (Kirtikar and Basu, 1935). The original Sanskrit name Agnijwala or Tamra-pushpi appears to be derived from the bright red colour of the flower and the bark. Locally (In Gujarat) it is known as Dhavdi (Shome et al., 1981; Khare, 2004). The bark of the plant, characteristically cinnamon-brown coloured and smooth, peels off in fibres and the young shoots are terete, often clothed with fine white pubescence. The leaves are 1.5-13 x 0.8-4 cm, opposite or sub-opposite, decussate, sometimes in whorls of 3, sessile. Flowers are brilliant red.

Considering the above, the objectives set forth are:

- Review of Literature for;
  - Reported anti-inflammatory activity of some medicinal plants
  - Reported hepatoprotective activity of some medicinal plants
  - *Woodfordia fruticosa* and their reported activities
- Pharmacognostic study of *Woodfordia fruticosa* flowers
- Physicochemical study of *Woodfordia fruticosa* flowers
- Phytochemical study of *Woodfordia fruticosa* flowers
- Anti-inflammatory study of *Woodfordia fruticosa* flowers
- Hepatoprotective study of *Woodfordia fruticosa* flowers
- Toxicity study of *Woodfordia fruticosa* flowers