CHAPTER - 2

LITERATURE REVIEW

2.1 Inflammation

Inflammation, a defensive reaction of tissues to infection or injury and is characterized by swelling, heat, redness and pain (Coussens and Werb, 2002). Inflammation is triggered by the release of several chemical mediators from injured tissues as well as from migrating cells. The specific chemical mediators may vary with the type of inflammatory process and include histamine (amines), serotonin, lipids such as prostaglandins and small peptides such as kinins (Patil et al., 2012). There are various components of an inflammatory reaction that can contribute to the associated symptoms include edema formation, leukocyte infiltration and granuloma formation which represents typical features of inflammation process (Hajhashemi et al., 2009; Nualkaew et al., 2009).

The inflammatory responses could be divided into two categories: acute and chronic. Acute inflammation is a short-lasting (i.e. few days) immunological process that in most cases is beneficial to the host due to the clearance of the pathogenic stimulation by the activated innate and adaptive immune cells. In contrast, chronic inflammation is an inflammatory response of prolonged duration (i.e. weeks, months, or even indefinite) whose extended time course is provoked by the persistence of the causative inflammatory stimulus (Tzianabos et al., 2002). Chronic inflammation could lead to cellular proliferation – a process that in and of itself increased the risk for aberrant cell proliferation and, ultimately, development of cancer (Grivennikov et al, 2010).
addition, there are many diseases characterized by a chronic inflammatory pathological process in which the underlying cause remains unknown, as in IBD’s such as ulcerative colitis and Crohn’s disease (Bouma and Strober, 2003).

2.2 Inflammatory bowel disease

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn’s disease, is a chronic intestinal disorder resultant from a dysfunctional epithelial, innate and adaptive immune response to intestinal microorganisms. The pathogenesis of IBD remain unclear, but imbalance between proinflammatory cytokines, such as, tumor necrosis factor-α (TNF-α), Interleukins (IL-1, IL-6 and IL-12), Interferon-γ (IFN-γ) and anti-inflammatory cytokines such as IL-4, IL-10 and IL-11 are believed to play a central role in modulating the inflammation process (Ardizzone and Porro, 2005). Its clinical course is unpredictable and presents remissions and exacerbation, characterized by rectal bleeding and diarrhoea leading to disruption of the epithelial barrier, and the formation of epithelial ulceration. The different responses to transient intestinal injury in genetically susceptible versus genetically resistant hosts were shown in Figure.2.1.

The development of an abnormal immune and inflammatory response occurs, which is mediated predominantly by activated neutrophils, monocytes and macrophages and characterized by an enhanced formation of reactive oxygen and nitrogen species (Martin et al., 2006). Furthermore, these can activate diverse signaling pathways which lead to the activation of transcription factors such as nuclear factor kappa (NF-κb) or activator protein-1 (AP-1), modulating a number of steps in the inflammatory cascade. These include production of pro-inflammatory cytokines as tumor necrosis factor alpha (TNF-α), Interleukin (IL)-1, Interferon (INF)-γ, IL-12 and IL-6 in different cell-types, degranulation of
neutrophils, as well as the expression of inflammatory mediators include inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 (Collino et al., 2006; Pecchi et al., 2009). Therefore, the imbalance between pro-inflammatory and anti-inflammatory cytokines and inflammatory proteins expression including COX-2 and iNOS, which are expressed as an early response to pro-inflammatory mediators and mitogen stimuli, plays an important role in the pathophysiology of this disease (Talero et al., 2008). Moreover, recent studies have reported that activation of mitogen-activated protein kinases (MAPKs) such as c-Jun N-terminal kinase (JNK) plays an important role in the intestinal inflammation in patients with IBD (Roy et al., 2008).

**Figure 2.1** Different responses to transient intestinal injury in *genetically susceptible* versus genetically resistant hosts
2.3 Ulcerative Colitis

UC (also termed as Colitis ulcerosa) is a form of Inflammatory Bowel Disease, specifically the large intestine or colon will be affected. The main symptom of this active disease is diarrhea mixed with blood, of gradual onset. UC has similarities to CD, another form of IBD. The anatomic distribution of UC is depicted in Figure 2.2. The disease is more prevalent in northern countries of the world, as well as in northern areas of individual countries or other regions (Rutter et al., 2004). Although UC has no known cause, there is a presumed genetic component to susceptibility. The disease may be triggered in a susceptible person by environmental factors. Treatment with anti-inflammatory drugs, immunosuppression, and biological therapy targeting specific components of the immune response is used. Colectomy (partial or total removal of the large bowel through surgery) is occasionally necessary, and is considered to be a cure for the disease.

Figure 2.2 Anatomic distribution of Ulcerative colitis
2.4 Oxidative stress and Ulcerative colitis associated carcinogenesis

Oxidative stress develops during inflammatory reactions because of the inflammatory cells, activated macrophages and neutrophils which produce a large amount of free radicals. It is well-known that, oxidative stress in the inflamed tissue can pave the way for malignant tumors, and that it is a major pathogenetic factor for the well-established correlation between inflammatory diseases and cancer. Oxidative stress has long been associated with the pathogenesis of chronic inflammatory bowel disease (IBD)-related colorectal cancer. Both Ulcerative Colitis and Crohn’s disease could lead to colorectal cancer if it persists for long duration. The risk of developing colorectal cancer from UC is estimated as 2.5% after 20 years, 7.6% after 30 years, and 10.8% after 40 years (Rutter et al., 2004). The pathogenesis of UC and CD is of multifactorial nature as genetic and immunologic factors, alterations in the colonic barrier function, altered colonic microflora, and, furthermore, nutrition and psychosocial factors are also involved.

The activated inflammatory cells will induce oxidant-generating enzymes, e.g. NADPH oxidase, myeloperoxidase and inducible nitric oxide synthase and this leads to production of high concentration of several ROS and RNS (Toullec et al., 2010). Low concentrations of hydrogen peroxide can stimulate cellular proliferation and survival in different tumor cells. Furthermore, ROS can activate the MAPK signaling pathway leads to cell proliferation and also involved in promoting vascular angiogenesis. ROS can also increase blood supply by vasodilation and by elevating the concentration of inducible nitric oxide (iNOS), which produces the vasodilator nitric oxide (NO) (Visconti and Grieco, 2009). The proposed role of oxidative stress and increased cellular turnover in ulcerative colitis associated carcinogenesis is represented in Figure.2.3.
2.5 Mediators of inflammation and cancer

The complex biological process of inflammation is usually tightly regulated: one mediator initiates and maintains inflammation, whereas another one shuts down the process. During chronic inflammatory condition, an imbalance between these two mediators occurs, resulting in cellular or tissue damage. A wide variety of mediators are known to activate or regulate the inflammatory pathways as well as inflammation associated carcinogenesis pathways which includes cytokines, interleukins, tumor necrosis factor-alpha (TNF-α), cyclooxygenase (COX-2), prostaglandins, chemokines, inducible nitric oxide synthase (iNOS) and nitric oxide (NO). Some of the important inflammatory pathways involved in chronic disease were shown in Figure 2.4 (Reuter et al., 2010). The inflammation induced altered signaling pathways...
contributing to carcinogenesis is depicted in Figure 2.5. Mechanisms by which inflammation contributes to process of carcinogenesis include:

(a) induction of chromosomal instability,
(b) alterations in epigenetic events & inappropriate gene expression,
(c) enhancement of cell proliferation,
(d) evasion from apoptosis,
(e) Stimulation of intratumoral neovascularization,
(f) invasion through tumor-associated basement membrane,
(g) stirring up the metastatic movement (Reuter et al., 2010).

**Figure 2.4** Some of inflammation regulatory cell signaling pathways involved in chronic disease
Figure 2.5 Overview of Inflammation induced altered signaling pathways contribute to carcinogenesis (Modified from Kundu et al., 2012)

2.6 Cancer and its prognosis

Cancer (malignant neoplasm) is defined by uncontrolled growth and spread of cells that may affect almost any tissues. Researchers divide the causes of cancer into two groups: first is cancers due to environmental cause and another one due to hereditary genetic cause. Common environmental factors that leading to cancer include: diet, obesity and tobacco, infections, radiation, environmental pollutants and lack of physical activity (Anand et al, 2008). Carcinogenesis is a multi-step process by which the cancer cells transforms from normal cells as shown in Figure.2.6. Cell reproduction is an extremely complex process that is
firmly regulated by various classes of genes, including oncogenes and tumor suppressor genes. Hereditary or acquired abnormalities in these regulatory genes can lead to the development of cancer (Nguven et al., 2009).

**Figure 2.6** The multi-step process of carcinogenesis

### 2.7 Tumor metastasis

Metastasis, the spread of cancer (malignant) cells from a primary tumor to distant sites, poses the biggest problem in the treatment of cancer and is the main cause of death of cancer patients. It occurs in a series of discrete steps, which have been modeled into a process termed as “metastatic cascade”. The understanding of metastasis has advanced tremendously in the last two decades (Eccles and Welch, 2007). The classical view on the metastatic cascade, starting from a primary, epithelial, neoplastic lesion includes:

1. EMT and breach of the basement membrane barrier;
2. dissociation of tumor cells from the bulk tumor;
3. invasion of the neighboring tissue;
4. Intravasation into pre-existing and newly formed blood and lymph vessels;
5. transport through vessels;
6. extravasation from vessels;
7. Establishment of disseminated cells (which can stay dormant for a prolonged period of time) at a secondary anatomical site;
8. outgrowth of micrometastases and macrometastases/secondary tumors.

The overview of the different steps of metastasis were depicted in Figure.2.7. When the area of cancer cells at the originating site is clinically detectable, it is termed as primary tumor. Some cancer cells also acquire the ability to penetrate and infiltrate surrounding normal tissues in the local area and form a new tumor. The newly formed "daughter" tumor in the adjacent site within the tissue is called a local metastasis (Gupta and Massague, 2006, Hanahan and Weinberg, 2000; Pantel and brakenhoff, 2004).

![Figure 2.7 Overview of different steps involved in metastatic cascade](image)

**Figure 2.7** Overview of different steps involved in metastatic cascade
2.8 Immunomodulation

The process that can alter the immune system of an organism by interfering with its function and enhancing or suppressing the immune reaction is called as Immunomodulation. The agents which stimulates or suppress the immune cells is known as immunomodulators. The drug enhancing the immune system is named as an immunostimulative drug which primarily implies stimulation of non-specific system, that is, granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances (Vinothapooshan et al., 2011). The process is known as immunostimulation. Immunomodulatory agents may selectively activate cell mediated or humoral immunity. The primary target of the immunomodulatory compounds is believed to be the macrophages, which plays a key role in the generation of an immune response. The immune system is known to be involved in the patho-physiologic mechanism of many diseases (Tatiya et al., 2007).

Immunosuppressants suppress the immune response and could be used for the control of pathological immune response in auto immune diseases (Vinothapooshan et al., 2011, Archana et al., 2011). The major drawbacks of current cancer therapeutic practices such as chemotherapy and radiation therapy include mucosal ulceration, alopecia, pulmonary fibrosis, cardiac toxicity, hepatic toxicity and bone marrow suppression resulting in cytopenia (Devasagayam and Sainis, 2002). Drugs that could alleviate these side effects will be useful as an adjuvant in cancer therapy (Siveen and Kuttan, 2010).

Many chemicals as well as natural plant products are used to enhance or suppress the immune system respective to the illness. In addition natural adjuvants, synthetic agents, antibody agents, antibody reagents are used as immunomodulatory agents. Nevertheless these are major limitations to the
general use of these agents such as increased risk of infection and generalized effect throughout the immune system (Makare et al., 2001). Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases especially when host defense mechanism has to be acquired under the conditioned immune responsiveness (Lily et al., 2005). Use of medicinal plant products for treatment of various acute and chronic diseases is gaining increasing importance around the globe (Singh et al., 2006; Carrasco et al., 2009; Latorre et al., 2009; Chen et al., 2009).

2.9 Treatment strategy of cancer

The best strategy for struggling with cancer is prevention - i.e., making changes in life-styles (smoking and poor diet) to reduce cancer risk. Current strategy for treating cancer involves surgery, radiation or drugs either single or in combination (Stoppler, 2009).

a) Surgery:

Surgical treatment involves excision of tumor, the most frequently employed form of tumor therapy worldwide. In recent years, surgery combined with other treatment approach such as chemotherapy and radiation therapy has enhanced the effectiveness of surgical treatment. Meanwhile the side effects of the surgery depends up on the location of the tumor, patient's health, type of operation and other factors.

b) Radiation therapy:

Radiation therapy involves the exposure of body to ionizing radiations like x-rays and γ-rays to selectively target the cancer tissue. Different types of radiation therapy include external beam radiation therapy (Eg. X-ray tubes, cobalt gamma rays and linear accelerators), brachytherapy (caesium-137, iodine-
125, or iridium-192) and radiopharmaceuticals that target specific tissues. The potential toxic side effects of the radiation therapy include inflammation of the esophagus that leads to dysphasia, bone marrow suppression, radiation injury to lung may produce pneumonitis, pulmonary fibrosis causing hypoxia and dyspnea, cataract (if eyes are irradiated), infertility or sexual dysfunction and skin changes. Since radiation is itself capable of causing cancer, secondary malignancies (e.g., leukemia, thyroid cancer) may be induced by radiation therapy.

c) **Chemotherapy:**

Chemoprevention is a relatively new approach with a concept of using naturally occurring or synthetic agents that slow down the progression or inhibit the process of carcinogenesis, thereby lowering the risk of developing clinically significant disease. Mostly chemopreventive agents suppress the progression of premalignant cells are believed to do so by modulating cell proliferation and/or differentiation for example inhibitors of cyclooxygenase-2 and anti-estrogens (for example Tamoxifen) (Hong and Sporn et al., 1997). But these agents has been suggested to be administered chronically (prolonged usage) to individuals with an increased risk of developing cancer. In this modality even minor adverse side effects would be unacceptable (Wattenberg, 1995). Like radiotherapy, chemotherapy can injure normal tissues especially tissues that contain cells that divide frequently such as bone marrow, gastro intestinal tract, hair follicles, and gonads. The common side effects of chemotherapy include vomiting, nausea, suppression of white blood cells and production of platelets (myelosuppression), hair loss, diarrhea and decreased spermatogenesis/ovarian follicle formation (Hail, 2005).

Cyclophosphamide (CTX) is one of the most frequently used alkylating anti-neoplastic drug for the treatment of many cancers. The most
severe dose-limiting toxicity of CTX is fulminant, usually of pain. Other toxic side effects of CTX are hemorrhagic cystitis, hematopoietic depression, gonadal dysfunction, nausea, alopecia, gastrointestinal toxicity, renal toxicity and anti-diuresis (Slavin et al., 1975). Alkylating agents have a common property of dissociating a positive charged electrophilic alkyl group, capable of attacking negatively charged electron rich, nucleophilic sites on most of the biological molecules and also the first compounds among others to be identified as useful in cancer chemotherapy. Initial activation reaction of CTX is carried out by microsomal oxidation system in liver produces 4-hydroxy CTX, a cytotoxic metabolite, which diffuses from hepatocytes into plasma and distributed throughout the body. 4-hydroxy CTX is then further converted to other cytotoxic metabolites such as acrolein and phosphoramide mustard (Grochow, 1996).

2.10 Oxidative stress and antioxidant system

Oxidative stress may be defined as the imbalance between free radical generation and antioxidant defenses. Free radical is an atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, which is capable of independent existence. Free radical species are easily formed, highly reactive and unstable, only becoming stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule and thus causing a cascade of damage which leads to disease (Sies et al., 1997). The most common ROS include: the superoxide anion (O_2^-), the hydroxyl radical (OH^-), singlet oxygen (^1O_2) and hydrogen peroxide (H_2O_2).

The cell membrane is one of the most susceptible sites to ROS damage. Free radicals can react with fatty acids present in the cell membranes and form lipid peroxides. Accumulation of these lipid peroxides can lead to production of carcinogenic agents like melonaldehyde. Cell membrane damage
via lipid peroxidation can permanently impair fluidity and elasticity of the membrane, which leads to rupture of cells. Another main target for free radicals attack is proteins. Overproduced free radicals can react with proteins to oxidize and then cross-link them. Radical-protein reactions can impair the function of important cellular and extracellular proteins like enzymes and connective tissue proteins permanently. DNA is also highly susceptible to free radical attacks. An oxygen radical interaction with DNA can break its strands or delete a base. This DNA damage can be a lethal event for an organism (Kehrer et al., 1993).

Cellular redox balance is maintained by a powerful antioxidant system that “neutralizes” ROS. It consists of SOD, catalase, the glutathione system (glutathione, glutathione reductase, peroxidase and transferase), the thioredoxin system (thioredoxins, thioredoxin peroxidase and peroxiredoxins), vitamin E and C. It is generally accepted that glutathione plays a central role in maintaining redox homeostasis. Reduced glutathione (GSH) has a multifaceted role in the antioxidant defense mechanisms. It acts as a direct scavenger of ROS by reacting with singlet oxygen, hydroxyl radicals and superoxide radicals, it is a co-substrate for peroxide detoxification by glutathione peroxidases, for conjugation by glutathione-S-transferases, can reduce protein disulfides and regulate the thiol/disulfide status of the cell through disulfide exchange reactions. During all these reactions, the oxidized form of glutathione (GSSG) is formed and is afterwards converted back to GSH by the glutathione disulfide reductase. The GSH/GSSG ratio provides an estimate of cellular redox buffering capacity (Schafer and Buettner, 2001).

2.11 Acacia ferruginea

Acacia is the second largest genus in the family Leguminosae, comprising >1200 species. Traditional healers in different regions in India have
routinely used *Acacia* species for treating various cancer of the mouth, bone and skin (Kalaivani and Mathew, 2011). Among acacia species, we had chosen *A. ferruginea* extract as potential agent to inhibit inflammation and cancer. The vernacular names and taxonomic classification of *A. ferruginea* were represented in Table 2.1 and Table 2.2 respectively.

**Table 2.1.** Vernacular names of *Acacia ferruginea*

<table>
<thead>
<tr>
<th>Tamil</th>
<th>Cimai-velvel, Chimaivelvel, Karambai</th>
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<tbody>
<tr>
<td>English</td>
<td>Rusty Acacia</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Karivelam, Thimai-velvelam, Vanni</td>
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<tr>
<td>Hindi</td>
<td>Khaiger, Kanta, Chira, Kaigu, Banni,</td>
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<tr>
<td>Telugu</td>
<td>Anachandra, Anasandra, Inupa tumma</td>
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<td>Gujarati</td>
<td>Khaiger</td>
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<tr>
<td>Kannada</td>
<td>Banni, Banue, Kiri banni</td>
</tr>
<tr>
<td>Marathi</td>
<td>Dhavi-khair, Pandhra khair</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Arimedah, Brahmashtalya, Dvijapriya</td>
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</tbody>
</table>

**Table 2.2.** Taxonomic classification of *Acacia ferruginea*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
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<tr>
<td>Class</td>
<td>Eudicots</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosids</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td>Mimosaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Acacia</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>ferruginea</em></td>
</tr>
<tr>
<td>Binomial name</td>
<td><em>Acacia ferruginea</em> DC</td>
</tr>
<tr>
<td>Synonym(s)</td>
<td><em>Mimosa ferruginea</em> (DC.) Roxb.</td>
</tr>
</tbody>
</table>
2.12 Occurrence and Botanical Description

The generic name ‘acacia’ is derived from the Greek word ‘akis’, which means point or barb. *A. ferruginea* is native to India, Nepal and Sri Lanka. It is found in Peninsular India, from Gujarat to Gunjam in the east, parts of Andhra Pradesh and moderately common in Maharashtra, Karnataka, eastern slopes of the Western Ghats and scattered in thorny forests. The branches are armed with conical prickles. In India, flowers appear from March to May when the tree foliage is very scanty; pods ripen from November to February (The Wealth of India Raw Materials, CSIR, 1985).

2.13 Plant morphology

*A. ferruginea* is normally a smallish, drought-resistant, deciduous tree, not more than 12 m tall (Figure.2.8). Branches slender, armed with conical prickles; spine persist on bole until it reaches about 15 cm DBH. Primary roots are long, thin, tapering, wiry, yellow to brown. Leaves: Leaves alternate; prickles twin, infra-stipular, slightly curved. The leaves are glabrous with a large gland on the petiole. Flowers: Flowers pale yellow in numerous lax axillary spikes about 14 cm long, which are often panicled at the end of the branches. Pod 8 to 18 cms long and 2 cms broad; dark brown, contain a dry sweetish pulp, glabrous, reticulately veined, 4 to 7 seeded and tardily dehiscent. Seeds 0.5-0.7 x 0.35-0.5 cm, flat ovate, oblong, distinctly stalked, and this is a diagnostic feature, greenish to brown (Orwa et al., 2009).

2.14 Traditional claim

Traditionally, bark decoction from *A. ferruginea*, in conjunction with ginger is frequently used as an astringent for the teeth (Suresh and Rao, 1999),
also as anti-diarrhoeal, haemostatic; used for treating excessive mucous discharges, haemorrhages, stomatitis, irritable bowel syndrome, antileprotic drug (Rajanna et al, 2011) and also used to treat skin disease mainly scabies (Das, 1983). To our knowledge, very few experimental studies have been assessed in relation to pharmacological aspects of *Acacia ferruginea*. Hence we aimed to screen the phytochemicals present in it and to evaluate the protective effect against inflammation, experimental ulcerative colitis, cancer and tumor metastasis using murine models.

![Figure 2.8 Morphology of *Acacia ferruginea*](image)

Figure 2.8 Morphology of *Acacia ferruginea*