1. INTRODUCTION

The importance of medicinal herbs, spices and medicinal plants as herbal remedies is continuously being lost as a result of deforestation and lack of awareness. This has resulted in loss and limited availability of many valuable medicinal herbs and thus the precious information is lost. The history of treatment of diseases with medicinal herbs is very old. In old times when modern medicine was not in practice, people became ill and suffered from various ailments. In absence of modern medicinal remedies people were treated with herbal medicine derived from herbs and spices. A number of medicinal herbs and spices find place in daily uses and many others are used as herbal remedies. The use of herbal remedies for curing some minor ailments like common cold, cough, etc. is preferred over modern medicine. The engagement of herbal sector in the production of herbal health care formulations, herbal based cosmetic products and herbal nutritional supplements throughout world has raised industrial demand for the medicinal plant resources. The use of traditional medicine for primary health care in rural areas in developing countries, developed countries and where modern medicines are predominantly used estimates about 60 per cent of the world's population (Kamboj, 2000). The sources of traditional medicine are medicinal plants, minerals, and organic matter, but the herbal drugs are prepared from medicinal plants only.

In India the use of plants as a source of medicine has been inherited and is an important component of the Indian health care system. In Indian systems of traditional medicine, many practitioners formulate and dispense their own recipes; hence proper documentation and research is required. There is a steady growth in the use of herbal medicine in western world also, with approximately 40% of population reported using herbs to treat medical illnesses in 2003 (Bent, 2004). The growth is exponential in public, academic and government sectors in the use of traditional medicine due to
adverse drug reactions and economic burden of modern system of medicine (Dubey et al., 2004). Although the herbal products do not have drug regulatory approval for their safety and efficacy, but their traditional use will be helpful for selection, preparation and indications for use of herbal formulation. Further efficacy of any drug is established by the common use. The historical information provides source to identify and study specific plant species with potential to be used in a particular disease. The plant identified for traditional medicine is checked in a systematic approach through experimental and clinical validation for its efficacy in a similar way as is done in modern medicine. The toxicity studies in animals are also carried out to establish potential adverse effects. The tests for checking efficacy through experimental screening method of traditional and new herbal products are important to establish the principle active component and appropriate extract of the plant (Chakravarty, 1993). There is a need to establish the pharmacological activities for identifying and comparing the various preparations for potency.

Many synthetic drugs are used for the treatment of inflammatory disorders, but none of them is without serious adverse effects and are thus highly unsafe for use in humans. Out of the different synthetic drugs used against inflammation, 90% are reported to produce drug related toxicities, adverse reactions and iatrogenic effects complicating the treatment process (Rossi, 2006; Lanas, 2009; Kandulski et al., 2009). Hence, anti-inflammatory treatment has observed a shift from synthetic to natural therapy. Reports suggest that the market constitutes 83% worldwide use of herbal drugs in the treatment of inflammatory diseases and expected value in the forthcoming years is around more than 95% due to less side effects and increased acceptability of these preparations (Anonymous, 1996; Boullata and Nace, 2000; Bent and Ko, 2004). In this perspective, the present study was carried out on Gentiana
*kurroo* Royle and *Artemisia amygdalina* Decne for evaluating their anti-inflammatory potential. This study may prove vital to establish the medicinal efficacy of these plants and thus find some way out for treatment of inflammatory diseases with less adverse effects.

**1.1. Inflammation**

Inflammation is a protective process that is essential for preservation of integrity of organism in the event of physical, chemical and infectious damage (Figure 1.1). Often, it is found that inflammatory response to severe lesions, erroneously damages normal tissue (Lunardelli et al., 2006). The harmful stimuli such as pathogens, damaged cells or irritants lead to complex biological response of vascular tissues characterized by redness, joint pain, swollen joint that is warm to touch, its stiffness and loss of joint function. This response may be acute or chronic. Under chronic state it becomes a causative factor in pathogenesis. Being a self-defense reaction in its first phase, inflammation is regarded as the main therapeutic target and a preferred choice for treatment of disease and alleviating the symptoms.

![Inflammation - causes, effects and consequences](image-url)
Inflammation in general may be acute or chronic type:

Acute inflammation is the initial response of body triggered by infection or tissue injury followed by increased movement of blood components - plasma and leukocytes, especially granulocytes to the infected site (Kumar et al., 2003; Majno and Joris, 2004). This involves a cascade of biochemical events which propagates and matures inflammatory response, activating local vascular system, the immune system and various cells within the injured tissue. The response to inflammation has been best characterized for microbial infections (particularly bacterial infections), where it is triggered by receptors such as Toll-like receptors (TLRs) and NOD (nucleotide-binding oligomerization-domain protein)-like receptors (NLRs) of the innate immune system (Barton, 2008). The acute inflammatory response is said to be successful when it results in elimination of infectious agents followed by a resolution and repair phase. The lipid mediators like lipoxins, resolvins and protectins switch from pro-inflammatory to anti-inflammatory agents and result in transition from inflammation to resolution. The inhibition of neutrophil recruitment and promotion of monocyte recruitment by lipoxins which remove dead cells have a crucial role in the resolution of inflammation. Resolvins and protectins as well as transforming growth factor-β and growth factors produced by macrophages, also play essential role in initiation of tissue repair (Serhan and Savill, 2005; Serhan, 2007).

The inflammatory process persists when acute inflammatory response fails to eliminate the pathogen, and thus acquires new characteristics. The infiltrated neutrophils are replaced with macrophages, also with T cells in case of infection. When combined effect of these different cells is still insufficient, the formation of granulomas and involvement of tertiary lymphoid tissues occurs leading to chronic inflammatory state (Kumar et al., 2003; Drayton et al., 2006). The inflammatory response in chronic
state goes out of proportion resulting in damage to the body. There can be other reasons for development of chronic inflammatory state other than the persistent pathogens, like tissue damage due to autoimmune responses (immune response against self antigens) or non-degradable foreign bodies. The examples are different types of allergies and many autoimmune diseases viz: rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus etc. The systemic chronic inflammatory state mechanisms are generally poorly understood, but it is also not true that they fit the classical pattern of acute to chronic inflammation transition. Different models have been designed to test drugs for their efficacy against the acute and chronic inflammation. The acute models are designed to test drugs which are modulating erythema, changes in vascular permeability, leukocyte migration, measurement of local pain, local analgesic action and rat paw edema (Barbosa-Filho et al., 2006). Chronic models are designed to find drugs modulating disease process induced by sponge, pellet implants, granuloma pouches and adjuvant induced arthritis. (Lewis, 1989).

1.1.1. The inflammatory pathway

In general the inflammatory pathway consists of:

Inducers and Sensors – Inducers serve as the signals for the initiation of the inflammatory response. Specialized sensors are activated by these signals leading to the production of specific sets of mediators.

Mediators and effectors – The mediators alter functional states of tissues and organs (effectors of inflammation) as such that they adapt themselves to the conditions indicated by the particular inducer of inflammation. Hence each component determines the type of inflammatory response. The components involved in different pathways of inflammation will be discussed in detail in the following sections. The pathway of inflammation with examples is shown in figure 1.2.
1.1.2. Inducers and sensors of inflammation

Inflammation is induced by the molecules known as inducers which can be exogenous or endogenous in nature (Figure 1.3).

1.1.2.1. Exogenous inducers of inflammation

Exogenous inducers are molecules which come from environment and are foreign to the body. These can be classified into two groups: microbial and non-microbial. Microbial inducers in turn are of two types: pathogen-associated molecular patterns (PAMPs) and virulence factors. PAMPs, is a limited and defined set of conserved molecular patterns that are carried by all microorganisms of a given class (whether pathogenic or commensal) (Medzhitov and Janeway, 1997). PAMPs are known as such, that the host in response has evolved a corresponding set of receptors (known as pattern-recognition receptors) that detects their presence. The virulence factors which
form second class of microbial inducers are restricted to pathogens. The adverse effects of these factors on host tissues are responsible for initiating inflammatory response. Specialized sensors detect typical activities of virulence factors. An example of Gram positive bacteria can be given which produce the pore-forming exotoxins detected by the NALP3 (NACHT-, leucine-rich repeat- and pyrin-domain-containing protein) inflamasome sensitive to the efflux of $K^+$ ions results from pore formation (Mariathasan et al., 2006).

Figure 1.3: Flowchart of different types of inducers of inflammation

Non-microbial exogenous inducers of inflammation include allergens, irritants, foreign bodies and toxic compounds (Majno and Joris, 2004). Certain allergens may mimic the virulence activity of parasites and as such are detected; others may result in irritation of mucosal epithelia. Both types of allergens largely induce the similar inflammatory response because defense against both types relies on clearance and expulsion mediated by mucosal epithelia. The sensors for most of the allergens are
unknown. The other exogenous inducer may be the foreign bodies that are indigestible particles. These may cause phagosomal membrane damage in macrophages. They are too large to be phagocytosed by macrophages. Regardless of its large size to be phagocytosed or disruption of phagosomal membrane, activation of the NALP3 inflammasome (a sensor) occurs when a macrophage encounters foreign bodies, (Dostert et al., 2008). The examples of foreign bodies are silica and asbestos particles which are notorious for elicitation of an inflammatory response.

1.1.2.2. Endogenous inducers of inflammation

The signals produced by stressed, malfunctioning or otherwise damaged tissues of body induce inflammation. Since these signals are produced within the body, so are known as the endogenous inducers of inflammation. These signals may belong to various functional classes depending on nature and degree of tissue anomalies where they act but their identity and characteristics are poorly defined. Acute tissue injury in one common way is detected through sensing the desequestration of molecules or cells that are normally compartmentalized in intact cells and tissues. During necrosis, for example, the plasma membrane integrity is disrupted, and various cellular constituents including uric acid, K⁺ ions, ATP, several members of the S100 calcium-binding protein family (S100A8, S100A9 and S100A12) and HMGB1 (high-mobility group box 1 protein) are released (Bianchi, 2007; Rock and Kono, 2008). The Hageman factor/factor XII which is a plasma-derived regulator of inflammation activates when it contacts with collagen and many other components of the extracellular matrix (ECM). After activation it acts as a sensor of vascular damage and four proteolytic cascades are initiated which generate inflammatory mediators: the coagulation cascade, the kallikrein–kinin cascade, the complement cascade and the fibrinolytic cascade. Activation of platelets by contacting collagen produces different inflammatory
mediators, including serotonin and thromboxanes (Majno and Joris, 2004). The endogenous inducers discussed so far are involved with tissue injury in acute inflammatory responses.

The endogenous inducers more relevant to chronic inflammatory conditions includes crystals of calcium pyrophosphate dihydrate and monosodium urate, oxidized lipoproteins (such as high-density lipoproteins and low-density lipoproteins) and GEs (advanced glycation end products). The facilitation of crystal formation occurs in certain connective tissues which provide an appropriate surface for crystal nucleation. The inflammatory conditions in gout and pseudogout arises due to monosodium urate and calcium pyrophosphate dihydrate crystal formation in joints and periarticular tissues respectively (Rock and Kono, 2008). On reaching a certain size these crystals are detected and treated by macrophages in essentially the same way as foreign bodies. These particles are Phagocytosed and activates the NALP3 inflammasome and subsequently caspase-1 substrates are produced which includes the interleukin 1 (IL-1) family (Martinon et al., 2006; Dostert et al., 2008).

The breakdown products of ECM which are generated during tissue damage or malfunction form other group of endogenous inducers of inflammation. In this context the glycosaminoglycan hyaluronate is the best-studied component of ECM. Under normal conditions, hyaluronate is an inert high-molecular-weight polymer. In tissue injury low-molecular-weight fragments are formed during its breakdown. These fragments are inflammatory and promote a tissue-repair response through activation of TLR4 (Jiang et al., 2005). ROS are also thought to lead to this conversion (Jiang et al., 2007). Therefore to initiate inflammatory response several endogenous pathways are ROS dependent. The more important thing about many endogenous inducers of
inflammation is that they probably exert the appropriate activity \textit{in vivo} only in certain combinations and presumably only in context of damaged or malfunctioning tissues.

1.1.3. Mediators and effectors of inflammation

Many tissues and organs which serve as the downstream effectors of inflammatory pathway are altered in their functionality by numerous inflammatory mediators whose production is triggered by inducers of inflammation. These mediators which may be derived from plasma proteins or secreted by cells have common effects on blood vessels and recruitment of WBC’s. According to the biochemical properties inflammatory mediators can be classified into seven groups (Kumar et al., 2003; Majno and Joris, 2004).

**Group I:** It includes the vasoactive amines (serotonin and histamine) produced in an all-or-none manner by degranulation of platelets and mast cells. They increase or decrease vascular permeability through vasodilation or vasoconstriction, as per the circumstances. At times their release can be highly detrimental in sensitized organisms. The immediate consequence may be anaphylactic shock causing vascular and respiratory collapse.

**Group II:** This group includes the vasoactive peptides. These peptides are generated in the extracellular fluid by proteolytic processing of inactive precursors (e.g., kinins, fibrinopeptide A, fibrinopeptide B and fibrin degradation products) or are already stored in an active form in secretory vesicles (for example, substance P). Substance P causes mast cell degranulation and is released by sensory neurons. Thrombin or plasmin and Hageman factor generate other vasoactive peptides through proteolysis which result in increased vascular permeability (either directly or by stimulating the release of histamine from mast cells). Hageman factor functions as both a sensor of vascular damage and an inducer of inflammation and thus plays a key role in coordinating these
responses. The Hageman factor through the activation of kallikrein–kinin cascade activates its main product i.e., bradykinin, which acts on vasculature, and is a potent pro-algesic (pain-stimulating) substance. Pain sensation alerts the organism to abnormal state of damaged tissue and thus has an important physiological role in inflammation.

**Group III:** The various pathways of complement system produce the complement fragments C3a, C4a and C5a (also known as anaphylatoxins). These fragments affect the vasculature by promoting monocyte and granulocyte recruitment more likely by C5a and to a lesser extent C3a and C4a. They also induce mast-cell degranulation which ultimately affects vascular permeability.

**Group IV:** The phospholipids present in the inner leaflet of cellular membranes are the sources of lipid mediators (eicosanoids and platelet-activating factors). For example, the phospholipid phosphatidylcholine is acted upon by the cytosolic phospholipase A2 after activation by intracellular Ca\(^{2+}\) ions and generates arachidonic acid and lysophosphatidic acid. These two are precursors of the above mentioned two classes of lipid mediators. Eicosanoids are metabolized from arachidonic acid either by lipoxigenases generating leukotrienes and lipoxins or by cyclooxygenases (COX1 and COX2), responsible for the production of prostaglandins and thromboxanes (**Majno and Joris, 2004**). Lipoxins (and dietary ω3-fatty-acid-derived protectins and resolvins) cause inhibition of inflammation but favour resolution of inflammation, and thus promote the tissue repair (**Serhan, 2007**). The prostaglandins PGE\(_2\) and PGI\(_2\), in turn, increase vascular permeability with PGE\(_2\) also being hyperalgesic and a potent pyrogen (**Higgs et al., 1984**). The acetylation of lysophosphatidic acid generates the second class of lipid mediator, platelet-activating factors. The several processes like vasodilation and vasoconstriction, platelet activation and recruitment of leukocytes occurring during
inflammatory response are activated by this lipid mediator class (Kumar et al., 2003; Majno and Joris, 2004).

**Group V:** This group includes molecules secreted by different cells of body. These secreted molecules are known as cytokines and when involved in inflammation are specified as the inflammatory cytokines. These include the tumour-necrosis factor-α (TNF-α) and different types of the interleukins like IL-1 and IL-6 as pro-inflammatory and IL-10 as the anti-inflammatory molecule. These cytokines are produced during inflammatory response by different leukocytes but majorly by macrophages and lymphocytes. They play important roles during inflammation by affecting the vasculature, adhesion of neutrophils to endothelial cells etc.

**Group VI:** This group includes the different chemokines which are produced in response to inducers of inflammation. Chemokines act as cell signalling molecules. During inflammation they are helpful in controlling the neutrophil extravasation and chemotaxis or directed movement of leukocytes towards the affected tissues.

**Group VII:** Lastly a number of proteolytic enzymes have diverse roles in inflammation which includes elastin, cathepsins and matrix metalloproteinases. These enzymes degrade ECM and basement-membrane proteins and thus play their part in inflammation. These proteases play important roles in different processes, including tissue remodelling, leukocyte migration and host defense. But it is still not clear which type of an inflammatory trigger dictates the type of mediator induced.

The mediators not only act on the target tissues but also cause the production of other mediators also. The hierarchy of mediators underlying this logic will be important to understand. Although tissues and cells act as effectors of an inflammatory response which depends on the type of inflammatory mediator, but the response to certain mediators like IL-1 and TNF-α is ubiquitous. The study of response of these mediators
will be very much useful in understanding the process of inflammation. These mediators are always present in the body and their levels change with the change in state of tissue or organ where they are present. They have diverse roles in different tissues and cell types. Although the inflammatory mediators show the most obvious effect on the vasculature and on leukocyte migration but they show equally important role on the maintenance of tissue homeostasis by affecting the metabolic and neuroendocrine functions (Tumbull and Rivier, 1999). From above discussion, we conclude that inflammation reflects a more general role in the control of tissue homeostasis and helps body to adapt to toxic conditions.
Chapter 1

1.2. Plant Profile

The current research work was undertaken on two medicinal plants - *Gentiana kurroo* Royle and *Artemisia amygdalina* Decne.

1.2.1. *Gentiana kurroo* Royle

**Taxonomy:**

Kingdom: *Plantae*

Class: *Dicotyledonae*

Order: *Gentianales*

Family: *Gentianaceae*

Genus: *Gentiana*

Species: *kurroo*

Common name: English: Indian Gentian

Kashmiri: Nilkanth

**Habitat:** In moist grassy slopes in temperate regions in the altitude of 2,000-4,000m.

**Description:** A small, decumbent, tufted herb with perennial root stock, stout, giving out numerous arching stems, 5-30 cm. Basal leaves are long lanceolate, usually 10-12 cm long; stem 2-3 pairs, narrow, linear about 2 cm long. Flowers-handsome, blue often spotted with white. Usually two or more on each stem, but usually solitary, funnel shaped (*Chauhan, 2006*).

**Ethnopharmacy:** Karu is used as a bitter tonic, stomachic and febrifuge. It is considered to be an antihelminthic, blood purifier, carminative, diaphoretic, digestive, useful in hepatic disorders and urinary infections (*Kirtikar and Basu, 1935*) and is a well known remedy for flattening of horses (*Kaul, 1997*).
1.2.2. *Artemisia amygdalina* Decne

**Taxonomy:**

- **Kingdom:** Plantae
- **Class:** Unknown
- **Order:** Asterales
- **Family:** Asteraceae
- **Genus:** Artemisia
- **Species:** amygdalina

**Common name:** Kashmiri: Veer Tethwan

**Habitat:** Population restricted to a small area in lower Jhelum Valley, occurs in sandy, relatively loose and moist soil along the foot hills in almost open sub-alpine situations. (Dar et al., 2006).

**Description:** *Artemisia amygdalina* Decne. is a Perennial herb, large glabrous; rhizome solid, hard, woody, 2.5-3.5cm in diameter, blackish-brown, almost straight-slightly curved; stem erect, 2-3m in height, 0.5-1.5cm in diameter, leafy, prominently grooved, bears dense, small, leafy branches and branchlets. The terminal tapering portion of stem bears a number of flowering heads; flowering heads in terminal branched raceme, ovoid, 0.5-1.5cm in length and 0.4-0.6cm in diameter; involucre bracts oblong, obtuse, glabrous with papery margins; outer florets female, fertile; disc florets hermaphrodite, fertile, yellow, tubular (Dar et al., 2006).

**Ethnopharmacy:** This plant has a strong folklore claim of having antihelminthic, anti-diabetic and anti-inflammatory activity (Ashraf et al., 2010; Sivagnanam et al., 2012). It has other pharmacological actions such as protecting liver, lowering the blood pressure, eliminating fever, sedation and is also used for curing gastrointestinal ailments (Qaisar, 2006; Sivagnanam et al., 2012).