2.1 INFLAMMATION

At a basic level, the acute inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of infection or injury (Kumar et al., 2003). This response has been characterized best for microbial infections (particularly bacterial infections), in which it is triggered by receptors of the innate immune system, such as Toll-like receptors (TLRs) and NOD (nucleotide-binding oligomerization-domain protein)-like receptors (NLRs) (Barton, 2008). This initial recognition of infection is mediated by tissue resident macrophages and mast cells, leading to the production of a variety of inflammatory mediators, including chemokines, cytokines, vasoactive amines, eicosanoids and products of proteolytic cascades. The main and most immediate effect of these mediators is to elicit an inflammatory exudate locally: plasma proteins and leukocytes (mainly neutrophils) that are normally restricted to the blood vessels now gain access, through the postcapillary venules, to the extravascular tissues at the site of infection (or injury). The activated endothelium of the blood vessels allows selective extravasation of neutrophils while preventing the exit of erythrocytes. This selectivity is afforded by the inducible ligation of endothelial-cell selectins with integrins and chemokine receptors on leukocytes, which occurs at the endothelial surface, as well as in the extravascular spaces (where newly deposited plasma proteins form a provisional matrix for the binding of leukocyte integrins) (Pober and Sessa, 2007). When they reach the afflicted tissue site, neutrophils become activated, either by direct contact with pathogens or through the actions of cytokines secreted by tissue-resident cells. The neutrophils attempt to kill the invading agents by releasing the toxic contents of their granules, which include reactive oxygen species (ROS) and reactive nitrogen species, proteinase 3, cathepsin G and elastase (Nathan, 2006). These highly potent effectors do not discriminate between microbial and host targets, so collateral damage to host tissues is unavoidable. A successful acute inflammatory response results in the elimination of the infectious agents followed by a resolution and repair phase, which is mediated mainly by tissue-resident and recruited macrophages (Serhan and Savill, 2005). The switch in lipid mediators from pro-inflammatory prostaglandins to lipoxins, which are anti-inflammatory, is crucial for the transition from inflammation to resolution. Lipoxins inhibit the recruitment of neutrophils and, instead, promote the recruitment of monocytes, which remove dead...
cells and initiate tissue remodeling (Serhan and Savill, 2005). Resolvins and protectins, which constitute another class of lipid mediator, as well as transforming growth factor-β and growth factors produced by macrophages, also have a crucial role in the resolution of inflammation, including the initiation of tissue repair (Serhan, 2007).

If the acute inflammatory response fails to eliminate the pathogen, the inflammatory process persists and acquires new characteristics. The neutrophil infiltrate is replaced with macrophages, and in the case of infection also with T cells. If the combined effect of these cells is still insufficient, a chronic inflammatory state ensues, involving the formation of granulomas and tertiary lymphoid tissues (Drayton et al., 2006). The characteristics of this inflammatory state can differ depending on the effector class of the T cells that are present. In addition to persistent pathogens, chronic inflammation can result from other causes of tissue damage such as autoimmune responses (owing to the persistence of self antigens) or undegradable foreign bodies. Unsuccessful attempts by macrophages to engulf and destroy pathogens or foreign bodies can lead to the formation of granulomas, in which the intruders are walled off by layers of macrophages, in a final attempt to protect the host (Kumar et al., 2003).

It should be noted that the mechanisms of infection-induced inflammation are understood far better than are those of other inflammatory processes. It is unclear how applicable knowledge of infection-induced inflammation is to other types of inflammation. Indeed, although infection-induced inflammation is vital, it might be a special case. The mechanisms of systemic chronic inflammatory states in general are poorly understood, but it is clear that they do not seem to fit the classic pattern of transition from acute inflammation to chronic inflammation.

The inflammatory ‘pathway’

The inflammatory response is coordinated by a large range of mediators that form complex regulatory networks. To dissect these complex networks, it is helpful to place these signals into functional categories and to distinguish between inducers and mediators of inflammation. Inducers are the signals that initiate the inflammatory response. They activate specialized sensors, which then elicit the production of specific sets of mediators. The mediators, in turn, alter the functional states of tissues.
and organs (which are the effectors of inflammation) in a way that allows them to adapt to the conditions indicated by the particular inducer of inflammation. Thus, a generic inflammatory ‘pathway’ consists of inducers, sensors, mediators and effectors, with each component determining the type of inflammatory response.

**Inducers and sensors of inflammation**

Inducers of inflammation can be exogenous or endogenous (Figure 2.1).

**Figure 2.1 Inducers of inflammation**

**Endogenous inducers of inflammation**

Endogenous inducers of inflammation are signals produced by stressed, damaged or otherwise malfunctioning tissues. The identity and characteristics of these signals are poorly defined. But they probably belong to various functional classes according to the nature and the degree of tissue anomalies on which they report.

One common (but not universal) theme in detecting acute tissue injury is the sensing of the desequestration of cells or molecules that are normally kept separate in intact cells and tissues. The sequestration of these components (for example, ligands and their receptors, or enzymes and their activators or substrates) is afforded by the various types of compartmentalization that occur in normal tissues. Important examples are sequestration bounded by cellular membranes (especially the plasma...
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membrane), basement membranes, the surface epithelium and the vascular endothelium. During necrotic cell death, for example, the integrity of the plasma membrane is disrupted, resulting in the release of certain cellular constituents, including ATP, K+ ions, uric acid, HMGB1 (high-mobility group box 1 protein) and several members of the S100 calcium-binding protein family (S100A8, S100A9 and S100A12) (Bianchi, 2007; Rock and Kono, 2008). ATP binds to purinoceptors (including P2X7) at the surface of macrophages, resulting in K+ ion efflux, and can cooperate with other signals to activate the NALP3 inflammasome (Mariathasan, 2008). ATP also activates nociceptors (which are sensory receptors), thereby reporting tissue injury to the nervous system (Julius and Basbaum, 2001). HMGB1 and S100A12 engage the receptor RAGE (advanced glycation end-product-specific receptor; also known as AGER), which (at least in the case of HMGB1) cooperates with TLRs to induce an inflammatory response (Hofmann et al., 1999; Park et al., 2006). S100A8 and S100A9 signal through TLR4 (Vogl et al., 2007). It should be noted that, although intracellular proteins are thought to be passively released when the plasma membrane of necrotic cells is disrupted, numerous intracellular proteins can be secreted by way of a non-canonical (endoplasmic-reticulum–Golgi-independent) pathway. A recent study has shown that this non-canonical secretion is mediated by activated caspase 1, implying that the secretion is regulated by inflammasomes (Keller et al., 2008). In light of this finding, it will be necessary to examine whether inflammatory intracellular proteins are passively released from necrotic cells or secreted by way of this caspase-1-dependent mechanism. These two possibilities are mutually exclusive for a given cell, because necrotic cells are metabolically inactive, whereas caspase-1-dependent secretion is an ATP-driven process. If caspase 1 is responsible for the secretion of intracellular proteins with inflammatory activities, this will shed a different light on the role of intracellular inflammatory proteins in initiating inflammation, as well as on the role of necrotic cell death. The prime example in this case is HMGB1, which has been shown to be secreted by macrophages stimulated with the TLR4 ligand lipopolysaccharide (Chen et al., 2004), apparently in the absence of necrotic cell death, suggesting that the non-canonical caspase-1-dependent secretory pathway might be involved.

In intact tissues, epithelial cells and mesenchymal cells are normally separated from each other by the basement membrane, and the disruption of this barrier results
in ‘unscheduled’ epithelial–mesenchymal interactions. These interactions indicate the presence of tissue damage and consequently initiate tissue-repair responses, but how these abnormal interactions are sensed is poorly understood. The surface epithelia separate the internal compartments from the external environment. In organs, such as the intestine, that are colonized by commensal microorganisms, the disruption of the epithelial barrier gives commensal microorganisms access to the TLRs on macrophages that reside in the lamina propria, resulting in TLR-mediated induction of tissue-repair responses in the intestine (Pull et al., 2005). In sterile organs with an epithelial lining, the desequestration of some non-microbial luminal components might have a similar role. Another remarkable example of the use of a desequestration strategy is the separation of the growth factor heregulin (also known as neuregulin 1) from its receptors (ERBB2, ERBB3 and ERBB4) in the airway epithelium (Vermeer et al., 2003). The tight junctions of the intact polarized epithelium separates heregulin, which is apically expressed, from its receptors, which are basolaterally expressed, thereby preventing their interaction. On epithelial injury, heregulin gains access to its receptors and initiates a tissue-repair response (Vermeer et al., 2003).

Finally, damage to the vascular endothelium allows plasma proteins and platelets to gain access to extravascular spaces4. A key plasma-derived regulator of inflammation, the Hageman factor (also known as factor XII), becomes activated by contact with collagen and other components of the extracellular matrix (ECM). Activated Hageman factor acts as a sensor of vascular damage and initiates the four proteolytic cascades that generate inflammatory mediators: the kallikrein–kinin cascade, the coagulation cascade, the fibrinolytic cascade and the complement cascade. Platelets are also activated by contact with collagen and produce various inflammatory mediators, including thromboxanes and serotonin (Majno and Joris, 2004).

The endogenous inducers that have been discussed so far are involved in acute inflammatory responses to tissue injury. Another class of endogenous inducer is more relevant to chronic inflammatory conditions. This class of inducer includes crystals of monosodium urate and calcium pyrophosphate dihydrate, AGEs (advanced glycation end products) and oxidized lipoproteins (such as high-density lipoproteins and low-density lipoproteins). The formation of such crystals is facilitated in certain connective tissues, which provide an appropriate surface for crystal nucleation (Rock
The formation of monosodium urate and calcium pyrophosphate dihydrate crystals in the joints and periarticular tissues, for example, is responsible for the inflammatory conditions known as gout and pseudogout, respectively (Rock and Kono, 2008). When these crystals reach a certain size, they are detected by macrophages and treated in essentially the same way as foreign bodies. Phagocytosis of these particles triggers the activation of the NALP3 inflammasome and subsequently the production of caspase-1 substrates, including members of the interleukin 1 (IL-1) family (Martinon et al., 2006; Dostert et al., 2008).

AGEs are products of the non-enzymatic glycation of long-lived proteins, such as collagen. These products can result in the crosslinking of the proteins they are attached to, leading to gradual deterioration of the function of these proteins. In addition, AGEs are recognized by their receptor, RAGE, which has inflammatory activity either alone (Hofmann et al., 1999) or in combination with TLRs (Yan, 2007). AGEs can accumulate under hyperglycaemic and pro-oxidative conditions, including type 1 and type 2 diabetes, and ageing (Brownlee et al., 1988). ROS, produced by phagocytes, also have a role in converting high-density lipoproteins and low-density lipoproteins into inflammatory signals by oxidizing their lipid and protein components (Navab et al., 2006). Another group of endogenous inducers of inflammation consists of breakdown products of the ECM that are generated during tissue malfunction or damage. The best-studied component of the ECM in this context is the glycosaminoglycan hyaluronate. In normal conditions, hyaluronate is present as an inert high-molecular-weight polymer. Tissue injury promotes its breakdown into low-molecular-weight fragments, which are inflammatory, activating TLR4 and promoting a tissue-repair response (Jiang et al., 2005). This conversion is also thought to be ROS dependent (Jiang et al., 2007). Thus, several endogenous pathways that initiate the inflammatory response depend on ROS.

The list of endogenous inducers of inflammation is growing, but the scientific literature on this subject contains many discrepancies. This is largely due to the technical difficulties that are associated with characterizing this class of signal. A common reason for incorrectly identifying a factor as an inducer results from contamination of recombinant proteins with traces of microbial ligands for TLRs or NOD proteins. More importantly, many endogenous inducers of inflammation presumably exert the appropriate activity in vivo only when...
present in certain combinations and perhaps only in the context of malfunctioning or
damaged tissues. For example, ischaemia (local lack of blood supply), hypoxia,
increased concentrations of ROS and altered ECM components are all commonly
associated with tissue damage or malfunction but are not reproduced in tissue-culture
conditions, which are commonly characterized by supra-physiological nutrient and
oxygen concentrations. In addition to the inducers associated with infection and tissue
damage, there is probably another, currently unidentified, class of inducer
that triggers the inflammatory response in tissues that are malfunctioning or are under
stress. These signals report on the homeostatic status of tissues and induce adaptive
changes that involve some hallmarks of the classic inflammatory response.

Exogenous inducers of inflammation

Exogenous inducers can be classified into two groups: microbial and non-
microbial. There are, in turn, two classes of microbial inducer: pathogen-associated
molecular patterns (PAMPs) and virulence factors. The first class of microbial
inducer, PAMPs, is a limited and defined set of conserved molecular patterns that is
carried by all microorganisms of a given class (whether pathogenic or
commensal)(Medzhitov and Janeway, 1997). PAMPs are defined in the sense that the
host has evolved a corresponding set of receptors (known as pattern-recognition
receptors) that detect their presence.

The second class of microbial inducer comprises a variety of virulence factors
and is therefore restricted to pathogens. In contrast to PAMPs, they are not sensed
directly by dedicated receptors. Instead, the effects of their activity, particularly their
adverse effects on host tissues, are responsible for triggering the inflammatory
response. Typical activities of virulence factors can be detected by specialized
sensors. For example, the pore-forming exotoxins produced by Grampositive bacteria
are detected by the NALP3 (NACHT-, leucine-richrepeat- and pyrin-domain-
containing protein) inflammasome, which is sensitive to the efflux of K+ ions that
results from pore formation (Mariathasan et al., 2006). Similarly, the proteolytic
activity of proteases produced by helminthes is sensed by basophils by an unknown
sensor (Sokol et al., 2008). Notably, this sensing mechanism can be inadvertently
activated by functional mimics, so allergens that are proteases can trigger the pathway
that is usually induced by helminthes (Sokol et al., 2008). An alternative way of
sensing virulence activity is non-specific and even more indirect, through detecting
the effects on cell death and tissue damage. In this case, the actual inducers of the inflammatory response are endogenous products of damaged cells and tissues. Importantly, the inflammatory responses that are induced by these two sensing mechanisms of virulence activity differ in their specificity, because the former is characteristic of pathogens (and in some cases, pathogen classes), but the latter is not. These inflammatory responses are likely to have different characteristics, and it will be interesting to investigate whether they result in distinct physiological and pathological outcomes.

It should be emphasized that microbial inducers of inflammation are not necessarily derived from pathogens. Commensal bacteria provide an important source of inflammation inducers that are detected by TLRs (Rakoff-Nahoum et al., 2004). The activation of TLRs by these bacteria is actively suppressed by multiple mechanisms. An example of this is the lethal TLR-dependent inflammation that develops in mice that lack A20, one of the crucial negative regulators of TLR signaling (Turer et al., 2008). Exogenous inducers of inflammation that are of non-microbial origin include allergens, irritants, foreign bodies and toxic compounds. Certain allergens are detected because they mimic the virulence activity of parasites (as mentioned earlier); others can act as irritants on the mucosal epithelia. The inflammatory response induced by both types of allergen is largely similar because defence against parasites and environmental irritants relies on expulsion and clearance mediated by the mucosal epithelia. The sensors for allergens are largely unknown. Foreign bodies are indigestible particles that either are too large to be phagocytosed or cause phagosomal membrane damage in macrophages. Silica and asbestos particles are notorious examples of foreign bodies that elicit an inflammatory response. Their large size and resistance to removal, as well as a lack of self markers (such as CD47) that are normally present on autologous cells and prevent their phagocytosis (by engaging inhibitory receptors), point to an abnormal occurrence in the tissues. The ‘missing self’ recognition presumably triggers a ‘phagocytic reflex’ in macrophages, but the large size or the shape of foreign particles results in ‘frustrated phagocytosis’: that is, a phagocytic cup is formed but cannot close to form a phagosome. If a foreign body is too large for a phagocytic cup to be formed, the macrophage forms a granuloma around this body instead. The sensor that triggers this reaction in macrophages is unknown. In some cases, macrophages can fuse with each other to
form ‘giant cells’ that encapsulate the foreign body. The encapsulation of foreign objects is an ancient defensive strategy, which is also found in *Drosophila melanogaster*, in which lamellocytes (macrophage-like cells) encapsulate parasitoid wasp eggs to protect the host (Rizki and Rizki, 1992). Regardless of whether a foreign body is too large to be phagocytosed or disrupts the phagosomal membrane, when a macrophage encounters foreign bodies, the NALP3 inflammasome (a sensor) is activated (Dostert et al. 2008).

**Mediators and effectors of inflammation**

Inducers of inflammation trigger the production of numerous inflammatory mediators, which in turn alter the functionality of many tissues and organs — the downstream effectors of the inflammatory pathway. Many of these inflammatory mediators have effects in common on the vasculature and on the recruitment of leukocytes. These mediators can be derived from plasma proteins or secreted by cells (Kumar et al., 2003). The cellular mediators can be produced by specialized leukocytes (particularly tissue-resident macrophages and mast cells) or by cells present in local tissues. Some mediators (such as histamine and serotonin) are preformed and stored in the granules of mast cells, basophils and platelets. Others are preformed and circulate as inactive precursors in the plasma. The plasma concentration of these mediators can increase markedly as a result of increased secretion of the precursors by hepatocytes during the acute-phase response. Other mediators are produced directly in response to appropriate stimulation by inducers of inflammation. Inflammatory mediators can be classified into seven groups according to their biochemical properties (Kumar et al., 2003): vasoactive amines, vasoactive peptides, fragments of complement components, lipid mediators, cytokines, chemokines and proteolytic enzymes.

**First**, vasoactive amines (histamine and serotonin) are produced in an all-or-none manner when mast cells and platelets degranulate. They have complex effects on the vasculature, causing increased vascular permeability and vasodilation, or vasoconstriction, depending on the context. The immediate consequences of their release by mast cells can be highly detrimental in sensitized organisms, resulting in vascular and respiratory collapse during anaphylactic shock.

**Second**, vasoactive peptides can be stored in an active form in secretory vesicles (for example, substance P) or generated by proteolytic processing of inactive precursors in
the extracellular fluid (for example, kinins, fibrinopeptide A, fibrinopeptide B and fibrin degradation products). Substance P is released by sensory neurons and can itself cause mast-cell degranulation. Other vasoactive peptides are generated through proteolysis by the Hageman factor, thrombin or plasmin and cause vasodilation and increased vascular permeability (either directly or by inducing the release of histamine from mast cells). As mentioned earlier, the Hageman factor has a key role in coordinating these responses, and it functions as both a sensor of vascular damage and an inducer of inflammation. The Hageman factor activates the kallikrein–kinin cascade, and the main product of this cascade, bradykinin, affects the vasculature, as well as having a potent pro-algesic (pain-stimulating) effect. Pain sensation has an important physiological role in inflammation by alerting the organism to the abnormal state of the damaged tissue.

Third, the complement fragments C3a, C4a and C5a (also known as anaphylatoxins) are produced by several pathways of complement activation. C5a (and to a lesser extent C3a and C4a) promote granulocyte and monocyte recruitment and induce mast-cell degranulation, thereby affecting the vasculature.

Fourth, lipid mediators (eicosanoids and platelet-activating factors) are derived from phospholipids, such as phosphatidylcholine, that are present in the inner leaflet of cellular membranes. After activation by intracellular Ca2+ ions, cytosolic phospholipase A2 generates arachidonic acid and lysophosphatidic acid, the precursors of the two classes of lipid mediator listed above, from phosphatidylcholine. Arachidonic acid is metabolized to form eicosanoids either by cyclooxygenases (COX1 and COX2), which generate prostaglandins and thromboxanes, or by lipoxygenases, which generate leukotrienes and lipoxins. The prostaglandins PGE2 and PGI2, in turn, cause vasodilation, and PGE2 is also hyperalgesic and a potent inducer of fever (Higgs et al., 1984). Lipoxins (and dietary ω3-fatty-acid-derived resolvins and protectins) inhibit inflammation and promote resolution of inflammation, and tissue repair. The second class of lipid mediator, platelet-activating factors, are generated by the acetylation of lysophosphatidic acid and activate several processes that occur during the inflammatory response, including recruitment of leukocytes, vasodilation and vasoconstriction, increased vascular permeability and platelet activation (Kumar et al., 2003).
Fifth, inflammatory cytokines (tumour-necrosis factor-α (TNF-α), IL-1, IL-6 and many others) are produced by many cell types, most importantly by macrophages and mast cells. They have several roles in the inflammatory response, including activation of the endothelium and leukocytes and induction of the acute-phase response.

Sixth, chemokines are produced by many cell types in response to inducers of inflammation. They control leukocyte extravasation and chemotaxis towards the affected tissues.

Seventh, several proteolytic enzymes (including elastin, cathepsins and matrix metalloproteinases) have diverse roles in inflammation, in part through degrading ECM and basement-membrane proteins. These proteases have important roles in many processes, including host defence, tissue remodelling and leukocyte migration.

It should be noted that it is unclear to what extent the nature of an inflammatory trigger dictates the type of mediator induced. In addition, many (but not all) mediators not only have direct effects on target tissues but also themselves induce the production of additional mediators.

It will be important to understand the logic underlying this hierarchy of mediators. The effectors of an inflammatory response are the tissues and cells, the functional states of which are specifically affected by the inflammatory mediators. Responsiveness to certain inflammatory mediators (such as TNF-α and IL-1) is almost ubiquitous, although these mediators have distinct effects in different tissue and cell types. Although the most obvious effect of inflammatory mediators is to induce the formation of an exudates (through their effects on the vasculature and on leukocyte migration), many inflammatory mediators have other, equally important, effects on neuroendocrine and metabolic functions and on the maintenance of tissue homeostasis in general (Turnbull and Rivier, 1999). These functions of inflammatory mediators reflect a more general role for inflammation in the control of tissue homeostasis and in adaptation to noxious conditions.
2.2 DIURETICS

By definition, diuretics are drugs that increase the rate of urine flow; however, clinically useful diuretics also increase the rate of excretion of Na\(^+\) (natriuresis) and of an accompanying anion, usually Cl\(^-\). NaCl in the body is the major determinant of extracellular fluid volume, and most clinical applications of diuretics are directed toward reducing extracellular fluid volume by decreasing total-body NaCl content. A sustained imbalance between dietary Na\(^+\) intake and Na\(^+\) loss is incompatible with life. A sustained positive Na\(^+\) balance would result in volume overload with pulmonary edema, and a sustained negative Na\(^+\) balance would result in volume depletion and cardiovascular collapse. Although continued administration of a diuretic causes a sustained net deficit in total-body Na\(^+\), the time course of natriuresis is finite because renal compensatory mechanisms bring Na\(^+\) excretion in line with Na\(^+\) intake, a phenomenon known as diuretic braking. These compensatory, or braking, mechanisms include activation of the sympathetic nervous system, activation of the renin-angiotensin-aldosterone axis, decreased arterial blood pressure (which reduces pressure natriuresis), hypertrophy of renal epithelial cells, increased expression of renal epithelial transporters, and perhaps alterations in natriuretic hormones such as atrial natriuretic peptide (Ellison, 1999).

Diuretic drugs increase urine output by the kidney (i.e., promote diuresis). This is accomplished by altering how the kidney handles sodium. If the kidney excretes more sodium, then water excretion will also increase. Most diuretics produce diuresis by inhibiting the reabsorption of sodium at different segments of the renal tubular system. Sometimes a combination of two diuretics is given because this can be significantly more effective than either compound alone (synergistic effect). The reason for this is that one nephron segment can compensate for altered sodium reabsorption at another nephron segment; therefore, blocking multiple nephron sites significantly enhances efficacy.

Some of the diuretics like ethacrynic acid and dicholothiazide suppress the development of proliferative inflammation (Zverev et al., 1985, Zverev, 1986). Prandota, 2006 demonstrated that furosemide, a loop diuretic, exhibit anti-inflammatory effect through inhibition of production and release of cytokines IL-6, IL-8, TNF\(\alpha\) from peripheral mononuclear cells, which may have a beneficial effect on the local inflamed tissues.
2.2.1 VASCULAR EFFECTS OF DIURETICS

Diuretics are believed to improve symptoms of congestion by several mechanisms. Loop diuretics induce hemodynamic changes that appear to be independent of their diuretic effect. They act as venodilators and, when giving intravenously, reduce right atrial and pulmonary capillary wedge pressure within minutes (Larsen, 1988; Stampfer et al., 1968). This initial improvement in hemodynamics may be secondary to the release of vasodilatory prostaglandins (Kramer et al., 1999). Studies in animals and humans have demonstrated that the loop diuretic furosemide directly dilates veins; this effect can be inhibited by indomethacin, suggesting that local prostaglandins may contribute to its vasodilatory properties (Pickkers et al., 1997). In the setting of acute pulmonary edema from myocardial infarction, Dikshit et al. measured an increase in venous capacitance and decreasing pulmonary capillary wedge pressure within 15 min of furosemide infusion, while the peak diuretic effect was at 30 min (Dikshit et al., 1973). Numerous other investigators have found similar results (Raftery, 1994). Other loop diuretics, such as bumetanide, have been reported to have differing effects (Kelly, 1994). There have also been reports of an arteriolar vasoconstrictor response to diuretics when given to patients with advanced heart failure (Francis et al., 1985). A rise in plasma renin and norepinephrine levels leads to arteriolar vasoconstriction, resulting in reduction in cardiac output and increase in pulmonary capillary wedge pressure. These hemodynamic changes reverse over the next several hours, likely due to the diuresis. The vasoconstrictor response to loop diuretic administration occurs more commonly in patients treated chronically with loop diuretics (Francis et al., 1985). In this situation, chronic stimulation of the renal renin/angiotensin/aldosterone axis may prime the vascular system to vasoconstriction. It is likely that different diuretics have complex and multifactorial actions on the vascular system.

2.2.2 NEUROHORMONAL EFFECTS OF DIURETICS

Diuretic drugs stimulate the renin–angiotensin– aldosterone (RAA) axis via several mechanisms. Loop diuretics stimulate renin secretion by inhibiting NaCl uptake into macula densa cells. Sodium/chloride uptake via the loop diuretic sensitive Na+–K+–2Cl− cotransport system is a central component of the macula densa-mediated pathway for renin secretion (Skott and Briggs, 1987). Blocking Na+–K+–2Cl−...
2Cl\(^-\) uptake at the macula densa stimulates renin secretion directly, leading to a volume–independent increase in angiotensin II and aldosterone secretion. Loop diuretics also stimulate renal production of prostacyclin, which further enhances renin secretion. All diuretics can also increase renin secretion by contracting the extracellular fluid (ECF) volume, thereby stimulating the vascular mechanism of renin secretion. ECF volume contraction also inhibits the secretion of atrial natriuretic peptide. Among its other effects, atrial natriuretic peptide inhibits renin release. Interestingly, the combination of aggressive vasodilator therapy and diuresis to achieve improved hemodynamic parameters in turn led to diminished neurohormonal activation (Johnson et al., 2002).

### 2.3 PLANT PROFILE

**Figure 2.2 Pergularia daemia**

**Botanical name:** *Pergularia daemia* (Forsk.) Chiov  
**Synonyms:** *Pergularia extensa* (Jacq.) N. E. Br., *Daemia extensa* (Jacq.) R. Br., *Asclepias daemia* (Forsk.)  
**Family:** Asclepiadaceae  
**Vernacular name:**  
- **San:** Kurutakah, Uttamarani  
- **Guj:** Nagala dudhi, Amara-dhudheli  
- **Hin:** Utaran, Sagovani  
**Habitat:** Throughout the hotter parts of India, upto 900 m
**Description:** Pergularia is a perennial, small, twining herb. It has hairy stems with milky juice and broad, egg-shaped leaves. It has greenish yellow or dull white, small flowers in tiny clusters and fruits reflexed in pairs, covered with spinous outgrowths. Fruits (follicles) lanceolate, long-pointed, about 5 cm long, covered with soft spines and seeds are pubescent, broadly ovate. Flowering may occur each year between August and January in central India, with fruits maturing from October to February. In central Indian deciduous forests, the stems typically die down in February and reappear with the onset of the rainy season.

**Taxonomy classification**
- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Super division: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Asteridae
- Order: Gentianales
- Family: Asclepiadaceae
- Genus: Pergularia
- Species: *P. daemia* (Forsk) Chiv

**Parts used:** Whole plant

### 2.3.1 ETHNOPHARMACOLOGICAL INFORMATION

Aerial parts of plant used as an emetic (Elango et al., 1985; Singh et al., 1980), expectorant (Arseculeratne et al., 1985), expectorant (Elango et al., 1985; Singh et al., 1980; Seshadri and Vydeeswaran, 1971), anthelmintic (Dutta and Ghosh, 1974a), emmenagogue (Berhault, 1971), antiseptic (Arseculeratne et al., 1985), and antivenin (Selvanayahgam et al., 1994) and used to facilitate parturition (Gupta et al., 1946), while used in Ayurvedic medicine for delayed childbirth (Ghatak and De, 1961), amenorrhea (Dutta and Ghosh, 1974a), asthma, snakebite, rheumatic swellings (Singh et al., 1980) and used to treat post-partum hemorrhage (Ghatak and De, 1961). Latex of this plant used for boils and sores (Girach et al., 1994). Dried leaf used as an emetic.
Phytopharmacological action of *Pergularia daemia* with special reference to its actions and mechanism of action as diuretic and anti-inflammatory agent

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(Mittal et al., 1962), antirheumatic (Kakrani and Saluja, 1994) and used for bronchitis (Mittal et al., 1962), amenorrhea, dysmenorrheal (De laszlo and Henshaw, 1954; Dutta and Ghosh, 1974b), asthma (Elango et al., 1985), healing cuts and wounds (Pushpangadan and Atal, 1984), while used to treat whooping cough (Reddy et al., 1989) and to facilitate parturition (Dutta and Ghosh, 1974b). Fresh leaf used as fish poison (Watt and Breyer-Brandwijk, 1962), while leaf juice used for amenorrhea, dysmenorrheal, catarrhal infections, infantile diarrhea (Elango et al., 1985) and used reduce the body pain (Reddy et al., 1988; John, 1984). Dried root used as an abortifacient (Kokwaro, 1976), emetic, bronchitis (Mittal et al., 1962) and used for cough, asthma and constipation (Watt and Breyer-Brandwijk, 1962), while fresh root used as an abortifacient (Kokwaro, 1981; Kokwaro, 1981) and used to treat gonorrhea (Samuelsson et al., 1991). Shoots used to treat whooping cough (Nagaraju and Rao, 1990). Stem bark has been used to treat malaria (Kohler et al., 2002) and twig used as an antipyretic and appetizer (Gill and Akinwumi, 1986).

### 2.3.2 PHARMACOLOGICAL REVIEW

The strong insecticide activity against *Periplaneta Americana*, *Blatella germanica* and *Oncopeltus fasciatus* of aqueous extract of entire plant was studied by Heal et al., 1950 at a dose level of 40 ml/kg. Gupta et al., 1946 reported alkaloid fraction of the whole plant was showed uterine stimulant effect on female cat (0.75 mg/animal, IV), while the same fraction was exhibited intestine smooth stimulant activity on cat (3mg/kg, IV) and also hyperglycemic activity on monkey (3mg/kg, IV). Oral dose of 3 mg/ kg on cat was showed gastric secretary stimulation activity, while Dutta and Ghosh, 1947a reported showed uterine stimulant effect on female rat with ethanol (95%) extract of entire plant. In 1959 Rakhit *et al.*, reported the aqueous extract of whole plant was showed uterine stimulant effect on female guinea pig. The toxicity (LD50) of aqueous ethanolic extract of whole plant and it was showed more than 1 gm/kg on mouse. Hypotensive activity of the extract showed active at 50 mg/kg, i.v. in dog, while antispasmodic activity of the extract tested on guinea pig ileum and hypothermic activity of the extract showed active at dose level of 500 mg/kg, i.p, in mouse (Dhar *et al.*, 1973). Ogunlana and Ramstad, 1975 reported the 50% methanol (1:1) extract of flower and leaf inactive against various bacteria including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis* and *Proteus species* by broth culture method. Prakash et al.,
1978 demonstrated safety of ethanolic extract of leaf at a dose of 100 mg/kg on pregnant rat, while Runnebaum et al., 1984 reported the ethanol extract of leaf not showed anti-implantation and abortifacient effect on female pregnant rat at a dose level of 200 mg/kg. The 80% ethanolic extract of whole plant exhibited antibacterial against Proteus mirabilis, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, agar disc diffusion method and 1 to 3 mg of the extract showed cardiovascular effects on heart of frog, while the high dose of extract was blocked heart. The extract 10 mg/kg showed uterine stimulant effect on female guinea pig, while 1 to 2.5 mg of the extract exhibited smooth muscle stimulant activity on guinea pig ileum (Elango et al., 1985). Valsaraj et al., 1997 studied the antibacterial activity of ethanol (80%) extract of leaf and stem and exhibited activity against various bacteria strain such as Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli and Staphylococcus aureus at a concentration of 25 mg/ml, while ethanol (95%) extract of plant inactive against Mycobacterium tuberculosis by agar plate method. Qureshi et al., 1997 demonstrated inhibitory nature of P. daemia extract against keratinophilic fungi. Sathish et al., 1998 demonstrated that ethanolic and petroleum ether leaf extract posses analgesic activity, while ethanolic extract, petroleum ether extract and butanolic extract posses antipyretic activity. In 2000 Perumal Samy and Ignacimuthu studied the antibacterial activity of various extracts of leaf by disc diffusion method and found methanol extract active against B. subtilis, S. aureus and E. coli at 10 mg/ml concentration, while Srinivasan et al., 2001 reported the 0.3 ml of aqueous extract of whole plant was inactive against various bacteria and fugal such as Chromobacterium violaceum, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis Staphylococcus aureus, Salmonella paratyphi, Salmonella typhi, Bacillus subtilis, Aspergillus niger, Aspergillus flavus, Aspergillus fumigates and Candida albicans. In 2001 Sadik et al., reported that ethanolic extract of P. daemia and its steroidal fraction at 200 mg/kg showed significant anti-implantation and abortifacient activity (Sadik et al., 2001a), further Sadik et al., 2001b demonstrated that alkaloidal fraction of ethanolic extract has great potential to prevent fertilization in female mice (antifertility activity). Hukkeri et al., 2001 established the anti-inflammatory activity of ethanolic leaf extract and its butanolic fraction. In 2002 Wahi et al. confirmed that aqueous and alcoholic extract has significant anti-diabetic activity in alloxan induced
hyperglycemia on rats, while Kohler et al. reported lipophilic fraction obtained from stem bark was showed antimalarial activity against Plasmodium falciparum. Suresh Kumar and Mishra, 2006 presented that ethanol extract obtained from aerial parts of plant at an oral dose of 200 mg/kg showed hepatoprotective properties by significant protective effect through lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and total cholesterol and increasing the levels of total protein and albumin levels. Whereas in 2007 Suresh Kumar and Mishra showed the hepatoprotective effect of acetone and ethanol sub fractions of ethanolic fraction obtained from total alcoholic extract against carbon tetrachloride- induced liver damage in rats. Flyman and Afolayan, 2007 studied the implication of the mineral ratios of P. daemia in human diets. The Ca/Fe, Ca/K, Ca/Mg, and Ca/Zn ratios were 1.7, 0.3, 1.6, and 9.7, respectively. The Fe/Zn ratio was 5.6, while P/Ca ratio was 0.

2.3. 3 PHYTOCHEMICAL REVIEW

Sankara Subramanian and Nair 1968; Sinha and Dogra, 1985; reported to contain hyperoside (flavonol) in dried stem, while flavonoids and saponins in fresh shoots and flowers. Dutta and Ghosh, 1947a,b accounted to contain daemia extensa polypeptide, daemia extensa glucoside, Inorganic salts such as KCl and KNO₃ in entire plant. In 1962 Mittal et al. documented to contain various cardenolide such as calotoxin, calotropagenin, dihydrocalotropagenin, calotropin and uscharidin in seed, while coroglaucigenin, corotoxigenin, uscharidin and uzarigenin in stem Seshadri and Vydeeswaran, 1971 reported to contain calactin, calotropin, corotoxigenin, daucosterol and sucrose in root. Rakhit et al., 1959 isolated betaine, hentriacontane and pentacosanoinacid from entire plant, while reported to contain magnesium and potassium carbonate, daemia extensa polypeptide, Ca, Mg and K oxalate. In 1998 Anjaneyulu et al. isolated lupeol-3-beta trans crotonate and oleanolic acid acetate from dried whole plant. Further, entire plant and root reported to contain β-sitosterol, lupeol, lupeol acetate, α, β-amyrin and its acetate (Anjaneyulu et al., 1998; Seshadri and Vydeeswaran, 1971; Rakhit et al., 1959; Raman and Barua, 1958). Sathish et al., 1998; reported the presence of triterpenes, saponins cardenolides and alkaloids. The chemical investigation of hexane extract of the whole plant yielded new trierpen ester,
leupol-3-β-transcrotonate along with the acetate of α-amyrin, β-amyrin, oleanolic acid and β-sitosterol (Jalalpure et al., 2002).

2.4 ANIMAL MODEL REVIEW

Inflammation is the reactive state of hyperemia and exudation from blood vessels with consequent redness, heat, swelling and pain which a tissue manifests in response to physical or chemical injury or bacterial invasion. Inflammation was characterized two thousand years ago by Celsus by the four Latin words: Rubor, calor, tumor and dolor.

The inflammatory process involves a series of events that can be elicited by numerous stimuli, e.g., infectious agents, ischemia, antigen-antibody interactions, chemical, thermal or mechanical injury. The response is accompanied by the clinical signs of erythema, edema, hyperalgesia and pain.

Mediators of inflammation

- Peptide Autacoids
  - Bradykinin
  - Substance – P
  - Tachykinins
  - Neurokinins

- Amine autacoids
  - Serotonin
  - Histamine

- Lipid derived autacoids
  - Eicosanoids
    - Prostaglandins
    - Thromboxanes
    - Leukotrienes

- Other Mediators
  - Cytokines – Interleukins, TNF α
  - C-Reactive Protein
  - Lymphokines
  - Gastrin
Review of Literature

- Somatostatins
- Vasoactive Intestinal Peptide (VIP)

Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms:

- An acute, transient phase, characterized by local vasodilatation and increased capillary permeability.
- A subacute phase, characterized by infiltration of leukocytes and phagocytic cells.
- A chronic proliferative phase, in which tissue degeneration and fibrosis occur.

Accordingly, anti-inflammatory tests have to be divided into those measuring acute inflammation, subacute inflammation and chronic repair processes (Vogel, 2002; Tunner, 1965).

Methods for testing acute and subacute inflammation are:

- Paw edema in rats
- Vascular permeability
- Pleurisy tests
- Granuloma pouch technique

The proliferative phase is measured by methods for testing granuloma formation, such as:

- Cotton wool granuloma
- Glass rod granuloma

Furthermore, methods for testing immunological factors:

- Adjuvant arthritis in rats (various modifications)
- Passive cutaneous anaphylaxis
- Arthus type immediate hypersensitivity
- Delayed type hypersensitivity

In different models, the inflammation has produced by different inducers by releasing inflammatory mediators. Each is having different mechanism of action for
producing inflammation either by increased in vascular permeability, the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair.

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer’s yeast, formaldehyde, dextran, egg albumin, kaolin, sulfated polysaccharides like carrageenan or naphthoylheparamine. For producing edema, histamine, xylene, arachidonic acid, phorbol myristate acetate, oxozolone, croton oil and formalin are also used.

For evaluating the most effective and widely used model for inflammation is carrageenan-induced paw edema, Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea moss, Chondrous crispus. Its use as an endemogen was introduced by Winter et.al. 1962 Carrageenan initially releases histamine and serotonin followed by release of prostaglandins, protease and lysosomes producing edema.

By producing air pouch in air pouch model, formation of exudates is there with migration of leukocytes and interleukins. Angiogenesis, nitric oxide synthesis and Kinin release are said to be the main causes of the granuloma. Angiogenesis is a chronic inflammatory state, which facilitates migration of inflammatory cells to the inflammatory site and supplies nutrients and oxygen to tissue to granulation tissue. Therefore the suppression of Angiogenesis in granulation tissue is important to suppress the development of chronic granulation tissue (Ghosh et al., 2000). Nitric oxide synthesis (NO), by inducible nitric oxide synthase (iNOS), increases in inflammation and leads to cellular injury. Kinins cause vasodilatation, increase vascular permeability and WBC migration in the early stages of the inflammation and are also responsible for, collagen formation in the later stages of inflammation. It may also be responsible for the vascular flushing that occurs in the carcinoid syndrome. The kinins formation is also implicated in the endotoxin shock, hereditary angioneurotic edema, anaphylaxis, arthritis and in acute pancreatitis.

In cotton pellet induced granuloma (Goldstein et al., 1976) and in glass rod granuloma, the foreign body like cotton or glass rod when implanted in the skin of animal is producing undifferentiated connective tissue around it indicating state of
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inflammation. The amount of newly formed connective tissue is measured by weighing the dried pellet after removal as an index of the extended severity of the inflammation (Hicks, 1969). This model is the indication of the proliferative phase of the inflammation of the microphages, neutrophils, fibroblasts and collagen formation which are basic source for the granuloma formation; therefore decrease in the granuloma formation indicates the suppression of the proliferative phase (Kavimani et al., 1999).

In acetic acid induced vascular permeability, acetic acid causes dilation of arterioles and venules and increased vascular permeability by releasing inflammatory mediators such as histamine, prostaglandins and leukotrienes are released following stimulation of mast cells. Myeloperoxidase (MPO) in neutrophils indicates intensity of inflammation (Whittle, 1964).

Complete Freund’s adjuvant induced arthritis has been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance after the study of pathophysiological and pharmacological control of inflammatory process, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (Butler et al., 1992; Besson and Guilbaud, 1988). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agent in this model and in rheumatoid arthritis in humans. Secondly, Freund’s adjuvants are commonly used because they produce a stronger, longer lasting immunogenic response compared to other adjuvants. They are easy to use water-in-oil emulsions. Freund's Complete Adjuvant is the form that contains killed cells of *Mycobacterium butyricum* to enhance the immune response. CFA administration produced arthritis in two phases an acute periarticular inflammation followed by a phase of bone involvement (Jacobson et al., 1999). Freund’s adjuvant into the rat paw induces inflammation as primary lesion with a maximum after 3 to 5 days. Secondary lesions occur after a delay of approximately 11 to 12 days which are characterized by inflammation of non-injected sites (hindleg, forepaws, ears, nose and tail), a decrease of weight and immune responses. Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented or diminished by immunosuppressive agents.

Diuretics are one of the most commonly used in modern therapeutics. They are widely used in treatment of congestive heart failure, essential hypertension, acute and chronic renal failure, nephritic syndrome, edema of varied origin and glaucoma.
Diuretic is any drug that increases the flow of urine. Diuretics promote the removal from the body of excess water, salts, poisons, and accumulated metabolic products, such as urea. They serve to rid the body of excess fluid (edema) that accumulates in the tissues owing to various disease states. Diuretic drugs increase urine output by the kidney (i.e., promote diuresis). This is accomplished by altering how the kidney handles sodium. If the kidney excretes more sodium, then water excretion will also increase. Most diuretics produce diuresis by inhibiting the reabsorption of sodium at different segments of the renal tubular system. The currently used methods for screening of diuretics are based on the effects of drugs on water and electrolyte metabolism in rats. The choice of this test and application of its results in the development of clinically useful diuretics has been justified by introduction of benzothiazides (Parmar and Shiv Prakash, 2006). Lipschitz test is one of the classical methods introduced by Lipschitz in 1943 for the evaluation of diuretic activity. It is based on water and sodium excretion in test animals as compared to test animals with high dose of urea. Kau et al. (1984) recommended a method for screening diuretic agents in the rat using normal saline (4% body weight) as hydrating fluid. Further studies of diuretic agents have ascertained it beneficial to pre-treat or “prime” the test animal with assorted fluids. The administration of saline has been found to be requisite to produce a graded response in the male rat with escalating dosage of aminophylline (McColl et al., 1956). Excretion of electrolytes is as important as the excretion of water for treatment of peripheral edema and ascites in congestive heart failure as well as for treatment of hypertension. Potassium loss has to be avoided. As a consequence, saluretic drugs and potassium-sparing diuretics were developed. The diuresis test in rats was modified in such a way that potassium and chloride as well as osmolality are determined in addition to water and sodium. Ratios between electrolytes can be calculated indicating carbonic anhydrase inhibition or a potassium sparing effect (Vogel, 2002).