2.1 Concept of Stress.

Stress is the term often used to describe distress, fatigue and feelings of not being able to cope. The term ‘stress’ has been derived from the Latin word ‘stringer’ which means to draw tight. The term was used to refer the hardship, strain, adversity or affliction. It refers both to the circumstances that place physical or psychological demands on an individual and to the emotional reactions experiences in these situations. Although, the adverse effects of stress on physical health and emotional well being are increasingly recognized, there is little agreement among experts on the definition of stress: -

According to Hans Selye (1976), a pioneer in stress research, stress is caused by physiological, psychological and environmental demands. In 1936, Selye coined the term ‘The General Adaption Syndrome (GAS)’. He proposed that the stress response is comprised of three phases. First is the alarm or emergency phase, during which a person recognizes a potential threat to his or her well-being. Depending on the nature of the threat, an emotion arises concomitantly with activation of the autonomic nervous system and hypothalamic-pituitary-adrenal axis in preparation for the second, or adaptation, phase. This phase is characterized by physiological adjustments capable of supporting the behavioral sequence best suited to adapt to the threat. It could also culminate in withdrawal or a depression-like response, depending on the extent to which a person might feel helpless. Selye believed that if the stressor persisted, it would result in the depletion of chemicals responsible for maintaining homeostasis, the third phase. Illness or even death might ensue. According to Lazaras, (1976): stress occurs when there are demands on the person, which taxes or exceeds his adjustive resources. According to Spielberger, (1979): the term stress is used to refer to a complex psycho- biological process that consists of three major elements- stressor, perception and anxiety. The process is initiated by a situation or stimulus that is potentially harmful or dangerous stressor. If a stressor is interpreted as dangerous or threatening, an anxiety reaction will be elicited.
2.1.1. Physiology of stress response.

It’s a physiological response induced by variety of physical, chemical, and psychological stimuli characterized by activation of the HPA axis leading to increased levels of adrenal hormones such as glucocorticoid, epinephrine, and norepinephrine (Whitnall et al., 1993). As threat or danger is perceived, the sympathetic nervous system and brain pituitary-adrenal axis are activated which enable a person to engage in an emergency referred to as the fight or flight response by Walter Cannon. The activation of the sympathetic nervous system leads to release of nor-epinephrine from adrenergic nerve terminals, including those in the spleen, thymus, and other lymphoid tissues (R. Ader et al., 1990). This arousal system also recruits emotion, which in some instances may manifest itself as anxiety. Stressful situations also stimulate various areas of the hypothalamus, including the paraventricular nucleus. Stimulation of this nucleus results in the secretion of corticotrophin releasing hormone (CRH). CRH in turn stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH) which circulates in the blood-stream and stimulates the adrenal cortex to secrete cortisol. Cortisol is a glucocorticoid (a naturally occurring steroid); almost every cell in the body has glucocorticoid receptors. Stimulation of this system results in increased glucose availability, increased blood flow and increased behavioral responsiveness in the face of stressful situations. Although the short-term effects of glucocorticoids are essential, the long-term effects are damaging and include damage to muscle tissue, increased blood pressure, diabetes mellitus, etc. In addition, excess cortisol inhibits inflammatory responses and the activity of macrophages, which are normally released into the bloodstream by the thymus gland to kill invading bacteria. A weakened immune system in turn makes us more vulnerable to infection and to other diseases. Many other neuroendocrine mediators are also increased or decreased in the peripheral circulation during most stress responses, thereby exposing cells of the immune system to increased or decreased concentrations. For example, concentrations of glucocorticoids (predominantly cortisol in humans and corticosterone in rodents), catecholamines (epinephrine and norepinephrine), endogenous opiates, ACTH, bombesin (Anisman et al., 1998), and prolactin (PRL) increase in response to a wide variety of stressor. In contrast, concentrations of GH, melatonin, and testosterone decrease in
response to stress. Both the major conduits via which emotions are translated into physiological outcomes, the autonomic nervous system and the neuroendocrine system, are capable of profoundly influencing health through the immune system. Each is essential to our survival yet has the potential to hasten our demise if activation occurs at an inappropriate time or is of too great an intensity. The autonomic nervous system can be sub-divided into the sympathetic and parasympathetic branches. While there are exceptions, the sympathetic and parasympathetic branches tend to work in opposition to each other in order to maintain an optimal physiologic state for the circumstances. Thus, when arousal results in increased heart rate, respiration rate, and inhibition of salivation, these changes are most likely the result of sympathetic arousal. Once the threat has passed, it is the parasympathetic branch that lowers heart and respiration rate and stimulates salivation. The two branches also can affect both the distribution of white cells within the immune and circulatory system, and their level of biological activity.

2.1.2 The hypothalamic-pituitary-adrenal axis.

The hypothalamic-pituitary-adrenal axis (HPA or HTPA axis), also known as the limbic-hypothalamic-pituitary-adrenal axis (LHPA axis) and, occasionally, as the hypothalamic-pituitary-adrenal-gonadotropic axis, is a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal (or suprarenal) glands. The interactions among these organs constitute the HPA axis, that controls reactions to stress and regulates many body processes, including, the immune system. A wide variety of species, from the most ancient organisms to humans, share components of the HPA axis. It is the common mechanism for interactions among glands, hormones, and parts of the midbrain that mediate the general adaptation syndrome (GAS). Cortisol is an essential hormone in humans (corticosterone in rodents), and without it, not only one can suffer from chronic fatigue, but also one’s ability to deal with stress is severely compromised. As its name implies, one of its major functions is to convert stored energy in the form of glycogen into usable glucose. It also converts protein into amino acids and triglycerides into free fatty acids. Each of these may be needed in order to provide
metabolic support for the coping strategy chosen (Vander et al., 1990). Excess cortisol/corticosterone can interfere with just about every biological system in the body. In addition to the changes subsequent to sympathetic activation, the additional energy substrates and molecular building blocks released into the blood stream can contribute to arteriosclerotic plaques. (Sapolsky, 1998; Black, P.H., & Garbutt, L.D. 2002). Along with other factors, many of which occur subsequent to chronic stress, the probability of myocardial ischemia can rise dramatically. Excessive cortisol can impair the memory, and normal functioning of the gastrointestinal system (Cahill et al.,1998). It can wreak havoc on the immune system by suppressing the it thereby causing an increased susceptibility to colds and other illnesses, an increased risk of cancer, the tendency to develop food allergies, an increased risk of an assortment of gastrointestinal issues (because a healthy intestine is dependent on a healthy immune system), and possibly an increased risk of autoimmune diseases.

Fig.2.1 Activation of Hypothalamic pituitary adrenal axis (HPA axis) following stress exposure.
2.2. Immune system.
The immune system is remarkably adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic micro organisms. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders. The term immunity is derived from Latin word “immunis”. Historically, immunity meant protection from diseases and more specifically infectious diseases. The cells and the molecules responsible for the immunity constitute the Immune system, and their collective and coordinated response to the introduction of foreign substance is called the Immune Response. The physiologic function of the immune system defense against infectious microbes. All those physiological mechanism that endow animal with the capacity to recognize materials as foreign to it and to neutralize, eliminate or metabolize them with / without injury to its own tissues. The definition of immunity can be simply stated as “body’s ability to defend itself against viruses, toxins, bacteria, fungi, Yeast and parasites.” Cellular elements of the immune system include lymphocytes, macrophages, monocytes, eosinophils, neutrophils, basophils, mast cells, and dendritic cells. Most of these cell types do not reside permanently at any particular anatomical location. Rather, they re-circulate via the blood and, in the case of lymphocytes, via the lymphatic circulation. Even the most sessile of these cells, the dendritic cells and macrophages, can be induced to migrate to sites of inflammation or to regional lymph nodes to function as antigen presenting cells. In addition to routine recirculation of immune system cells in their ‘patrol’ function, most cells of the immune system can be attracted to sites of infection or inflammation and accumulate there to participate in events that lead to the elimination or isolation of microbes and to tissue repair (Selye,1970; Baggiolini, 1998; Guo, et al., 2002).
It can be divided into two components.

- Innate immunity
- Acquired immunity
2.2.1. Innate Immunity.

The term, innate immunity, refers to the basic resistance to disease that a species possesses. It is usually the first line of defense against infection. This prevents entry of micro-organisms into tissues or, once they have gained entry, eliminates them prior to the occurrence of disease. One's innate immunity are the barriers that keep harmful materials from entering your body and form the first line of defense in the immune response. The principal components of innate immunity are (1) physical and chemical barriers such as epithelia and anti-microbial substances produces at epithelia surface such as the stomach acid, mucous (traps microorganisms and small particles), the cough reflex, and enzymes in tears and skin oils. (2) phagocytic cells (neutrophils, macrophages and natural killer cells (NK) cells; (3) blood proteins including members of the complement system and other mediators of inflammation (4) proteins called cytokines that regulate and coordinate many of the activities of the cells of immunity. The mechanisms of innate immunity are stimulated by structures that are common to groups of related microbes and may not distinguish fine differences between foreign substances. The pathogenicity of microbes is in part related to their ability to resist the mechanisms of innate immunity. If an antigen gets past the external barriers, it is attacked and destroyed by other parts of the immune system. Innate immunity also includes those things that make humans resistant to many of the diseases of animals. The inflammatory response (inflammation) is part of innate immunity. It occurs when tissues are injured by bacteria, trauma, toxins, heat, or any other cause. Chemicals including histamine, bradykinin, serotonin, and others are released by damaged tissue causing blood vessels to leak fluid into the tissues, resulting in localized swelling. This helps to isolate the foreign substance from further contact with body tissues. These chemicals also attract white blood cells that surround, engulf, and destroy foreign substances. This process is called phagocytosis, and the cells are collectively referred to as phagocytes. The characteristics of innate immunity response include the following:

1. Responses are broad spectrum (non-specific)
2. There is no memory or lasting protective immunity.
3. Present from birth.
2.2.2. Adaptive Immunity: Adaptive immunity is a highly evolved defense mechanism that is stimulated by exposure to infectious agents and increases in magnitude and defense capabilities with each successive exposure to particular microbe. As this form of immunity develops as a response to infection and adapts to the infection, it is called adaptive immunity. Because of its extraordinary capacity to distinguish among different even closely related microbes and macromolecules, adaptive immunity is also called specific immunity or acquired immunity. Adaptive and innate immunity do not operate independently of each other, they function as a highly interactive and cooperative system, producing a total response more effective than either of them could produce alone. It displays four characteristic attributes:

1. Antigenic specificity
2. Diversity
3. Immunologic memory

Innate and Adaptive immune responses are components of an integrated system of host defense in which numerous cells and molecules function co-operatively. The innate immune response to microbes stimulates adaptive immune response and influences the nature of adaptive responses and in turn adaptive immune responses use many of the effector mechanisms of innate immunity to eliminate microbes and they often function by enhancing the antimicrobial activities of the defense mechanisms of the innate immunity.

There are two types of Adaptive Immune Responses:

- Humoral or Antibody mediated immunity
- Cell mediated immunity

Humoral immunity is mediated by molecules in the blood called antibodies that are produced by cells called B-lymphocytes. These antibodies specifically recognize microbial antigens, neutralize the infectivity of the microbes and target microbes for elimination by various effector mechanisms. Humoral immunity is the principal defense mechanisms against extracellular microbes and their toxins because secreted antibodies can bind to these
microbes and toxins and assist in their elimination. Intracellular microbes such as viruses and some bacteria survive and proliferate inside phagocytes and other host cells where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the lysis of infected cells. Cell mediated immunity also referred to as cellular immunity is mediated by cells called T lymphocytes.

### 2.2.3 Effectors of the immune system.

The bone marrow is the site of generation of all circulating blood cells in the adult, including immature lymphocytes and is the site of B cell maturation. During fetal development, the generation of all blood cells called hematopoiesis, occurs naturally in blood islands of the yolk sac and the para-aortic mesenchyme and later in the liver and spleen. All the blood cells originate from a common stem cell that becomes committed to differentiate along particular lineages (i.e., erythroid, megakaryotic, granulocytic, and lymphocytic). The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines. Many of these cytokines are also called as colony stimulating factors (CSFs) because they are originally assayed by their ability to stimulate the growth and development of various leukocyte or erythroid colonies from marrow cells. Although, immune responses are mediated by a variety of cells, the leukocytes derived from stem cells in the bone marrow, play the central role.

(a) **T-lymphocytes.**

T lymphocytes called so as their cell maturation is taking place in the thymus gland, differentiate into two subsets helper T lymphocytes and cytolytic (or cytotoxic) T lymphocytes (CTLs). CD4+ T cells are activated by antigens (generally proteins or peptides) after their processing by antigen-presenting cells. These cells may be dendritic cells, macrophages or B cells. CD4+ T cells recognize antigens processed through the exogenous pathway by APCs expressing MHC class II molecules leading to the differentiation of CD4+ T cells into the functional subsets T helper 1 (Th1) and Th2. Th1 cells mediate the killing of organisms responsible for a variety of intracellular infections through their production of IFN-gamma. The Th2 cell subset mediates the production of specific antibodies by sensitized B-cells through the production of IL-4. CD8+ T cells recognize antigens that are
processed through the endogenous pathway and presented by antigen presenting cells expressing MHC class I molecules. CD8⁺ T cells mediate their effector function by producing IFN-gamma and TNF-α. They can also kill their target cells through a direct cytolytic mechanism. These processes concern protective immunity against intracellular infections but the same mechanisms apply to tumor cells following sensitization of the immune cells to tumor-associated antigens (Offringa R et al., 2000).

(b) B-lymphocytes.
These cells are primarily involved in antibody production and antigen presentation to T cells. B cells originate from lymphoid stem cells in the fetal liver and the bone marrow. B-lymphocytes are thymus-independent cells that express intrinsically produced immunoglobulins on their external membranes and upon stimulation by antigen differentiate into plasma cells that produce and secrete large numbers of antibody molecules.

![Fig.2.2 Origin and differentiation of different immune cells.](image)
(c) **Natural killer cells.**

They are large granular lymphocytes, providing innate protection by killing tumor cells and cells infected with intracellular pathogens. These cells display a low-affinity surface receptor for the Fc fragment of IgG (CD16; e.g. CR3) and CD56. Natural killer cells account for 10-15% of blood lymphocytes and are found in low numbers in the peripheral lymphoid system. Natural killer cells regulate certain aspects of T and B cell activation and hematopoiesis, and they defend against certain tumors and intracellular infections by killing the involved cells. In contrast to cytotoxic T cells, the NK cell-mediated cytotoxicity neither requires previous sensitization nor is MHC-restricted. The cytotoxicity of NK cells is increased after exposure to cytokines such as IL-2 (Wang et al, 2000). NK cells also mediate antibody-dependent, cell-mediated cytotoxicity via the CD16.

(d) **Monocytes.**

Monocytes are the circulating cells, which upon migration into tissue differentiate into macrophages and play an important role in phagocytosis and killing of microorganisms. Following activation, monocytes release cytokines and other mediators, starting a cascade of proinflammatory responses (Havemann et al., 1999). They are professional antigen presenting cells, which process antigens and present them to T cells and B cells via MHC class II receptors and thus initiate the adaptive immune response.

(e) **Neutrophils.**

Neutrophils are the main components of polymorphonuclear granulocytes and the most abundant of the leukocytes. These are the first circulating phagocytic cells recruited to the site of infection and inflammation to ingest, kill, and digest pathogens. Responsible for the first line of defense, they migrate to the inflammatory site and kill the invading microorganisms by releasing proteolytic enzymes and oxygen radicals. Their capacity to synthesize cytokines implies their role in immune responses (Cassatella MA et al., 1995).

(f) **Co-stimulatory molecules.**

Numerous costimulatory molecules have been identified playing an important role in the initiation of immune response by T and B lymphocytes. Full activation of naïve T cell
requires two signals; the first signal is antigen displayed by antigen presenting cells (APCs) in the form of peptides bound to histocompatibility molecules; the recognition of antigen by T cell provides specificity to the response. The second signal is co stimulatory signal is antigen non specific and is provided by the molecules on APCs that engage particular co-stimulatory receptors on T cells. Therefore co-stimulation is crucial to the development of an effective immune response. Activation of T cells without co-stimulation may lead to T cell anergy, T cell deletion or the development of immune tolerance. In particular, CD28 is one of the molecules expressed on T cells that provide co-stimulatory signals required for T cell activation and plays an important role in enhancement of proliferation and cytokine production by T cells, as well as in the preventing T-cell anergy, thus identifying it as a key second signal for T-cell activation. Signals generated by the binding of CD28 to its ligands CD80 or CD86, found on antigen presenting cells, cooperate with TCR-dependent signals leading to cell proliferation and Th1/Th2 cytokine production (Kuchroo et al., 1995)

(f) Th1/Th2 cytokines.

Cytokines are proteins or peptides, some of which have sugar molecules attached (Blotta, et al., 1997). They are a large group of molecules and include the interferons (IFNs), interleukins (ILs), and various colony-stimulating factors (CSFs). Also included are the tumor necrosis factors (TNFs) and transforming growth factors (TGFs), thought to be particularly important in mediating inflammatory and cytotoxic reactions (Blotta, et al., 1997). The Th1/Th2 balance hypothesis emerged in the late 1980s, stemming from observations in mice of two subtypes of T-helper cells differing in cytokine secretion patterns and other functions (Mosmann TR et al., 1986). They suggested these “Th1” and “Th2” cells were “important regulators of the class of immune response.” The concept subsequently was applied to human immunity, and 10 years after the original discovery the effects of Th1 and Th2 in disease became a major research focus (Mosmann TR et al., 1989) (Abbas AK et al., 1996). The Th1/Th2 hypothesis rests largely on the cytokine patterns (chemicals released by these cells that promote cell-to-cell communication) of these two cell types. Th1 cells and the pathway they dominate are heavily reliant on interferon-gamma (IFN-gamma), and to a lesser extent interleukin-2 (IL-2) and interleukin-12 (IL-12). Th2 cells are most heavily reliant on interleukin-4 (IL-4) and sometimes interleukin-5 (IL-5) as
well. Th1 and Th2 cells also can differ in the arrays of receptors on their outer surface that they use to respond to cytokines and other messenger substances. Th1 cells lead the attack against intracellular pathogens such as viruses, raise the classic delayed-type hypersensitivity (DTH) skin response to viral and bacterial antigens, and fight cancer cells. Th2 cells are believed to emphasize protection against extracellular pathogens such as multicellular parasites. On the negative side, the Th1 pathway is often portrayed as being the more aggressive of the two, and apparently, when it is over reactive, can generate organ-specific autoimmune disease (e.g., arthritis, multiple sclerosis, type 1 diabetes) (Singh et al., 1999). The Th2 pathway is seen as underlying allergy and related IgE-based disease, and predisposing to systemic autoimmune disease (Dent, 2002). Th1 and Th2 responses are mutually inhibitory. Thus, IL-12 and IFN-gamma inhibit Th2, and, vice versa, IL-4 and IL-10 inhibit Th1 responses. Type 2 cytokines, such as IL-4 and IL-10, promote humoral immunity by stimulating the growth and activation of mast cells and eosinophils, the differentiation of B cells into antibody-secreting B cells, and B-cell immunoglobulin switching to IgE. Importantly, type 2 cytokines inhibit macrophage activation, T-cell proliferation, and the production of proinflammatory cytokines. Thus, IL-4 and IL-10 are considered the major anti-inflammatory cytokines (Fearon, D.T. & Locksley, R.M., 1996) (Mosmann, T.R. & S. Sad. 1996).

Fig: 2.3 A simplified model of the possible interactions between polarized T helper 1 (Th1) and Th2 responses. The model shows the cytokines produced by each cell type and how they positively (indicated by plus sign) and negatively (indicated by a minus sign) regulate each other.
2.3. Stress related disorders.

Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression and anxiety, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension ulcerative colitis and immunosuppression (Elliott and Eisdorfer, 1982). If stress persists after the initial fight or flight reaction, the body's reaction enters a stage where, the activity of the sympathetic nervous system declines and epinephrine secretion is lessened, but corticosteroid secretion continues at above normal levels. The body is unable to cope this and there is likely to be breakdown of bodily resources (Rubin, Paplau, & Salovey, 1993). It is in this stage that there may be a reduction of the levels of epinephrine and norepinephrine in the brain, a state related to depression (Rubin, Z.; Peplau, L.A.; & Salovey, P, 1993). Stressful life events are related to the risk of infected individuals developing an illness (Cohen et. al. 1998). Traumatic stressful events may trigger either behavioral or biological processes that contribute to the onset of disease. Chronic stress has been associated with increased reports of illness. Long-term exposure to chronic stress may facilitate the development of illness during exposure to stress (Cohen, Kessler & Gordon, 1995). Exposure to chronic stress may results in permanent or at the very least long-term psychological, biological, or behavioral responses that alter the progression of illness (Cohen et. al. 1998) found that those who had either a work related or interpersonal chronic stressors (defined as stress lasting one month or longer) had an increased risk of developing colds compared to those who had no chronic stressor. In addition, the longer the stress endured, the more likely a person was to become ill.

2.3.1. Stress induced immunological alterations.

Conceptualizations of the nature of the relationship between stress and the immune system have changed over time. Selye’s (1975) finding of thymic involution led to an initial model in which stress is broadly immunosuppressive. Early human studies supported this model, reporting that chronic forms of stress were accompanied by reduced natural killer cell cytotoxicity, suppressed lymphocyte proliferative responses, and blunted humoral responses to immunization (Cohen, Miller, & Rabin, 2001; Herbert & Cohen, 1993). Diminished immune responses of this nature were assumed to be responsible for the heightened
incidence of infectious and neoplastic diseases found among chronically stressed individuals (Andersen, Kiecolt-Glaser, & Glaser, 1994; Cohen & Williamson, 1991). Dhabhar and McEwen (1997, 2001) proposed that acute fight-or-flight stressors should instead cause redistribution of immune cells into the compartments in which they can act the most quickly and efficiently against invaders. They found that during acute stress, T-cells are selectively redistributed into the skin, where they contributed to enhancement of the immune response. In contrast, during chronic stress, T cells were shunted away from the skin, and the immune response to skin test challenge was diminished (Dhabhar & McEwen, 1997). On the basis of these findings they proposed a biphasic model in which acute stress enhances, and chronic stress suppresses the immune response.

Fig. 2.4. HPA axis and immune cell interactions in response to stress.
The most well-known model hypothesizes that chronic stress elicits simultaneous enhancement and suppression of the immune response by altering patterns of cytokine secretion (Marshall et al., 1998). Th1 cytokines, which activate cellular immunity to provide defense against many kinds of infection and some kinds of neoplastic disease, are suppressed. This suppression has permissive effects on production of Th2 cytokines, which activate humoral immunity and exacerbate allergy and many kinds of autoimmune disease. This shift can occur via the effects of stress hormones such as cortisol (Chiapelli et al., 1994). Th1-to-Th2 shift changes the balance of the immune response without necessarily changing the overall level of activation or function within the system. Because a diminished Th1-mediated cellular immune response could increase vulnerability to infectious and neoplastic disease, and an enhanced Th-2 mediated humoral immune response could increase vulnerability to autoimmune and allergic diseases, this cytokine shift model also is able to reconcile patterns of stress-related immune change with patterns of stress-related disease outcomes (Marshall et al., 1998).

Cortisol, the main effectors of the HPA limb of the stress system, modulate immune responses in numerous ways, including through gene expression, transcription, translation, post-translational processing, protein secretion, and cell progenitor proliferation and differentiation. Cytokine inhibition accounts for many of the inhibitory effects on the immune response during stress. Glucocorticoids inhibit cytokine production by altering mRNA stability at the level of gene expression. Their actions may be mediated directly (Type 1 mechanism) or indirectly (Type 2 mechanism) (Bamberger et al., 1996). For example, glucocorticoids directly decrease transcription of the genes for interleukin-6 (IL-6) and interleukin-1β, thus decreasing their production by immune cells. In contrast, the immune response is indirectly suppressed by glucocorticoids through inhibition of pro-inflammatory transcription factors such as nuclear factor-κ B (NF-κ B) and activating protein-1 (AP-1).
2.3.2. Stress induced biochemical and behavioral alterations.

Chronic unpredictable stress, apart from causing immunosuppression can induce several biochemical and behavioral alterations like glucose intolerance, increase in lipid peroxidation and decrease in glutathione, gastric ulcerations, behavioural depression, cognitive deficits, male sexual dysfunction, fatigue etc (Bhattacharya, 1998; Bhattacharya et al., 2000; Muruganandam et al., 2002). Stress of any kind results in a progressive deterioration in most of the endocrine functions and enhances exogenous or endogenous stress associated with an impairment of hepato- pancreatic function (Kumar et al., 1992; Zbigniew, 1994), and results in a significant elevation of the activities of GPT, GOT, ALP, bilirubin, (Singh, et al., 2005) triglycerides in serum and liver glycogen and GSH, indicating considerable alteration in normal physiological functioning of the body.

2.4. Stress and wound healing.

Cutaneous injury initiates a set of relatively sequential events which leads to repair of the wound (Clark, 1991; Thomas et al., 1995; Martin, Hopkinson-Woolley, & Mc Cluskey, 1992). Healing progresses through three general stages: (a) an inflammatory stage which consists of platelet aggregation, blood coagulation, and migration of inflammatory cells into the wound site; (b) a proliferative phase which involves the migration and proliferation of keratinocytes, fibroblasts, and endothelial cells, leading to re-epithelialization and granulation tissue formation; and (c) a long remodeling phase that may last several years. Although there is quite a bit of overlap between these stages, the latter phases are strongly dependent upon the initial events of the healing process (Hubner et al., 1996). It has been shown that the invasion of granulocytes (PMNs) into the wound tissue is needed for the subsequent recruitment and differentiation of macrophages (Clark, 1991). Within minutes of tissue damage, PMNs are attracted to the wound area by preformed mediators released from damaged keratinocytes at the wound margin (Thomas et al., 1995). Once at the wound site, PMNs function as professional phagocytes and also produce pro-inflammatory and chemotactic cytokines to stimulate the extravasation of macrophages into the area (Lawrence & Diegelmann, 1994). Apart from phagocytosing effect neutrophils and potentially dangerous microorganisms,
macrophages also produce growth factors like Keratinocyte growth factor (KGF) and vascular endothelial cell growth factor (VEGF) and proinflammatory cytokines (IL-1 alpha, and TNF-alpha), which are essential for efficient wound healing, e.g., stimulation of fibroblast and keratinocyte growth, synthesis and breakdown of extracellular matrix proteins, fibroblast chemotaxis, (Leibovich & Ross, 1975).

Because the later stages of wound healing are dependent upon these early inflammatory steps, dysregulation of neutrophil and macrophage recruitment and function can have a significant impact on the overall healing process. Disruption of this regulated process can impact healing and have deleterious health consequences. The interference of stress in the process of tissue repair is reported in the literature (Friesel, R. E., and Maciag, T, 1995; Bock, O., Mrowietz, U., Keloids, 2002). Upon chronic stressor stimulus, the hypothalamic-pituitary-adrenal (HPA) axis releases electrical and chemical stimuli capable of inducing the formation of substances such as cortisone, adrenalin, and nor-adrenaline; these hormones suppress or excite cells involved in tissue repair, such as fibroblasts, neutrophils, macrophages, and lymphocytes, which naturally induce a delay in tissue repair (van Zuijlen et al., 2002; Rahban, S. R., and Garner, 2003). For example, chronically stressed human subjects (Alzheimer’s caregivers) who have comparatively low inflammatory responses showed a delay in healing compared to age-matched control patients (Kiecolt-Glaser et al., 1995). Implicated in this delay in healing were physiological changes associated with chronic stress which disrupts the normal physiologic equilibrium and activates the hypothalamic–pituitary–adrenal axis (HPA). Among the products of the HPA axis are glucocorticosteroid (GC) hormones which affect many components of the inflammatory response. Leukocyte production of proinflammatory cytokines such as IL-1, IL-6, and TNF-alpha is suppressed by GC (Snyder & Unanue, 1992). Furthermore, the phagocytic activity of activated granulocytes and macrophages is likewise suppressed by stress (Shurin, et al., 1994). Therefore, modulation of the pro-inflammatory response, which is an integral stage of wound repair, may influence normal healing kinetics.
2.5. Herbals as stress busting agents.

Various attempts have been made to counter the aversive effects of stress, ranging from exercise, yoga, and meditation to antistress drugs, particularly the anxiolytic benzodiazepines (BDZ). However, despite claims to the contrary, these non-pharmacological and pharmacological methods appear to have limited utility (Mason, 1975) and the answer to this perplexing problem of countering stress induced perturbations of physiological homeostasis can be from the plant kingdom. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety profile besides being economical, effective and their easily available (Atal CK, Kapoor BM, 1989; Siddiqui HH, 1993). According to a survey (1993) of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh (Siddiqui HH, 1993; WHO survey, 1993) and this is very high percentage of people by any means.

Ayurveda, one of the most ancient medical systems widely practiced has a sound philosophical, experiential and experimental basis. Rasayana therapy, one of the eight branches of Ayurveda, consists of nourishing and rejuvenating drugs with multiple applications for longevity, memory enhancement, immunomodulation and adaptogenic steroidal lactones and their glycosides as immunomodulators (Patwardhan, 2000). Ayurveda, classifies remedies that prevent disease and counteract aging as rasayana or rejuvenation remedies (Davydov and Krikorian, 2000; Auddy et al., 2003; Bhattacharya and Muruganandam, 2003). The concept of treating stress-related conditions with medicinal plants is familiar to most traditional healing models throughout the world (WHO, 2002). Though conventional medicine does not use these plant remedies, primarily citing lack of research, the popular health market has been selling botanical products to treat stress and fatigue in the United States since the 1960s. Thus, despite the difficult conceptual grasp required, a literary history of the treatment for stress exists in many cultures and can be explored for the development of novel drugs or therapeutics. The remedies of these ethnomedical models of medicine can provide leads to further drug research and a comparison to known antistress agents/adaptogens. For example, a preliminary survey using ethnobotanical reports has located 65 potential plant adaptogen species belonging to 44 plant families. In 1943, the People’s Commissars Council of the
Union of the Soviet Socialist Republics charged its scientists with the task of finding tonic substances to strengthen the health of workers in the Russian defense industry during World War II (Panossian, 2003). Thus began the effort to find remedial substances that would increase the protective state of resistance during conditions of stress. N.V. Lazarev (1946; 1962) showed that ingestion of certain plant extracts could improve stress markers in laboratory animals, such as cognitive function or oxidative damage. Between 1950 and 1960 these plant remedies were termed as *adaptogens* and three criteria were set to describe their remedial action (Brekhman, 1969).

An adaptogen that is an antistress agent, should be innocuous and cause minimal disturbance to the normal physiological function of an organism, should have nonspecific (i.e., should increase resistance to a wide range of stressors), and it should have a normalizing action irrespective of the direction of the preceding pathological changes (i.e., be able to normalize either high and low physiological responses to stressors) (Brekhman and Dardymov, 1969). Many herbs covered by Ayurveda as Rasayana, influence the balance of certain neurotransmitters exert antistress and adaptogenic effect. Apart from these preventive uses, antistress, adaptogenic drugs have more therapeutic value in stress related disorders. Many adaptogens have been labeled immunostimulant, immunomodulator, biological response modulator, or immunopotentiator most likely in the attempt to give these remedies a more specific mechanism of action or perhaps to fit them into the conventional medical paradigm. The initial dysfunction of the HPA axis has been shown to involve dysregulation of catecholamines, dysregulation of glucocorticoids, dysregulation of cytokines, receptor desensitization, fatigue, anxiety, anorexia, altered cognitive performance, and decreased sexual behavior (McEwen, 2002). Adaptogen remedies have been shown to ameliorate immune dysfunction by lowering corticosterone level (Kim et al., 1999), thus reducing the damage caused by excess cortisol. Other adaptogen remedies act directly by stimulating macrophages and lymphocytes (Cai et al., 1998) or otherwise reversing immunosuppression caused by stress (Wagner et al., 1994). Thus, in this study an attempt has been made to identify a plant molecule having the potential to restore the disturbances caused by stress on immunological, biochemical and behavioral levels.