Review of Literature
I. Stress

Living organisms survive by maintaining an immensely complex dynamic and harmonious equilibrium, or homeostasis, which constantly challenge or outright threaten by intrinsic and extrinsic disturbing forces or stressors. Counteracting/ reestablishing forces to maintain the steady state required for successful adaptation, or adaptational responses, consisting of an extraordinary repertoire of physical or mental reactions that attempt to counteract the effects of the stressors in order to reestablish homeostasis. In this context, stress can be defined as a state of disharmony, or threatened homeostasis.

The adaptive response can be specific to the stressor or can be generalized and nonspecific. The latter can be stereotypic and generally occur only if the magnitude of the threat to homeostasis exceeds a certain threshold. These contemporary concepts regarding stress have evolved over the past millennia.

1. Chronological developments

The concept of stress in the modern sense is not easily found but defined in the traditional texts of Indian culture and tradition such as Charak Sanhita, Patanjali’s Yogasutras and Bhagawat Gita. However, a number of concepts developed by ancient Indian scholars relate to appear similar to the phenomenon of stress. Some of these, for example, are dukha (pain, misery or suffering), klesa (afflictions), Kama or trisna (desires), atman and ahamkara (self and ego), adhi (mental aberrations) and prajnaparadha (failure or lapse of consciousness). It is interesting to note that the body-mind relationship, characteristic of modern stress studies, is emphasized in the Ayurvedic (Indian) system of medicine.

In the beginning of the classic era, Heracleitus was the first to suggest that a static, unchanged state is not the natural condition, but rather that the capacity to undergo constant change was intrinsic to all things. Shortly afterward Empedocles proposed the corollary idea that all matter consisted of elements and qualities in a dynamic composition or alliance to one another, and that balance or harmony was a necessary condition for the survival of the living organisms. One hundred years later, Hippocrates equated health to a harmonious balance of elements and qualities of life and disease to a systematic disharmony of these elements. Hippocrates also suggested that the disturbing forces that reduced the disharmony of disease derived from natural rather than supernatural sources and that counterbalancing or adaptive forces were of a natural origin as well. On years of the Renaissance, Thomas Sydenham extended the Hippocratic concept of disease as a systematic disharmony brought about by disturbing forces, when
he suggested that an individual's adaptive response to such forces could itself be capable of producing pathological changes. Walter Cannon later coined the term "homeostasis" and extended the homeostatic concept to emotional as well as physical parameters. He also described the "fight or flight reaction" and linked the adaptive response to stress with catecholamine secretion and actions.

1.1 Views of Hans Selye

In the 1930's, Hans Selye borrowed the term "stress" from physics and hypothesized that a constellation of stereotypic psychological and physiological events occurring in seriously ill patients represented the consequences of a severe, prolonged application of adaptation responses. To him stress, for better or for worse, was a constant influence in day-to-day existence. He recognized that stress, plays a very significant role in the development of all types of disease. He identified three stages of the body's response to a stressor and called the entire process whereby stress influences the body as the General Adaptation Syndrome (GAS) and, in effect, redefined Sydenham's concept of diseases of adaptation.

1.1.1. General Adaptation Syndrome

When the body meets a stressor, an adjustment takes place in an effort for the body to regain its equilibrium (homeostatic state). Dr. Hans Selye's General Adaptation Syndrome is one of the most widely accepted and has been widely held as a comprehensive theory regarding stress and its effect on the human system. The three stages, known as the General Adaptation Syndrome or GAS were represented in Figure:1

![Diagram of the General Adaptation Syndrome](image)

**Duration of Exposure to Stress**

Figure 1. Stages of General Adaptation Syndrome (GAS)
a. Alarm or Reaction Stage

In the alarm stage, the body becomes aware of a stressor. This stage includes an initial ‘shock phase’ in which the resistance is lowered, and a counter-shock phase in which defensive mechanisms becomes active. During this stage, the body prepares to take emergency action to defend itself against or escape from the danger. It is in this stage that the physiological "fight or flight" response happens. This stage is characterized by autonomous excitability, adrenaline discharge, increased heart rate and blood pressure, muscles tense, gastro-intestinal ulceration and other physiological reactions. Depending on the nature and intensity of the threat and the condition of the organism, the periods of resistance vary and the severity of symptoms may differ from ‘mild invigoration’ to ‘disease of adaptation’ (Pestonjee, 1997).

b. Resistance Stage

After the initial reaction to the stressor, the body attempts to restore its equilibrium (homeostatic state) and the bodily signs characteristic of the alarm reaction disappear. The adaptation occurs primarily because the nervous and endocrine systems help the body deal with the stressor. Maximum adaptation occurs during this stage. Resistance increases to levels above normal as indicated in Figure 1. In the case of short-term stressors, the resistance state continues until the stressor ceases (Pestonjee, 1997). If exposed to long term or chronic stress, the body eventually loses its ability to adapt and moves to the third stage, the stage of exhaustion.

c. Exhaustion Stage

The stage of exhaustion occurs if the stressors continue as mentioned above or if the stressors return repeatedly. If the stress continues in these two cases, the body’s ability to resist appropriately diminishes resulting in the depletion of adaptation energy, failure of the immune system to function properly, and stress related disease would occur. Signs of the alarm reaction reappear, and the resistance level begins to decline irreversibly and the organism collapses (Pestonjee, 1997).

Selye believed that daily lives are influenced by two different kinds of stress: pleasant stress contributing to “wellness” and unpleasant stress contributing to disease and sickness. He made it clear that not all states of stress were noxious when he coined the terms ‘eustress’ and ‘distress’ (Chrousos and Philip, 1992.)
Hence, he believed that mild, brief, and controllable states of challenged homeostasis could actually be perceived as pleasant or exciting and could be positive stimuli to emotional and intellectual growth and development. It was the more severe, protracted, and uncontrollable situations of psychological and physical distress that Selye believed led to frank disease states.

2. Types of stressors

In contrast to Selye, Mason in 1971 described stress as a multi hormonal response pattern organized in a rather specific or selective manner depending on the particular stimulus. Later on, the definition of stress has been constantly changing as its multifactorial nature has been recognized and the role of stressors mediating stress response has been identified. Stressors can be defined as conditions that endanger, or are perceived to endanger, the survival of individual (Van de Kar and Blair, 1999). In general, these stressors can be grouped into three broad categories.

I  Psychological stressors based on learned response to the threat of an impending adverse condition (Fear, anxiety, exposure to a novel or uncontrollable environment)

II  Stressors that consist of a physical stimulus have a strong psychological component (Pain, foot shock, Immobilization).

III. Stressors which challenge cardiovascular homeostasis (hemorrhage, orthostatic stress/ upright tilt exercise and heat exposure) and many other manifested in humans daily as shown in table-1

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<thead>
<tr>
<th>Psychological/Psychosocial Stress</th>
<th>Physical Stress</th>
<th>Internal Stress</th>
<th>External Stress</th>
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<tr>
<td>Unemployment</td>
<td>Early menopause</td>
<td>Chronic illness</td>
<td>Pollution</td>
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<td>Financial problems</td>
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<td>Competitive life</td>
<td>Insomnia</td>
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<td>Disagreement</td>
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<td>Loss of love ones</td>
<td>Alteration in circadian rhythm</td>
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3. **Physiology of stress response**

Over the past few years, our understandings to the neuroendocrine and brain circuits that are linked to the stress response have increased dramatically. Exposure to the hostile conditions (Usually referred to as stressors) results in a series of coordinated responses organized to enhance the probability of survival. These coordinated responses, often referred to as "stress responses", are composed of alterations in behavior, autonomic function and the secretion of multiple hormones including adrenocorticotropic hormone (ACTH) and cortisol/corticosterone, adrenal catecholamines, oxytocin, prolactin and rennin (Van de kar and Blair, 1999). Some of the changes associated with the stress response include:

1. Mobilization of energy to maintain brain and muscle function.
2. Sharpened and focused attention on the perceived threat.
3. Increased cerebral perfusion rates and local cerebral glucose utilization.
4. Enhanced cardiovascular output respiration, and redistribution of blood flow. Increasing substrate and energy delivery to the brain and muscles.
5. Modulation of immune function.
6. Inhibition of reproductive physiology and sexual behavior.
7. Decreased feeding and appetite.

In the specialized situation of fluid loss due to hemorrhage, responses also include water retention through both renal and vascular mechanisms (Habib et al., 2001; Sapolsky, 2000).

These neuroendocrine responses are considered important survival mechanisms during exposure to life threatening stimuli. Many brain structures are involved in the response to psychologically and physically stressful stimuli. Activation of the hypothalamic-pituitary adrenal axis (HPA axis) leads to a rapid secretion of (Adreno Corticotropic Hormone) ACTH from corticotrophs in the anterior pituitary and to increase in circulating glucocorticoids (Aguilera et al., 1996). Initially it was thought that corticotrophin releasing factor (CRF) is the sole means of ACTH release from the pituitary gland. Later on it was established that CRF is the primary but not the only regulator of ACTH release from pituitary gland. CRF plays a prominent role in mediating the effect of stressors on the hypothalamic-pituitary-adrenocortical axis and in coordinating the endocrine, autonomic, behavioral and immune responses to stress. In
addition to CRF, various other factors have also been shown to induce ACTH secretion during stress. These intrinsically weaker secretagogues include Arginine vasopressin (AVP), oxytocin, Angiotensin-II, cholecystokinin, vasoactive intestinal peptide, and catecholamines. However, most of them have been shown to depend upon the presence of CRH for this modulatory action in HPA axis activation. Among these, the physiological importance of AVP in the regulation of stress response becomes important, because of co-localization and co release of CRF and AVP in mPVN and because of synergistic peptides of both peptides on the corticotrops. Although AVP is a weak ACTH stimulator by itself, it markedly potentiates the effects of CRH (Vale et al., 1981; Van de Kar and Blarr, 1999; Gonzalo et al., 2003).

Specifically because of their potency and their range of actions, glucocorticoids have to be maintained within optimal range either too little or too much is deleterious to the organism. Therefore, the HPA axis is well controlled to produce rapid and optimal responses, which terminate promptly cessation of stress. This is achieved by multiple, nested, negative feedback loops, primarily mediated by the steroids themselves.


The end hormones of the HPA axis are glucocorticoids. Glucocorticoids are synthesized by the cells of the zona reticulosa in the adrenal cortex as shown in Fig. 2. Cholesterol is cleaved to pregnenolone, which undergoes dehydrogenation and subsequent hydroxylations to form corticosterone.

Fig: 2

Biosynthetic pathway of steroidogenesis
The neurons of the medial parvocellular division of the Para ventricular nucleus of the hypothalamus synthesize corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) and project to the external layer of the median eminence (Akil et al., 1995). AVP is a potent synergistic factor with CRH in stimulating ACTH secretion, although AVP has negligible ACTH secretagogue activity on its own (Lamberts et al., 1984). There is a reciprocal positive interaction between CRH and AVP in the hypothalamus, with each neuropeptide stimulating the secretion of the other. Activation by stressors leads to the release of these peptides into the portal blood, whereby these secretagogues are carried to the anterior pituitary. On binding with the CRH and AVP receptors in the anterior pituitary, ACTH and related peptides, derived from the common precursor proopiomelanocortin (POMC) are released into the total circulation. ACTH activates the release and biosynthesis of glucocorticoids (corticosterone in rodents and cortisol in primates) by the cells of the adrenal cortex (Akil et al., 1995).

In non-stressful situations, both CRH and AVP are secreted in the portal system in a circadian, pulsatile fashion, with a frequency of about two to three secretory episodes per hour (Engler et al., 1989). Under resting conditions, the amplitude of the CRH and AVP pulses increase to a maximum in the early morning hours, resulting in ACTH and cortisol secretory bursts in the general circulation (Horrocks et al., 1990; Chrousos et al., 1998b; Tsigos et al., 2002). These diurnal variations are perturbed by changes in environment and stressful conditions.

During acute stress, the amplitude and synchronization of the CRH and AVP pulsations in the hypophyseal portal system markedly increases, resulting in increases of ACTH and cortisol secretory episodes (Tsigos et al., 2002). Depending on the type of stress, other factors such as AVP of magnocellular neuron origin, angiotensin II and various cytokines and lipid mediators of inflammation are secreted and act on the HPA axis and potentiate its activity (Holmes et al., 1986; Phillips, 1987).

Glucocorticoids should be maintained within an optimum range, as extremes of levels can be deleterious (Munck et al., 1984). The HPA axis is therefore, well controlled to produce quick responses to activation which terminate at the cessation of the activation. This is accomplished with the help of multiple, nested feedback loops (Keller-Wood et al., 1984). The subsequent rise in the level of glucocorticoids by the binding of ACTH to its receptor resulting in the conversion of cholesterol to pregnenolone exerts a negative feedback. The most rapid mechanism, called fast feedback, occurs within minutes of activation. This mechanism monitors the rate of rise of the steroids in the
circulation rather than their absolute level and assists in turning off the CRH/AVP secretion. The site of action is suprapituitary (Keller-Wood et al., 1984) at hypothalamic or supra hypothalamic levels (DeKloet, 1991; Jacobson and Sapolsky, 1991). The genomically mediated feedback, with a much slower time course, is thought to act on the pituitary and cause a reduction in the transcription of the genes coding for CRH, AVP, ACTH and POMC (Autelitano et al., 1990) and also at the level of higher nerve centers.

Both these mechanisms are activated simultaneously, but come into effect at different time spheres. Fast feedback is more sensitive, rapid and brain mediated and sets the magnitude and duration of each response. The genomic feedback is slower but has more profound effects at multiple levels of the HPA axis and establishes the range of the stress responsiveness.

**Fig-3** Schematic representation of the hypothalamus-pituitary-adrenal (HPA) axis. Bold arrows indicate the hormonal cascade that triggers the release of corticosterone from the adrenal glands. Dashed arrows represent the feedback actions of corticosterone on the level of the pituitary and PVN which involves GR activation (negative feedback) and at the level of the hippocampus (negative and/or positive feedback) which can be a synergistic activity of co localized MRs and GRs in addition to specific MR and GR effects.

5. **Glucocorticoid receptors**

Corticosteroids interact with specific receptor proteins in target tissues to regulate the expression of corticosteroid-responsive genes, thereby changing the levels and array of proteins synthesized by various target tissues. Most of the effects of corticosteroids
are not immediate but become apparent after several hours due to the time required for changes in gene expression and protein synthesis. Although glucocorticoids predominantly act to increase the expression of target genes, there are several well-documented examples to show that they also decrease the transcription of some target genes (Schimmer and Parker, 2001). Immediate actions of glucocorticoids are mediated by membrane bound receptors. Glucocorticoids (GC) being lipophilic molecules hormones readily pass through the plasma membrane of all cells in the body. If a cell possesses a GC receptor (GR), that cell can be a target for action. Using receptor binding and audiography, it was found that there are two types of binding sites that recognize corticosteroids (Reul and Dekleot, 1985). Type I receptor has a higher affinity to cortisol/corticosterone than Type II, whereas Type II has more affinity to aldosterone than Type I. These receptors have been cloned and identified as the glucocorticoid receptor which corresponds to Type II binding (Hollenberg et al., 1985) and the mineralocorticoid receptor, which in the kidney recognizes aldosterone and in the brain, corresponds to Type I binding (Arriza et al., 1987; Patel et al., 1989). The glucocorticoid receptor is fairly evenly distributed throughout the central and peripheral tissues and is found in high concentrations in the spleen and thymus (Lowy, 1989; Miller et al., 1988). They are unevenly localized in the brain and are found in both neurons and glial cells as shown by in-vitro cytosol binding assays (Reul and Dekleot, 1985) and immunocytochemistry. (Fuxe et al., 1985) Greatest immunostaining was present in the hippocampal pyramidal and granular neurons and also in brain regions known to be involved in the integrative regulation of the stress response. The type-II receptor exhibits higher affinity for synthetic glucocorticoids such as dexamethasone, than for corticosterone

5.1 Glucocorticoid receptor localization and Activation.

The glucocorticoid receptor resides primarily in the cytoplasm in an inactive form until it binds to the glucocorticoid steroid ligand. Steroid binding results in receptor activation and translocation to the nucleus. The inactive GR is found as a complex with other proteins including heat shock protein (HSP) 90 (Dalman et al., 1989; 1991), a member of the heat-shock family of stress induced proteins, HSP70 and a 56000 dalton immunophilin, one of the group of intracellular proteins that bind immunosuppressive agents. HSP90 facilitates the folding of the glucocorticoid receptor into an appropriate
conformation that is essential for ligand binding, through interactions with the steroid-binding domain (Fig. 4).

**Fig. 4**

![Diagram of steroid receptor signaling](image)

- GR  →  **Glucocorticoid receptor.**
- GRE  →  **Glucocorticoid response element.**
- HSP  →  **Heat Shock Proteins.**

Once the glucocorticoid receptor binds to its ligand it dissociates from its associated proteins and translocates into the nucleus, where it interacts with specific DNA sequences within the regulatory regions of the affected genes. These sequences are termed as Glucocorticoid Responsive Elements (GREs) and they provide specificity to the induction of gene transcription. The mechanisms by which GR activates transcription are complex and are not completely understood. They appear to involve the interaction of the GR with transcriptional cofactors and with proteins that make up the basal transcription apparatus. The activated receptors also inhibit, through protein-protein interactions, other transcription factors, such as c-jun, c-fos and NF-kB, which are positive regulators of the transcription of several genes involved in the growth and activation of immune and other cells (Scheinman *et al.*, 1995; McKay *et al.*, 1999). Further, glucocorticoids change the stability of messenger RNAs and therefore, the translation of several glucocorticoid-responsive proteins, as well as the electrical potential of neuronal cells.
5.2 Receptor independent mechanism for corticosterone specificity

Aldosterone and corticosterone bind to the mineralocorticoid receptor with equal affinity (Funder, 1992). The specificity of the mineralocorticoid receptor for aldosterone is maintained in spite of higher circulating levels of corticosterone, with the help of a type-2 isozyme 11 β-hydroxysteroid dehydrogenase. This enzyme metabolizes glucocorticoids such as corticosterone to receptor-inactive 11-keto derivatives. Aldosterone escapes metabolism by this enzyme, as its predominant form in a physiological setting is the hemiacetal derivative, which is resistant to 11 β-hydroxysteroid dehydrogenase actions. In the absence of this enzyme, the mineralocorticoid receptor is swamped by corticosterone, leading to severe hypokalemia and mineralocorticoid-related hypertension.

5.3 Physiological actions of glucocorticoids

Their diverse effects include alterations in carbohydrate, protein and lipid metabolism, maintenance of fluid and electrolyte balance and preservation of the normal function of the cardiovascular system, the immune system, the kidney, skeletal muscle, the endocrine system and the nervous system. In addition, glucocorticoids ready the organism for changes in energy and metabolism required for coping with stressful factors such as noxious stimuli and environmental changes that disturb its homeostasis.

a. Carbohydrate and protein metabolism.

Glucocorticoids protect glucose dependant tissues such as the heart and brain from starvation by stimulating the liver to form glucose from amino acids and glycerol and by stimulation the deposition of glucose as liver glycogen. They diminish the utilization of glucose, increase protein breakdown and activate lipolysis thereby providing amino acids for gluconeogenesis in the periphery. The direct consequence of this effect is the increase in blood glucose levels (Bhattacharya et al., 2000).

b. Lipid metabolism

Two effects of corticosteroids on lipid metabolism are established. The first is the redistribution of body fat that is seen in disorders of hypercorticism, such as Cushing’s syndrome. The second is the permissive facilitation of the effect of other agents, such as growth hormone and β-adrenergic receptor agonists, in inducing lipolysis in adipocytes, with a resultant increase in free fatty acids following glucocorticoid administration. There is an increased fat distribution in the back of the neck, face and supraclavicular area, coupled with a loss of fat in the extremities (Rai et al., 2003).
c. Electrolyte and water balance

Glucocorticoids exert effects on electrolyte and water balance largely due to the permissive effects on tubular function and actions that maintain the glomerular filtration rate. They also play a permissive role in the renal excretion of free water. Glucocorticoids exert multiple effects on Ca\(^{2+}\) metabolism (Schimmer and Parker, 2001). They interfere with Ca\(^{2+}\) uptake by unknown mechanisms and there is an increased Ca\(^{2+}\) excretion at the level of the kidney. These effects collectively lead to decreased total body Ca\(^{2+}\) stores.

d. Cardiovascular system

The most prominent effects on the cardiovascular system are due to mineralocorticoid-induced changes in renal Na\(^{+}\) excretion. Glucocorticoids increase expression of adrenergic receptors in the vascular wall. So they enhance vascular reactivity to vasoactive substances. Hypoadrenalism leads to hypotension and decreased response to vasoconstrictors such as nor epinephrine and angiotensin II. Conversely, excess glucocorticoid secretion leads to hypertension (Williams et al., 1998).

e. Skeletal muscle

Adequate concentrations of corticosteroids are required for the normal function of skeletal muscle and diminished work capacity is a sign of adrenocortical insufficiency (Schimmer and Parker, 2001). Excess glucocorticoids over prolonged periods cause skeletal wasting termed as steroid myopathy.

f. Formed elements of blood

Glucocorticoids exert minor effects on hemoglobin and erythrocyte content of blood. The administration of glucocorticoids leads to a decreased number in the circulating lymphocytes, eosinophils, monocytes and basophils and an increase in circulating polymorphonuclear leukocytes as a result of increased release from the marrow, diminished rate of removal from the circulation and increased demargination from vascular walls (Schimmer and Parker, 2001). Glucocorticoids have an ability to activate programmed cell death in certain lymphoid tissues.

g. Anti-inflammatory and immunosuppressive actions

Glucocorticoids can prevent or suppress inflammation in response to multiple inciting events, including radiant, mechanical, chemical, infectious and immunological stimuli. Although the use of glucocorticoids as anti-inflammatory agents does not address the underlying cause of the disease, the immunosuppressive action is of great clinical utility and has made these drugs among the most frequently prescribed agents.
Glucocorticoids inhibit the production of factors that are critical in generating the inflammatory response (Chrousos, 1992), including lymphokines, prostaglandins, interleukins, leukotrienes, bradykinin and platelet activating factor. They decrease the release of vasoactive and chemoattractive factors, lipolytic and proteolytic enzymes, extravasation of leukocytes to areas of injury and fibrosis. Glucocorticoids are also very useful in treating diseases that arise from undesirable immune reactions.

**h. Central Nervous System**

Improved awareness of the distribution and function of the steroid receptors in the brain had led to increasing recognition of direct effects of corticosteroids on the CNS, including effects on mood, behaviour, learning, memory and brain excitability. Some recent findings on the effect of glucocorticoids on CNS are:

- **Neurogenesis**

  Studies in young rats have shown that the formation of the dentate gyrus is regulated by corticosteroids (Gould, 1994). It has been shown that administration of exogenous cortisol to increase the level of circulating glucocorticoids on the fifth day of the hypo responsive period causes a reduction in granule cell death on the sixth day. Similarly, depletion of the glucocorticoids by performing adrenalectomy causes an increase in cell death. This shows that low levels of adrenal steroids in this period are important in enabling cell death to occur. To determine whether corticosteroids also affect cell birth, the uptake of tritiated thymidine by granule cells in rats was quantified (Gould et al., 1991). Cortisol treatment resulted in a decrease in thymidine labeled cells, which counteracted the density of pyknotic cells such that there was no apparent change in granule cell number. Similarly, adrenalectomy in young animals resulted in an increase in thymidine labeled cells (Yehuda et al., 1989). In adult rats, cortisol administration suppresses neuronal birth, while; adrenalectomy causes increased neurogenesis (Cameron et al., 1994). This conclusively proves that there is glucocorticoid mediation in cell birth, migration and death in both young and adult rats. Instead of focusing on glucocorticoids as a brake on the proliferation of precursor cells, impeding cellular renewal, they can also be regarded as the “curator” of old granule cells, which have a long-acquired synaptic architecture and can, constitute a living museum of past experiences (Kitraki et al., 1995).

- **Memory and Learning**

  Memory and spatial learning in rats have been found to be impaired by lesions in the hippocampus, particularly in the dentate gyrus. In the past, this has been attributed to
increases in glucocorticoid levels associated with aging and/or stress. In reality, the equivalent of maximum learning, in the form of hippocampal primed burst potentiation, is obtained in the adrenalectomized rat in the presence of blood concentrations of the hormone in the stress range, whereas above these levels, learning is progressively impaired. Most of the investigations with respect to spatial learning and memory are performed on the experimental models – Morris Water Maze (MWM) and Radial Arm Maze (RAM). Adrenalectomized rats with dentate gyrus damage did not perform as well as the control animals on the MWM (Conrad et al., 1993). They also made more errors than the sham animals in the RAM (Vaher et al., 1994). The shortfall in the RAM performance can be reversed by aldosterone treatment indicating that the impairment can be attributed to adrenal steroid insufficiency. Cortisol administration to adrenalectomized rats increases both basal and kainic acid induced glutamate levels in the hippocampus. The shortfall in the RAM performance can be reversed by aldosterone treatment indicating that the impairment can be attributed to adrenal steroid insufficiency. Altogether, various findings indicate that the extent of lifetime exposure to glucocorticoids determines the extent of age-related decline in hippocampal neurogenesis and consequently age-related cognitive dysfunctions.

5.4 Some recent findings about corticosterone and Brain functions.

The hippocampus, an important integration center for learning and memory in the mammalian brain, undergoes neurological changes in response to a variety of stimuli that are suggestive of ongoing synaptic reorganization. Studies using radio immuno-cytochemistry and con-focal microscopy, increased synaptophysin expression in CA1, CA3 and the dentate gyrus, regions that exhibit synaptic plasticity in response to glucocorticoid exposure. This study demonstrate that changes in the expression and distribution of synaptic proteins provide another measure of synaptic plasticity in the rat hippocampus in response to glucocorticoid exposure, changes that may accompany or contribute to neuroanatomical, neurochemical, and behavioral changes observed in experimental models of type 1 diabetes (Grillo et al., 2005).

Studies on the role of corticosteroids in regulating the expression of the glial glutamate transporters glial glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST) in rat primary astrocytes suggested a potential role for glucocorticoids in regulating GLT-1 gene expression during central nervous system development or pathophysiological processes including stress (Zschocke et al., 2005).

6.1 Corticotropin Releasing factor (CRF)

CRF, a primary regulator of ACTH secretion is a 41-amino acid peptide is generated by cleavage of the 196-amino-acid C-terminus of pro-CRF (Vale et al., 1981). The actions of CRF are mediated through receptor subtypes designated CRF1 (Vita et al., 1993), CRF2 (Reul and Holsboer, 2002) and CRF3 (Arai et al., 2001). CRF1 receptors are relatively selective over CRF2 receptors. Those are widely distributed in the central nervous system and are involved sensory information processing and motor controls (Sanchez et al., 1999). The CRH (corticotrophin releasing hormone) system is widespread throughout the brain but is best characterized in the Paraventricular nucleus (PVN) of the hypothalamus and one of the principal components of general adaptation syndrome. CRF mRNA express in the cerebral cortex, amygdala and hippocampus (Bittencourt and Sawchenko, 2000) as well as in periphery like adrenal gland, testis, spleen and thymus (Dautzenberg and Hauger, 2002). CRF and noradrenergic neurons are stimulated by serotonin and acetylcholine and inhibited by glucocorticoids, gamma-amino butyric acid (GABA), ACTH and opioid peptides (Aghajanian and Vandermaelen, 1982; Calogero et al., 1988; Stratakis and Chrousos, 1995).

CRF has been hypothesized to be an integrator of multiple components of the stress response (Vale et al., 1981) and elicits activation of autonomic nervous system, arousal, anxiety like behaviors, suppression of the immune system and eating behaviour (Carrasco and Van de Kar, 2003). These central actions of CRF are appropriate to facilitate “fight or flight” responses (Kalin et al., 1994). Expression of both CRF and CRF1 receptor mRNA in the parvicellular neurons of the hypothalamic paraventricular nucleus is increased substantially and rapidly (with in the first 5min) by stress (Imaki et al., 2001). Studies examined the correlation between cellular activation and increased levels of c-fos mRNA, and CRF1 receptor levels in different brain areas highly involved in the neuroendocrine response to stress such as parvicellular hypothalamic paraventricular nucleus (Imaki et al., 2001), which is blocked by antagonist treatment. Peptide and non-peptide CRF receptor antagonists reduce the behavioral and neuroendocrine effects of different acute and chronic stressors in rodents (Griibel, 1999). It is also proposed that CRF may act as a neurotransmitter or neuromodulator in extrahypothalmic circuits to integrate brain multi system responses to stress (Preil et al., 2001).
6.2 Vasopressin.

Vasopressin is a nine-amino-acid neuropeptides that is synthesized in different hypothalamic nuclei (Miller et al., 1988) is released directly into the systemic circulation and regulates the hypothalamic –Pituitary-adrenocortical axis (Mouri et al., 1993). In the anterior pituitary, vasopressin potentiates the effect of CRF on ACTH release (Swanson and Sawchenko, 1983). The biological effects of vasopressin are mediated by activation of V1a, V1b (also called V3) receptors and V2 receptor (Birnbaumber, 2000). The V1b receptor is mainly involved in the stimulating effect of vasopressin on ACTH secretion in the pituitary (DeKeyzer et al., 1994; Hernando et al., 2001). Vasopressin is also critically involved in a variety of brain functions, such as the generation of emotions, learning and memory (Ebner et al., 2000). Its importance in stress system lies in the synergetic control of the ACTH secretion.

6.3 Serotonin.

Serotonin (5-HT) is widely distributed in the brain and involved in mood and impulse control, have major influence on the regulation of neuro-endocrine function. Serotonergic neurons located in the midbrain raphe innervate the hypothalamus (Fink et al., 1998). Seven families (5-HT1-7) of serotonin receptors have been cloned (Hoyer et al., 1994). Dysfunction of serotonergic neurotransmission has been associated with several mood disorders, including depression, anxiety, panic disorder, obsessive-compulsive disorder and eating disorders (Graeff et al., 1997; Mora et al., 1997) via 5-HT1A and 5-HT2A receptors (Blier, 2001). Evidence supporting a role for serotonin in stress was obtained in micro dialysis studies examining changes in extracellular levels of serotonin in different brain Ares, including hypothalamus, amygdale, frontal cortex and raphe nuclei, after exposure to several stressors (Funada and Hara, 2001; Fujihino et al., 2002). Insulin injection in fasted rats, exercise and immobilization produced an increase in brain tryptophan availability and serotonin levels in the hypothalamus. Raised tryptophan hydroxylase activity through glucocorticoid receptors was also observed an effect prevented by adrenalectomy. Also immobilization or restraint stress has been associated with increased synthesis/metabolism of serotonin in some limbic regions (Shimizu et al., 1989; Dunn, 2000). Injection of the 5-HT2 receptor antagonist ketanserin into the amygdale inhibits the effect of photic stress on ACTH release and thus was speculated, that some of the effects of stressors are mediated via serotonergic mechanisms (Feldman et al., 1998). In addition to the role of 5-HT1A and 5-HT2A
receptors on the release of stress hormones, acute stress either increases or decreases hippocampal and/or cortical 5-HT1A receptor binding (Raghupathi and Mc Gonigle, 1997). Chronic fluoxetine treatment with (daily for 14 days) reversed the stress-induced defecation and suppression of exploring behavior and shortened the duration of stress induced freezing behavior but no changes were found on stress-induced disorders (Zhang et al., 2000). Serotonergic neurons also interact with CRF neurons (Kirby et al., 2000; Van Pett et al., 2000). CRF1 receptor knockout mice show a decreased corticosterone response to stress, but enhanced hippocampal serotonergic neurotransmission (Penalva et al., 2002).

6.4 Catecholamines.

Nor epinephrine and dopamine are broadly distributed catecholamines in brain (Shih et al., 1999). The locus coeruleus –Nor adrenaline (LC-NE)/sympathetic systems are located in the brain stem. The hypothalamus as a major integrative center of the neuroendocrine response also receives innervations of nor epinephrine containing neurons (Habib et al., 2001) of origin from caudal nucleus (A2 cell group) (Palkovits et al., 1999). The pharmacological classification of noradrenergic receptors includes alpha (α1 and α2) and beta (β1, β2 and β3) receptors. A general activation of the nor epinephrine neurons has been described in response to different stressors (Sound, restraint, hypoglycemia and swimming) in rats (Abercrombie and Jacobs, 1987, 1988; Morilak et al., 1987) Activation of LC-NE system leads to release of NE from extraordinarily dense network of neurons throughout the brain, resulting in enhanced arousal and vigilance as well as increase in anxiety. A neither enhanced turnover in nor epinephrine metabolism was also described during exposure to stress in rats (Korf et al., 1973). Also, in vivo micro dialysis revealed that the release of endogenous nor epinephrine in the hippocampus of rats submitted to restraint and intermittent tail shock stress (Abercrombie and Jacobs, 1988). Neither tyrosine hydroxylase activity, the neither rate-limiting enzyme in nor epinephrine synthesis is increased in response to repeated intermittent stress paradigm involving foot shock and noise stress. Reciprocal neural connections exist between CRF neurons in the hypothalamic Para ventricular nucleus and nor- adrenergic neurons in the locus coeruleus (Habib et al., 2001). Synaptic contacts were observed between CRF-Immunoreactive terminals and locus coeruleus dendrites (Curtis et al., 1997; Van Bockstaele et al., 2001). Both α and β adrenergic receptor agonists stimulates the secretion of ACTH, β endorphin and
proopiocelanocortin (al-Damluji, 1988; Whitnall, 1993). Infusion of α adrenergic antagonist inhibits stress-induced ACTH release (Szafarczyk et al., 1987). Recently authors also reported that intra-locus coeruleus microinfusion of CRF (3-100ng) increased the nor epinephrine levels in the frontal cortex in a dose dependent manner (Curtis et al., 1997).

The dopaminergic system is very sensitive to stress. It has been found that stress increased dopaminergic activity in the CNS, especially in the mesolimbic (nucleus accumbens) and mesocorticol (prefrontal cortex) pathways (Cabib and Puglisi-Allegra, 1994; Gresch et al., 1994). Recently decrease in dopamine activity has been reported in the nucleus accumbens after forced swimming (Rosetti et al., 1993). These results suggest that the dopaminergic response to stress might be sensitive to particular stressor its characteristics. Chronic exposure to various kind of stressor enhanced dopamine release through D2 receptors (Gil and Armario, 1998).

6.4.1 Catecholamines and HPA axis regulation.

Catecholamines often act in concert with activation of the HPA axis. For example, paralleling the increase of GC-hormone production from the adrenal cortex, activation of the HPA axis also results in catecholamine production from the adrenal medulla (Carrasco and Van de Kar, 2003). Cells in the adrenal medulla synthesize and secrete nor-epinephrine and epinephrine. In humans, 80% of the catecholamine output of the medulla is epinephrine (Goldfien, 2001). Nor-epinephrine is released from sympathetic nerve fibers in direct approximation with target tissues. If acutely activated, these catecholaminergic systems can provide the body with a needed ‘boost’ to deal with an immediate threat; the typical and most obvious effect of stress induced epinephrine and nor epinephrine is the establishment of the primitive mammalian fight or flight reaction, in which there is increased heart rate and increased blood flow to skeletal muscles. If the sympathetic adrenal medullary axis is chronically activated, these molecules can deregulate immune function. A link from the sympathetic nervous system to the immune system is supported by the observations that nor-adrenergic sympathetic nerve fibers run from the CNS to both primary and secondary lymphoid organs (Felten et al., 1999).

6.4.2 Mechanism of action for catecholamines

Catecholamines mediate their effect on target tissues through adrenergic receptors. These are G-protein coupled receptors that can be divided into two subgroups,
the α and β adrenergic receptors. β adrenergic receptors function as intermediaries in transmembrane signaling pathways that involve receptors, G-proteins and effectors (Gilman, 1987). The β2 adrenergic receptor is a seven membrane spanning, serpentine receptor embedded in the plasma membranes of many cell types, including macrophages and T lymphocytes. Once bound to ligand, the β2 adrenergic receptor communicates with the cytoplasm by stimulating the activation of a G-protein complex. This G protein is formed from three distinct protein subunits, α, β and γ. When in its inactive form; the three G-protein subunits are bound together in a heterotrimeric complex. In its inactive state, the G α - subunit is bound to guanosine diphosphate (GDP); when active, it binds the triphosphate form (GTP). When β-adrenergic receptor activates the G protein as a result of binding of a catecholamine, the α subunit releases GDP and binds GTP. Once this happens, the GTP-bound a subunit loses affinity for the receptor and for the β and γ subunits, dissociates from them, and subsequently activates adenylate cyclase. In turn, adenylate cyclase catalyzes the synthesis of cAMP from ATP; this reaction involves the release of the β and γ phosphates from ATP and the linking of the surviving a phosphate (still attached to the 50 hydroxyl of ribose) to the 30 hydroxyl as well as, forming cAMP (Gardner, 2001; Simonds, 1999).

One major cellular effect of the activation of the cAMP cascade is the stimulation of transcription after phosphorylation of transcription factors by cAMP-dependent protein kinase A (PKA) (Barradeau, et al., 2002). One such transcription factor, called CREB (cAMP response element binding protein) binds to the conserved consensus cAMP response element, TGACGTCA, present in the promoter regions of responsive genes. CREB stimulates basal transcription of cAMP response element-containing genes and mediates induction of transcription on phosphorylation. For example, a conserved palindromic cAMP response element has been identified in the promoter of various genes regulated by cAMP (i.e. IL-6). After phosphorylation on Ser133 by PKA, CREB binds as a homodimer to this palindromic element and stimulates elevated transcription. As such, the expression of numerous immune response genes can be modified by elevated catecholamine production during times of stress (Shaywitz and Greenberg, 1999).

7. Pathophysiology of dysregulated HPA axis.

It has been proposed that nearly two thirds of the ailments that are seen by physicians are either induced by, or related to stress (Sabban and Kvetnansky, 2001).
The stress response is meant to be of acute or limited duration. The time limited nature of this process renders the accompanying antigrowth, antireproductive, catabolic and immunosuppressive effects temporarily beneficial. Thus, chronicity of this response leads to several pathogenic manifestations (Selye, 1936). Hyperactivity of the HPA axis leads to melancholic depression, dysphoric hyperarousal and relative immunosuppression (Tsigos et al., 2002). The chronically active stress system also causes depressed patients to sustain other disorders like osteoporosis, features of a metabolic syndrome, atherosclerosis, innate and T-helper 1-directed immunosuppression and certain infectious and neoplastic diseases (Chrousos, 2000). Hypoactivation of the stress system also results in pathological hypoarousal. Patients exhibit atypical depression, chronic fatigue and other such states. CRH hyposecretion is also seen in hypothyroidism (Chrousos et al., 1998). Cushing syndrome is caused by increased cortisol production due to bilateral adrenal hyperplasia caused by hypersecretion of ACTH. Addison’s disease is due to progressive degeneration of the adrenal glands. The cause is usually due to idiopathic atrophy or autoimmune factors (Williams et al., 1998). Some of the important stress induced disorders are summarized below

7.1 Behavioral disorders

Stress is linked with number of behavioral disorders like anxiety, mood and depression. However, the best-studied case of a stress axis interface with a psychiatric illness is that of major depressive disorders (MDD) (Harro and Oreland, 2001). Depressive disorders are common in humans and are believed to be influenced and/or induced by a wide variety of factors including biological, environmental and genetic ones. The correlation between stress and depression has face validity, not only because being profoundly depressed is unquestionably stressful, but also because an external stressful even often precipitates depressive episodes (Post, 1992). Recent findings indicate that the “catecholaminergic hypothesis” (Schildkraut, 1995) and the serotonergic hypothesis (Maes and Meltzer, 1995) of major depression. Neuromodulators like βendorphin or other putative neurotransmitters/neuromodulators, such as GABA, neuropeptides (such as neuropeptides Y, substance P and corticotrophin releasing factor and growth factors may also be involved, albeit not centrally in manifestation of depressive behaviour (Nestler, 1998; Ressler and Nemero, 1999).
7.2 Gastric ulcers

Ulceration is a common occurrence in human population in the present day society where stress and tension arising from various socioeconomic factors are unavoidable. Prolonged anxiety, emotional stress, burns and trauma are known to cause severe gastric irritations (Prestov et al., 1994). Increased gastric motility, Vagal over activity and various neuroendocinological factors have been shown to be involved in the stress ulceration. Chao has also shown that factors other than vagal stimulation could also contribute to stress ulceration. Stress resulted in ulcer incidences are due to involvement of hyper-activation of Para ventricular nucleus (PVN) in hypothalamus. This causes a decrease in mucosal blood flow and hyper contractility through its descending projections, which influences the activity of vagal efferent. Vagal activity leads to increase in gastric muscular contraction and compression of blood vessels. This leads to the ischemia, which is one of the major factors in stress- induced gastric ulcers (Zhang and Zheng, 1997).

7.3. Hypertension and heart diseases.

Stress has been postulated to be involved in etiopathogenesis of hypertension and coronary heart disease. A growing body of evidence also indicates that elevation in hematocrit and hemoglobin are present in situations involving both physical and mental stressors and have been identified as an independent risk factor for the development of a number of diseases, including hypertension, coronary heart disease and stroke (Roy et al., 2001). Persistent high catecholamine levels are also associated with increased vascular endothelial turnover and permeability to calcium and lipoproteins, increased blood velocity, abnormal blood flow patterns and atheroma formation which also accounts for a number of cardiovascular disorders (Cruickshank and Smith, 1989). Furthermore increased works load in response to stress and leads to an increase in oxidative challenge in heart muscles thereby increasing the probability of coronary heart disease and stroke (Hu et al., 2000).

7.4. Metabolic disorders.

Stress has been associated with alteration of number of cellular, molecular and morphological changes resulting in metabolic disorders such as diabetes and lipid metabolism (Reagan et al., 2000). Stress has been reported to cause glucose intolerance and subsequently to diabetes of particular importance, high levels of glucose can produce
permanent chemical alterations in proteins and increase lipid peroxidation in a variety of experimental models of hyperglycemia (Folmer et al., 2002). Various forms of acute stressful stimuli have been generally accepted to cause a hyperglycemic response. The CNS and HPA axis plays an important role in regulation of hepatic glucose and lipid metabolism via the sympathetic nervous system and cytokines (Gotoh et al., 2001). Some of the important brain regions and mediators involved in hyperglycemic response during stress is shown in Fig. 5.

Fig. 5.

7.5. Senescence and Brain Degenerative Processes

Various studies have implicated the role of corticosteroid in the involvement of normal brain aging, leading to the formation of the glucocorticoid hypothesis of age related hippocampal neurodegeneration (Landfield et al., 1994). Astrocyte hypertrophy measurement revelations are in consonance with the above-mentioned hypothesis (Landfield et al., 1978). Cortisol levels and adrenal weight are positively correlated with hippocampal aging. When young rats are injected daily with cortisol for three months, they exhibit the hippocampal cell loss associated with aging (Sapolsky, 1985). Adrenalectomy attenuates hippocampal cell loss in aging rats (Landfield, 1981). The Type I receptor is fully occupied during high and low adrenal steroid levels. This implies that the Type II (GR) receptor mediate hippocampal damage during aging. GR receptor plasticity is diminished with aging. Aged rats exposed to chronic mild stress showed less down regulation of the GR as compared to young rats. Following adrenalectomy, young rats exhibit up regulation of the GR, while no such plasticity is seen in old rats.
(Landfield et al., 1994). However, an interesting point must come into consideration when dealing with senescence. Some degree of stress is present under conditions involving bodily or psychic suffering depending on the way the stressor activates the HPA axis (Fig. 5). A homeostatic adjustment is attempted (Alema et al., 1995; Casolini et al., 1993) with an adequate transformation in the cognitive and neuroendocrine-hormonal regulatory activity of these receptors. This phenomenon results not from a disinhibition of adrenal secretion but an increased neuroendocrine-competent cellular sub stress in the hypothalamus (Raadsheer et al., 1995). It is an attempt at adaptation, aimed to neutralize the inflammatory effect of the variously advanced degenerative processes in the aging brain.

Some degree of stress is constantly generated as an adaptive process.

As regards neuronal death, two predominant types of cell death in the CNS have been characterized in oncosis and apoptosis. There is a selective destruction of the granule cells of the dentate gyrus 3-4 months following adrenalectomy (Sloviter et al., 1989). This finding has also been reported in humans (Machien et al., 1990). Nissl staining revealed that the cell loss was bilateral and first appeared at the lateral end of the
inner blade of the dentate gyrus (see Fig. 6 for structural anatomy of hippocampus). The septal and middle thirds of the dentate gyrus exhibited a greater and more complete degeneration.

**Fig. 6.**

---

**Basic structural anatomy of hippocampus**

In order to determine whether glucocorticoids have a direct role in the adrenalectomy-induced neurodegeneration, the effects of short-term adrenalectomy were seen (Gould et al., 1990). It was observed that at both three and seven days post adrenalectomy, pyknotic cells were seen and the number of pyknotic cells increased with time as compared to the sham animals. In adrenalectomized rats, there is a trend towards decrease in the total dendritic material. The degenerative changes are prevented by cortisol treatment (Gould et al., 1990). The specific type of cell death that granule cells undergo in response to adrenalectomy has the morphological characteristics of apoptosis.

**II. Oxidative Stress**

Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as *Pro-oxidants*. On the other hand compounds and reactions disposing off these species, scavenging them, suppressing their formation or opposing their actions are called antioxidants. In normal cell, there is an appropriate pro-oxidant-antioxidant balance. How ever this balance can be shifted towards the prooxidants when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called oxidative stress and can result in serious
cellular damage if the stress is massive or prolonged. Oxidative stress is implicated in the etiopathogenesis of a variety of human diseases (Frei, 1994; Peterhans, 1997; Beck and Levander, 1998; Domenico et al., 1998). Common element in such diverse human disorders as ageing, neurodegeneration, cancer, arthritis and many others is the involvement of partially reduced forms of oxygen.

1. Major targets of Oxidative attack

Oxidative stress induced by free radicals disrupts the equilibrium of biological systems by damaging their major constituent molecules, including proteins, lipids and DNA, leading eventually to cell death. Polyunsaturated fatty acids within cell membranes and lipoproteins are particularly susceptible to oxidative attack (lipid peroxidation), often as a result of metal ion-dependent hydroxyl radical formation. Following initiation by a single radical, if oxygen is present, long chains of lipid peroxides may be formed by a rapid free radical chain reaction causing serious disruption of cell membrane function (Aust et al., 1985).

Proteins exposed to free radical attack may fragment, cross-link or aggregate. The consequences include interference with ion channels, failure of cell receptors and failure of oxidative phosphorylation. Free radical-induced damage to DNA may cause destruction of bases and deoxyribose sugars or single and double strand breaks (Birnboim and Kanabus, 1987; Aruoma et al., 1989).

2. Antioxidants

Antioxidants can be defined as substances whose presence in relatively low concentrations compared to that of an oxidizable substrate, significantly inhibit or delay the oxidation of that substrate (e.g. Lipid/Protein/DNA). Primarily they function as blockers of radical processes. Antioxidants may be enzymes that catalyze the breakdown of free radicals, those that prevent the participation of transition metal ions in free radical generation and free radical scavengers (Maxwell, 1995).

2.1. Antioxidant Enzymes:

The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase exist to catalyze the reduction of oxidants primarily in the intracellular environment (Fig.6). These enzymes are present in mitochondria and cytosol (Larson, 1988). The enzyme superoxide dismutase (SOD) catalyzes the conversion of \( \cdot \text{O}_2^- \) into \( \text{H}_2\text{O}_2 \). Catalases remove hydrogen peroxide, are found in peroxisomes in most of the
tissues, and probably serve to remove peroxide generated by peroxisomal oxidase enzymes. Glutathione peroxidases are major enzymes that remove hydrogen peroxide generated by SOD in cytosol and mitochondria, by oxidizing the tripeptide bearing a thiol group, glutathione (GSH) into its oxidized form (GSSG).

**Fig. 7.**

- **Antioxidant Enzymes**
  (Catalyzing breakdown of free radicals.)
- **Preventative Antioxidants**
  (Sequestration of metal ions.)
- **Scavenging Antioxidants**
  (Chain Breaking.)

**Classification of Antioxidants**

**a. Superoxide Dismutase (SOD)**

Many one-electron processes have been described that convert O₂ to its radical anion reduction product, `O_2^-`, superoxide (Fig. 7). Superoxide dismutases catalyse the conversion of `O_2^-` to H₂O₂ and oxygen.

\[
2 \cdot O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2
\]

This reaction is quite rapid even without enzymatic catalysis at ordinary physiological pHs (at a rate approximately four orders of magnitude less at pH 7.4. SOD is a powerful enough catalyst to increase the rate of the reaction by several orders of magnitude at physiological pHs. Superoxide, like H₂O₂, is not directly reactive toward most organic compounds (at least not as an oxidant), but it probably gives rise to more reactive oxygen species of higher potential toxicity (Halliwell, 1991).

**b. Catalase**

A long-known metalloenzyme, catalase is one of the most efficient protein catalysts known; it promotes the redox reaction
Catalase

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is probably an intracellular precursor for more reactive oxidants such as \( \cdot \text{OH} \). It is relatively stable (Half life $10^{-5}$ sec), poorly reactive, non-radical oxygen species, which easily crosses cell membrane and attacks different sites by converting into \( \cdot \text{OH} \) (Halliwell, 1991).

c. Glutathione Peroxidase

Glutathione Peroxidase (GSH-Px) also reduces peroxides like catalase. The selenium-dependent GSH-Px reduces H$_2$O$_2$ as well as organic hydroperoxides. The selenium independent GSH-Px accepts hydroperoxides as substrate:

\[ \text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O} \]

Glutathione (GSH) is found in very high concentrations in many cells. It reacts with many oxidants such as H$_2$O$_2$ to form the oxidized form, a disulphide known as GSSG. GSH also reacts without enzyme catalysis (GSH-Px) with many other potentially damaging intracellular oxidants such as $^1\text{O}_2$, $\cdot \text{O}_2^-$, and \( \cdot \text{OH} \) (Halliwell, 1991).

2.2 Preventive Antioxidants: Sequestration of Metal Ions

Many transition metals have variable oxidation numbers, e.g., iron has Fe$^{2+}$ and Fe$^{3+}$ ions and copper has Cu$^+$ and Cu$^{2+}$ ions. Changing between oxidation states involves accepting and donating single electrons, e.g.,

\[ \text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+} \]
\[ \text{Cu}^{2+} + e^- \rightarrow \text{Cu}^+ \]

Transition metal ions are remarkably good promoters of free radical reactions (Larson, 1988; Halliwell, 1991). They act as a template for the formation of the highly destructive hydroxyl radical by.

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{OH} + \text{OH}^- + \text{Fe}^+ \ldots \text{Fenton reaction} \]
\[ \cdot \text{O}_2^- + \text{H}_2\text{O}_2 \xrightarrow{\text{Fe or Cu ions}} \text{OH} + \text{OH}^- + \text{O}_2 \ldots \text{Haber-Weiss reaction} \]
These are important reactions that should not disseminate in the transformation of H₂O₂ into hydroxyl radical (\cdot OH). \cdot OH is extremely reactive. Although it is uncertain whether \cdot OH or iron-oxygen complex is the ultimate reactive initiating species, it is evident that safe storage of iron ions is crucial (Halliwell, 1987). Two thirds of the 4 g iron present in an adult is stored in hemoglobin. Ten percent of the iron is found in myoglobin and a small portion in iron-containing enzymes and in the transport protein transferrin. The remainder is present in intracellular storage proteins such as ferritin and hemosiderin (Halliwell, 1987).

**Fig. 8.**

The interaction of free radicals and antioxidants

The free plasma concentration of iron is therefore almost nil, but any free metal ions that escape during cell death or turnover are rapidly sequestered to prevent redox activity. This is achieved by the iron-binding proteins transferrin and lactoferrin, and the copper-containing protein caeruloplasmin (Bast et al., 1991). Albumin binds copper ions. \cdot OH formed around the albumin-bound copper immediately reacts with the albumin...
molecule. It has been suggested that albumin functions as a sacrificial antioxidant in this way (Halliwell, 1988).

2.3. Scavenging (‘chain breaking’) Antioxidants

Scavenging or ‘chain breaking’ antioxidants react preferentially with free radicals before more vital structures can be attacked. In scavenging free radicals these molecules are themselves sacrificed (oxidized). Abstraction of electrons by radicals often forms another radical, which could theoretically cause radical chain reactions. The so-called ‘chain-breaking’ antioxidant molecules are oxidized to products with insufficient reactivity to propagate radical chains. The chain breaking molecules are often divided into those which are water-soluble, including ascorbate and urate, and those that are lipid-soluble, such as the tocopherals and carotenoids. Their actions are not mutually exclusive. Ascorbate can regenerate tocopherol (vitamin E) from its oxidized radical form and indirectly contributes to lipid antioxidant defense (Packer et al., 1979).

3. Stress Induced Oxidative Stress

Damage by reactive oxygen species has been reported to be involved in some emotional stress models (Guiaeva et al., 1988; Deviatkina et al., 1989; Sosnovsky et al., 1992; Liu et al., 1994; Liu et al., 1994; Liu et al., 1996). Experimental evidence shows that this oxidative damage occurs principally in the mitochondria (Liu et al., 1996). Evidence has been shown to support the idea that stress produces oxidants, and that the oxidative damage in stress could contribute to the degenerative diseases of ageing, including brain dysfunction (Liu et al., 1996).

The stress system coordinates the adaptive response of the organism to real and perceived stressors. The main components of the stress system as stated earlier are corticotrophin-releasing hormone (CRH), locus-eruleus-norepinephrine/autonomic (LC/NE) systems, and their peripheral effectors; the hypothalamic-pituitary-adrenal (HPA) axis and the limbs of the autonomic system (Chrousos and Philip, 1992; Chrousos, 2000).

3.1. Major Sources of Free Radical generation in Stress

a. Glutamate toxicity

Glutamate can be directly toxic to neurons via two different processes, (i) the classical pathway that is mediated by specific glutamate receptors (Choi, 1992) and (ii)
the induction of an imbalance in the intracellular antioxidant system (e.g., depletion of intracellular glutathione) (Murphy et al., 1989). Both pathways of the glutamate toxicity can induce the generation of reactive oxygen species at one point with hydrogen peroxide or superoxide as reactive intermediates (Coyle and Puttfarcken, 1993). Increase in the release of glutamate and aspartate is associated with stress (Moghaddam, 1993; Lowy et al., 1993). The excitatory neurotransmitter glutamate interacts with N-methyl-D-aspartate (NMDA) receptor present on the postsynaptic cell. But under certain conditions (e.g., ischemic), the enhanced release of glutamate causes over activation of NMDA receptors resulting in increased calcium (Ca$^{2+}$) influx causing over stimulation of nitric oxide synthase (NOS) activity. Chronic stress has also been demonstrated to increase the expression of inducible nitric oxide synthase in rat brain cortex (Olivenza et al., 2000). These lead to the generation of more number of nitric oxide molecules. Nitric oxide interacts with superoxide radicals causing formation of peroxynitrite anions (ONOO$^-$) that causes heavy damage to lipid molecules of the cell membranes (Olanow, 1992; Bettahi et al., 1996; Jenner, 1996; Finch and Cohen, 1997).

b. Byproducts of Enzyme action

Several enzymes expressed in the brain, including monoamine oxidase (MAO), tyrosine hydroxylase, and L-amino oxidase produce H$_2$O$_2$ as a normal byproduct of their activity (Coyle and Puttfarcken, 1993).

\[
\begin{align*}
\text{Mixed Function} \\
\text{Oxidase} \\
\text{Substrate} + O_2 & \rightarrow Product + H_2O_2 \\
\text{Xanthine} \\
\text{Oxidase} \\
\text{Xanthine} + O_2 & \rightarrow Uric\ acid + \cdotO_2^- + H_2O_2
\end{align*}
\]

c. Glucocorticoid toxicity

Corticosteroids have an effect on the activity of the stress-adaptive and stress-responsive Hypothalamic-pituitary-adrenal system, which is mainly regulated by hippocampus (Holsboer and Barden, 1996). Glucocorticoids have been found to enhance oxidative stress-induced hippocampal cell death. Hippocampal neuronal cell’s pretreatment with glucocorticoids enhanced their oxidative cell death induced by the oxidative stressors (amyloid $\beta$ protein and glutamate). This increases vulnerability of
neurons that appeared after glucocorticoid pretreatment was found to be specifically mediated via activated glucocorticoid receptors, because this effect could be blocked by the specific glucocorticoid receptor antagonist RU486 (mifepristone) (Behl, 1998).

4. The Susceptible Brain

The central Nervous system shows increased susceptibility to oxidative stress because of its high oxygen consumption rate (20% of the total oxygen inhaled by the body) that accounts for the increased generation of oxygen free radicals and reactive oxygen substrates like superoxide radical (O$_2^-$), singlet oxygen (↑O$_2$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (•OH).

![Diagram of Brain and Free radical damage due to Physiological Stress]

Brain and Free radical damage due to Physiological Stress.
Brain has a low level of anti oxidative defense system. The concentration of various anti oxidative enzymes like SOD, GPX, GRd, and catalase (Zhang and Zheng, 1997) is low in brain. The Glutathione (GSH), concentration is also very much reduced in the brain when compared to other various organs in the body (Makara and Haller, 2001). In addition to these factors, brain has high concentration of ascorbate and iron in certain regions, which provide favorable environment for the generation of oxygen free radicals. Brain is also enriched with polyunsaturated fatty acids (PUFA) that render them susceptible to oxidative attack. The interplay of all these factors that contribute to enhance the oxidative stress is outlined in Fig. 9.

The increased level of oxidative stress seen in the brain is thus the major contributory factor for the development of neurodegenerative disease like Parkinsonism and Alzheimer’s disease in aged individuals (Reiter, 1995; Joseph et al., 1998).

III. Importance of Herbal Drugs.

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. The world health organization has estimated that up to 80% of the world’s population depends on traditional medicine for primary health care because such drugs are easily available at low cost, comparatively safe due to their negligible side effects and moreover people have faith in such remedies.

There has been a dramatic revival in recent years in the use of herbal preparations for the treatment of a wide range of ailments (Eisenberg et al., 1998). Some products are being recommended for use as specifics to treat particular illnesses or conditions much like conventional synthetic medications are prescribed in North America, Western Europe and the rest of the economically developed world (Blumenthal et al., 1998).

Nevertheless, there are a fair number of traditional medical systems that have incorporated routine use of adjuvant preparations or treatments into their health care delivery systems. All of these preparations are used to keep body in proper tune so to speak. In the traditional –Hindu medical (Ayurvedic) system of India they are called Jamu, in fungshui system of china in which one seeks to balance between man and nature to created a harmonious environment they are called Zi bu or huifu (Meaning a tonic and restorative respectively in Mandarin and in Russian Tonizirayachie sredstva (Meaning tonic substances). Each of these connotes or means that which makes new again, or that
which helps restore one's youth full state of physical and mental health as well as helps expand a state of happiness.

1. **Plant drugs known for anti-stress effects.**

Since the introduction of the concept of ‘adaptogen’ several plants have been investigated starting with *Eleutherococcus senticosus* (Siberian ginseng), *Panax ginseng*, *Raponticum carthamoides* and *Rhodiola rosea*. These were used as tonics in folk medicine, and were found to fulfill the criteria laid down by Brekhman and Dardymov and therefore qualified as adaptogens. These initial studies opened a vast arena for research and in India work has been carried out on plants such as *Acanthopanax sessiliflorum* and *Rhodiola rosea* from Russia, *Albizia julibrissin* and *Cicer arietinum* from Japan, *Codonopsis pilosula* and *Panax ginseng* from China as well as *Withania somnifera*, *Ocimum sanctum*, *Hoppea dichotoma*, *Alium sativum* and *Emblica officinalis* from India (Rege *et al.*, 1999).

Anti-stress drugs depending on their offering non-specific resistance were classified to be adaptogens. Adaptogens confers their action by mediating through diverse modules and were proved to be effective against chronic stress induced adverse effects on immunity, behaviour cognition etc which were there by separated from benzodiazepine anxiolytics despite having significant anti-stress activity (Costa and Guidotti, 1996). According to the literature among the number of plants *Panax ginseng*, *Panax quinquefolium*, *Elutherococcus senticosus*, *Ginko biloba* and *Withania somnifera* were scientifically proven for their anti-stress activity by various authors.

**1.1 Ginseng**

Ginseng is the root of perennial herbs of *Panax quinquefolium* L., which grow in unites states and Canada and *Panax ginseng* (Araliaceae). The former is known as American ginseng and the latter, designated Korean or Chinese ginseng. Ginseng has been used in eastern Asia for more than 5000 years as a tonic and resotorative, promoting health and longevity. Traditionally, ginseng use has been divided into two categories: short term, to improve stamina, Concentration, healing process, stress resistance, vigilance and work efficiency in healthy individuals and long term, to improve well being in debilitated and degenerative conditions especially those associated with old age (Nocerino *et al.*, 2000). According to current understanding, the adaptogenic effect of the drug is ascribed to the ginsenosides or panaxosides. The chief glycone are the ginsenosides Rb1 and Rg1. Pharmacological examinations using ginseng extracts,
ginseng fractions and ginsenosides have revealed, besides adaptogenic effects, anabolic and nootropic properties. Indeed, ginseng enhances resistance to X-Irradiation, Viral and tumor load, temperature stress; hyperbaric hyperoxia and physical exercise and increases swimming time in rats (Carbral deOliveira et al., 2001; Grandhi et al., 1994). Brekhman, a pioneer in the experimental studies of ginseng, used the term adaptogen to describe the ability of ginseng to increase resistance to physical, chemical and biological stress and to build up general vitality (Brehm and Dardymov, 1969). Many of these activities have been attributed to corticosteroïd-like action. CRH and ACTH hormone studies have suggested that ginsenosides may augment adrenal steroidogenesis via an indirect action on the pituitary gland (Hiai et al., 1979). Even though ginseng got an enormous beneficial and health promoting properties, ginseng has been documented with number of adverse effects like nervousness, diarrhea, insomnia, euphoria (Zhu et al., 1999), hypertension and hypotension, gastrointestinal upset (Kim et al., 1995), Vaginal bleeding (Greenspan, 1983) and skin eruptions (Lei and Chiou, 1986). The long-term effects of the use of ginseng effect on primarily central nervous system excitation and arousal termed as ginseng abuse syndrome. Ginseng abuse syndrome is characterized by hypertension together with nervousness sleeplessness, skin eruptions, edema and diarrhea. There is also evidence that ginseng could cause Stevens-Johnson syndrome (Dega et al., 1996).

1.2. Ginkgo biloba.

Ginkgo biloba (G. biloba) is one of the widely used Chinese plants for its medicinal properties for several thousand years. At present, it is among the most commonly used phytopharmaceutical. It has been used in the treatment of various common geriatric complaints including vertigo, short-term memory loss and lack of attention or vigilance. Standardized extracts of G. biloba leaves, EGb 761, contains 22-26% flavanoid glycosides; 2.5-4.5% ginkgolides A, B, C and J 2-4% bilobalide; less than 10ppm, in particular less than 1 ppm, alkylphenol compounds; and less than 10% proanthocyanidins (Puebla-Perez et al., 2003).

The standardized extract of G. biloba has been shown to have beneficial effects on cerebral vascular disorders (Clostere, 1999), MAO inhibitory (Pardon et al., 2000) and immune stimulating properties (Puebla-Perez et al., 2003). G. biloba has received attention as potential cognitive enhancers (Ward et al., 2002) for the treatment of Alzheimer's disease and other cognitive disorders. (Mantle et al., 2000; Oken et al.,
Pretreatment with *G. biloba* induced the normalization of mitochondrial respiration, a diminution of cerebral oedema, correction of the accompanying ionic perturbations and practically total functional restoration, revealed by a normal neurological index (Oken *et al*., 1998; Pratico and Delanty, 2000). The standardized extract egb 761 inhibits the formation of reactive oxygen species, and posse’s platelet-activation factor (PAF) antagonist properties, reduced level of nitric oxide in macrophages (Kobuchi *et al*., 1997).

Findings suggest that *G. biloba* extract may also facilitate the successful behavioral adaptation to stressors or noxious stimulants (Ward *et al*., 2002). The extract of *G. biloba* has been shown to inhibit the corticosterone secretion in rats via acting directly at the level of adrenal gland through diminution of the number of peripheral benzodiazepine receptors as well as may have a direct hypothalamic or supra-hypothalamic action which has drawn attention for anti-stress properties and has been proposed to revert back some of stress induced perturbations (Marcilhac *et al*., 1998). The anti-oxidant, MAO inhibitory and corticosterone regulatory actions.

### 1.3 *Withania somnifera L.*, Solanaceae [Aswagandha, Indian ginseng]

Ashwagandha is a small shrub is widely cultivated throughout India and is still immensely popular in traditional Ayurvedic and folk medicine. However it is the roots of Ashwagandha that are considered to be medicinally tonic, adaptogenic and strengthening. It is said to “protect the organism from illness through maintaining the healthy balance of the physical energy. Traditionally, they are recommended for indigestion, heart disease, arthritis, lumbar pain, to lower fevers, and as a general strengthening medicine for children and for people recovering from illness.

The root contains the steroid lactone Withaferin A and related withanolides beside various alkaloids. The sitoindosides IX and X represent C-27-glycowithanolides, the sitoindosides VII and VIII, acylesterylglycosides. The extracts of *Withania somnifera* seeds, significantly improved the protection against stomach ulcers and adrenal gland weight that were induced by stress as well as oral intake over 3 days and this extract (60mg/Kg) effected a weakening of the milk-induced leucocytosis in mice. Similar anti-Stress effects were shown by the sitoindosides VII and VIII; the induction of stomach ulcers through stress was hindered by a pre-treatment with sitoindoside VII or VIII. The mice showed a distinct shortening of the duration of immobility, after giving sitoindoside VII and VIII (i.p.) in forced swimming stress. This anti-depressive effect is
apparently caused through a diminishing of the stress effect, or through intervention in the monoamine metabolism of the brain. Examinations showed that stress effects in rats led to a significant increase of the dopamine receptors in the corpus striatum and this effect can be suppressed through pre-treatment with *Withania somnifera* or with *Panax ginseng* extracts. The oral application of sitoindosides IX and X has been reported for the improvement in learning and memory patterns in mice. Sitoindosides VII, VIII, IX and X have been reported as adaptogenic active substances of *Withania somnifera*, in spite of diverse steroidal structures.

1.4. **Eleutherococcus senticosus**

Taiga root, originating from Siberia was screened to replace the expensive ginseng root. The chief constituents are phenyl propane compounds (syringin=eleutheroside B). Lignanes (Eleutheroside E (D)), Cumarins (Isofraxidin-7-o-glucoside) and polysaccharides along with sterins, oleanolic acid essential oil and sugar. The importance of *Eleutherococcus senticosus* in various pathologies is clearly evident from its array of therapeutic activities (Davydov and Krikorian, 2000).

The adaptogenic effect of *Eleutherococcus* during and/or after extensive exercise and at high altitude could be explained as the protective antioxidant effect of vitamin E and other anti-oxidants contained in plant extracts. That is to say, under such external “stresses” there is increased production of oxygen species as the result of compensation to the lack of oxygen in the outside environment. *Eleutherococcus* shows an aphrodisiac effect in animals and has suggested that it should have the same “invigorative” and tonic effect on people. In addition to this the anti-stress effect of eleutherococcus extracts have been demonstrated in animal experiments, through a raised protection from the typical organic changes during the alarm phase, as described by Selye (Brekhman and Kirillov 1969). Improved resistance occurred in diverse models, with regard to a series of stressors. In experiments single doses from 2.0 upto 16.0 ml of the extract (p.o.) no side effects have been observed. The tolerance of the *eleutherococcus* extract was very good. Only a very few patients complained of side effects of a mild nature such as headaches, raised blood pressure, sleeplessness (Gaffney *et al.*, 2001).

1.5 **Bacopa monniera (Brahmi)**

It is a well-known nootropic plant available all over India and equally reputed for its central and peripheral protective effects. Vast literature is available indicating its pharmacological properties ranging from anti-stress, memory enhancement, anti-
inflammatory and anti-oxidant properties (Russo and Borelli, 2005). The memory enhancement properties were the mostly investigated properties and were attributed to the saponin content (Sing et al., 1992; 1997; Bhattacharya, 1999). Rai et al. (2003a) have demonstrated the anti-stress properties of standardized Bacopa extract during alarm phase response in rats.

1.6 Ocimum sanctum.

Ocimum sanctum (OS), popularly known as ‘Tulsi’ in Hindi and ‘Holy Basil’ in English and is considered sacred for its medicinal properties in Indian sub-continent. OS contains a number of chemical, constituents that interact in a complex way to elicit their pharmacological responses.

a. Plant description

The plant is distributed and cultivated throughout India. It is an erect, much branched softly pubescent. Under shrub, 30-60cm high with red or purple subquadranular branches. Leaves are simple, opposite, elliptic, oblong, obtuse or acute, with entire, subserate, or dentate margins, pubescent on both sides, minutely gland dotted, with slender, hairy petioles. Flowers are purplish in elongate racemes in close whorls, stamens exerted, upper pair with a small bearded appendage at the base fruits nutlets, smooth, not mucilaginous when wetted. The plant is bitter and acrid. The whole plant of as has medicinal value, few of them are aromatic, stomachic, demulcent, diaphoretic, digestive, diuretic, expectorant, febrifuge, vermifuge and alexiteric properties. Mostly leaves and sometimes the seeds are also used.

In several ancient systems of medicine including Aired, Greek, Roman, Sridhar and Inane, Osmium sanctum has vast number of therapeutic applications such as in cardiopathy, haemopathy, leucoderma, asthma, bronchitis. Catarrhal fever. otalgia. hepatopathy. vomiting, lumbago. hiccups, ophthalmia. gastropathy, genitourinary disorders. ringworm, verminosis and skin diseases etc.

b. Chemical constituents

O. sanctum leaves contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. Ursolic acid has been isolated from the OS leaves (Balanehru and Nagarajan, 1991; Nair et al., 1982). Nair et al. (1982) also isolated apigenin, luteolin, apigenin7 -a-glucuronide, luteolin-7 -a-glucuronide, orientin, and mollandstin. Isolation of two flavonoids, orientin and vicenin from the aqueous leaf extract of OS is also reported Umadevi et al. (1998). Kelm (2000) have extracted, and purified phenolic
compounds from the fresh leaves and stems of OS such as cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid and appreciable quantities of eugenol. The structures of these compounds have also been elucidated. Norr and Wagner (1992) identified vicenin-2, rosmarinic acid, gal uteolin, cirsilineol gallic acid, gallic acid methylester, gallic acid ethyl ester, protocatechic acid. vanillic acid, 4-hydroxybenzoic acid, vanillin, 4-hydroxybenzaldehyde, caffeic acid, chlorogenic acid from the ethanolic extract of OS. They also detected 2-phenylpropaneglucoside 1 and 2. The leaves of as are also known to contain traces of zinc, manganese and sodium (Samudralwar and Garg, 1996). Seeds of OS possess the fatty oil (17.82%) consisting 6.9% palmitic acid, 2.1 % stark acid, 15.7% linolenic acid, 66.1 % linoleic acid and 9% oleic acid. The unsaponifiable matter yielded a small quantity of sitosterol. Three insoluble bromoglycerides were crystallized on direct bromination of the oil in dry ether (two dilinoleno-linolins melting at 157°C and 145°C respectively and a linolenodilinolin melting at 80°C (Nadkarni and Patwardhan, 1952). The fixed oil content of seeds may vary depending on the geographical sources.

C. Pharmacological profile studied for Ocimum sanctum to till date.

- **Antimicrobial activity**
  
The aqueous leaf extract showed insecticidal activity and antibacterial activity against Gram positive and Gram-negative bacteria, however it was not effective against *Shigella* and *Salmonella*, *Staphylococcus citreous*, *E coli* and *Aspergillus niger*. At relatively high concentration, the extract showed antimycotic activity against *Trichophyton mentagrophytes* and *Pestalotiopsis mangiferae* (Gupta and Vishwanathan, 1955).

  The antimicrobial properties of the whole extract of as and its principal component eugenol (4-allyl-2methoxy-phenol) were tested on NRRL-2999. Both the substances could inhibit aflatoxin production. The results also suggested possible use of as extract to control infestation of aflatoxin producing moulds in food industry (Jayshree and Subramanyam, 1998).

- **Hypoglycemic and hypolipidaemic activity**
  
  Holy basil leaves obtained from two closely related species, as and *Eclipta alba* possessed similar therapeutic values and was used for treating diabetes, arthritis and bronchial asthma (Dhar et al., 1968; Palit et al., 1983; Giri et al., 1987). Experimental studies in albino rats showed the efficacy of basil leaves in decreasing blood glucose in
hyperglycemic rats and rabbits (Sarkar and Panth, 1989). In the latter study, seeds were found to be less effective than leaves. Further, oral administration of ethanolic as leaf extract potentates the action of exogenous insulin in normal rats. The activity of the extract was 91.55 and 70.43% of that of tolbutamide in normal and streptozotocin induced diabetic rats respectively (Chattopadhyay, 1993). However, in a comparative study OS leaf extract was found to have the least potent blood sugar lowering activity than Catharanthhu roseus, Gymnema sylvestre and Azadirachta indica (Chattopadhyay, 1999).

To explore further evidence, effect of treatment with holy basil leaves on fasting and post-prandial blood glucose and serum cholesterol levels in humans were assessed through a randommixed placebo controlled crossover single blind trial in patients of non insulin dependent diabetes mellitus (NIDDM) (Agarwal and Singh, 1996). The results of the trial indicated a significant decrease in fasting and post-prandial blood glucose levels during treatment as compared to placebo and the findings suggested that basil leaves might be prescribed as an adjunct to dietary therapy and as a drug treatment in mild to moderate NIDDM.

Tulsi leaf powder supplementation at 1% dose level showed significant hypoglycemic and hypolipidaemic effects in diabetic rats with could be associated with the essential oil, eugenol present in OS leaf powder (Rai et al., 1997). In addition there could be some other active insulinogenic ingredients present in OS leaf powder, bringing the blood sugar level down in the diabetic rats. Significant lowering in serum total cholesterol, triglycerides, phospholipids, cholesterol levels and a significant increase in HDL-cholesterol and total fecal sterol contents of rabbits was observed by oral administration of fresh leaves for 4 weeks at two dose levels of 1 an d2 %W/W mixed in the diet (Sarkar et al., 1994).

Trasina, an ayurvedic herbal formulation containing Ocimum sanctum as one of the ingredients showed little effect on blood sugar concentrations and islet superoxide dismutase activity (SOD) in euglycemic rats, in 100and 200mg/kg PO doses administered once daily for 28 days. However, these doses of Trasina caused a dose related decrease in streptozotocin hyperglycemia and attenuation of streptozocin induced decrease in islet SOD activity (Bhattacharya, 1997).
- **Radioprotective activity**

  Uma Devi and coworkers have extensively worked on radioprotective activity of OS and was recently reviewed (Umadevi, 2001). Hydro alcoholic extract of OS has provided radio protective effect when given intraperitoneally before a whole body exposure to 11 Gy of Co (Prakash and Gupta, 2000).

  The aqueous extract exerted protective effect against radiation induced chromosome damage in mouse bone marrow and modified bone marrow radio sensitivity, which could be attributed to its free radical scavenging activities. (Ganasoundari *et al.*, 1997.) The two isolated flavonoids from as leaves, orientin and vicenin showed better radioprotective effect as compared with synthetic radio protectors, WR-2721 and MPG (2-mercaptoptyropionyl glycine). Both the flavonoids showed a significant protection against chromosome aberration in mice, the activity of vicenin being significantly greater than orientin without systemic toxicity. Free radical scavenging appeared to be the likely mechanism of radiation protection by these flavonoids. In a separate *in vitro* system, orientin and vicenin provided almost equal protection against radiation induced lipid peroxidation (LPO) in mouse liver and a significantly greater free radical inhibiting activity than DMSO. They showed no pro-oxidant activity at the tested concentrations (Umadevi *et al.*, 1998).

  The combination of as leaf extract with WR-2721 resulted in higher bone marrow cell protection and reduction in the toxicity of WR-2721 at higher doses, suggesting that the combination would have promise for radioprotection in humans (Ganasoundari, 1998).

  At an optimum dose of 50mg/kg, I.P administration of orientin and vicenin, provided protection to mice against death from gastrointestinal and bone marrow syndrome before whole body exposure to 11Gy γ-radiation. Survival and duration of protection were better with vicenin than orientin. (Umadevi *et al.*, 1999).

- **Antilcerogenic activity**

  The fixed oil of OS was found to possess significant antiulcer activity against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress induced ulceration in experimental animal models. Significant inhibition was also observed in gastric secretion and aspirin induced gastric ulceration in pylorus ligated rats. The oil had lipooxygenase inhibitory, histamine antagonistic and anti secretory effects, which could probably contribute to its antiulcer activity (Singh and Majumdar, 1995). Recently
(Dharmani et al., 2004) has demonstrated the anti ulcerogenic and ulcerhealing properties of ethanolic of OS. The studies were carried out in Cold restraint, asprin, pyloric ligation and histamine induced ulcer models. The ulcer healing activity was demonstrated in chronic acetic acid induced ulceration. The anti-ulcer effect of OS was said to be due to its anti secretory and cytoprotective effect.

- **Antifertility activity**

Antifertility activity of as leaves has been reported in rats, mice and rabbits. In one of the above studies benzene extract was more effective than petroleum ether extract or other extracts. Benzene extract of fresh as leaves in male rats indicated significant reduction in sperm count, sperm motility and weight of testis (Kasinathan et al., 1972; Seth et al., 1981; Khanna et al., 1986; Batta and Santhakumari, 2000).

Long term feeding (up to 3 months) of Tulsi leaves (200 and 400 mg/kg) to adult male and female albino rats along with normal diet decreased sperm count, sperm motility and the weight of male reproductive organs. The mating behaviour of both male and female rats was inhibited severely. In some animals where mating took place only during the initial phase of the treatment, pregnancy was carried to term with birth of normal pups. However, short term oral administration of as leaf extract to the rats in the graded doses of 100, 150, 200 and 400 mg/kg body weight along with normal diet for 15 days continuously, decreased the Sexual behaviour. A significant decrease in sexual behavior score was noticed when the dose was increased to 200 and 400 mg/kg. It suggested that Tulsi plant could not be exploited for contraceptive use since it depresses mating behavior and does not cause azoospermnia (Kantak and Gogate, 1986)

- **Hepatoprotective activity**

The cold-water extract at 3g/100g body weight dose when fed orally for 6 days was found to be effective against carbon tetrachloride (0.2 ml/100 g subcutaneously) induced liver injury (necrosis, fatty degeneration and hydroid degeneration) in albino rats (Seethalaksmi et al., 1982). Similar effect was observed with 70% ethanolic extract.

- **Immunomodulatory activity**

Steam distilled extract from the fresh leaves showed modification in the humoral immune response in albino rats, which could be attributed to such mechanisms as
antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs (Mediratta, 1988).

- **Anti-oxidant activity**

Uma Devi has recently reviewed the antioxidant activity of OS (Umadevi, 2001). Antioxidant properties of flavonoids from different sources have been reported (Hussain et al., 1987 Hu et al., 1995, Saija et al., 1995). It was thought (Shimoji et al., 1994, 1996) of a probable relationship between radioprotective and antioxidant activity which was also confirmed by Ganasoundari (1998) and co-workers. Ursolic acid isolated from as offered remarkable protection against lipid peroxidation in isolated liver and heart microsomes *in vitro*. The compound did not induce lipid peroxidation by itself and thus improved therapeutic application. It also provided mild protection as compared to strong protection by oleanolic acid against adriamycin induced lipid per oxidation (Balanehru and Nagarajan 1991,1997).

Protective role of aqueous leaf extract against radiation-induced lipid per oxidation, glutathione and allied antioxidant enzymes in liver of mice was observed (Umadevi, 1998) as leaf extract exhibited significant antioxidant activity against several paradigms of oxidative stress induced by a variety of techniques in different rat tissues, which was comparable to that of vitamin E (Bhattacharya et al., 2001). Recently cyclooxygenase inhibitory properties of as have also been reported along with its antioxidant properties (Maulik et al., 1997; Nair et al., 1982).

- **Chemo preventive and anti-carcinogenic activity**

The antiproliferative and chemopreventive activity of as aqueous leaf extract and seed oil was studied using 3-(4,5- dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on HeLa cells. Significant antiproliferative activity was observed only with high concentrations (83.33 and 250g/ml) of seed oil. Leaf extract and low doses of seed oil did not affect the proliferation of the cells (Samudralwar and Garg, 1996). Oral supplementation of maximal tolerated dose of 1.0ml/kg body weight against 20-methylcholanthrene (MCA) induced fibrosarcoma tumours in Swiss albino mice reduced the cumulative tumour incidence and tumour volume. Increased survival rate and delay in tumour incidence was also observed. Liver enzymatic (superoxide dismutase, catalase, glutathione-S-transferase) and non enzymatic antioxidants (reduced glutathione) and lipid peroxidation end-product, malondialdehyde levels were
significantly modulated with oil treatment suggesting that the potential chemopreventive activity of the oil is partly attributable to its antioxidant properties. The chemopreventive efficacy of 100 -11kg seed oil was comparable to that of 80 mg/kg of vita. Min E60. The oil also showed significant chemopre. Preventive activity against 7,12-dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice. This was evidenced by significant reduction in incidence of papillomas, average number of papillomas/mouse, increased survival and modulation of level of reduced glutathione and lipid peroxidation, and activity of superoxide dismutase, catalase and glutathione peroxidase in papillomas by oil supplementations, and histopathological parameters (Prakash, 2000).

Anticarcinogenic potential of OS has significantly decreased the incidence of benzo(a)pyrene induced neoplasia of stomach and 3'-methyl-4-dimethylaminoazobenzene induced hepatomas in rats. Topical treatment of as leaf extract in DMBA induced papillomagenesis significantly reduced the tumour incidence, average number of papillomas/mouse and cumulative number of papillomas in mice. Topical application of the extract significantly elevated reduced glutathione content and glutathione-S-transferase (GST) activity. The chemopreventive action of as leaf extract is probably through the induction of hepatid extrahepatic GST in mice. Elevated levels of reduced glutathione in liver, lung and stomach tissues in as extract supplemented mice were also found as compared to untreated control mice (Prashar and Kumar, 1995).

Ethanolic leaf extract also had significant modulatory influence on carcinogen metabolizing enzymes (cytochrome P450, cytochrome b5 and aryl hydrocarbon hydroxylase, glutathione-S-transferase and glutathione levels in mice (Banerjee et al., 1996).

- **Psychopharmacological activity**

  The ethanolic leaf extract of as was screened for psychopharmacological activities. The extract prolonged the time of lost reflex (the time interval between the loss and regaining to righting reflex after administration of pentobarbital sodium at a dose of 40 mg/kg Lp. in mice), decreased the recovery time and severity of electroshock and pentylenetetrazole induced convulsions. It also decreased apomorphine induced fighting time and ambulation in open field studies. Using a behavioral despair model involving forced swimming in rats and mice, the extract lowered immobility in a manner comparable to imipramine. This action is blocked by haloperidol and sulpiride, indicating a possible action involving dopaminergic neurones. In a similar study, there
was a synergistic action has been reported when extract was combined with bromocriptine, a potent D2-receptor agonist (Sakina et al., 1990).

- **Adaptogenic activity.**

  The ethanolic leaf extract of *O. Sanctum* was screened for antistress activity against acute and chronic noise induced changes in plasma corticosterone level in albino rats (Sembulingam et al., 1997). Changes in leukocyte count during noise induced stress and the normalization by OS were reported (Archana and Sembulingum, 2000).

  Methanolic and ethanolic extracts tested for anti stress activity in swimming endurance model increased physical endurance during swimming, prevented stress induced ulcers and milk induced leucocytosis in rats and mice, respectively, indicating induction of non-specifically increased resistance against a variety of stress induced biological changes by OS in animals (Bhargava and Singh, 1981).

  Modulation of stress induced biochemical perturbations by Eugenol and OS were studied in rats. There was no significant effect on blood glucose and urea levels. But the authors observed lowering effect on stress-induced increase in cholesterol, lactate dehydrogenase and alkaline phosphatase levels (Sen et al., 1992). From these experimental findings, authors suggested the involvement of neurotransmitters for the anti-stress activity of OS.

  Studies conducted to till date proves the multifunctional nature of *Ocimum sanctum* was conformed that the anti-stress effect is due to the cumulative effort of all the constituents extracted with specific solvents used in the study but a complete study of fractions and the major active constituents is lacking. Recently Gupta et al. (2002) has reviewed the validation of traditional claim as medicinal plant and suggested for directing the efforts towards isolation and characterization of the active principles and elucidation of the structure activity relation ship in view of its potential anti-oxidant, immunomodulatory, anti-inflammatory, anti-cancer and a plethora of other diseases.

**1.6 Evolvulus alsinoides.**

*Evolvulus alsinoides* (Linn) (Family:Convolvulaceae) commonly known as *Shankhpuspi* is found throughout India ascending to 6000ft in the Himalayas. It is well known for its therapeutic effect on brain disorders like insanity, epilepsy, memory enhancement, nervous debility, Scrofula in Indian Ayurvedic system of medicine (Nadkarni, 1982, Chatterjee et al., 1990). Pharmacological studies on leaves and whole
plant of *Shankhpuspi* have indicated anti-ulcer (Asolkar et al., 1992) and the leaf extract has been used to treat whitlow in the fingers and toes (Bhatt et al., 1999).

Free radicals play an important role in the progression of several brain related disorders and in view of its usage for central disorders in Ayurveda some investigators focused on anti-oxidant properties of *Evolvulus alsinoides*. *In-Vitro* and *ex-vivo* studies conducted by Auddy et al. (2003) revealed the potential anti-oxidant properties of *Evolvulus alsinoides*.

- **Anti-oxidant properties**

  Ethanolic extract and aqueous infusion of leaves was tested for Lipid peroxidation and ABTS radical scavenging properties in plasma and brain homogenate of rat. Ethanolic extract at a dose of 10-100µg/ml and aqueous infusion at a dose of 50-400µg/ml were effective in scavenging ABTS radical and preventing lipid peroxidation.

  Cytotoxicity studies were conducted in PC12 cell lines for both Ethanolic extract, aqueous infusion and were found to be non-cytotoxic at a concentration ranging from 25µg/ml- 1mg/ml. Both the extracts were again considered for *Ex-vivo* studies for the effect on rat plasma TBARS and ABTS radicals and found to be significantly effective. The authors also concluded from their study that aqueous extract is having potential anti-Oxidant capacity compared to the ethanolic extract of *Evolvulus alsinoides*.

- **Immunomodulatory properties**

  Immunomodulatory properties of *Evolvulus alsinoides* were evaluated by Lilly et al. (2003) in adjuvant induced arthritic (AIA) rat model. AIA is an erosive autoimmune polyarthritis involving both humoral and cell mediated immune responses that resemble human rheumatoid arthritis (RA). In this study the dose was optimized and was found to be effective at 25mg/kg body weight of rat. The Aqueous extract was effective in reducing AIA by lypmocytesuppression and Macrophage inactivation as reflected in lymphocyte proliferation assay and Nitric-oxide release estimation by activated macrophages and was concluded to be having potential immunosuppressive properties.

  In our literature survey not much scientific validation of *Evolvulus alsinoides* was reported for its various therapeutic activities as recorded in Ayurvedic texts. So, this plant is considered to screen the crude extracts and isolated compounds for anti-stress and anti-oxidant potential which may provide scientific basis for activities reported in Ayurvedic texts.
2. Set backs of existing herbal anti-stress agents.

Benzodiazepines appeared to be effective against acute stress but failed to prevent the consequences of chronic stress. In addition, the problems of tolerance and physical dependence exhibited by benzodiazepines, on prolonged use, limit their utility (McCarty, 1989). An answer to this vexing problem was provided when Brekhman and Dardymov reported that some plant-derived agents could induce a state of non-specific increase of resistance to diverse aversive assaults, which threaten to adversely affect internal homeostasis. They put forth specific criteria that need to be fulfilled for a substance to qualify as an adaptogen (Brekhman II, Dardymov IV, 1969). Thus an adaptogen must-

- Produce a nonspecific response, *i.e.* increase the power of resistance against multiple (physical, chemical or biological) stressors.
- Have a normalizing influence, irrespective of the direction of change from physiological norms caused by the stressor, and
- Be innocuous and not influence normal body functions more than required.

When used appropriately, these adaptogens appears to be relatively safe but in few studies which provides information regarding theirs and interactions with other drugs. Among the well known adaptogens *Panax ginseng* has been studied most widely and has been extensively investigated experimentally and clinically for its asters attenuating properties, hence interaction and side effects are well known. It is considered a food supplement, not a drug, in many countries. The documented side effects include hypertension, diarrhea, sleeplessness, mastalgia, eruptions and vaginal bleeding. Siegel indicated the long-term effects of the use of ginseng abuse syndrome (Siegel *et al.*, 1979). Ginseng abuse syndrome is characterized by hypertension together with nervousness, sleepless ness skin eruptions, oedema and diarrhea. There is also evidence that ginseng could cause Stevens Johnson syndrome (Nocerino *et al.*, 2000).

The studies with these plants leave no doubt, at least, in animal experiments that they are capable of modulating stress related changes. However, even after spending over 30 years of research efforts on these on these herbal remedies, none of them has been successfully introduced in the modern medical market under the heading adaptogens. Most of these plant drugs have remained as putative adaptogens or some have made a niche for themselves as immunostimulants, anabolic agens are antioxidants. Moreover the unwanted side effect of the known adaptogens is in favous antioxidants. Most of
these plant drugs have remained as putative adaptogens or some have made a niche for themselves as immunostimulants, anabolic agents or antioxidants. Moreover the unwanted side effect of the known adaptogens is in favour to look for newer adaptogen, which meets the Lazrev concept of adaptogens. Therefore, there is need for an affective adaptogenic substance, which could replace Panax ginseng in the therapy of stress-induced disorders. Thus this field needs more focused research for the developments, to try, identify and solve, if possible, the problems in evaluation of adaptogens. This allows us in the future to develop a drug, which could be characterized more specifically as an adaptogen.

**Objectives of the present study**

In view of the complexity of stress response and the real need of an anti-stress agent from plant origin, which offers nonspecific resistance, the following objectives were chosen.

1. **Behavioral and Biochemical characterization of Stress response in Different stress models of rats.**
   - Standardization of Acute, Chronic and Chronic unpredictable stress models in rats.
   - Characterization of stress response by measuring Plasma glucose, creatine kinase and corticosterone in plasma of rats and behavioral changes.
   - Evaluation of free radical load during stress response in plasma and brain regions as indicated by lipid per oxidation and anti-oxidant enzyme changes in various stress models considered under study.
   - Effect of stressors and the time duration on Monoamine (Noradrenaline, Dopamine and 5-HT) metabolism in brain regions involved in stress response.
   - Effect of stress on glucocorticoid receptor expression changes in various brain regions of rats.

2. **Evaluation of Ocimum sanctum and Evolvulus alsinoides for anti-stress activity.**
   - Preparation of crude extracts and their preliminary screening for anti-stress and in-vivo antioxidant activity during stressful conditions.
   - Bioassay guided fractionation and evaluation of fractions from active crude extract for anti-stress activity.
   - Isolation and Chemical Characterization of active constituents from bioactive fractions.
3. **Evaluation of Ocimum sanctum and Evolvulus alsinoides for learning behavior.**

- Screening of crude ethanolic and aqueous extracts of *Ocimum sanctum* and *Evolvulus alsinoides* for learning behavior by Morris water maze and passive avoidance tests.

4. **Evaluation of Pure compounds Isolated from active fractions of Ocimum sanctum and Evolvulus alsinoides for anti-stress activity.**

- Antistress effect evaluation as indicated by the alterations in glucose, CPK and corticosterone on stress induced plasma changes.
- Effect of pure compounds on Brain monoamines during stress full conditions.
- Effect on Glucocorticoid receptor density changes during stressful conditions.