Discussion
Physiological adaptation during stressful conditions is defined as a biochemical change in an organism that results from exposure to certain environmental conditions or stressors and generates a more effective response to their survival. Stress, including both eustress and distress (roughly meaning challenge and overload respectively), may be the result of negative or positive events. Whilst eustress is essential to life (in the sense, for example, that exercise is required to avoid muscularatrophy), distress can cause disease. Eustress, however, raises the levels of adrenaline and prostaglandins in the body, which in turn increases the heart-rate, respiration and blood pressure and places greater physical stress on the body organs. Long-term stress can induce heart disease, high blood pressure, stroke and other psychosomatic disorders (Chrousos et al., 1992; Fink et al., 2000).

The insufficiency in understanding the etiology of the stress induced disorders and their simulation is the major obstacle in creating animal models of stress. The only approach, which has been used, is to try to reproduce one or more symptoms of that disorder in animals. How ever a novel model should offer an advantage with respect to the older ones in order to understand the biology and physiology of the illness and mechanisms. Stress based animal models represents an indispensable preclinical approach to human pathology, since, the clinical data point towards the major role of stress experiences (Life events) in the development of expression and exacerbation of behavioral disturbances. Thus, several models, in which primarily physical, psychological or a combination of both have been used to investigate stress. The common stressors employed in different studies include cold, heat, restraint, immobilization, foot shock or tail shock, insulin induced hypoglycemia, glucoprivation with 2-deoxy-D-glucose, hemorrhage, forced run or swim, emotional stressors (e.g. Exposing predators) and different social stressors that are based on housing (e.g. Isolation stress, crowding, mixed sex colonies, introduction of an intruder, etc). Among the swimming, isolation, fasting, restraint and immobilization stressors, immobilization stressor was found to impart effect on various behavioral and physiological profiles indicating its unique importance (Glavin et al., 1999). Apart from this, stressors employed should approve for animal ethics. On the basis of the literature available and considering the above facts, we designed our study to include various stressors to explore the effect of stress on rats.
**Effect of stress on important Physiological markers in plasma.**

Originally, stress was considered to be a non-specific phenomenon. But various findings demonstrated that different types of stressors elicit specific responses (Sabban and Kvetnansky, 2001; Gilad and Gilad, 2002). In this study, we used acute stress (AS) Chronic stress (CS) and Chronic unpredictable stress (CUS) to explore the stress-induced manifestations on certain behavioral and physiological parameters, which reflect the healthy state of living organism. The distinction between AS and CS rises from its intensity. AS procedure was employed in order to investigate changes induced by a short acting severe stressor and represents the reaction to an immediate threat, commonly known as the fight or flight response where as the stress lasting for several days or weeks fall with in the concept of chronic stress. The distinction between CS and CUS falls in its total difference in behavioral and physiological consequences as studied earlier (Katz et al., 1981; Marti and Armario, 1998). When animals repeatedly subjected to the same stressor, as done by CS in our study, the decline of the initial response towards the same stressor might be a result from the contribution of two different mechanisms of adaptation. In which, adaptation to homotypic stressor have proposed to be due to reduced emotional activation, peripheral and central biochemical habituation (Dronzak et al., 2004; Bobbert et al., 2005). In CUS, exposure to a heterotypic stressor results in more intense behavioral and biochemical disturbances indicating its severity and damaging effects. Chronic stress-induced plasticity in the acute stress response is important for stress adaptation, but may also contribute to pathophysiological conditions associated with stress. Thus, understanding the neural mechanisms underlying such adaptations may help us understand the etiology of such disorders, and contribute to the future development of more effective treatment or prevention strategies.

Validity of the method used in our experiments is demonstrated by the biological effects induced by it i.e. gastric ulcerations, adrenal gland weight, plasma corticosterone, glucose and creatinine kinase levels. All these parameters have been conclusively shown to be stress-dependent (Natelson et al., 1981). In all the experiments in our study, the last stressor employed was immobilization to avoid the stressor dependent specific alterations.

Stress response has been reported to activate the paraventricular nucleus of the hypothalamus (Zhang and Zheng, 1997), which in turn activates autonomic and endocrine responses thus inducing ulcers (Henke, 1979) by weakening the defensive factors and the
hyperactivity of the aggressive factors in stomach. This includes increase of acid and pepsin, decrease in mucous secretion, alterations in adrenal steroids and catecholamines, followed by ischemia and excessive oxidative damage which forms the basis for initial damage of gastric mucosa this initial damage is further exacerbated by prostaglandin synthesis. Some of these events, if not all, appear to be adequately controlled by the central nervous system in response to stress (Glavin et al., 1991; Mayer, 2000; Bousta et al., 2001). In our investigations, the incidence of ulcer increased with the increase in the stress severity from AS to CUS. These findings are in conformity with the earlier reports of Natelson et al. (1998).

Studies on stress in rats have generally indicated a transient activation of the pituitary adrenal system, as measured by increased adrenal size with subsequent increase of plasma corticosterone level and other correlates of adrenal activation (Vernikos et al., 1982). Stress induced adrenal hypertrophy was observed in all the three stress models and this finding could be attributed to the activation of the HPA axis, which is highly responsive to stress and is one of the principal mechanism by which an organism mobilizes its defense against stress events (Makara and Haller, 2001; McEwen, 2000). The prolonged activation of HPA axis resulted in an increase in adrenal hypertrophy, the maximum being observed during CUS. The sympathetic nervous system in response to stress results in increased secretion of corticosterone from adrenal cortex and epinephrine from adrenal medulla (Selye, 1950; Walker et al., 1986). These hormones are deemed to be essential protecting the body against stress conditions; thus helping to cope with stress. The increase in the adrenal gland (size) and the circulating corticosterone is a marker for the activation and involvement of the HPA axis during stress. The adrenal gland weight increased proportionally to the intensity of the stress. However the increase in corticosterone was inconsistent in relation to stress intensity. The initial increase in corticosterone level during AS, may be related to novelty of the stressor, which in turn triggers the activation of the HPA axis, to the maximum due to flight or fight response (Selye, 1950; Walker et al., 1986). During CS the level of the corticosterone was not increased to the extent as that of AS, which was due to the adaptation of the animals against the repeated stress and depletion of reserve stores of glucocorticoids in the adrenals. This could possibly explain the reason for less corticosterone secretion in CS compared to AS, although adrenal hypertrophy was observed. Moreover, the enhanced secretion of the corticosterone during the AS is the result of mobilization of the stored corticosterone from the cells of adrenal cortex to the circulation. Whereas, it has been
speculated that hypertrophy supports the increased mRNA transcription and translation of synthesis of glucocorticoids. Evidences suggest that the medial basal hypothalamus is involved in the extra pituitary regulation of compensatory adrenal growth, triggered during stress, as reflected by an increase in the adrenal gland weight (Holzworth and Dallman, 1979). This is due to the consequences of low corticosteroid level accompanied by a compensatory increase in ACTH secretion (Akana et al., 1983). In case of CUS the increased secretion of the corticosterone was maintained by the simultaneous synthesis and subsequent secretion of the corticosterone. The stored glucocorticoids are released with the first stress signal but with the increased demand during the chronic stress, a hypertrophy take place in order to compensate the glucocorticoids demand. Out of the various factors, desensitized feedback loop during intense stressful conditions has already been reported for various neuropsychiatric disorders like depression and anxiety (Arborelius et al., 1999; Carmine and Andrew, 2001; Kazushige et al., 2001).

Stress conditions are known to increase the blood glucose level due to the release of corticosteroids (Mason, 1968). Increased activity of the HPA axis leads to the activation of the pathways necessary the increased energy demand within the body, during crisis; the ultimate goal is to increase the circulating blood glucose (Brindley and Rolland, 1989). The blood glucose is the first available substrate, which is rapidly metabolized to produce energy in the form of ATP. In our study AS induced hyperglycemia which makes easy availability of substrate and increased production of energy in order to escape the threat to life which conforms with the studies in which, exogenous administration of corticosterone produced hyperglycemia (Wass et al., 1996).

After depletion of the immediate available carbohydrate source, in case of CS and CUS there is no increased glucose levels in plasma this is mainly due to, the body is utilizing the secondary source of non-carbohydrate origin for glucose i.e. lipids and proteins which is slow and rate limiting and the level of glucose is maintained at physiological quantities (Black and Garbutt, 2002; Rai et al., 2003c).

Stress hormones not only stimulate and ensure supply of glucose, but they also increase creatine kinase (CK) activity at a time when there is an increased energy demand (Adelbert, 2000). CK activity increased maximum in the rats after AS exposure whereas, a reduced CK activity when compared to AS was observed following CUS but higher compared to non-stress control. However, no significant change was observed after CS exposure. The increase of CK activity was crucial to meet the acute energy demand during AS to overcome the stress-induced challenges. Hyperactive CK, maintain
the flow of ATP during stress (Davydov and Shvets, 2002). Intracellular phosphocreatine is an essential component of energy metabolism in muscles and brain, since it acts as a store of high-energy phosphate which can be converted into ATP by creatine kinase (Zorzano et al., 2000), thus maintaining the CK reaction in a state of equilibrium, keeping ADP and ATP almost constant is crucial for the proper functioning of a variety of cellular ATPases (Markus and Kaddurah-Douk, 2000). The CK system may be important in stabilizing the ATP level and energy metabolism in myocardium and other skeletal muscles of rats during stress, and disturbances of CK activity during stress may results in ischemia due to non-availability of ATP (Davydov and Shvets, 2002).

Following CUS exposure a reduced CK activity compared to AS group shows partial resistance and the other energy compensatory mechanisms playing role as described earlier. Whereas, no change observed in CK activity following CS exposure shows complete adaptation against stress in rats.

**Stress and Behavior**

A number of publications emphasizes on the behavioral alterations induced by stress, such as depression (Yadid et al., 2000), multiple mood disorder, anxiety (McEwen et al., 2002). Stress-induced depression has been studied extensively and has also been used as the marker for the stress intensity. In our study, we observed that stressors elicited a unique response pattern which could be easily understood from their respective effect on behavioral profile i.e. horizontal activity, total distance traveled and stereotypy counts. A complete decrease in the behavioral activity was observed when rats were subjected to AS, CS and CUS. There is no much difference in the behavioral response between the models. However the behavioral changes observed were retractable when observed 24hrs after the cessation of stress indicating the transient effect rather than a permanent alteration as observed in more sever models like stress for several months, damaging particular region etc. So, it is always essential to observe the neurotransmitter changes whose constant alterations during stress full conditions will lead to a permanent psychopathological out comes.

**Stress and neurotransmitter response**

The role of important monoamines like Noradrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) is well established in modulating various behavioral and biochemical responses during stressful conditions (Tsigos and Chrousos, 2002; Gonzalo et al., 2003). Stress stimulates the sympathoadrenal system, causing activation of the monoamine synthesis and their metabolizing enzymes (Torres et al., 2002.). In our study,
immobilization for 150 min has resulted in a decrease of nor adrenaline in all the brain regions. In the acute stress, the selective depletion of NE might be due to the preferential and higher utilization of NE from this brain region during the stressor (Tanaka et al., 1982). However, NE levels depleted during acute stress can be returned to normal levels after the cessation of the acute stressor (Hellriegel and Mello, 1997). In accordance with the acute immobilization stress used in our study, acute stress full treatments such as cold restraint, mild foot shock stress (Herman et al., 1982) and conditioned fear (Deutch et al., 1986) increase dopamine and 5-HT in frontal cortex and hypothalamus (Thierry et al., 1976; Dunn, 1984). A decrease in the level of dopamine is observed in hippocampus only. These neurotransmitter changes are well associated with transient functional and behavioral changes like learning and memory that observed normally during acute stress full conditions in animals. Decrease of dopamine in hippocampus has a different significance due to its functional nature. Hippocampus is nested with excess of acetylene choline when compared to other regions and has a functional significance of coordinating cognition and reward. Normal function of memory is facilitated by acetylene choline. Acute stress decreases the acetyl cholinesterase activity which is an important acetylcholine metabolizing enzyme (Das et al., 2005). Due to this, the effects of acetylcholine are enhanced. Recent studies on acute nicotine exposure showed decrease of dopamine in hippocampus is mediated by muscarinic receptors (Shermann et al., 2005).

Chronic unpredictable stress has a different neurotransmitter, biochemical and behavioral responses depending on time duration and intensity of the stressor (Hellriegel and Mello, 1997; Gamrao et al 2003; Bekris et al., 2005). Most of the chronic studies of comparable intensity to Chronic unpredictable stress (CUS) that used in our study increased dopamine level in cortex and hypothalamus and decreased levels in hippocampus. 5-HT was observed to be increased in hypothalamus, hippocampus and decrease in cortex (Torres et al., 2000, Gamrao et al., 2003). In contrast to these studies NA, DA and 5-HT were significantly depleted in Cortex, Hippocampus and Hypothalamus respectively. In the present study, rats were continuously exposed to two different types of stressors (unpredictable) and were sacrificed immediately after the last stress regimen (immobilization). The probable reasoning for the depletion of these monoamines lies in various factors that play in concert. Corticotropin-releasing factor (CRF) and norepinephrine (NE)-containing neurons in the brain are activated during stress, and both have been implicated in the behavioral responses. NE neurons in the
brain stem can stimulate CRF neurons in the hypothalamic paraventricular nucleus (PVN) to activate the hypothalamic-pituitary-adrenocortical axis and may affect other CRF neurons. CRF-containing neurons in the PVN, the amygdala, and other brain areas project to the area of the locus coeruleus (LC) and CRF injected into the LC alters the electrophysiologic activity of LC-NE neurons. Neurochemical studies have indicated that CRF applied intracerebroventricularly or locally activates the LC-NE system, and microdialysis and chronoamperometric measurements indicate increased NE release in LC-NE terminal fields (Palamarchouk et al., 2000). The reciprocal interactions between cerebral NE and CRF systems have been proposed to create a "feed-forward" loop. It has been postulated that a sensitization of such a feed-forward loop may underlie clinical depression (Curtis and Valentino, 1991). The role of CRF in sensitizing the NE system is conformed and in majority of studies, repeated stress has been shown to decrease the behavioral and the neurochemical responsivity (Dunn et al., 2004). 5-HT is another neurotransmitter whose diagnostic identity has been proven by various behavioral and pharmacological studies in anxiety /aggression driven depression (Vanpraag, 2004) and in stress induced transient aggressive behavior which on prolongation leads to depression. The major degradation product of 5-HT, 5-HIAA, is found in the CSF as well as in the brain itself. 5-HIAA in lumbar CSF originates partly in the brain, partly in the spinal cord. Both animal (Mignot et al., 1985) and human (postmortem) studies have revealed a close correlation between brain and CSF 5-HIAA and 5-HT. Furthermore, the 5-HIAA concentration in the brain is to a large extent a function of 5-HT metabolism. Therefore, CSF 5-HIAA can be considered as an indicator (albeit it a crude indicator) of 5-HT metabolism in (certain parts of) the brain. Low CSF 5-HIAA, thus, suggests lowering of 5-HT metabolism in (certain parts) of the central nervous system (CNS). Subsequently, this tentative conclusion was supported by several lines of evidence. First, the abundant data that the various classes of antidepressants as well as electroconvulsive treatment improve the efficiency of 5-HTergic trans-mission, particularly of 5-HT1A receptor-mediated transmission. This happens either by sensitization of post-synaptic 5-HT2 receptors or by desensitization of presynaptic 5-HT2 receptors that normally reduce the release of 5-HT in the synaptic cleft or inhibit the firing rate of the 5-HT nerve cell (Blier and De Montigny, 1994). A second group of data is derived from the so-called tryptophan-depletion strategy (Young et al., 1985). Trypto-phan is an essential amino acid and the precursor of 5-HT. A shortage of tryptophan will lead to a deficiency of 5-HT. Such a shortage is experimentally generated by ingesting a mixture of amino acids,
devoided of tryptophan and rich in competing amino acids, *i.e.* amino acids competing with tryptophan for the same transport mechanism from the blood stream into the CNS. This leads to rapid decrease of tryptophan in the blood stream, lowering of 5-HIAA in the CSF (Delgado et al., 1989) and in animals leads to substantial lowering of brain 5-HT (Moja et al., 1989) had further demonstrated the role 5-HT in depressive behavior. These findings are in support of our data and demonstrate that sustained stress can cause changes in the 5-HT systems similar to those found in a subtype of depression, and associated with instability of anxiety and aggression regulation. The conclusion that stress may cause depression, or phrased probably more accurately, may cause particular depressive features, in particular anxiety and aggression, seem justified. The normal activation of limbic dopamine neurotransmission caused by stress is thought to exacerbate the hyperdopaminergic state associated with schizophrenia or mimic the activation of dopamine elicited by drugs of abuse, thus reminding or "priming" the addict of the high associated with drug taking. An alternative theory is that the vulnerability to relapse involves a maladaptive response to stress. Therefore, stress does not merely exacerbate or mimic a particular state rather, the stress response per se is abnormal and contributes to the expression of symptoms. An experimental example of this theory that involves the possible contribution of stress to relapse in recovering addicts. Recent basic and clinical findings suggest that addiction may involve reduced efferent activity in brain regions of the frontal cortex. Notwithstanding the structural and functional differences between rodent and primate cortices, experimental induction of reduced medial prefrontal cortex (PFC) efferent activity in the rat changes the acute dopaminergic response to stress in that a decrease, rather than an increase, in dopamine release in the nucleus accumbens is observed. Thus, in contrast to mimicking a euphoric state that is generally associated with dopamine activation, in a vulnerable brain with reduced PFC efferent activity, stress may in fact produce a limbic hypo-dopaminergic condition that causes a profound dysphoric state, leading to drug-seeking behavior to normalize dopamine neurotransmission. Added to our finding of decreased dopamine in FC, HIP and HT in CUS recent chronic studies suggest that chronic exposure to high CRF levels induces region-specific responses in dopamine projection areas. While these changes proved to be reversible, it is possible that a stronger or more protracted CRF activation could contribute to the pathogenesis and/or progression of disorders of the dopaminergic systems, particularly in vulnerable individuals (Izzo et al., 2005).
Adaptive significance of monoaminergic response during chronic stress (CS) is well demonstrated in animals subjected to single type of (Homotypic) stressor for a brief period of time. Frontal cortex and hypothalamus has shown a normal response for nor adrenaline and 5-HT. There is a statistically insignificant increase of DA in FC and a significant increase of 5-HT in hippocampus an important marker of adaptation. (Haleem et al., 2002). Stress administered repeatedly decreases 5-HT1A receptor binding, especially in the hippocampus, which may be a consequence of altered serotonergic activity in this structure. (Flugge, 1997). Recent lesion studies have shown that repeated, but not acute, exposure to an inescapable stressor results in a cascade of regionally selective changes in the expression of mineralocorticoid and glucocorticoid receptors in the dorsal hippocampus which are largely abolished by selective lesions of the 5-HT fibres that project to the hippocampus from the median raphe nucleus (Storeg et al., 2005). These results support the hypothesis that 5-HT release in the hippocampus, evoked by repeated exposure to an inescapable stressor, may play a pivotal role in the neurobiological mechanisms that underpin habituation to this form of stress.

Although repeated restraint stress is commonly employed in stress studies, there is a growing appreciation of the fact that rats can quickly habituate to the repeated application of the same stressful stimulus. In contrast, application of different stressful stimuli at irregular intervals would be expected to reduce the degree of habituation or desensitization that would result in response to any particular stimulus. This has been demonstrated directly in studies where in repeated unpredictable stress has been shown to produce biochemical and behavioral changes not seen with repeated predictable stress. As a result, unpredictable stress has been proposed to represent a better animal model to study the long term consequences of stress.

**Stress and Free radical homeostasis.**

Studies as reported by Tannebaum et al. (2002) focused on the role of stress in the increase of allostatic load (wear and tear imposed on the organism) with increased susceptibility to diseases. These observations indicates the importance of considering other subtle factors like reactive oxygen species (ROS) during stress response which are at least in part responsible for the aggravation of stress induced disorders. ROS are produced continuously during regular cellular aerobic metabolism and were controlled by super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). When the oxidative load increase the anti-oxidant homeostasis is disturbed and results in a considerable cellular damage such as per oxidation of membrane lipids, oxidation of
proteins and damage to DNA (Wolf, 2002). Recent studies indicate considerable impact of stress on anti-oxidant defense homeostasis (Manoli et al., 2000, Kaur and Kaushik, 2003, Sahin and Gumusulu, 2004) and the differential regulation by various anti-psychotics (Parikh et al., 2003). These studies suggested the possible role of neurotransmitters in free radical induced damage (Venuracci, et al., 1999, Carpagnano et al., 2003). Except a few in vitro studies indicating the prooxidant effect of phenol containing neurotransmitters (Siraki and Brein, 2002) there are no considerable animal studies addressing the changes in monoamine levels and anti-oxidant enzymes in brain regions. The adverse effects of neurotransmitters on the underlying oxidative biochemical mechanisms are often overlooked which add up to the ambiguity surrounding neurotransmitters and oxidative load in stressful conditions. In view of this we evaluated the neurotransmitter response and subsequent free radical damage in important brain regions viz. Frontal cortex (FC), Hippocampus (HP), Hypothalamus (HT) and Striatum (ST) involved during acute and chronic stress responses. Acute immobilization has not induced lipid per oxidation or produced any significant changes in antioxidant enzyme levels and glutathione. In our study, in rats subjected to CUS there is an increased lipid peroxidation, decreased SOD, CAT, glutathione and increased GPX in plasma and all the brain regions considered under the study. The contributing factors for oxidative damage during stressful conditions are hyper secretion of glucocorticoids (Lehman et al., 2002), increased oxygen uptake (Yamamoto et al., 2003), xanthine oxidase (Granger et al., 1988), inducible nitric oxide synthase (iNOS) activity and depleted glutathione levels which disrupts mitochondrial electron transport chain (Jose et al., 2000). All these factors culminate to increase the free radical load which, has been demonstrated to cause lipid peroxidation in plasma (Liu et al., 1994), brain (Liu et al., 1996), liver, heart and intestine (Kaur and Kaushik, 2003). Apart from these well-established factors, It has been reported that the production of free radicals in the brain is due to catecholamine metabolism and elevated catecholamine levels may undergo autooxidation, in which electrons are generated that in turn can produce ROS. High metabolic rate of monoamines is one of the contributing factors for increased oxidative damage during CUS. Catabolism of NA, DA and 5-HT is facilitated by intraneural enzyme MAO (Monoaminooxidase) which conducts oxidative deamination of a variety of amine neurotransmitters, such as adrenaline, nor adrenaline, dopamine and 5-HT in the central nervous system, as well as the neuroactive and vasoactive peripheral tissues (Kopin, 1994). The by product of MAO activity is hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) which is
only a weak oxidizing and reducing agent. Despite its poor reactivity, \( \text{H}_2\text{O}_2 \) plays a pivotal role among the reactive oxygen species which are continuously generated in-vivo. In CS model there is a brief but insignificant increase in lipid per oxidation in the hippocampus when compared to the normal rats and an increase, of SOD, CAT and decrease of GPx in all the brain regions was observed when compared to CUS indicating less “free radical load”. All the observations in the three models were well correlated with the Plasma total anti-oxidant capacity. Considering the recent findings and our study it is conformed to be the stressor intensity added with the perturbed neurotransmitter response and metabolism that synergize the oxidative damage during stress full conditions (Sahin and Gumusulu, 2004). Neurotransmitter intervention in oxidative damage should be considered seriously. However probing into the receptor mediated neurotransmitter regulation and simultaneous oxidative damage mechanisms during stressful conditions can yield more insight into the neurotransmitter mediated oxidative damage.

**Effect of Stress on glucocorticoid receptor expression**

The physiological effects of glucocorticoid are mediated by both mineralocorticoid receptors (MR) and glucocorticoids receptors (GR). Both GR and MR are involved in negative feedback regulation of HPA axis at the level of mPFC and HP regions. An additional distinction between MR and GR is that MR has a 4- to 10-fold higher affinity than GR for the endogenous corticosteroids. MR is predominantly occupied (upward of 90%) by endogenous hormone, even under the lowest levels of basal secretion, whereas GR only becomes occupied by hormone as corticosterone increases during times of stress or during the circadian rise in basal secretion and get delocalized with acute corticosterone raise (deKloet and Reul, 1987).

It has been demonstrated that disruption of integrity of HPA axis leads to attenuation of feed back suppression resulting in consistent elevation of plasma corticosterone (Holsboer, 1996). GR protein and mRNA are down regulated by chronic stress (Herman et al., 1995). Such regulatory mechanisms of GR at the feedback sites of the brain due to severe stress may cause attenuation of the glucocorticoids negative feedback to corticoids, which is one of the important causes for anxiety and depressive disorders (Kim and Gorman, 2005). In our study AS has significantly decreased the GR protein levels as observed by the western blott analysis in all the brain regions. In CS there is no significant difference in the glucocorticoid receptor expression profile even
though there is an increase in the Plasma corticosterone. In CUS model there is a significant decrease of GR expression profile in hippocampus compared to other regions of brain. Under Severe stress conditions, hippocampal GR’s appear to be sensitive to elevated glucocorticoids receptor levels (Jacobson and De Kloet, 1991). The pure compounds were later studied for their effect on GR expression during stressful conditions in hippocampus only.

Effect of Ocimum sanctum and Evolvulus alsinoides on learning and memory Behavior

Memory is one of the important indices of efficient brain function and is controlled by the brain limbic system, which is intimately involved in the stress response. (Fuchs and Flugge, 2003). Stressful conditions adversely affect cholinergic system and results in learning deficits, but the cholinergic response to the stressful stimuli is variable and depends on the type and duration of the stressor (Pullia et al., 1996). There is substantial clinical evidence that muscarinic receptor blockade by drugs like scopolamine results into disruptions of behavioral inhibition, working (short-term) memory, retrieval from reference (long term memory), attention and decisional processes movement and strategy selection and altered sensory processing (Fibiger, 1990). In view of the potential implication memory deficit during stress full conditions all the extracts and fractions of Ocimum sanctum (OS) and Evolvulus alsinoides (EA) were evaluated for memory enhancement in scopolamine induced dementia and Morris water maze test.

Control mice showed significant increase in TL on second trial compared to first trial (acquisition) which shows a successful memory response. Scopolamine treated mice failed to show increase in TL on second trial (retention) indicating deficit in memory (amnesia). However, in mice pretreated with EA at a dose of 100mg/kg body weight orally even after scopolamine treatment, a significant increase in TL was observed on second trial (retention). Prevention of scopolamine induced amnesia by ethanolic extract of EA demonstrated the potential anti-amnesic effect.

In Morris water maze test rats were trained to locate the plat form in water maze and later the latency time to locate the plat form was noted in which decreased latency time was considered to be the marker for memory enhancement. In this test rats were treated with 100mg/kg body before subjecting to water maze performance in which Ethanolic extract of EA was effective.
In both the studies extracts and fractions of OS did not show any effect on memory enhancement. Our study provides a brief scientific evidence for the memory enhancement properties of EA as reported in ayurvedic texts.

**Anti-oxidant effects of Crude extracts of Ocimum sanctum (OS) and Evolvulus alsinoides (EA)**

With the damaging role of free radicals in stress full conditions being clear anti-stress therapy should therefore include either free radical scavenging enzymes or agents, which are capable of augmenting the activity of major antioxidant enzymes (Cheeseman and Scater, 1993). In our study, Whole plant Ethanolic extracts of *Ocimum sanctum* (OS) and *Evolvulus alsinoides* (EA) when administered orally at a dose of 200mg/kg body weight, 7 days, 1 hr prior to Chronic unpredictable stress (CUS) regimen, has normalized the stress induced alterations of SOD, CAT and GPx anti-oxidant defense enzymes in plasma and frontal cortex, hippocampus, hypothalamus and striatum of brain, indicating the reduction in free radical load as marked by reduced lipid per oxidation, increased total plasma anti oxidant capacity (TAC) and glutathione levels.

In our study aqueous extract of OS and EA has failed to show anti-oxidant effect during CUS. But, numerous studies reported the peripheral anti-oxidant capacity of aqueous and alcoholic crude extracts of OS and they suggested it to be due to the flavanoid content of the crude extracts (Nair et al., 1982; Chattopadhyay et al., 1992; Maulik et al., 1997; Umadevi, 2001). In all the reported studies the plant material was directly subjected to aqueous extraction. But, In our study a greater yield of the pure compound is required. So, In view of the feasibility of isolating pure compounds from the extracts of Ethanol and Dicholoromethane rather than water, the plant material of both OS and EA was first extracted with ethanol and subsequently extracted with water which can be the important reason for its infectiveness and how ever the central protective effects were mostly conferred to ethanolic extract (Sakina et al., 1990; Sembulingam et al., 1997; Bhargava and Singh, 1981; Sen et al., 1992).

In Case of EA *In-Vivo* and *ex-vivo* studies conducted by Auddy et al. (2003) is the first study to reveal the potential anti-oxidant properties in which, authors have compared between ethanol and aqueous extracts and concluded that aqueous extract is having potent anti-oxidant properties. We have a completely different pattern which can be due to the *in-vivo* testing conditions and also the anti-amnesic property was observed by the ethanolic extract in scopolamine induced dementia. We can hereby conclude that the central protective effects of EA as reported in Ayurvedic texts (Chatterjee et al.,
1990) can be attributed at least partially due to the anti-oxidant capacity of the ethanolic extract rather than aqueous extract.

**Anti-stress effects of standard drug *Panax quinquefolium***

In our study commercially available standardized root powder of *Panax quinquefolium* containing total of 7.01% ginsenosides (Rg: 0.13%, Re: 1.32%, Rb₁ <0.10%, Rc : 1.49, Rb₂: 0.14%, Rd :0.54%, others:1.17%) was used to as a standard to compare the anti-stress effects of crude extracts and pure compounds. This was selected due to its widely accepted position as an “adaptogen” and scientifically proven herb for offering non-specific resistance during stressful conditions. Most pharmacological actions of ginseng are attributed to ginsenosides. More than twenty ginsenosides have been isolated (Gillis, 1997), and novel structures continue to be reported, particularly from *Panax quinquefolium* and *Panax japonicus* (Yoshikawa *et al.*, 1998) Ginsenosides are potent because Ginsenosides (except Ro) belong to a family of steroids named steroidal saponins (Ota *et al.*, 1987). They have been named ginsenoside saponins, triterpenoid saponins, or dammarane derivatives under previous classifications. Ginsenosides possess the four trans-ring rigid steroid skeleton, with a modified side chain at C-20 (Shibata *et al.*, 1985). The classical steroid hormones have a truncated side chain (progesterone, cortisol, and aldosterone) or no side chain (estradiol and testosterone). Many steroids have a b-OH group at C-3; ginsenosides (for example, Rb₁, Rb₂, Rc, and Rd) usually have a sugar residue attached to the same site (Shibata *et al.*, 1985). Sugar moieties are cleaved by acid hydrolysis during extraction, or by endogenous glycosidases to give the Pharmacological Effects of Ginseng. Steroids possess numerous physiological activities, partly due to the nature of the steroid skeleton. The trans-ring junctions of the skeleton allow substituent groups, which interact with receptors, to be held in rigid stereochemically defined orientations (Banhoop, 1994). In addition, the steroid skeleton endows the whole molecule with a favored structure to allow, for example, insertion into membranes (Bastiaanse *et al.*, 1997). Recent work showed that Rg₁ is a functional ligand of the nuclear glucocorticoid receptor (Lee *et al.*, 1997 Chung *et al.*, 1998). Ginsenosides are amphiphilic in nature (Banhoop, 1994), and have the ability to intercalate into the plasma membrane. This leads to changes in membrane fluidity, and thus affects membrane function, eliciting a cellular response. There is evidence to suggest that ginsenosides interact directly with specific membrane proteins. Moreover, like steroid hormones, they are lipid-soluble signaling molecules, which can traverse the plasma membrane and initiate genomic
effects. In our study oral administration of PQ powder at a dose of 100mg/kg was effective in normalizing AS and CUS induced changes in mean ulcer score, adrenal gland weight plasma glucose, Creatine kinase and corticosterone levels. These are in well accordance the reported activities of ginseng during alarm phase reaction of stress full conditions (Rai et al., 2003a). Crude extracts and fractions of the plants considered in our study were compared for their potency.

*Panax quinquefolium* (PQ) was effective at a dose of 100mg/kg body weight and normalized the acute stress induced increase in dopamine in frontal cortex and hypothalamus. It also normalized the elevated 5-HT levels in Frontal cortex, hippocampus and hypothalamus. Acute stress depleted nor-adrenaline levels are restored in frontal cortex and hippocampus and has not shown any effect in hypothalamus. In chronic unpredictable model (CUS) PQ administration restored the levels of 5-HT in cortex, hippocampus and Hypothalamus. The levels of nor-adrenaline were increased significantly in hippocampus and an insignificant increase was observed in frontal cortex. PQ did not show any dopaminergic activity during CUS conditions and its actions are mostly confined to hippocampus and frontal cortex. PQ is well known for its memory enhancing effects and its site of action being hippocampus Petkov (1978) found that ginseng administration (50 mg/kg) led to increases in brainstem dopamine and norepinephrine and increases in serotonin in the cortex. This action was abolished by administration of either a serotonin receptor agonist or a specific serotonin antagonist, suggesting that serotonergic transmission was involved in the memory-enhancing effect. It has also been shown that ginseng total saponin can modulate dopaminergic activity at both presynaptic and postsynaptic dopamine receptors and can block behavioral sensitization induced by psychostimulants such as morphine (Kim et al., 1995b), cocaine (Kim et al., 1995a), methamphetamines (Kim et al., 1998c), and nicotine (Kim et al., 1999a,b). The latter authors suggest that these effects are mediated by the inhibition of drug-related dopamine release by the action of ginseng total saponins on presynaptic dopamine terminals. Wang et al. (1995) also found that both root and stem/leaf saponins improved learning and raised the levels of biogenic monoamines in normal rats' brains. Ginseng has also been shown to attenuate pentylenetetrazole-induced decreases in rat brain monoamine oxidase, possibly accounting for its demonstrated antianxiety effect in rodents during stress full conditions (Bhattacharya and Mitra, 1991).
Anti-stress effects of crude extracts and fractions of *Ocimum sanctum*.

Fresh juice of Ocimum sanctum (OS) was screened for anti-stress activity in acute stress (AS) model. Fresh juice of Ocimum sanctum (OS) at a dose of 1ml/ kg body weight was effective in reducing AS induced hyperglycemia, increased Creatine kinase (CK) and Corticosterone in plasma. But has not shown any normalizing effect on stress induced ulceration and adrenal hypertrophy. With this study, whole ethanolic extract (EtOH/W) Ethanolic extract (EtOH), Dichloromethane (DCM), Butanolic fraction of ethanol extract (BuOH) and Aqueous fraction (Aq) were screened for anti-Stress activities in Acute and Chronic unpredictable stress models. Crude extracts of DCM, EtOH, EtOH(W) , fractions of BuOH and AQ were administered orally at a dose of 200mg/kg body weight prior to the stress session.

Stress induced ulcers are mainly due to the initial acid secretion and further aggravated due to the activation of inflammatory mediators and oxidative stress. OS is well known for its effect against stress induced ulceration. In our study all the extracts and fractions excluding Aq fraction were effective in reducing the stress induced intestinal hemorrhages. Various studies demonstrated the anti-ulcer effect of Ethanolic extract of OS in more intense and specific ulcer models (Singh and Majumdar, 1996, Dharmani et al., 2004). These studies attributed the anti-ulcer activity of OS due to its anti-secretary, cytoprotective and anti-oxidant principles which may directly or indirectly effecting the ulcer causative factors.

All the extracts and fractions except Aq were effective in reducing acute stress induced hyperglycemia. The hypoglycemic effect of OS is well demonstrated by various authors in a more specific models in which oral administration of ethanolic 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 *Catharanthus roseus*, *Gymnema sylvestre* and *Azadirachta indica* (Chattopadhyay 1993).

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 randomized 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-pandial 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 and suggested that it could be associated with the essential oil, eugenol present in OS leaf powder (Rai et al., 1997). All these results attributed the hypoglycemic effect to the active ingredients present in the crude extracts, which was further explored in our study.

DCM, EtOH, EtOH(W), BuOH and Aq extracts were screened for their effect on stress specific markers like adrenal gland weight, Plasma corticosterone and Plasma CK levels in AS and CUS models. DCM, EtOH, EtOH (W) and BuOH extracts were effective in reducing AS and CUS induced adrenal hypertrophy and elevated levels of Plasma corticosterone. However, in case of CK, DCM and EtOH(W) were effective in normalizing AS and CUS elevated Plasma CK levels whereas EtOH and BuOH were effective in normalizing the AS elevated CK levels only. Aq fraction was not effective in AS and CUS on our tested parameters. Out of all the extracts and fractions, DCM and EtOH extracts were very potent in all the stress parameters supporting the reported findings for the anti-stress potential of OS (Sembulingam et al., 1999, Bhargava and Singh 1981; Sen et al., 1992; Samson et al., 2005).

**Anti-stress effect of crude extracts and fractions of *Evolvulus alsinoides***

Crude extract of whole plant of *Evolvulus alsinoides* (EA) in Ethanol (EtOH) and water (Aq) were first screened for anti-stress effects on the stress markers considered in our study in acute stress (AS) and Chronic unpredictable stress models (CUS). Ethanolic and Aqueous extracts were found to be effective in reducing AS induced ulceration, hyperglycemia, adrenal hypertrophy, plasma CK and corticosterone. In CUS model Ethanol extract was effective in all the tested parameters but Aq was effective in reducing CUS induced increase in adrenal gland weight and CK levels but failed to normalize CUS induced ulceration and Plasma corticosterone levels. Since there was a promising anti-stress and anti amnesic effect of ethanolic extract we further proceeded with the fractionation of Ethanol extract with Dichloromethane (DCM), Butanol (BuOH) and water (Aq). Out of all the fractions screened BuOH was found to effective in normalizing the AS and CUS induced ulceration, increase in Adrenal gland weight, Plasma CK, Corticosterone and AS induced hyperglycemia. While DCM extract was effective in stress induced ulceration only. However Aq fraction failed to produce effect
on any of the stress parameters considered in our study. Since not much work was done with EA, the observed anti-stress effects can be explained based on the pure compounds and their chemical nature from the active fraction. We further continued for the isolation of pure compounds from active BuOH fraction and their bioactivity was evaluated.

**Effect of Pure compounds isolated from *Ocimum sanctum* and *Evolvulus alsinoides* on stress induced peripheral and central changes.**

*Ocimum sanctum* and *Evolvulus alsinoides* are well known in Ayurveda, the Indian traditional system of medicine for their central and peripheral effects. These plants are rich in flavanoids and several other unknown compounds, which contribute to their reported pharmacological effects. Basils contain a wide range of essential oils rich in phenolic compounds (Phippen and Simon, 2000) and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins. Various aspects of the stress alleviating potential of the crude extracts of OS have been established. We were able to isolate some well-known and widely reported compounds in their pure form from the bioactive fractions. Ursolic acid (OS/A) Eugenol glycoside (OS/B), Flavanone glycoside (Os/C) and spingolipid (OS/D) were isolated from *Ocimum sanctum* where as Caffeic acid EA/A, Flavanone glycoside EA/B and Erythritol EA/C from *Evolvulus alsinoides*. These compounds were evaluated for their peripheral and central anti-stress effects at a dose of 40mg/kg body, which is one fifth of the dose of effective extract and were compared with *Panax quinquinifolium*.

**Ursolic acid (OS/A)**

Ursolic acid and its derivatives are constituents of numerous plants which are having diversified phylogenetic origin and taxonomic position. It has been isolated from the protective wax-like coatings of apples, pears, cranberries, prunes, and other fruits. Seaweed’s are rich in ursolic acid derivatives. Ursolic acid [(3b)-3-Hydroxyurs-12-en-28-oic acid] rarely occurs without its isomer oleanolic acid [(3b)-3-Hydroxyolean-12-en-28-oic acid] they may occur in their free acid form or as glycones for triterpenoid saponins which are comprised of a triterpenoid aglycone linked to one or more sugar moieties. Several ursolic acid derivatives, both natural and synthetic, have been reported (Liu, 1995). Ursolic acid is known for several pharmacological effects like anti-cancer, antiulcer, antihyperlipidemic, anti-inflammatory, hepatoprotective, antimicrobial and antiviral effects (Liu, 1995; Zaletova et al., 1987; Chattopadhyay et al., 2002a).
In our study ursolic acid was effective in normalizing AS induced ulceration, adrenal hypertrophy, hyper glycemia, increased plasma creatinine kinase and corticosterone. In CUS, it effectively decreased the ulceration and adrenal hypertrophy but was ineffective in normalizing plasma creatine kinase and corticosterone levels.

Ursolic acid is having a good anti-oxidant potential which is responsible for its anti-ulcer activity. It is well known for its corticosteroid like effects and directly acts like dexamethasone thereby inhibits the stress induced changes (Tsuruga et al., 1991; Safayhi et al., 1997; Najid et al., 1992; Diaz et al., 2000; Somova et al., 2003).

In our study, ursolic acid reduced the acute stress induced elevation of 5-HT and DA levels in cortex and hypothalamus. In hippocampus there is no effect on decreased dopamine levels, but was effective in reducing the elevated 5-HT levels. Ursolic acid did not show any effect on the depleted levels of neurotransmitters in CUS model. However, there is no effect of ursolic acid in NA levels in both AS and CUS models.

There are two possible explanations for the behavior of Ursolic acid in chronic conditions. The dexamethasone like effect of ursolic acid is partly substantiated by its effects in CUS conditions in which there is no effect on plasma corticosterone, CK and the neurotransmitters. Because, in CUS, due to the increased stressor intensity, the HPA axis is desensitized and the dexamethasone like effects are suppressed which is normally observed in depression. Ursolic acid may be acting feed back loop at the level of pituitary. But, recent report of Chttopadyaya et al. (2003) where ursolic acid rich fraction of the crude extract of Mallotus peltatus was tested for analgesic and anti-depressant activity. The authors attributed their findings to the ursolic acid or in combination with β-Sitosterone another component rich in the methanolic crude extract they studied. How ever their observations are based on behavioral parameters. From neurochemical point of view in CUS conditions there are no many reports regarding the direct action of ursolic acid on neurotransmitters.

But, while considering the decreased adrenal gland weight and glucocorticoid receptor expression we tend to consider the anti-inflammatory activity for which ursolic acid is tested in different models, the activity is mainly due to its glucocorticoid like effects (Manez et al., 1997) with this given possibility, in our study ursolic acid down regulated the Glucocorticoid receptor density in hippocampus of rats subjected to CUS, which clearly indicates its potentiation of glucocorticoid effects. The direct effect of ursolic acid on glucocorticoid receptors was clearly demonstrated by its down-regulation
of MMP-9 gene through the nuclear translocation of glucocorticoid receptor in HT1080 human fibro sarcoma cell lines (Cha et al., 1998).

With this we can say that the differential role of ursolic acid/its profile of dexamethasone like activities depend on the duration and intensity of the stressors which contribute majority to the anti-stress effects observed in crude extracts. The possible neuroprotective effects of ursolic acid might be attributed to its anit-oxidant properties by increasing endogenous anti-oxidant enzyme levels (Kitani et al., 1999) or by directly combating the free radical load (Shih et al., 2004) rather than its direct action on neurotransmitter regulation

- **K-116 (OS/B)** \((4\text{-allyl}-1\text{-O-β-D-glucopyranosyl}-2\text{-hydroxy benzene})\) (Eugenol glycoside)

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum* L., has been found to be largely responsible for the therapeutic potentials of Tulsi. The spicy fragrance of cloves comes largely from eugenol, the principle ingredient in oil of cloves. Eugenol is used not only in perfumes and mouthwashes, but also as an insect attractant and a dental analgesic. It can be oxidized to form vanillin, which has a vanilla scent. We have isolated Eugenol in its glycoside form which was reported earlier in OS and other sources (Heidrun et al., 1992, Yoshio et al., 1978).

Eugenol and its glycosides are widely known for their anti-oxidant, dental analgesic, anti-inflammatory and anti-microbial properties (Tominaga et al., 2005; Chami et al., 2005; Kong et al., 2001).

Oral administration of OS/B at a dose of 40mg/kg body weight prior to stress session was effective in reducing AS and CUS induced ulceration, hyperglycemia, adrenal hypertrophy, Plasma CK levels and Plasma corticosterone.

In our study OS/B showed anti-stress effect at the neurotransmitter level regulating the Dopamine and 5-HT monoamines in AS and CUS models, OS/B was effective in decreasing the AS induced elevated levels of DA in frontal cortex and Hypothalamus and was ineffective in increasing the decreased dopamine levels in Hippocampus. Whereas effective in decreasing the AS induced elevated 5-HT levels in all the brain regions. In CUS, OS/B was effective in restoring the depleted dopamine and 5-HT levels in hippocampus and Nor-adrenaline, DA and 5-HT levels in Hypothalamus, where as, ineffective in the frontal cortex. OS/B has not shown any effect on the NA in all the brain regions in AS and in CUS, OS/B was effective in restoring the nor-
adrenaline levels in hypothalamus only. The decreased plasma concentration of corticosterone was well correlated with the restored Glucocorticoid receptor levels in hippocampus of rats subjected to CUS.

But, Apart from OS, Eugenol is also reported as a major active constituent in rhizoma acori graminei, a medicinal herb that has been used for epilepsy and forgetfulness in the East Asia for centuries and demonstrated to decrease Aβ1-40-induced cytotoxicity on PC-12 cells in vitro suggesting its central effects (Irie et al., 2004b). Our study substantially supports the anti-depressant effects of Eugenol during stressful conditions (Irie et al., 2004a) and its actions are mostly being mediated through 5-HT and DA.

**K-171-OS/C** [3,4,5-trihydroxy-6-[5-hydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-7-yloxy]-tetrahydro-pyran-2-carboxylic acid.] Flavone glycoside (OS/C)

Flavonoids are a class of water-soluble plant pigments. Flavonoids are broken down into categories, though the issue of how to divide them is not universally agreed upon. One system breaks flavonoids into isoflavones, anthocyanidins, flavans, flavonols, flavones, and flavanones. One of the undisputed function of flavanoids and other polyphenols is their anti-bacterial activity due to the presence of phenolic groups (Harborne and Grayer, 1994; Harborne, 1999). Some of the best-known flavonoids, such as genistein in soy, and quercetin in onions, can be considered subcategories of categories. Although they are all structurally related, their functions are different. Flavonoids also include hesperidin, rutin, citrus flavonoids, and a variety of other supplements. Flavonoids are found in a wide range of foods. For example, flavanones are in citrus, isoflavones in soy products, anthocyanidins in wine and bilberry, and flavones in apples and tea. The flavanoid we were able to isolate was Apigenin glycoside (OS/C) belonging to the sub class flavones of flavanoids. Flavonoids are generally available in herbaceous families, e.g. Labiatae, Umbelliferae, Compositae. Some of the well known flavones include Apigenin (Apium graveolens, Petroselinum crispum). Luteolin (Equisetum arvense) known for diverse pharmacological activities. In our study OS/C was administered orally at a dose of 40mg/kg body weight. OS/C was effective in reducing AS induced hyperglycemia, and adrenal hypertrophy. It was effective in decreasing ulcer incidence, elevated plasma creatine kinase and corticosterone levels. But was ineffective in reducing CUS induced adrenal gland hypertrophy.
OS/C was effective at 40mg/kg dose in normalizing the acute stress induced increase 5-HT in frontal cortex, hippocampus and hypothalamus. It was also effective in reducing the elevated dopamine levels in frontal cortex and Hypothalamus but was ineffective in normalizing the depleted dopamine levels in hippocampus. However OS/C did not show any effect on AS depleted Nor-adrenaline levels.

In CUS model, OS/C failed to produce any effect on depleted nor-adrenaline levels in all the brain regions. But, was effective in restoring dopamine and 5-HT significantly in frontal cortex and hippocampus but did not show any effect on Hypothalamus. From the peripheral and neurochemical data obtained in our study, the anti-stress effects of OS/C are mainly due to its serotonin and dopamine modulation in cortex and hippocampus.

Flavanoids like apigenin were known to exhibit benzodiazepine like activity which were found in Passiflora coerulea and Matricaria chamomilla (Medina et al., 1990; Viola et al., 1995). Apigenin from Matricaria chmomilla was tested for GABA mediation. In this study the authors demonstrated that the inhibitory effects of apigenin were not antagonized by GABA antagonist (Avallone et al., 2000). Their study suggests the possibility of other neurotransmitter systems for the central inhibitory effects of apigenin. How ever not many studies were conducted in this aspect. In western blott analysis OS/C was effective in restoring the depleted glucocorticoid receptor density. The neurochemical profile and glucocorticoid receptor data supports the role of Apigenin component for the anti-stress and anxiolytic effects of Ocimum sanctum.

- **K-117 OS/D [Sphingo lipid] Sphingolipid (OS/D)**

OS/D when hydrolyzed was obtained fatty acid methyl ester together with long chain base (LCB) and methyl glucopyranoside and was categorized to be spingolipid. However this compound failed to produce any anti-stress effect in both the models in important parameters of stress considered in our study.

**Anti-stress effects pure compounds isolated from Evolvulus alsinoides. (EA).**

- **K-014 EA/A- 3,4-dihydroxy cinnamic acid**

Caffeic acid (3,4-dihydroxycinnamic acid) is one of the most common phenolic acids, frequently occurs in fruits, grains and dietary supplements for human consumption as simple esters with quinic acid or saccharides, and are also found in traditional Chinese herbs. A variety of pharmacological activities of various Caffeic acid derivatives reported
Discussion

till date includes antioxidant, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antivirous and antitumor properties (Jiang et al., 2005).

This compound is first time isolated from Evolvulus alsinoides caffeic acid was tested for anti-stress effect at a dose of 40mg/kg body weight. It was effective in normalizing the AS and CUS induced peripheral changes in mean ulcer score, plasma glucose, creatine kinase and corticosterone levels. But, has not shown any effect on stress induced adrenal hypertrophy in both AS and CUS models. It was effective in reducing the AS induced increase in dopamine levels in cortex and hypothalamus and restored the depleted levels of dopamine in hippocampus. Caffeic acid normalized the AS elevated levels of 5-HT in all the brain regions.

In CUS, caffeic acid was ineffective in normalizing the depleted levels of neurotransmitters where as It has not shown any effect on nor-adrenaline levels in the entire brain region in both AS and CUS models. Whereas caffeic acid was ineffective in normalizing the CUS induced decrease of glucocorticoid receptor density in hippocampus.

Caffeic acid and its derivatives obtained from different plant sources are experimentally proven for various pharmacological effects (Lu and Yinrong, 2002). The central effects were not still completely explored. From our present study caffeic acid cannot be attributed with complete anti-stress effects because it is effective only in the acute stress model at neurotransmitter level. However, previous pharmacological studies have shown that caffeic acid can activate the \( \alpha \)-adrenoreceptor system (Cheng and Liu, 2000) and inhibit the production and release of nitric oxide (NO) (Soliman and Mazzio, 1998; Yokozawa and Chen, 2000). It has been suggested that these systems in the brain may contribute to stress and depression. In our study caffeic acid has not shown any effect on Nor-adrenaline levels in all the brain regions during acute stress full conditions. The results of our study are in support with the study conducted by Takeda et al., 2002 in which caffeic acid increased the swimming retention time (anti-depressant effect) during swimming despair test with out affecting the monoamine uptake or monoamine oxidase activity. Even though the monoamine hypothesis in various stress related pathologies like depression is very well known, caffeic acid definitely have its central effects being mediated directly or indirectly by controlling less well established neurotransmitters like NO and Histamine. Hence, the central effects of EA can be partially attributed to this component.
The peripheral anti stress effects shown in our study compensated by the neurotransmitter data in AS and in view of the complexity of stress response, caffeic acid can be seriously considered for exploring its central effects and its influence on other central mediators.

K-015 [EA/B] - 2-[4,5-dihydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-7-] glucose

EA/B was tested at 40mg/kg body weight for its anti-stress properties. This compound was effective in reducing stress-induced hyperglycemia, mean ulcer severity, and plasma creatine kinase levels. But was found to be ineffective in reducing adrenal hypertrophy in AS and CUS models and normalizing the CUS induced elevated levels of corticosterone.

EA/B in AS model was effective in normalizing the stress induced elevated levels of Dopamine in cortex, hypothalamus and increased the depleted levels of dopamine in hippocampus. It was also effective in decreasing the elevated levels of 5-HT in all the brain regions. However in CUS model EA/B was ineffective in producing any changes in the depleted monoamine levels and also has no effect on NA in all the brain regions in AS and CUS models. EA/B was also in effective in producing any considerable changes in glucocorticoid receptor density in brain regions of rats subjected to CUS. In particular this molecule is not very well known for central effects but belongs to the popular class of multifunctional molecules flavonoids (Flavonone) and in major contributes to the anti-oxidant activities and other central effects of EA.

K-019 (EA/C) Erythritol

EA/A is ineffective in all the parameters considered for anti-stress activity in both acute and chronic unpredictable stress models.