DISCUSSION

Noise as a Stressor

Noise is one of the most widespread sources of environmental stress in living environments (Wallernius, 2004). WHO has declared noise to be an international health problem when it exceeds 90 dBA (Ramsey, 1982). Hence, the noise level used in this study, 100 dBA, is a definite stressor and this level was chosen as comparable with the noise frequently detected in discos and some industrial workplaces (Cohen et al., 1981).

The treatment for stress needs a substance that maintains the body equilibrium in different circumstances. The physiological system, which plays a major role in maintaining this equilibrium, has been described differently by the individual systems of Medicine. Some of the herbal remedy for stress has been scientifically proved and it action on various organs including central nervous system.

Herbal management techniques provide a desired relief to such unavoidable stressors. These natural stress relievers are said to be devoid of dreadful side effects. According to the concept of Siddha science, which is one of the traditional systems of Medicine in India, nature and man are essentially one. Nature is man and man is nature. Man is said to be the microcosm and Universe is the macrocosm, because what exists in the world exists in man. Man is nothing but a miniature world containing the five basic elements of the various principles which constitute the minerals, vegetable and the animal kingdom. Siddha concepts describes that the Universe originally consisted of atoms which contributed to the five element, viz., earth, water, fire, air and ether. These atoms correspond to the five senses of the human body and they are the fundamental of all the corporeal things is the world. A suitable proportion of these five

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elements in combination with each other produce a healthy person. Any change in environment is known to act as stimulus and bring about changes in the body systems. Hence it is believed that any imbalance in these five elements can be corrected form naturally available herbs. One such herb with widespread applications is *Acorus calamus*. The study of AC is based on the reports available in Siddha literature and the findings of the present study have vindicated these reports.

**Changes in corticosterone and LPO after noise stress**

Exposure to noise stress led to an increase in plasma CORT levels in the rats in this study. The elevation of steroid level after exposure to a number of stressors has been reported (Miki et al., 1998; Poll et al., 2001). An immediate increase in plasma CORT levels after restraint stress was observed in Lewis rats (Lowry et al., 2003). This indicates that the increase in CORT level is a common stress-induced phenomenon. One day noise stress has been shown to significantly elevate the plasma CORT level in rats (Sembulingam et al., 1997). It has been well established that the CORT level is an indicator of stress intensity and greater HPA axis activation in response to stress (Sandstrom, 2005). Activation of the HPA axis leads to a rapid secretion of ACTH in the anterior pituitary and to an increase in circulating glucocorticoids (Aguilera et al., 2001). The marked increase in plasma CORT level in noise stress exposure may lead to stimulation of hormones such as the stress peptide CRH released from the hypothalamus (Nemeroff, 1996). CRH initiates the HPA axis in response to stress and stimulates a release of ACTH (Kemp et al., 1998) and then stimulates the release of glucocorticoids from the adrenal cortex (Fisher, 1989) (Figure 117).

There was a sustained increase in CORT levels even after 30 days noise stress in this study. Selye, (1952) has shown that if adaptation to stress fails, circulating corticosteroid levels will remain elevated for a prolonged period of time after the stress exposure. This may be due to the changes in the glucocorticoid receptor levels, which were associated with the altered glucocorticoid negative feed back sensitivity in rats (Meaney et al., 1996). This sustained elevation in circulating CORT has been
Figure 117

Hypothalamus Pituitary-adrenal (HPA) axis

Stress $(+)$

Hypothalamus

CRH $(+)$

Pituitary gland

ACTH $(+)$

Cortisol

Adrenal gland

Kidney
recognized as the prime factor that mediates the neuropathological effects and the negative health consequences of chronic stress (Sapolsky, 1996).

Noise (100 dBA/4h) exposure to one day, 15 days or 30 days causes oxidative stress and leads to elevation of LPO levels in all the brain regions as well as plasma LPO (Liu et al., 1994). Elevation in the CORT level accelerates the generation of free radicals (McIntosh and Sapolsky, 1996). A studies by Lin et al., (2004a,b) revealed that chronic dietary CORT supplementation could result in a significantly enhanced plasma LPO, suggesting that corticosteroids may be one of the reasons for the augmented production of ROS during chronic stress. Such correlation between corticosteroid and LPO levels could be observed. In this study, after 30 days of stress exposure LPO levels remained elevated in all the brain regions and the plasma LPO.

Effect of AC on the levels of corticosterone and LPO

Extracts of AC as well as α-Asarone treatment prevented the stress-induced increase in CORT levels. As the treatment of EAAC, MAC or α-Asarone (6 and 9 mg/kg) resulted in a similar response, it could be said that α-Asarone, the active principle of AC might be involved in preventing the stress-induced increase in plasma CORT levels. Corticosteroids has always been ascribed to be beneficial in stress situations, the return to normal levels observed after AC and α-Asarone treatment needs a debate to come to conclusion. Chronic stress CORT has been hypothesized to act on the brain in an excitatory rather than an inhibitory fashion and this would be expected to decrease the level of discomfort and anxiety induced by chronic stress (Dallman et al., 2004). According to Dhalla and Bhattacharya, (1968) AC acts as a tranquilizer-sedative action. The experiment of Menon and Dandiya, (1967) also showed that α-Asarone has got a tranquilizer action. In such cases, the animals threshold for stress may be altered and there by the animal might not have perceived the stress.
Changes in the antioxidant status after noise stress

The enzymatic (SOD, CAT, GPx and G6PD) and non-enzymatic (GSH, vitamin C, vitamin E) antioxidant systems are present in the brain to neutralize the ROS. When the production of free radicals is faster than their neutralization by anti-oxidative mechanisms, oxidative stress is induced (Sies, 1991). The oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. ROS levels in the cochlea were found to be significantly higher after 1 h exposure to 110 dBA noise (Ohlemiller et al., 1999a), persisting after the cessation of the exposure (Ohlemiller et al., 1999b). Brain is a major target of ROS toxicity due to its high oxygen consumption (Skaper et al., 1999). Further, brain is the tissue most vulnerable to oxidative damage, because of its high content of polyunsaturated fatty acids, relatively low antioxidant levels, non-replicatory nature of neuronal cells, and high levels of iron and copper (Floyd and Carney, 1992).

Enzymatic antioxidant levels

Activity of SOD in all the brain regions (cerebral cortex, cerebellum, midbrain, pons-medulla, hippocampus and hypothalamus) increased after noise stress exposure in this study. The increase activity of SOD after noise stress is similar to that observed by Aravind et al., (1998) and Ozguner et al., (1999), and also by Sahin and Gümüsülü, (2004) where different stressors were used. On the contrary, Kaushik and Kaur, (2003) showed a significant decrease in the SOD activity in the brain after chronic cold exposure. This may be due to cold-induced decrease in the rate of metabolism and suppression of enzyme activity and metabolism-induced free radical generation. Chaudiere and Ferrari-Iliou, (1999) observed a decrease in the activities of SOD and CAT after a restrain stress. Free radical generation could increase or decrease depending upon the duration of exposure and/or nature of the stressor.

The increased SOD activity is an indication that the brain’s antioxidant machinery is activated in response to excessive generation of free radicals (Bannister et al., 1987). Enhanced SOD activity catalyses the conversion of Superoxide (O$_{2}^{-}$) to hydrogen peroxide (H$_{2}$O$_{2}$), which is more toxic than the oxygen derived free radicals
and requires to be scavenged further by tissue thiols (glutathione redox pathway) and CAT (Fridovich, 1995).

The increase in SOD activity, observed in this study, indicates the possibility of the formation of increased production of H\textsubscript{2}O\textsubscript{2} that in turn could stimulate the second line of defense, which include CAT and GPx. These enzymes convert H\textsubscript{2}O\textsubscript{2} into water and molecular oxygen, rationalizing the cause for the elevation of these two during noise stress. In this current study, CAT and GPx activity was increased during one day and 15 days stress exposure. This observation was in agreement with an earlier report of Barichello et al., (2004). CAT and GPx are the most important endogenous antioxidant enzymes, and along with SOD constitute the defense mechanism of cells against damage due to ROS (Xiao et al., 2000). According to the study of Halliwell and Gutteridge, (1999) antioxidant enzymes are inactivated by excess of lipid peroxides and ROS. Activity of SOD is inhibited by excess of O\textsubscript{2}•−. \textsubscript{H}_2\textsubscript{O}_2. GPx and CAT (Pigeolet et al., 1990). This was revealed in the current study also after 30 days of noise stress exposure. A repeated stress exposure to generate excess of O\textsubscript{2}•− in turn inhibits GPx and CAT in all the brain regions after 30 days noise stress.

The ROS scavenging activity of SOD is effective only when it is followed by the actions of CAT and GPx, because the dismutase activity of SOD generates H\textsubscript{2}O\textsubscript{2} from the O\textsubscript{2}•−, which is more toxic than oxygen-derived free radicals and requires to be scavenged further by CAT and GPx (Bhattacharya et al., 2001). Apart from its own toxicity, H\textsubscript{2}O\textsubscript{2}, in the presence of iron, leads to the generation of toxic hydroxyl ions (Blake et al., 1987). Excess of SOD in relation to the activities of H\textsubscript{2}O\textsubscript{2} removing enzymes like CAT and GPx, are known to induce deleterious tissue effects (Blake et al., 1987). It appears that 30 days of noise stress-induced increase in LPO activity is caused by the increase in SOD activity with concomitant reduced CAT and GPx activity, result increased generation of H\textsubscript{2}O\textsubscript{2} and HO• which are not effectively scavenged and accumulate to induce increased LPO in all the brain regions (Kovacs et al., 1996).
Glucose-6-phosphate dehydrogenase (G6PD) is also known to have an antioxidant function (Stumpo and Kletzien, 1985) and is the regulatory enzyme of the pentose phosphate pathway. One of the main functions of this pathway is to supply reducing equivalents in the form of nicotinamide-adenine dinucleotide phosphate (NADPH), which is required for the maintenance of intracellular GSH concentrations (Gaetani et al., 1989). The vulnerability of G6PD is particularly important in the brain function, because it is the rate-determining enzyme in the glutathione recycling (Hashida et al., 2002). It has also been reported that G6PD is strongly inactivated by 4-hydroxy-2-nonenal, a toxic product of membrane peroxidation (Szweda et al., 1993; Ninfali et al., 2001). In this study, G6PD activity was decreased in all the brain regions after one day, 15 days and 30 days of noise stress exposure. G6PD insufficiency renders cells sensitive to oxidative stress (Kuo and Tang, 1999). This was well agreement with the present work when rats exposed to noise stress lead to oxidative stress. Further, the finding of Aspberg and Tottmar, (1994) where brain cells cultured under hyperoxic conditions show decreased activity of G6PD was well supports the present study.

**Effect of AC on enzymatic antioxidants after noise stress**

In this present study, EAAC, MAC or α-Asarone administration appears to mitigate noise stress-induced perturbation by tending to normalize SOD activity and reversing the effect of stress on CAT and GPx. These effects, therefore, leads to decrease in LPO levels in all the brain regions as well as the plasma LPO then results in attenuation of the adverse effects of all duration of noise stress. Moreover present observations are in agreement with Xuejiang et al., (1999), Shah and Vohora, (2002) and Salim et al., (2003) who have shown that the treatment of rats with herbal formulations/or plants extract resulted in the increased activity of these enzymes because of the ROS scavenging activity of these traditional drugs which might lead to the restoration of the depleted enzymes. The flavonoids present in plant are found to enhance free radicals scavenging enzyme activities like GPx and CAT or were capable
of preventing the stress-induced decrease of enzyme levels (Al-Qirim et al., 2002). Flavonoids have been shown to scavenge various ROS and have been implicated as inhibitors of lipid peroxidation (Mora et al., 1990). In this current study, also estimated flavonoid contents of AC which may minimize the ROS effect which was formed in excess during noise stress exposure. Therefore, further studies of the mechanistic properties of flavonoids are of potential importance in understanding and preventing ROS-linked diseases (Braca et al., 2003).

LPO has been shown to be involved in excitotoxicity of neuron which could probably be due to the relief of the voltage-dependent Mg^{2+} block of N-methyl-D-aspartate (NMDA) associated channels and damage of glutamate transporters (Mark et al., 1997; Keller et al., 1997; Madrigal et al., 2001). Since the treatment with EAAC, MAC and α-Asarone normalized the activity of SOD, GPx and CAT in all the brain regions. It could have, not only, prevented LPO but also the subsequent adverse effects mediated by NMDA receptors (Cho et al., 2002).

There is a direct correlation between plasma CORT and LPO level. (Ohtsuka et al., 1998; Rajashree and Puvanakrishnan, 1998) In this present study, treatment with EAAC, MAC and α-Asarone normalized the plasma CORT level. However, LPO level in all the brain regions as well as the plasma LPO was markedly decreased but not normalized indicating that few more factors may be responsible for the increase in the LPO. Another possible cause reported for the production of radicals and LPO in the brain was catecholamine metabolism such as dopamine and norepinephrine (Venarucci et al., 1999). Normally, elevated catecholamine may undergo auto-oxidation to generate electrons which in turn can produce ROS (Carpagnano et al., 2003). This may be true because Nankova et al., (1994) reported stress-induced increase in sympathetic arousal which stimulated and activated the catecholamine biosynthetic enzymes.

Compared to other doses α-Asarone 9 mg/kg effectively normalized the SOD, GPx and CAT activity in all the brain regions irrespective of stress duration. Though
treatment with EAAC, MAC and $\alpha$-Asarone 9 mg/kg could correct the enzyme activity leading to reduce the stress induced LPO level (Nishiyama et al., 1994). Although few regions showed LPO level, which was not normalized indicating that the stress-induced free radical formation may be faster than the scavenging system and difference in regional distribution of antioxidant enzyme in the various part of the brain. This was well in agreement with that of conclusion Manoli et al., (2000) and Baek et al., (1999) where in their study the vulnerability to oxidative stress in the brain is region specific.

From the foregoing, it is clear that noise stress was not adapted even after 30 days of noise stress exposure. EAAC, MAC and $\alpha$-Asarone 9 mg/kg doses were found to be effective in reducing the stress impact on the brain. Pharmacological effects of the medicinal plants extract related to its free-radical scavenging properties include inhibition of LPO, helping to maintain integrity and permeability of cell walls (Foster, 1996; Stoll et al., 1996) and protection of neurons against oxidative stress (Scif-el-Nasr et al., 1995; Oyama et al., 1996). Further, it is not clear whether the component $\alpha$-Asarone which was found to be effective in most of the regions but not all. Apart from this observation AC and their active component was effective in preventing noise stress-induced oxidative stress in neuron cell in the brain due to its antioxidant property (Acuna et al., 2002; Govindarajan et al., 2003) and neuronal protective role (Shukla et al., 2002; Cho et al., 2002).

In this study EAAC, MAC and $\alpha$-Asarone administration effectively prevented the decrease in G6PD activity during noise stress exposure and in turn regenerated GSH and NADPH which was depleted during noise stress. Recent work supports a pivotal role for G6PD in the cellular response to oxidative stress (Filosa et al., 2003). AC administration may be reducing oxidized glutathione to GSH indicating the increase in G6PD activity (Arivazhagan et al., 2000).
Non-enzymatic antioxidant levels

Non-enzymatic antioxidants systems also actively participate in protecting the brain cell from oxidative stress. They include GSH, vitamins C and E. Noise stress caused decreases in all these three non-enzymatic antioxidants in the brain regions. Many earlier studies have shown similar decrease in the non-enzymatic antioxidants after various stressors such as chronic immobilization stress (Madrigal et al., 2001; Sahin and Gumuslu 2004), cold stress and immobilization-cold stress exposure (Sahin and Gumuslu 2004).

GSH depletion determines the vulnerability to oxidant attack, which was observed in noise stress exposed animals. It has been shown that the glutathione status of a cell could be taken as the single most accurate indicator of the health of the cell and GSH maintains the cellular redox state of protein thiols and low molecular mass antioxidants like vitamin C and vitamin E (Meister, 1991; Haenen and Bast 1999). GSH as a redox regulator participated in the maintenance of oxidant homeostasis and the cellular detoxification of ROS in brain cells (Cruz et al., 2003). The acute depletion of intracellular GSH caused immediate cellular damage (Martensson et al., 1989; Meister, 1991). Loss of antioxidant ability due to GSH deficiency was directly related to mitochondrial damage in the brain (Jain et al., 1991). As mentioned earlier, LPO levels were found to be increased in the stress-exposed animals. The enhanced LPO may also be due to marked depletion of GSH content of the brain (Lewine, 1982). According to Tirmenstein et al., (2000) GSH must be depleted below a certain critical level in order to cause large increases in lipid peroxidation and necrotic cell death to occur.

The intracellular antioxidants GSH, vitamins C and E are interrelated with each other and they can be recycled. Vitamin C regenerates α-tocopherol, spares GSH, offsets GSH deficiency in cells, and works synergistically with GSH and other antioxidants (Jain et al., 1991; Meister, 1992; Rose and Bode, 1993). Vitamin C disrupts lipid peroxidation both directly, as an effective scavenger of $O_2^{•-}$, $H_2O_2$, ONOO•, HO• and other ROS, and indirectly, by regenerating vitamin E (Packer et al.,
GSH can spare ascorbate, and GSH is involved in recycling dehydroascorbate, the oxidized form of vitamin C, back into ascorbate (Meister, 1992; Wells and Xu, 1994 Arivazhagan et al., 2000). Because GSH and vitamin C perform many of the same functions, deficiency in one can be an offset of the other (Wells et al., 1990). Endogenous vitamin C level has been reported to decline under stress conditions (Acharya and Acharya, 1997; Cadet and Brannock, 1997). Decline in vitamin C levels in plasma, adrenal and all the brain regions were observed in the present study after one day, 15 days and 30 days of noise stress thereby supporting the earlier findings. Vitamin C deficiency results in depletion of tissue α-tocopherol (Arivazhagan and Panneerselvam, 2000). The decreased vitamin C content in the noise stressed rats might be one of the reasons for the observed decrease in vitamin E level. Vitamin E has been effective in blocking peroxyl-mediated chain reactions and in combination with ascorbate in scavenging O₂⁻ in lipid membranes.

Protein thiolation is apparently induced by different mechanisms that involve thiol/disulfide exchange or one or two electron oxidations of cysteinyln residue (Costa et al., 2003; Giles et al., 2003). The protein thiols are significantly decreased in the noise stress (100 dBA/4h for one day, 15 days and 30 days) due to oxidation of proteins. It is also justified by GSH, one of the major thiol substances, depletion of GSH can lead to protein denaturation and aggregation subsequent to protein thiol oxidation (Freeman et al., 1997). Similar results showing decreased protein thiols in brain due to oxidative damage, has been reported by Patsoukis et al., (2004). This was further strengthens the findings of this study which shows that noise exposure, irrespective of stress duration, caused a state of oxidative stress and lead to decrease in protein thiol levels (Patsoukis et al., 2004).

**Effect of AC on the non-enzymatic antioxidant system**

Treatment with EAAC, MAC or α-Asarone during noise stress of one day, 15 days and 30 days were effective in preventing depletion of GSH, vitamins C and E thereby inhibiting the LPO in noise stressed brain. Further, AC treatment restored
asma vitamin C levels the protein thiols in all the brain regions in addition to that of renal vitamin C, and it was shown that the pharmacological effects of the medicinal plants extract are related to its free radical scavenging properties that include inhibition of lipid peroxidation, helping to maintain integrity and permeability of cell walls (Foster, 1996, Stoll et al., 1996) and protection of neurons against oxidative stress (Seif-el-Nasr et al., 1995; Oyama et al., 1996).

Stress-induced decrease in GSH levels were not observed when the rats treated with AC extracts. GSH levels could be normalized in many brain regions by the treatment of AC but not in all the regions. In connection with this, LPO levels were also not normalized in all the brain regions after noise stress. There are two distinct mechanisms of protection that have been suggested; 1) increasing intracellular GSH and 2) directly lowering levels of ROS. Shukla et al., (2002) reported that extracts of AC treatment prevented the depletion of GSH in brain, in the same way it could prevent the GSH depletion during noise stress exposure. GSH provides a critical defense system for the protection of cells from many forms of stress (Maher, 2005).

Similar to GSH, the levels of vitamins C and E were also prevented from stress-induced changes in the animals treated with EAAC, MAC or α-Asarone. This could probably be due to either decreased oxidative stress or enhanced vitamin C synthesis in rat brain (Chatterjee et al., 1975). The other possible reasons for the observed increase in the levels of vitamin C apart from increased synthesis are (i) stabilization of vitamin C, (ii) reduction of dehydroascorbate to ascorbate, (Hughes and Wilson, 1977) and (iii) metabolic sparing of vitamin C by flavonoids (Zloch, 1973). Vitamin C disrupts lipid peroxidation both directly as an effective scavenger of ROS and indirectly by regenerating vitamin E (Packer et al., 1979; Meister, 1992; Rose and Bode, 1993) which continues to scavenge the free radicals (Hansen et al., 1991).
Effect of noise stress on memory

Noise stress exposure produced impairment in the spatial memory, increased the plasma CORT and decreased dendritic branch number in the CA1 and CA3 regions of hippocampus of male rats. These findings correlated well with many earlier reports where different stressors were used (Nishimura et al., 1999; Bowman et al., 2001; Ilyuva-Gomez et al., 2003).

In RAM test, when compared to the control, the noise-stress exposed animals showed significant increase in the number of reference memory errors, working memory errors and time taken to visit all baited arms in all the days tested. Our data correlates well with that of Jarrard, (1993), who reported that complete lesions of the hippocampus typically caused increase in both working and reference memory errors in the RAM. This spatial memory impairment was noted in all the different noise stress periods namely 1 st, 5 th, 10 th, 15 th, 20 th, 25 th and 30 th day. Increase in both errors and time taken to visit all baited arms in day one noise stress might be due to the anxiety state of the animals or psychological stress (Conrad et al., 2004). But increases in errors from day 5 to day 30 might be due to the elevated corticosterone levels (Conrad et al., 1996).

Stress has been known to impair brain function and increase the vulnerability of neurons to injury, especially in the hippocampus (Cochrane, 1991; Bondarenko et al., 1999; Gamaro et al., 2003). Effects of chronic CORT on the hippocampus are also well documented (Reagan and McEwen, 1997; Nair et al., 1998). In an earlier study, exogenous application of a high dose of corticosteroids had elicited neuronal atrophy in the hippocampus (Woolley et al., 1990). Stress-induced changes in both humans and rodents included the cognitive impairment and hippocampal atrophy (Patel and Finch, 2002).

Chronic restraint stress or daily exogenous administration of CORT caused extensive atrophy of CA3 pyramidal neurons in the hippocampus as well as an impairment in performance on hippocampally-mediated learning and memory tasks in
e RAM (Woolley et al., 1990; Watanabe et al., 1992; Luine et al., 1994; Magarinos and McEwen, 1995a,b; Magarinos et al., 1998; Sapolsky, 1999; McKittrick et al., 2000). Further, the apical dendritic retraction and debranching occur following 3 weeks of repeated restraint stress (Radley et al., 2004) and also induced apical dendritic atrophy along with spine synapse loss in this cortical region (Cook and Wellman, 2004). Prolonged intense psychosocial stress in vervet monkeys caused dendritic atrophy and neuron loss in the hippocampus (Uno et al., 1989). Hence, it is clear that increased CORT levels after the stress might have resulted in the damage to hippocampal neuron. It can also be suggested that the destruction of the hippocampal neurons may decrease the CORT receptors and there by affect the feed back mechanisms that result in the increased CORT levels in chronic stress. It has also been reported that the human subjects had 15% lower hippocampal volume without significant atrophy of the cerebral cortex. This selective loss of hippocampal volume is correlated with elevation of CORT level for 5 years (Lupien et al., 1998). Hence, it is clear that the prolonged elevation in the corticosterone level is not a beneficial one for the nervous system irrespective of the stressor and the species. Besides glucocorticoids, MDA receptors are also involved in plasticity, atrophy and in neuronal death in the hippocampus (McEwen, 1999). This also might have played a role in the atrophy of the neurons in hippocampus, which in turn led to memory impairment under noisy environment. Padovana et al., (2000) has suggested that hippocampal NMDA receptors could play a role in the development of behavioral changes induced by stress.

In this present study, 2nd and 3rd order of dendritic reduction were observed in hippocampal CA1 and CA3 pyramidal neurons in the golgi cox dendritic count after noise stress exposure. This might be due to elevated plasma CORT level even after 30 days of noise stress. The functional loss was indicated by the memory impairment in RAM, in terms of increase in errors of reference and working memory as well as in time taken to visit all the baited arms during the 30 days of noise stress exposure. Numerous studies in the past have dealt with the role of glucocorticoids on the processes of memory acquisition and consolidation in both animals and human (Lupien...
d McEwen, 1997). Gumuslu et al., (1997) further supported the fact that stress-induced significant dendritic atrophy and neuronal loss in the hippocampus in parallel with impaired spatial memory performance (Capel et al., 1983; Asahi et al., 1995). In this study, noise exposure led to oxidative stress in all the brain regions including hippocampus. This might be due to depletion in GSH and indicated by increased LPO. The finding of Cruz et al., (2003), which showed that the maintenance of normal GSH level was important for acquisition of spatial memory, correlates well with this study. GSH unavailability induced failures in hippocampal synaptic plasticity mechanisms that were related to spatial memory deficits.

Significant increase in AChE activity was noted in all the brain regions including hippocampus in rats exposed to one day, 15 days and 30 days noise stress. This might lead to decrease in acetylcholine in the synaptic cleft and a consequent increase in the cholinergic activity, which in turn leads to memory impairment (Yamaguchi and Kawashima, 2001). During one day noise stress exposure increase in AChE activity was noted in hippocampus in this study. This finding was similar to that of Sembulingam et al., (2003). A decrease in the levels of AChE activity in the chronic restrain stressed rats (Sunanda et al., 2000; Das et al., 2005) was reported. But the activity of AChE was till elevated even after 30 days stress exposure in our present study. The increased AChE activity observed after amyloid beta-peptide have been suggested to be mediated by oxidative stress-induced Ca$$^{2+}$$ influx in the cultured retinal cells (Melo et al., 2003). So, it is possible that the enhancement of AChE: activity observed after 30 days of noise stress in this study could have been mediated by oxidative stress (Hartmann and Mobius, 2003).

Effect of AC on stress-induced memory impairment

Treatment with EAAC, MAC or \(\alpha\)-Asarone along with one day, 15 days or 30 days of noise stress normalized AChE activity in all the brain regions. This might be due to the cholinesterase enzyme inhibitor activity of AC (Oh et al., 2004; Rahman et al., 2004), thereby it could counteract the elevated AChE during stress. Abe et al.,
(2003) reported that CNS AChE inhibitor improves cognitive performance in patients with the Alzheimer type of senile dementia of the. Since the treatment with EAAC, MAC or α-Asarone normalized the activity of SOD, GPx, CAT and GSH in all the brain regions as well as the level of plasma CORT after stress, it could have prevented LPO and the subsequent adverse effects mediated by NMDA receptors (Cho et al., 2002) thereby protecting the neuronal atrophy and memory loss. Furthermore, NMDA receptor-mediated neuroprotective action by α-Asarone may provide pharmacological basis for the traditional clinical applications of AC to treat memory impairment (Nishiyama et al., 1994a; Nishiyama et al., 1994b; Aruoma et al., 1998).

The memory improving activity of AC might be thus attributed to its antioxidant, neuroprotective, anti-acetylcholinesterase properties and NMDA receptor-mediated memory improving action under noisy environment. In this study AC and α-Asarone treatment normalized the errors of reference and working memory as well as the time spent to visit all baited arms during noise exposure. Further, the memory improvement was confirmed by increased hippocampal neuronal dendritic count after administration of AC and α-Asarone. Several studies have suggested that these increases in oxidative stress vulnerability and the resulting neuronal loss can be reduced through dietary supplementation of plant extracts that prevent brain atrophy as well as learning and memory impairments (Kanowski et al., 1996; Moriguchi et al., 1997; Nishiyama et al., 1997).

**Effect of noise stress on behavioral parameters**

The EPM test is one of the most widely used non-conditioned animal models of anxiety. It is well characterized and has been extensively validated pharmacologically as well as ethologically (Pellow, 1985; Pich et al., 1993). In animals, though, anxiety cannot be analyzed directly it can be studied using behavioural parameters, like entry and time spent in the open arm and head dipping, which are thought to be associated with that of emotional state (Liebsch et al., 1998). In animal models of anxiety that depend on exploratory ambulation such as the EPM, particular care has to be taken that
measures indicating anxiety-related behavior are not exclusively based on reduced locomotor activity (Dawson and Tricklebank, 1995). Thus, anxiety and locomotor activity are interlinked in the EPM (Gentsch et al., 1981; Courvoisier et al., 1996; Steimer et al., 1997).

The OFB test is also one of the most important procedures in animal psychology (Belzung and Dubreuil, 1998). In the OFB test three independent behavioral dimensions relating to motor reactivity, exploration and emotional reactivity was observed.

This current study analyses the anxiety level based on reduced open arm exploration and head dipping in the EPM with or without noise stress and AC treatment. Percentage of open arm entry, percentage of time spent in the open arm (Liebsch et al., 1998) and number of head dipping was markedly reduced. This indicates anxiety-related behavior, which was observed after one day and 30 days of noise stress exposure. In this study, one day, 15 days and 30 days of noise stress exposure reduced over all OFB activity as reflected by decreased the number of peripheral square entry, central square entry, rearing and grooming as well as increased immobilization time and number of fecal bolus. Especially decrease in the number of centre square entry after noise stress reinforces the higher anxiety level of animals. This study was well in agreement with Guimaraes et al., (1993) reported that two hours of either acute or repeated restraint stress-induced an overall reduction in general activity in the OFB and in the EPM. Harris, (1998) suggested acute exposure to restraint stress for 30 min induced an increase in motor activity without any effect on exploratory variables. This indicates that the behavioral alteration depends on the nature of the stressor, duration and intensity (Panakhova et al., 1984). NMDA receptors are involved in the anxiety behaviors, when rats treated with agonists of NMDA receptor induced anxiety (Wiley and BaBster, 1993). Administration of Diazepam, a benzodiazepine with clinical anxiolytic effects, produces anxiolytic-like effects in the EPM (Jardim et al., 2005).
Effect of AC on stress-induced behavioral changes

Wall and Messier, (2001) stated that a rat treated with an anti-anxiety drug would have a higher percentage of time spent in the open arms than a normal rats. This was true in this current study where in noise stress exposed animals treated with AC showed an increased activity in open arm in the EPM and increased number of center square entry as well as decreased immobilization time and fecal bolus in the OFB. The treatment with EAAC, MAC and α-Asarone increased percentage of open arm entry and percentage of time spent in the open arm indicating the AC may have an anxiolytic action. EAAC, MAC as well as α-Asarone (9 mg/kg) treatment during stress exposure normalized locomotor and emotional behavior in the OFB test by means of increased peripheral and central square entry as well as decreased immobilization time and fecal bolus even after stress exposure. According to the report of Menon and Dandiya, (1967) Asarone in AC possessed a tranquilizing effect and moreover it doesn’t deplete the 5-hydroxytryptamine. Extract of AC or α-Asarone treated animals have shown tranquilizing action (Bannerjee, 1967; Dandiya, 1968; Panchal et al., 1989; Belova et al., 1985). These studies support current observations because AC also has similar tranquilizing and anxiolytic action. Wiley et al., (1995, 1998) suggested that NMDA receptor antagonists are anxiolytic. If so α-Asarone might be acting through NMDA receptor (Cho et al., 2002) and this action of AC may be the cause for anxiolytic action. In the present study, AC significantly increased the percentage of time spent in the open arm and percentage of number of open arm entries without affecting motor activity similar to diazepam.

Effect of stress on certain molecular markers

Expression of c-fos mRNA in brain after noise stress

Fos is the protein product of the proto-oncogene c-fos and is expressed in response to hormone and neurotransmitter induced elevations in intracellular levels of calcium and cAMP (Morgan and Curran, 1991). Induction of c-fos does often correlate
with increased electrical and metabolic activity in cells and has been used as marker of neuronal activity. The c-fos mRNA genes are rapidly induced in the rat brain by a wide range of experiences, including those characterized as stressors (Herdegen and Leah, 1998). Cullinan et al., (1995) reported that there was widespread upregulation in c-fos expression in many brain areas due to stress. Acute stress is known to evoke a discrete pattern of c-fos expression in the brain.

In the present study, the c-fos mRNA was increased in the cerebral cortex, cerebellum, hippocampus and hypothalamus after 30 days of noise stress exposure compared to the control. This finding is well supported by many earlier investigators where different stressors were used (Yokoyama and Sasaki, 1999; Ostrander et al., 2003). It is conceivable that these brain regions are mostly involved in stress-mediated response.

Cortex and hippocampus have repeatedly been reported to be associated with stress using measures such as c-fos induction (Cullinan et al., 1995; Morrow et al., 2000). According to Campeau and Watson (1997), many brain regions displayed reliable c-fos mRNA induction in response to increasing levels of noise. Hypothalamus and few brain regions were found to display maximal c-fos mRNA induction in response to noise intensities (Burow et al., 2005). Important regions implicated in stress reactivity such as the prefrontal cortex and the hippocampus was not found to be particularly associated with increasing acute loud noise intensities (Campeau and Watson, 1997). Normally, basal c-fos levels in naive animals are low (Herdegen and Leah, 1998). However, in this study, expression of c-fos mRNA in the cerebral cortex, cerebellum, hypothalamus and hippocampus was observed after 30 days of noise stress and increased expression of c-fos mRNA in these regions might be due to long term noise exposure. Exposure of c-fos mRNA was used as a marker of regional brain activity during stress (Burow et al., 2005), and this expression correlated well with current study. Further, both unconditioned and conditioned stressors increased c-fos mRNA levels in the brain which correlated with stress-induced plasma CORT
oncentrations (Smith et al., 1992). In the present work, increased c-fos expression with plasma CORT level after noise stress was observed.

**Effect of AC on c-fos expression**

Treatment with EAAC or α-Asarone (9 mg/kg) during 30 days stress exposure was effective in preventing the enhanced c-fos expression in cerebral cortex, cerebellum, hippocampus and hypothalamus (Figure 119, 120). The restraint-induced increase in c-fos mRNA expression in the hippocampus and the morphological changes induced by chronic restraint stress are attenuated by treatment with NMDA antagonists (McEwen and Magarinos, 1997; Lino de Oliveira et al., 1997). This finding further strengthens the current study (Cho et al., 2002). In brain c-fos expression has been well correlated with anxiety (Boguszewski and Zagrodzda, 2005). In this current study, animals exposed to noise stress showed increased anxiety level and AC treatment during noise stress reduced the anxiety due to its tranquilizer action (Menon and Dandiya, 1967). This current study has been well agreement with McGregor et al., (2004) which showed that the benzodiazepine was able to decrease c-Fos expression in most hypothalamic areas.

**Hsp70 mRNA expression in brain after noise stress**

Heat shock proteins (Hsp) represent several families of cellular stress-response proteins, some of which are expressed constitutively and others expressed largely under conditions of stress (Welch, 1992). The well-characterized program of gene expression, leading to the synthesis of Hsp70 exerts cytoprotective functions (Sorger, 1991). Hsp70 mRNA studies in rat tissues showed a higher expression in neurons and nerve fibers than in glial cells (Kaul et al., 1997). Cell damaged proteins occupy chaperone-binding sites, and liberate the heat shock factor1 and this transcription factor is responsible for Hsp induction (Morimoto, 1999). In particular, induction of Hsp70 has been linked directly to the accumulation of improperly folded or denatured proteins in cells and may be involved in the repair of damaged proteins (Ananthan et al., 1986).
Expression of c-fos mRNA after 30 days of noise stress in the rat brain
(Ethidium bromide stained agarose gel showing RT-PCR amplified mRNA from discreet regions of the rat brain)

Figure 119

Lane 1 - DNA 100 bp marker
Lane 2 - Control - cerebral cortex
Lane 3 - 30 days noise stress
Lane 4 - EAAC + 30 days noise stress
Lane 5 - α-Asarone + 30 days noise stress
Lane 6 - Control - cerebellum
Lane 7 - 30 days noise stress
Lane 8 - EAAC + 30 days noise stress
Lane 9 - α-Asarone + 30 days noise stress

Figure 120

Lane 1 - DNA 100 bp marker
Lane 2 - Control - hippocampus
Lane 3 - 30 days noise stress
Lane 4 - EAAC + 30 days noise stress
Lane 5 - α-Asarone + 30 days noise stress
Lane 6 - Control - hypothalamus
Lane 7 - 30 days noise stress
Lane 8 - EAAC + 30 days noise stress
Lane 9 - α-Asarone + 30 days noise stress
Hsp70 gene encodes a major stress-inducible heat shock protein (Hsp70), which plays an important role in protecting cells from deleterious stresses. There are number of molecular signals in the cells that are known to induce the parallel expression of Hsp70 and ROS-mediated oxidative stress. *In-vitro* and *in-vivo* experiments have shown that the oxygen free radicals (hydroxyl, superoxide and nitric oxide) are inducers of heat shock response (Jornot et al., 1998; Malyshev et al., 1995; Bachelet et al., 2002). In this study, the cerebral cortex cerebellum, hypothalamus and hippocampus regions showed increased expression of Hsp70 after 30 days of noise stress (Figure 121, 122). The possible cause for the expression of excess Hsp70 may be due to the elevated levels of free radicals as indicated by the altered enzymatic and non-enzymatic antioxidant status. Thus, noise stress-induced oxidative stress might be responsible for the induction of Hsp70 in the rat brain. Upon oxidative stress, protein damage in the brain might be having a role in signaling the expression of Hsp70 (Thannickal and Fanburg, 2000; Singh and Kaur, 2006). It appears that the role of Hsp70 is to protect the oxyradical induced changes, as oxygen radical induced synthesis of stress proteins leading to tolerance to oxidative stress has been reported (Marini et al., 1996; McDuffee et al., 1997).

Since in the noise stress group formation of the increased free radical level was indicated by the elevated LPO and alteration in the scavenging enzymes the induction of Hsp may be due to the reduced the free radical attack on proteins (Papp et al., 2003). However, administration of sympathomimetic drugs and neuroendocrine hormones evoked an Hsp response in non-stressed animals indicating that various aspects regulate Hsp expression (Udelsman et al., 1994). Several reports suggest that inducible Hsp70 has a protective function in the CNS (Lowenstein et al., 1991; Rordorf et al., 1991) and over expression of Hsp70 has been shown to be neuroprotective (Akbar et al., 2001) during stress exposure.
Expression of Hsp 70 mRNA after 30 days of noise stress in the rat brain (ethidium bromide stained agarose gel showing RT-PCR amplified mRNA from discreet regions of the rat brain)

**Figure 121**

- Lane 1 - DNA 100 bp marker
- Lane 2 - Control - cerebral cortex
- Lane 3 - 30 days noise stress
- Lane 4 - EAAC + 30 days noise stress
- Lane 5 - α-Asarone + 30 days noise stress
- Lane 6 - Control - cerebellum
- Lane 7 - 30 days noise stress
- Lane 8 - EAAC + 30 days noise stress
- Lane 9 - α-Asarone + 30 days noise stress

**Figure 122**

- Lane 1 - DNA 100 bp marker
- Lane 2 - Control - hippocampus
- Lane 3 - 30 days noise stress
- Lane 4 - EAAC + 30 days noise stress
- Lane 5 - α-Asarone + 30 days noise stress
- Lane 6 - Control - hypothalamus
- Lane 7 - 30 days noise stress
- Lane 8 - EAAC + 30 days noise stress
- Lane 9 - α-Asarone + 30 days noise stress
et of AC on Hsp70 expression

AC and α-Asarone administration prevented the induction of Hsp70 expression in all the brain regions tested, indicating that the brain tissue was able to cope better with stress. Several antioxidants (quercetin, superoxide dismutase, vitamin C and curcumin) have been demonstrated to modulate the differential expression of Hsp in various stress conditions (Pinot et al., 1997; Kato et al., 1998). This study further strengthened current results that EAAC and α-Asarone treated animals showed a marked decrease in free radical formation and at the same time the expression of Hsp70 was much reduced in all these regions.

DNA fragmentation in rat brain due to noise stress

An increase in cytosolic calcium is related to cell oxidative processes (Maciel et al., 2001; Ernak and Davies, 2002) subcellular alterations are related to an imbalance in calcium homeostasis (Gesi et al., 2000), in line with this, noise exposure (110 dBA for 1 hr) has been reported to increase ROS (Ohlemiller et al., 1999a). For instance, calcium stimulates generation of ROS where they attack protein thiols, thereby opening permeability transition pores (Fagian et al., 1990; Kowaltowski et al., 1996). One crucial effect of ROS is known to be the oxidative damage of nucleic acids (Cross et al., 1987; Lemasters et al., 1992). The persistence of ROS mediated DNA alterations might lead to serious and long-lasting consequences, as suggested by the association between the persistence of ROS mediated DNA alterations and mutagenic events (Emerit, 1994). It is noteworthy that DNA single-strand breaks are usually repaired within 15 min and that DNA double-strand breaks are repaired within 2 hr (Plappert et al., 1997; Vijayalaxmi et al., 1993). Thus, such maintenance of genotoxic effects 30 days (100 dBA/4h/d) after noise exposure might be the consequence of a long-lasting clastogenic agent.

Our results on DNA damage might be interpreted as the output of the clastogenic effect of oxyradicals (Frenzilli et al., 2004). The negative effects of noise on cell structure and function were supposed to be, at least in part, mediated by the
crease of ROS (Lenzi et al., 2003). ROS levels in the cochlea were found to be significantly higher 1 hr after exposure to 110 dBA noise (Ohlemiller et al., 1999a), persisting after the cessation of the exposure (Ohlemiller et al. 1999b). In this respect, it is worthy to note that DNA is a main target of ROS toxicity (Cross et al., 1987; Emasters et al., 1992). The involvement of ROS might play a causal role in the induction and persistence of genetic damage related to loud noise exposure also in extra-auditory organs (Van Campen et al., 2002).

The DNA fragmentation analysis in this study revealed that on 31st day i.e., after 30 days of stress exposure (24 hrs after stress termination) when the DNA was analysed it showed an increase in the fragmentation. This was well agreement with earlier reports Frenzilli et al., (2004) reported that 12 hr of exposure to loud noise exposure produces a significant loss of DNA integrity in the rat adrenal gland. However, Manoli et al., (2000) reported that the chronic stress and high corticosterone levels induced neuronal death. In this study, elevation of corticosteroid along with elevated LPO and alteration in the anti oxidant scavenging enzymes indicate the free radical changes. It appears that stress-induced alterations within a biological system are multi factorial and likewise the effects induced by them are also at multi level in the multi organ system.

**Effect of AC on stress-induced DNA fragmentation**

Treatment with EAAC, MAC or α-Asarone to animals for 30 days did not alter the DNA integrity whereas administration of AC extracts and its active principle α-Asarone during stress exposure effectively prevented the noise stress-induced DNA fragmentation. Navasumarit et al., (2000) suggested that ethanol induced DNA strand break was prevented by pre-treating with vitamin C and vitamin E. It can be assumed that the normalization of the vitamins C and E level as well as enzymatic antioxidants in the rat brain by AC, in this regard may be at least in part prevented stress-induced the at brain DNA fragmentation.
Effect of AC on noise stress-induced Fas/FasL expression

Fas is a 45-kDa transmembrane molecule transmitting a programmed cell death signal following ligation with its natural ligand (Gu et al., 1995; Ferrari et al., 1998). FasL is a Type II membrane protein (Suda et al., 1993) and can be expressed on the cell surface or released in soluble form into the intercellular milieu (Tanaka et al., 1996). In some cell types, FasL can be stored preformed in granules, then rapidly released at the cell surface in response to external stimuli. Fas expression by normal neurons is not yet fully documented. Fas was reported to be undetectable in normal neurons and the basal expression of Fas is so low that it seems to be nonfunctional (Tan et al., 2001).

In this current study, Fas/FasL was not expressed in western blot analysis in control animals as well as EAAC or α-Asarone treated non-stressed animals (Figure 123, 124). According to Choi and Benveniste, (2004) in-vivo expression of Fas/FasL molecules seems to be restricted in the normal brain and these data have been consistent with this present study. However, inducible expression of Fas receptors in the CNS can cause direct or bystander damage of neurons. Therefore, the Fas/FasL system should be considered as inducing neuronal cell death in a variety of neurologic disorders (Dietrich et al., 2003).

In this study, 30 days of noise stress-exposed rats showed increase expression of Fas/FasL in the brain and also observed oxidative stress. Oxidative stress has received increasing attention as a possible contributor to many neurodegenerative of diseases (Facchinetti et al., 2002; Ferrer and Planas, 2003). A number of studies suggested that a possible connection between oxidative stress and increases in Fas/FasL expression in the brain (Vogt et al., 1998; de la Monte et al., 2000; Kwon et al., 2001; Facchinetti et al., 2002). Treatment with EAAC or α-Asarone prevented the Fas/FasL expression and this might be due to the reduction in the oxidative stress by AC (Figure 123, 124). However, further investigations are needed to delineate the relationship between the Fas/FasL system and oxidative stress in noise exposure.
Effect of AC and \( \alpha \)-Asarone on Fas receptor protein expression after noise stress

Figure 123

Lane 1 - Marker
Lane 2 - Control
Lane 3 - EAAC alone
Lane 4 - \( \alpha \)-Asarone
Lane 5 - Noise Stress
Lane 6 - EAAC+NS
Lane 7 - \( \alpha \)-Asarone +NS

Effect of AC and \( \alpha \)-Asarone on Fas ligand protein expression after noise stress

Figure 124

Lane 1 - Marker
Lane 2 - Control
Lane 3 - EAAC alone
Lane 4 - \( \alpha \)-Asarone
Lane 5 - Noise Stress
Lane 6 - EAAC+NS
Lane 7 - \( \alpha \)-Asarone +NS
AC and α-Asarone

The present work revealed that the action of AC as an antistressor, antioxidant, anxiolytic and memory improving effect in noisy environment may be due to one of its components α-Asarone. This was decided because among the extracts of AC, EAAC showed the better responses which contain more of α Asarone as substantiated by the Thin layer chromatography in this study. Therefore, it has been justified that HFXAC and DCMAC was not used for 30 days of noise stressed groups. In Thin Layer Chromatography of crude extracts of Acorus calamus (Figure 125) Hexane (Lane 2), Dichloromethane (Lane 3), Ethyl acetate (Lane 4) and Methanol (Lane 5) The same solvent system was used to analyze the pure compounds of AC, α-Asarone (Lane 1).

EAAC (50 mg/kg BW), MAC (50 mg/kg BW) and α-Asarone (9 mg/kg BW) when given alone did not produce any changes in the brain cortex morphology (Figure 126) and antioxidant status in all the brain regions. Interestingly, EAAC, MAC and α-Asarone treatment along with noise-stress prevented the cortical neuronal alteration which is observed in noise-stress exposed rat. Furthermore, morphology of liver not all the experimental groups showed any alteration, when these extracts are given to non-stressed and stressed rats, in parenchymal and sinusoidal pattern (Figure 127).

Moreover, it is relevant to point out that the maximum dosage of AC and α-Asarone used in this study only 50 mg/kg BW and 9 mg/kg BW respectively. Further, the LD$_{50}$ value of Acorus oil 221 ± 1.8 mg/kg BW (Dandiya and Cullumbine, 1959) and α-Asarone 225.5 ± 1.1 mg/kg BW (Yabiku, 1980) in rats indicates that the dosage used in this present work did not show any harmful effect even after 30 days administration to control animals.

AC as an adaptogen

AC prevented the stress-induced alterations in most of the parameters studied. In normal animals, its administration did not produce any significant change. Such type
TLC profile of *Acorus calamus* Linn Rhizome crude extract

Figure 125

UV at 254 nm  UV at 366nm  Ammonium Molybdate  10% Sulphuric acid in Methanol


TLC resolved in Hexane/ Ethyl acetate 6/4
Figure 127 (10X). Illustrates H&E stained parenchymal cell and sinusoidal pattern of rats liver in different experimental groups. Control rat liver cell morphology (A), EAAC (B), MAC (C) and α-Asarone (D) for 30 days treated rat cortex also shows normal cytoarchitecture, in noise-stress exposed rats for 30 days (E). Treatment with EAAC (F), MAC (G) and α-Asarone (H) along with noise stress for 30 days rats Liver parenchyma of all experimental group animals has not shown any significant changes.
of action in a herb is referred as adaptogens. According to Lazarev, an adaptogen is an agent that allows the organism to counter adverse physical, chemical or biological stressors by raising non-specific resistance towards such stress, thus allowing the organism to ‘adapt’ to the stressful circumstances (Wagner et al., 1994).

The concept of adaptogenic action was further elaborated by Brekhman and Dardymov, (1969) some 20 years later. They put forward specific criteria that a substance should meet in order to be regarded as an adaptogen.

- It must allow the normal functioning of the body
- It should normalize body function irrespective of the existing pathological conditions and must produce a non-specific response and therefore increase resistance against a variety of stressors.
- It should help in increasing the physical endurance (Rege et al., 1999)

Few scientists define that any anti stressor plant material substance that cause a state of nonspecific increased resistance are known as adaptogens (Brekhman and Dardymov, 1969). Based on this criteria AC can be considered as adaptogens.

Many animal studies have shown that various herbal adaptogens have protective effects against physically stressful experiences (Rege et al., 1999). Since the adaptogenic action is present in virtue, AC can be used as an antistressor. AC extracts were more effective in preventing stress-induced alterations compared to the action of α-Asarone. Thus it may be concluded that the protective role of AC is not only due to the presences of α-Asarone but also some other active components present in AC.