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Role of *Acorus calamus* and α-asarone on Hippocampal Dependent Memory in Noise Stress Exposed Rats

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Abstract: Stress is a condition or stimulus that threatens an organism's survival. Noise is an environmental stressor. It is well known that long term as well as acute exposure to noise led to oxidative stress. In the present study, it was investigated that the persistence of noise stress (100 dBA/4 h/d for 30 days) could cause memory impairment in rats and whether ethylacetate extract of AC EAAC (50 mg kg^{-1} b.wt.) and α-Asarone (9 mg kg^{-1} b.wt.). treatment can prevent or not. In order to understand the possible mechanism behind it, antioxidant status and acetylcholinesterase (AChE) activity in hippocampus was evaluated after rats were tested in Radial Eight-arm Maze (RAM). Heat shock protein 70 (hsp 70) expression in hippocampus was also evaluated to understand the intensity of stress level. Results showed that after noise stress exposure, time taken to visit all the baited arms, working and reference memory errors were increased in RAM. The superoxide dismutase, lipid peroxidation, AChE activity, hsp 70 were significantly increased with concomitant decrease in catalase, glutathione peroxidase activity and G6PD activity of non-enzymatic levels was observed in the 30 days noise stress exposed group. When rats were co-administrated with EAAC and α-Asarone prevents the noise stress induced alterations significantly. In Conclusion, noise stress induced oxidative stress, increased AChE activity, and over expression of hsp 70 in hippocampus region might have led to the impairment of spatial memory. EAAC and α-Asarone prevents this noise stress induced memory impairment.

Key words: *Acorus calamus*, α-Asarone, noise stress, spatial memory, hsp 70

INTRODUCTION

Stress is a condition or stimulus that threatens an organism's survival. Stress can be beneficial or detrimental to the survival of an organism, leading to behavioral or physiological changes (Herman and Cullinan, 1997). Noise is an environmental stressor. It is well known that long term as well as acute exposure to noise led to oxidative stress (Manikandan and Devi, 2005) and impaired immune response (Srikumar *et al*., 2006). In the initial stage of stress leads to the development of adaptive mechanisms, whereas repeated stress causes gradually exhaust and disadvantageous changes occur mostly due to the excessive activity of the Hypothalamo-Pituitary-Adrenal (HPA) axis (Seale *et al*., 2005) which causes overproduction of corticosteroids leads to impair cognition (Lupien and Lepage, 2001). The hippocampus has long been implicated in the formation of memory and critically dependent on cholinergic transmission. Impairment of spatial memory is likely to be associated with dysfunction of the cholinergic and serotonergic systems (Egashira *et al*., 2002; Mishima *et al*., 2002). The cholinesterase inhibitors have been shown to improve memory disorders in Alzheimer's disease patients (Rogers *et al*., 1998). Oxidative damage is considered a likely cause of age-related brain dysfunction, cognitive decline, age-related impairment in spatial learning and memory due to functional (D'Hooge and De Deyn, 2001) changes of the hippocampal formation (Geinisman *et al*., 2004). The hsp 70 has been studied extensively for its potential to protect the brain from several injuries. The Hsp 70 is up-regulated in response to different stress conditions in the brain, such as hypoxia, ischemia, hyperthermia and exposure to toxic compounds (Tu *et al*., 2004).

Several pharmacological agents and plant have been found to be effective in preventing stress induced the impairment of memory although the mechanism is not

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clearly explained. In the present study *Acorus calamus* Linn. (AC). Rhizome of AC, commonly known as sweet flag (Family: Araceae), is used in the Indian and Chinese systems of the herbal medicine for more than hundreds of years for its beneficial effects on learning performance, anti-aging, epilepsy, and neurosis (Vohora *et al*., 1990). From AC two active principles namely α-Asarone and β-Asarone have been isolated and studied (Wang *et al*., 2011).

The present study was aimed to investigate the memory improving action of ethyl acetate extract of AC (EAAC) and its active principal α-Asarone in the noise stress exposed rats were tested in radial eight-arm maze (RAM). Hippocampal depended spatial memory improvement was accessed by evaluating hippocampal antioxidant status and acetylcholinesterase activity. Besides, we also assessed the hsp 70 gene expression in hippocampus to understand the neuronal activity.

MATERIALS AND METHODS

Subject: Healthy adult male Wistar strain albino rats, weighing between 200-220 g (13-15 weeks of age), were used in this study. The rats were kept in the animal house with controlled ambient temperature (24±2°C), humidity and light (12 h/12 h light/dark cycle, lights on 06:00 a.m.) with food and water *ad libitum* The experiments were performed in accordance with the guidelines laid by the Animal Ethics Committee of Institute of Basic Medical Sciences (IAEC No. 08/012/03), University of Madras, Chennai, India.

Experimental protocol: Animals were divided into 6 groups and each group has 6 animals. The different groups and their experimental schedule are given in Table 1.

Preparation of extract: *Acorus calamus* was purchased from Tampcol Ltd., Chennai, India. It was identified and authenticated by The Director of Centre for Advanced Studies on Botany, University of Madras, India. The dried rhizome (100 g) of *Acorus calamus* Linn. was extracted with ethyl acetate (1:2 w/v) at room temperature. Ethyl acetate extraction was carried out, and repeated three times with the solvent and concentrated in a rotatory evaporator under reduced pressure, giving 2 g yield of extract, which was stored in refrigerator (4°C) until used (Giridharan *et al*., 2002).

Active principal of AC, α-Asarone was purchased form Fluka, Sigma-aldrich Ltd., St. Louis, MO, USA.)

The suspension of EAAC and α-Asarone was prepared by dissolving in 3% Tween 80 and 400 μL of the suspension was administered to the rats intraperitoneally half an hour prior to the noise stress procedure.

Noise stress procedure: White noise (all broad bands) was produced by a white noise generator and amplified by an amplifier (40 W) that was connected to a loudspeaker located at 30 cm above the animal cage. The intensity of the sound was measured by a sound level meter (Cygnet systems-D 2023 Serial No. F02199, India) and maintained at 100 decibel (dBA) intensity (Manikandan *et al*., 2006). Control rats were kept in noise-chamber for the same period of time without switching on the noise generator and then animals were returned back to the animal room.

Radial eight-arm maze test: Spatial learning and memory were tested by using eight-arm radial maze (RAM) apparatus as described previously by Spritzer *et al*. (2011). During behavioral training and testing, the body weight of the rats was maintained at about 75-85% of their free-feeding level. Prior to the experiment, all the rats for the maze experiment were given adaptation to the maze for 1 week. The adaptation and maze test were performed between 10 and 12 a.m. Rats were trained daily to find a piece of cereal in 4 of the 8 arms (1, 3, 4, and 7 arms). Initially, animals were allowed to freely explore the maze for 2 consecutive days with all arms baited with cereal. For training on the spatial task, each trial began with the placement of the animal on the central platform facing arm number one and ended when the rat had visited the four baited arms or after a period of 10 min. The following data were recorded, (1) Number of reference memory errors, i.e., each entry into a non-baited arm, (2) Number of working memory errors, i.e., re-entries into already visited baited arms and (3) Time taken to visit all the baited arms. Animals required 20-25 training sessions to reach the criterion of 0-2 errors. After the criterion (0-2 errors) was

Table 1: Different groups of animals and the experiment schedule

Groups	Drug injected (i.p.)	Volume/dose	No. of days	Noise stress
Control	3% tween 80	400 (μL day^{-1})	30	No
EAAC alone	Ethylacetate extract of AC (EAAC)	50 mg kg^{-1} 400 (μL day^{-1})	30	No
α-asarone alone	α-Asarone	9 mg kg^{-1} 400 (μL day^{-1})	30	No
30 days NS	Tween 80	400 (μL day^{-1})	30	Yes (4 h day^{-1})
EAAC+30 NS	EAAC	50 mg kg^{-1} 400 (μL day^{-1})	30	Yes (4 h day^{-1})
α-asarone+30 NS	α-Asarone	9 mg kg^{-1} 400 (μL day^{-1})	30	Yes (4 h day^{-1})

reached, the animals were subjected to noise stress. During the noise stress procedure, rats were tested for retrieval of the task. Retention of the task was examined on the 1st, 15, and 30th day of the noise stress exposure, after 1 h.

Specimen preparation: At the end of noise stress exposure and behavioral testing rats were sacrificed by cervical dislocation on 31st day. The brain was removed and placed in ice-cold saline. Hippocampus was dissected out in ice-cold (4°C) chamber according to the method of (Glowinski and Iversen, 1966). Using ice-cold Tris-HCl buffer (0.1 M, pH 7.4) 10% homogenate was prepared by motor driven Teflon-glass tissue homogenizer. The homogenate was centrifuged at 2000 rpm at 4°C for 15 min and the supernatant was used for the biochemical analysis.

Biochemical estimation: Protein was estimated by the method of Lowry *et al.* (1951). The activity of superoxide dismutase (SOD) was measured at the degree of inhibition of autooxidation of pyrogallol at an alkaline pH (Marklund and Marklund, 1974). The activity of catalase (CAT) was measured by the method of Sinha (1972). Glutathione Peroxidase (GPx) was assayed by measuring the amount of GSH consumed in the reaction mixture according to the method of Rotruck *et al.* (1973). The Reduced glutathione was assayed by development of relatively stable yellow color, when 0.2 M DTNB solution is added (Moron *et al.*, 1979). Vitamin C was measured by the method of Omaye *et al.* (1979). Vitamin E was estimated by the method of Desai, (1984). Glucose-6-phosphate dehydrogenase was estimated by Beutler *et al.* (1996). Lipid peroxidation (LPO) activity was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances, in the tissue homogenate according to the method of Ohkawa *et al.* (1979). The activity of Acetylcholinesterase (AChE) was assayed by the method of Ellman *et al.* (1961).

Reverse transcription-polymerase chain reaction (RT-PCR) for hsp 70: Total RNA from rat hippocampus was isolated according to the RNA isolation kit instructions (Prefect RNA™ Eckaryotic Mini Kit, Eppendorf AG, 22331, Hamburg, Germany). The level of hsp 70 mRNA in the hippocampus was quantified by RT-PCR with an endogenous internal standard, β-actin as described with slight modifications (Nakahara *et al.*, 2002; Zhou *et al.*, 2008). RT was performed on 1 μg total RNA for 90 min at 42°C in a 5 μL reaction mixture containing 25 mmol L^{-1} Tris-HCl (pH 8.3), 50 mmol L^{-1} KCl, 5 mmol L^{-1} $MgCl_2$, 2 mmol L^{-1} dithiothreitol, 1 mmol L^{-1} each deoxynucleotide, 10 U AMV reverse transcriptase, 10 U ribonuclease inhibitor, and 0.8 μg oligo (dT) primer. The RT was terminated by heating the sample at 95°C for 2 min. Twenty five multiplexed PCR was performed in a 20 μL reaction mixture containing 10 mmol L^{-1} Tris-HCl (pH 8.3), 50 mmol L^{-1} KCl, 1.5 mmol L^{-1} $MgCl_2$, 2% (v/v) dimethyl sulfoxide, 0.2 mmol L^{-1} each deoxynucleotide, 0.1 μmol L^{-1} each of 5' and 3' β-actin-specific primers, 1 μmol L^{-1} each of 5' and 3' hsp 70 specific primers, 25 ng of reversetranscribed total RNA, and 0.5 U Taq DNA polymerase. The PCR amplification was performed, denaturation at 95°C for 2 min, 3' Annealing at 55°C for 45 sec and extension at 72°C for 75 sec for 29 cycles. After 8 (hsp 70) cycles, 0.1 μmol L^{-1} each of β-actin primer pair was added to the reaction mixture, and PCR cycles were further continued. The primer sequences used for amplification of the coding regions of hsp 70 and β-actin were as follows: hsp 70.

Sense: 5'-GAGTCCTACGCCTTCAATATGAAG-3'.

Anti-sense: 5'-CATCAAGAGTCTGTCTCTAGCC AA-3' target sequence 347 bp, Accession No. L16764. β-actin, Sense5'-TCATGCCATCCTGCGTCTGGACCT-3'. Anti-sense5'-CCGGACTCATCGTACTCCTGCTTG-3' target sequence 582 bp; Accession No. V01217. To compare the amount of steady state mRNA, 5 μL of each PCR product was resolved in 2% agarose gel (stained with ethidium bromide). After electrophoresis, the gels were viewed under UV light, and digital images were captured on BioRad Gel Doc 2000 System. The levels of hsp 70 mRNA were calculated as the ratios of optical density of the PCR products to that of the β-actin PCR product.

Statistical analysis: All data were expressed as Mean±SEM. The statistical significance was evaluated using one-way ANOVA and Tukey's test. These were applied using SPSS version 18.0 (SPSS, Cary, NC, USA) and $p<0.05$ was considered to be statistically significant.

RESULTS

Number of reference memory error and working memory error in RAM: Number of reference memory error (Fig. 1) and working memory error (Fig. 2) increased in noise stress exposed group from 1st, 15, and 30th day. On the 0 day all rats in different groups did not showed any error. The errors were increased as the day in progress from first to 30th day when compared to control. Upon noise stress administration of α-Asarone could not prevented the noise stress induced increase in the errors on 1st day. However, from 15th day onwards rats showed similar

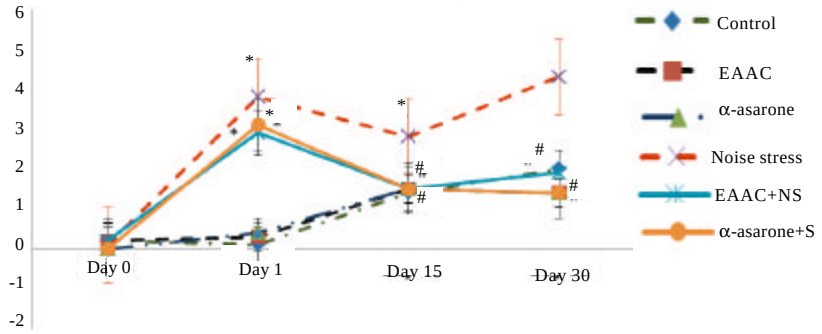

Fig 1: Effect of ethylacetate extracts of *Acorus calamus* and α-Asarone on reference memory error in rat hippocampus. Each line represents Mean±SEM of 6 rats. *Significant ($p<0.05$) vs. control #Significant ($p<0.05$) vs. 30 days noise stress

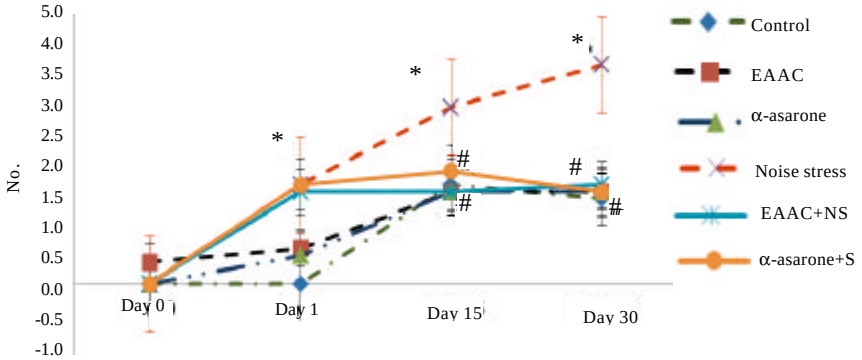

Fig. 2: Effect of ethylacetate extracts of *Acorus calamus* and α-Asarone on working memory error in rat hippocampus. Each line represents Mean±SEM of 6 rats. *Significant ($p<0.05$) vs. control. #Significant ($p<0.05$) vs. 30 days noise stress

number of reference and working memory errors as that of control rats. Treatment with EAAC markedly prevented the stress induced increase in reference and working memory errors in 15 and 30th day.

Time taken to visit all bitted arms in RAM: The data of time taken to visit all baited arms (sec) (Fig. 3) were increased in the noise stress exposed group from 1st, 15, and 30th day compared with control group. Administration of EAAC during noise stress exposure significantly reduced the time taken to complete all the baited arms after 1st day onwards when compare to control group, whereas α-Asarone administration was ineffective on first day. However, α-Asarone treatment prevented the stress-induced alterations in time taken to visit all baited arms from 15th day onwards.

Enzymatic and non-enzymatic antioxidant: The extent of oxidative damage was measured by assaying lipid peroxidation (Table 2). After the exposure of the noise stress LPO level were increased significantly in hippocampus, after 30 days of noise stress exposure, when compared to the control. After co-administration of EAAC (50 mg kg^{-1} b.wt.) and α-Asarone (9 mg kg^{-1} b.wt.) markedly prevented 30 days of noise stress exposed rat's LPO level, which was almost same as that of control.

The SOD activity in the hippocampus was significantly increased in 30 days of noise stress exposed group, when compare to control. Treatment with EAAC or α-Asarone effectively prevented stress induced increased SOD activity. The CAT and GPx activity in the hippocampus after 30 days of noise exposure were significantly decreased from control group (Table 2). Treatment with EAAC or α-Asarone during noise exposure the CAT activity was normalized. Whereas the GPx activity in 30 days stressed animals were not statistically differ from control.

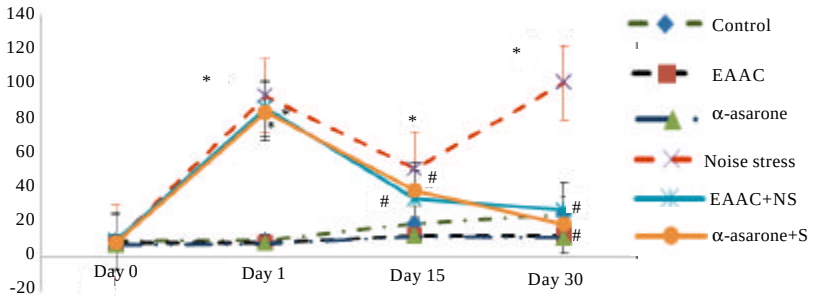

Fig. 3: Effect of ethylacetate extracts of *Acorus calamus* and α-Asarone on time taken to visit all bitted arm in rat hippocampus. Each line represents Mean±SEM of 6 rats. *Significant ($p<0.05$) vs. control, #Significant ($p<0.05$) vs. 30 days noise stress

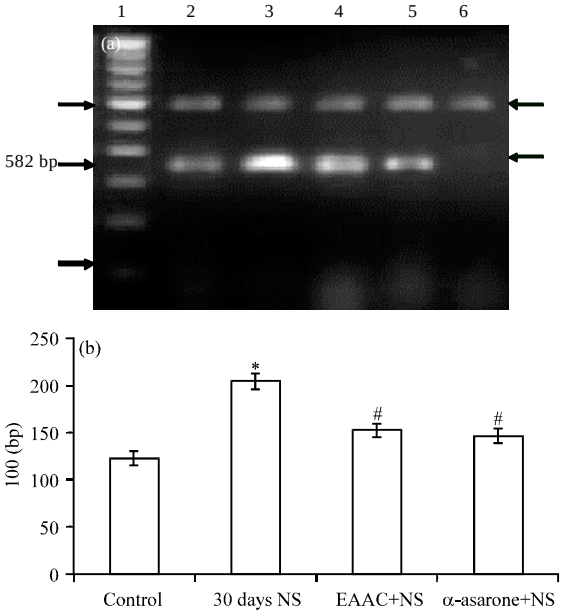

Fig. 4(a-b): Ethidium bromide-stained 2%agarose gel showing PCR products amplified from rat hippocampus RNA. Total RNA extracted from the control (lanes 2), 30 days noise stress (lanes 3), EAAC+ Noise stress (lanes 4), α-Asarone +Noise stress (lanes 5) and negative control (lane 6) of rat hippocampus were incubated in the presence of reverse transcriptase. The reverse transcription products were coamplified with Hsp 70 and α-actin primers. A DNA standard lane 1 is shown at the left of the gel with bands labeled in base pair lengths and (b) Effect of ethylacetate extracts of *Acorus calamus* and α-Asarone on Hsp70 expression in rat hippocampus. Each bar represents Mean±SEM of 6 rats. *Significant ($p<0.05$) vs. control, #Significant ($p<0.05$) vs. 30 days noise stress

The level of GSH, vitamin C, vitamin E and activity of G6PD after 30 days stress exposure were significantly decreased when compared to control (Table 2). Treatment with EAAC or α-Asarone along with noise stress markedly prevented stress-induced alteration and the level of these antioxidants were almost same as that of control.

The activity of AChE in hippocampus was given in the Table 2. Rats exposed to noise stress for 30 days AChE activity was increased significantly from control. Treatment with EAAC or α-Asarone along with stress exposure AChE activity was normalized in hippocampus.

Heat shock protein 70: The levels of hsp 70 mRNAs was calculated as the ratios of optical density of the PCR products to that of the β-actin PCR product. After 30 days of noise stress exposure a significant increase in hsp 70 mRNA (Fig. 4a) levels in the hippocampus (df = 3,

Table 2: Effect of ethyl acetate extracts of *Acorus calamus* (50 mg kg^{-1} b.wt.) and α-Asarone (9 mg kg^{-1} b.wt.) on enzymatic and non-enzymatic antioxidant levels in hippocampus after 30 days of noise stress exposed rat

				30 days noise stress		
	Veh cont.	EAAC	α-asarone	Stress	EAAC+stress	α-asarone+stress
LPO (df = 11, 60; F = 54)	1.28±0.02	1.27±0.04$^{\#}$	1.26±0.03$^{\#}$	2.29±0.02*	1.42±0.07$^{\#}$	1.33±0.03$^{\#}$
SOD (df = 11, 60; F = 24)	1.82±0.03	1.83±0.04$^{\#}$	1.80±0.02$^{\#}$	2.23±0.04*	1.85±0.06$^{\#}$	1.75±0.02$^{\#}$
CAT (df = 11, 60; F = 50)	5.88±0.05	5.80±0.04$^{\#}$	5.88±0.05$^{\#}$	4.54±0.03*	5.62±0.11$^{\#}$	5.74±0.04$^{\#}$
GPx(df = 11, 60; F = 170)	1.73±0.05	1.75±0.03$^{\#}$	1.78±0.04$^{\#}$	1.33±0.02*	1.81±0.08$^{\#}$	1.83±0.01$^{\#}$
GSH (df = 11, 60; F = 25)	0.43±0.01	0.43±0.01$^{\#}$	0.43±0.01	0.27±0.01*	0.39±0.01$^{\#}$	0.42±0.01$^{\#}$
Vitamin C (df = 11, 60; F = 26)	391.60±8.90	389.90±10.4$^{\#}$	392.60±9.30$^{\#}$	276.90±7.00*	370.6±14.40$^{\#}$	375.80±11.6$^{\#}$
Vitamin E (df = 11, 60; F = 57)	17.64±0.32	17.65±0.35$^{\#}$	17.96±0.61$^{\#}$	11.24±0.35*	17.19±0.38$^{\#}$	17.17±0.29$^{\#}$
G6PD (df = 11, 60; F = 16)	0.75±0.04	0.74±0.04$^{\#}$	0.76±0.05$^{\#}$	0.46±0.02*	0.74±0.03$^{\#}$	0.68±0.02$^{\#}$
AChE (df = 11, 60; F = 18)	9.63±0.40	10.05±0.33$^{\#}$	10.04±0.34$^{\#}$	12.47±0.27*	9.75±0.44$^{\#}$	10.05±0.34$^{\#}$

Data are expressed as Mean±SEM for 6 rats in each group, *Significant ($p<0.05$) vs. control, $^{\#}$Significant ($p<0.05$) vs. Respective noise stress group

20; F = 18.7) when compared to the control group. EAAC or α-Asarone treatment effectively prevents stress induced increase in hsp 70 mRNA level in rat hippocampus (Fig. 4b).

DISCUSSION

We have observed in this study after 30 days of noise stress exposure, the spatial memory was impaired, besides increase in free radical generation, AChE activity and increased hsp 70 expressions in rats' hippocampus, administration of EAAC and its active principle α-Asarone to chronic noise stressed rats prevented memory impairment.

In RAM test, the noise stress exposed animals showed significant increase in the number of reference and working memory errors and time taken to visit all the baited arms in the studied days. Increase in errors and time was observed in one day noise stressed rats might be due to the anxiety state of the animals or psychological stress (Conrad *et al*., 2004). Park *et al*. (2010) in his finding the restraint stress memory impairment developed only after 21 days in contrast with our study memory impairment was noted in day one onwards of noise stress exposure. Although, increase in errors in day 15 and 30 was also observed this might be due to the elevated corticosterone level which increase the corticosterone receptor in the hippocampus which leads to memory impairment (Vreugdenhil *et al*., 2001). Treatment with EAAC or α-Asarone caused reduction in the reference and working memory errors and time taken to visit all the baited arms in day one of noise stress exposure was observed, which may be due to anxiolytic action of *Acorus calamus*. During 15 and 30th day test period, retaining of memory might be due to memory improving action of AC (Nishiyama *et al*., 1994; Zhang *et al*., 1994) by preventing corticosterone elevation after noise stress (Manikandan *et al*., 2005).

Hippocampus plays a critical role in learning and memory, and hippocampal damages cause cognitive impairment (Ieraci and Herrera, 2007). In this study memory impairment after noise stress exposure was due to hippocampal damage by increased generation of free radical and AChE activity in hippocampus which was correlated well with earlier reports where different stressors was used (Bowman *et al*., 2001; Silva-Gomez *et al*., 2003). Hippocampal AChE activity was increased in 30 days noise stress exposed rats which was correlated well with Schloesser *et al*. (2013). Increase in the AChE activity leads to decrease in acetylcholine level in the synaptic cleft, results in decreased cholinergic activity, which in turn leads to memory impairment. Yamaguchi and Kawashima (2001) and Hornick *et al*. (2011) were reported that selective central nervous system AChE inhibitor, improves cognitive performance. In this study treatment with EAAC or α-Asarone normalized AChE activity, might be due to cholinesterase enzyme inhibitor activity (Oh *et al*., 2004) which leads to memory enhancement action (Shah and Goyal, 2010).

LPO has been shown to be involved in excitotoxicity of neuron due to the relief of the voltage-dependent Mg^{2+} block of NMDA associated channels (Madrigal *et al*., 2001). in the hippocampus (Nacher and McEwen, 2006). In the current study revealed that increased LPO level and imbalance of the enzymatic and non-enzymatic antioxidant levels after noise stress exposure were also observed in the hippocampus which is very well supported by Bhattacharya *et al*. (2001).

The depletion in GSH content impairs hydrogen peroxide clearance and enhancement of lipid peroxidation might have led to degeneration of hippocampus (Wullner *et al*., 1996). α-Asarone or EAAC treatment prevented the GSH depletion and thereby preventing elevation in LPO level which prevented Hippocampal damage there by retaining hippocampal depended memory by Govindarajan *et al*. (2003) and Manikandan and Devi (2005).

Vitamins C and E is an antioxidant that prevents the biological membranes damage from oxidative stress (Sahin and Gumuslu, 2004). Vitamin C regenerates vitamin

E from its oxidized form, as a result of which tocopherol continues to scavenge the free radicals (Hansen *et al*., 1991). A concomitant increase in vitamin E concentration of stressed rats with EAAC or α-Asarone administration in our study could be due to either decreased oxidative stress or enhanced ascorbic acid level (Muthuraman *et al*., 2011).

SOD converts superoxide anion ($O_2^{\cdot -}$) to H_2O_2. Enzymes CAT and GPx removes H_2O_2. Restoration of SOD and H_2O_2 removing enzymes protect the cell damage from radical elevation noticed after noise stress exposure (Miller *et al*., 2013). The activities of SOD, GPx, CAT and G6PD were restored after 30 days of noise stress in the EAAC or α-Asarone treated rats, demonstrates the hippocampal protective role of EAAC or α-Asarone in noise stress. EAAC or α-Asarone has already been reported to have potential antioxidant activity which supports well to minimizing stress induced changes in hippocampus (Govindarajan *et al*., 2003; Acuna *et al*., 2002).

In this study increased expression of hsp 70 after 30 days of noise stressed rats where observed in hippocampus. This expression of hsp 70 states that upon 30 days of noise stress have been unremitting free radical imbalance, led to oxidative stress and protein damage in the hippocampus (Fig. 4a, b) (Bachelet *et al*., 2002; Singh and Kaur, 2006). EAAC or α-Asarone administration to rats prevented the induction of hsp 70 expression in the hippocampus, indicating that the hippocampus was able to cope up better with 30 days noise stress induced oxidative stress in rats. Several antioxidants (N-acetyl cysteine, quercetin, superoxide dismutase, vitamin C and curcumin) have been demonstrated to modulate the differential expression of hsp 70 in various stress conditions (Kato *et al*., 1998). AC also has anti-stress effect, this was already been reported in our earlier studies which reinforce the antioxidant effect of EAAC and α-Asarone effectively modulated the expression of hsp 70 (Manikandan *et al*., 2005; Manikandan and Devi, 2005).

CONCLUSION

In conclusion, we observed that preventive action of EAAC or α-Asarone against memory impairment in loud noise exposure. It might be due to antioxidant, NMDA receptor blockade, AChE inhibitor and anti-stressor properties of EAAC and α-Asarone. Both EAAC and α-Asarone can be used as a remedy for stress-induced memory loss. Further studies are warranted to elucidate exact mechanism of action of *Acorus calamus* and its active principle α-Asarone on hippocampal neurons for their memory promoting action in other animals and also in human subjects, who are living in the noisy environment.

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