Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3719

EFFECT OF ACORUS CALAMUS LINN ON ASTROCYTES IN THE SUBGRANULAR ZONE OF THE DENTATE GYRUS IN THE HIPPOCAMPAL REGION IS NEUROPROTECTIVE.

Dr.Subamalani S[1], Dr.Sasikumar Arumugam[2*], Dr.Balaji Arumugam[3], Dr. Jamunarani Srirangaramasamy[4]

[1]Associate Professor, Department of Physiology, Tagore Medical College and Hospital, Chennai, India.600127.
[2*]Associate Professor, Department of Physiology, Arunai Medical College and Hospital, Tiruvannamalai, Tamilnadu, India.606603.
[3]Professor & Head, Department of Community Medicine, Arunai Medical College and Hospital, Tiruvannamalai, Tamilnadu, India.606603.
[4]Professor & Head, Department of Pathology, Bhaarath Medical College Hospital, BIHER, Selaiyur, Chennai 600073

***Corresponding Author**: Dr.Sasikumar Arumugam
*Associate Professor, Department of Physiology, Arunai Medical College and Hospital, Tiruvannamalai, Tamilnadu, India.606603. simmamsasi@gmail.com

Abstract
Our objective was to examine and uncover the neuroprotective properties of the plant Acorus calamus linn, also referred to as Vacha. Any form of stress is a major contributing factor to a number of ailments. In the future, a medication molecule with the ability to minimize cellular damage, boost antioxidant levels, and combat stress will be required. Thus, a total of 24 male Wistar albino rats—that is, 6 groups of animals—were used in the current investigation. Dimethyl sulphoxide (1 mL/kg/p.o./day) was given as a control. Stress: administered 1 mL/kg/p.o./day of dimethyl sulphoxide and underwent 6 hours of restraint per day. EE-ACL: Mammals ACL ethanolic extract (100 mg/kg/p.o./day) was received. Alpha-asarone (9 mg/kg/p.o/day) was administered 30 minutes prior to undergoing 21 days of restraint stress. Sigma Plot 13.0 was utilized for data analysis. According to the findings, rats given an ethanolic extract of Acorus calemus Linn and active principle alpha-asarone prior to stress demonstrated noticeably better spontaneous alteration behavior in the Y maze test. Additionally, the drug-treated groups' corticosterone levels were lower than those of the stress group. The amount of neurons in the dentate gyrus region of the hippocampal regions in drug-treated groups remained comparable to those in the control group, as indicated by toludine blue staining. In the drug-treated groups, glial fibrillic acidic protein immunoreactivity revealed newly proliferating astrocytes that looked like stars. The fibers were thin and had a regular course. The findings imply that the central nervous system is produced by phytochemicals, specifically those found in polyphenolic compounds, glycosides, flavonoids, alkaloids, and triterpenoids, have a relaxing effect . The rhizome of Acorus calamus linn, which is rich in these phytochemicals, effectively reversed changes brought on by stress by reducing neuroinflammation and enhancing cognitive function.

Introduction

Humanity faces several dangers and difficulties that may affect the brain and other important organs (De Kloet et al., 2005). One of these is stress, which is an inevitable part of modern living. The human central nervous system has evolved sophisticated adaptation mechanisms to deal with a variety of stressful stimuli and the myriad impacts they can cause (Vachon, 2018). The autonomic nervous system and the hypothalamo-pituitary-adrenal axis are two hormonal systems that are particularly significant in this context since they both function to mitigate the negative consequences of stress. The hypothalamo-pituitary-adrenal axis functions through elevated levels of adrenal cortical hormones, while the autonomic nervous system works by increasing catecholamine output (McEwen, 2007). The brain, particularly the hippocampus region, is an important target organ for adrenal corticosteroids (De Kloet et al, 2005; Joels et al., 2007). The dentate gyrus (DG) region of the hippocampus is known to be extremely sensitive to glucocorticoids (GCs) due to the high density of glucocorticoid receptors (GCr) present here (De Kloet et al., 1998). The DG has extensive connections with other brain regions such as the cortex, medial septum, and amygdala (McGaugh et al., 2004). Dentate neural progenitor cells (NPCs) have been shown to proliferate and give rise to new neurons throughout life in many species, including humans (Abrous et al., 2005).

Acute stress appears to benefit hippocampal cell proliferation and astrocytic fibroblast growth in this case (Elizabeth et al, 2013). Chronic or repeated exposure to stress episodes, on the other hand, has negative consequences such as the onset of cardiovascular disease, diabetes mellitus, depression, obesity, and the progression of neurodegeneration (McEwen, 2004). Chronic stress-like restraint for 21 days in a row for 6 hours causes morphological changes in the cornu ammonis (CA1,3) and DG regions of the hippocampus, as well as a reduction in neurogenesis (Gregus et al., 2005). These morphological changes play an important role in the decline of spatial learning and memory (Zhang et al., 2008).

The most common type of polyphenolic compound found in plants is flavonoids (Devaki et al., 2016). Emerging evidence suggests that polyphenol-containing phytochemicals may protect neurons and neuroglial cells from stress-induced damage in the central nervous system. Polyphenolic compounds not only reduce neuroinflammation, but they also improve cognitive function and promote neurogenesis (Shirwaikar et al., 2006).

For many years, the plant Acorous calamus linn (ACL), also known as sweet flag or sweet cane, has been widely used in Indian and Chinese medicine for its antioxidant, antiallergic, anti-inflammatory, and anti-aging properties (Mukherjee et al., 2007). According to in vitro studies, the ethanolic extract of ACL has antioxidant activity (Acuna et al., 2002). One of the major polyphenolic compounds flavonoid extracted from plant ACL has been shown to have neuroprotective properties. Dharini et al, (2012) investigated the effect of an ethyl acetate extract of ACL and its active component, Alpha -asarone (A-Asarone), on noise stress. Acorus and flavonoids are linked here.

According to the available literature review, there is no scientific evidence to demonstrate neuronal changes in the DG region of the hippocampus after exposure to restraint stress. There is little evidence to support the possibility of new neurons or the regeneration of damaged cells in the DG region after ACL treatment. As a result, the current study was designed to demonstrate the neuroprotective effect of native plant ACL in the brain, specifically the DG region, which has the greatest capacity for neurogenesis.

The current study was designed to assess the neuroprotective effect of ACL in restraint-induced stress for 21 consecutive days, 6 hours per day. The purpose of this study is to compare the impact of ACL's active principle alpha-asarone versus ACL's ethanolic extract on behavioral responses and biochemical changes in restraint-induced stressed rats.

To investigate the effect of ACL's active principle alpha-asarone and ACL ethanolic extract on histomorphometric and immunohistochemical changes in the DG region of the hippocampus.

It was also hoped that the EE-ACL and its active principle A-Asarone would reveal useful information about the structural changes attributed to the neurons of the hippocampus under stressful conditions and whether they would be reversed.

MATERIAL & METHODS

The current study sought to determine the neuroprotective effect of ACL in restraint-induced stress. For 21 days, animals were subjected to restraint-induced stress for 6 hours per day. The animal's stress level was determined biochemically by measuring corticosterone levels in plasma. The Y-maze test, a specialized apparatus for testing spatial memory and willingness to explore new environments, was used to assess behavioral changes (Yanjie et al., 2007). Toluidine blue staining was used to examine morphological changes in the DG region of the hippocampus, as well as astrocyte activity by Glial Fibrillic Acidic Protein (GFAP). A type III intermediate filament expressed by astrocytes is glial fibrillic acidic protein (GFAP). It reacts to trauma as well as to central nervous system disorders. If the stress causes cellular level damage, there is a rearrangement and up-regulation of astrocyte intermediate filaments during neurodegenerative disorders (Elly et al, 2015).

Animals

Twenty four male adult Wistar albino rats (Rattus norvegicus), weighing 180g to 220g were obtained from the Center for Laboratory Animal Research, Department of Research and Development, Saveetha Institute of Medical and Technical Sciences, Chennai (Institutional Animal Ethical Committee Ref. No. SU/CLAR/RD/037/2017 dated 25/08/17). The rats were housed in polypropylene cages with Alpha Dri plus bedding, standard food pellets and drinking water ad libitum, and 12hr light and 12hr dark schedule in 23°C ± 2°C. All animals were quarantined before starting the study.

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, Chennai, India.

Preparation of Ethanolic Extracts:

The shade dried rhizome (100 g) of ACL was ground to coarse powder, placed in a Soxhlet extractor containing 70% of ethanol and resulting extract was concentrated in a rotatory evaporator under reduced pressure. Extracts were stored in refrigerator (4 °C) until further use.

The active principle alpha asarone was purchased from Fluka, Sigma-Aldrich Ltd., St. Louis, MO, USA).

Corticosterone assay kit was purchased from Thermo-FisherScientific company, USA. Immunohistochemical antibodies (GFAP) were purchased from BIO SV, USA.

The suspension of ethanolic extract of ACL and alpha asarone for oral administration was prepared by dissolving it in 0.5% of Dimethyl Sulphoxide (DMSO)to the required volume.

Experimental groups:

The Wistar albino rats were divided into four groups of six animals each. Each group animals was housed in a separate cage labelled with the specific group name (Control, Stress, EE-ACL, A-Asarone)

Control: Received Dimethyl Sulphoxide (1mL/kg/p.o/day) for 21 days.

Stress: were subjected to restraint stress 6hrs daily for 21 days and received Dimethyl Sulphoxide ((1mL/kg/p.o/day) half an hour before subjecting to restraint stress

EE-ACL: Animals received ethanolic extract of ACL (100mg/kg/p.o/day) half an hour before subjecting to restraint stress (6hrs daily for 21 days)

A-Asarone: Received alpha-asarone (9mg/kg/p.o/day) half an hour before subjecting to restraint stress (6hrs daily for 21 days)

Restraint stress induction in rats

The apparatus we used for restraint was made of stainless-steel mesh and was 15cm long, 7 cm broad and 9 cm high. The restraint apparatus allowed the animal to stretch its legs, but would not

allow the animal to move or even turn within the restraint cage. The apparatus was used to cause stress for 6hrs per day (9.00am to 3.00pm) for 21 consecutive days. (Kumar et al, 2009)

Y Maze test

Spatial learning and spontaneous alteration task in the animals were studied using a Y-maze. The apparatus consists of three arms connected into a Y shape (40 x 8 x 15cm arms). The animal is placed in arm A facing away from the center and allowed to move through the apparatus for 5 minutes. Scoring consists of recording entry into each arm. Alteration is defined as entry into all three arms consecutively and the sequence of arm entries was manually recorded. The alteration percentage was then calculated as the ratio of actual to possible alterations. Number of triads = (total number of entries-2). Triad (set of three letters) containing all three letters is scored as alteration.
Percent alteration = [(number of alterations / total number of triads) *100].
Rats with less than 8 times of arm entries during the 5-min trial were excluded from the analysis (Sangeetha et al., 2017). (Wan et al., 2007)

Tissue processing procedure

The animals were sacrificed and brain tissue was harvested and placed in buffered formalin solution for 48hours for dehydration. The brain was sectioned coronally, giving two halves. We used one half for immunohistochemistry and the other for histology.
The dehydrated brains were cleaned and embedded in blocks using paraffin wax. The blocks were sectioned in 3µm thickness by microtome at the level of the hippocampus in the rat brain. The sections were mounted on glass slides and left to dry in a hot plate (40°C). The slides were dewaxed using xylene and this was followed by toluidine blue staining.
For IHC labelling, the tissue was washed three times for 5 min each with phosphate buffer solution (PBS) to remove sodium azide, then incubated for two hours with fluorescent conjugated secondary antibody (goat anti-mouse GFAP antibody, 1:200 in 0.3% Triton-X solution) at room temperature. Sections were then rinsed with double-distilled water and mounted onto slides using an anti-fade mounting medium. The slides were allowed to air dry in the dark at room temperature and then stored at 48ºC until used for analysis. Of the many slides prepared, five sections per animal were chosen randomly from each animal for the microscopic evaluation.

Histological examination (Cell counting and pathological score)

The slides were examined microscopically (Olympus-BX40) and histology studied using image-capture software (ImageJ software version 1.45). The following parameters were assessed in each section stained with toluidine blue. The number of viable cells within a $1mm^2$ area with normal size, shape, and regularly ordered arrangement, large cell bodies, no evidence of apoptosis, no pyknosis or no nuclear condensation were considered as live cells. The dark stained and ruptured neurons were excluded from counting. The number of viable cells in the DG region of the hippocampus was calculated from selected sections in a group (n=6) and was tabulated.
GFAP immunoreactivity in the hippocampus was quantified using a semiquantitative scale. The reactivity of astrocytes within a $1mm^2$ area of subgranular zone (SGZ) of DG regions were quantified. The immunoreactivity of GFAP were carried out by unbiased counting by 3 pathology experts.

Statistical Analysis

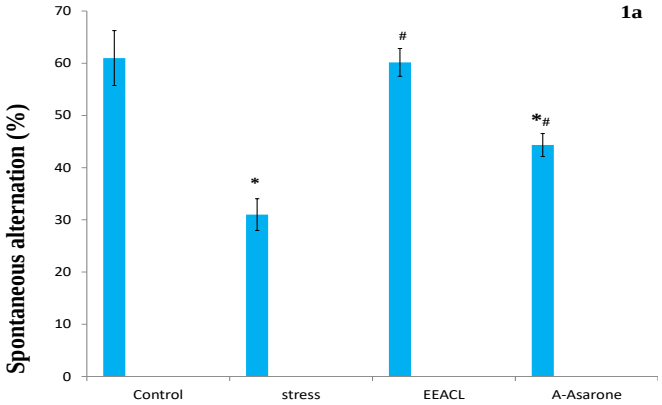
The data were expressed as mean ± SE and analysed using one way analysis of variance followed by post-hoc Tukey's test for multiple comparison of groups. All statistical analysis and graph plotting was carried out using Sigma Plot 13.0 (Sys stat software, USA).

Results

Y maze test

The spontaneous alteration behaviour (SAB) of the control and treatment groups are shown in Figure 1a. The rats exposed to restraint stress showed decreased spontaneous alteration when compared with control group. The groups treated with EE-ACL showed increased SAB when compared with the A- Asarone and stress groups with F=12.026, p<0.001.

Figure 1a: Results of spontaneous alteration in Y-maze test in different experimental groups.

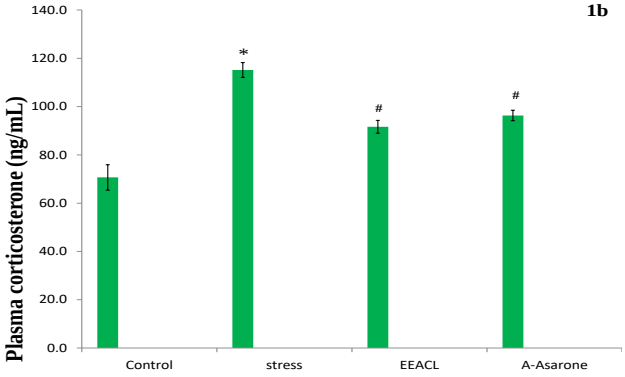


EEACL- Ethanolic extract of ACL, A-Asarone- Alpha Asarone
Values are expressed as mean ± SE(n= 6).
*-Significantly different from the control group.
#-Significantly different from stress group.

Plasma Corticosterone level

In stress group the corticosterone levels are significantly higher than the control group. The drug treated groups showed significantly lower levels of corticosterone than the stress group but higher than the control with F value = 19.809 and p< 0.001 The mean ± SEM is shown in Figure 1b.

Figure 1b Plasma corticosterone levels in different experimental groups.



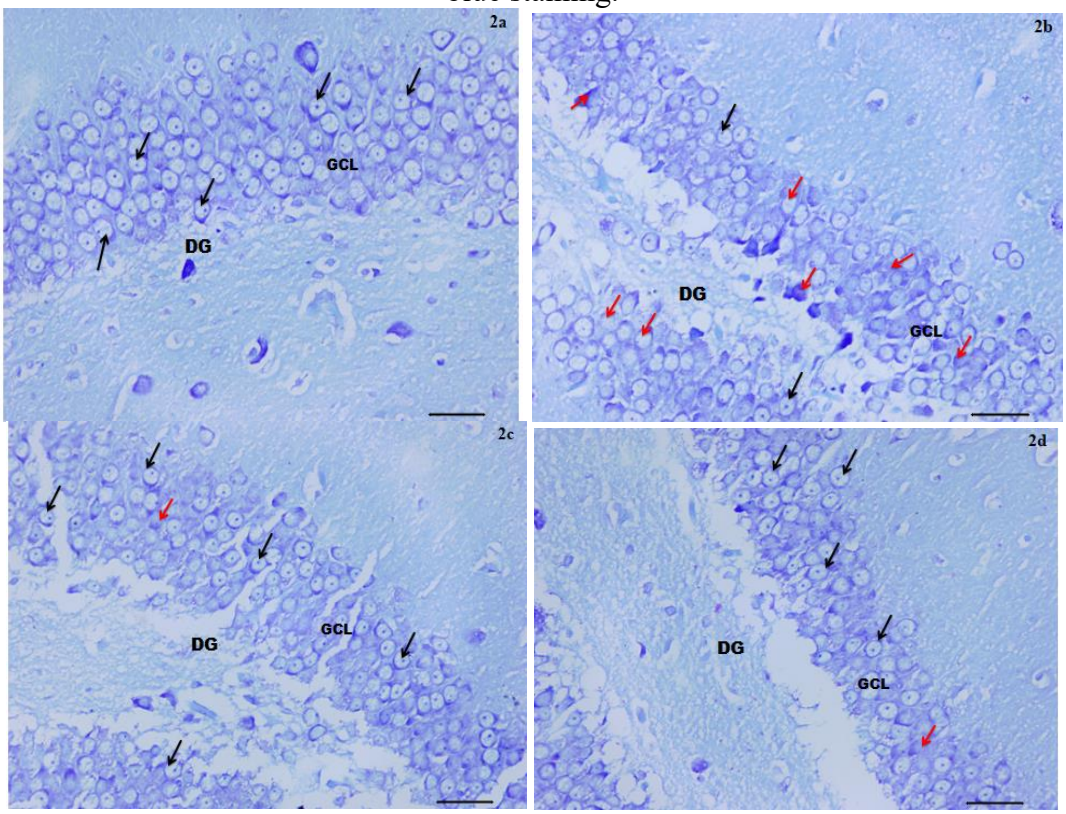
EEACL- Ethanolic extract of ACL, A-Asarone- Alpha Asarone
Values are expressed as mean ± SE(n= 6).
*-Significantly different from the control group.
#-Significantly different from stress group.

Morphology of hippocampal neuron in DG

The morphological changes in different groups are shown in Figure 2. Comparison of the total number of neurons in DG regions of the hippocampus, mean ± SEM, in Figure 3. Total number of

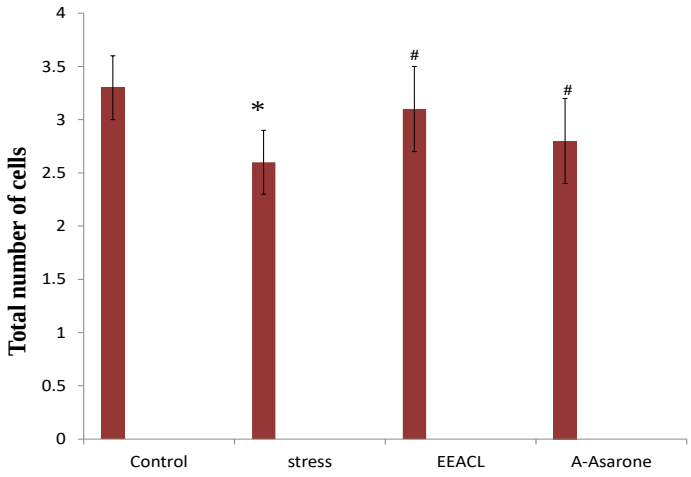
neurons in the stress group was found to be decreased compared to the control group in DG region. EE-ACL maintained the number of neurons in DG region similar to the control group and higher than stress group. A-Asarone group showed a decrease in number of neurons compared with the control group but not less on comparing with the stress group. This difference was found to be statistically significant with a p value<0.001 and F value is 9.603.

Figure 2: Histological changes in DG region of the hippocampus in various groups by toulidine blue staining.



A - control, B- stress, C- EE-ACL, D- A-Asarone. Black arrow in A –D is normal neurons and Red arrows- vacuolisation and condensation of nucleus in the cell body. DG- Dentate gyrus, GCL- Granular cell layer, ML- molecular cell layer. Scale bar- 50µm(40x magnification)

Figure 3 Histomorphometric analysis of total number of neurons in DG region of the hippocampus in various groups.

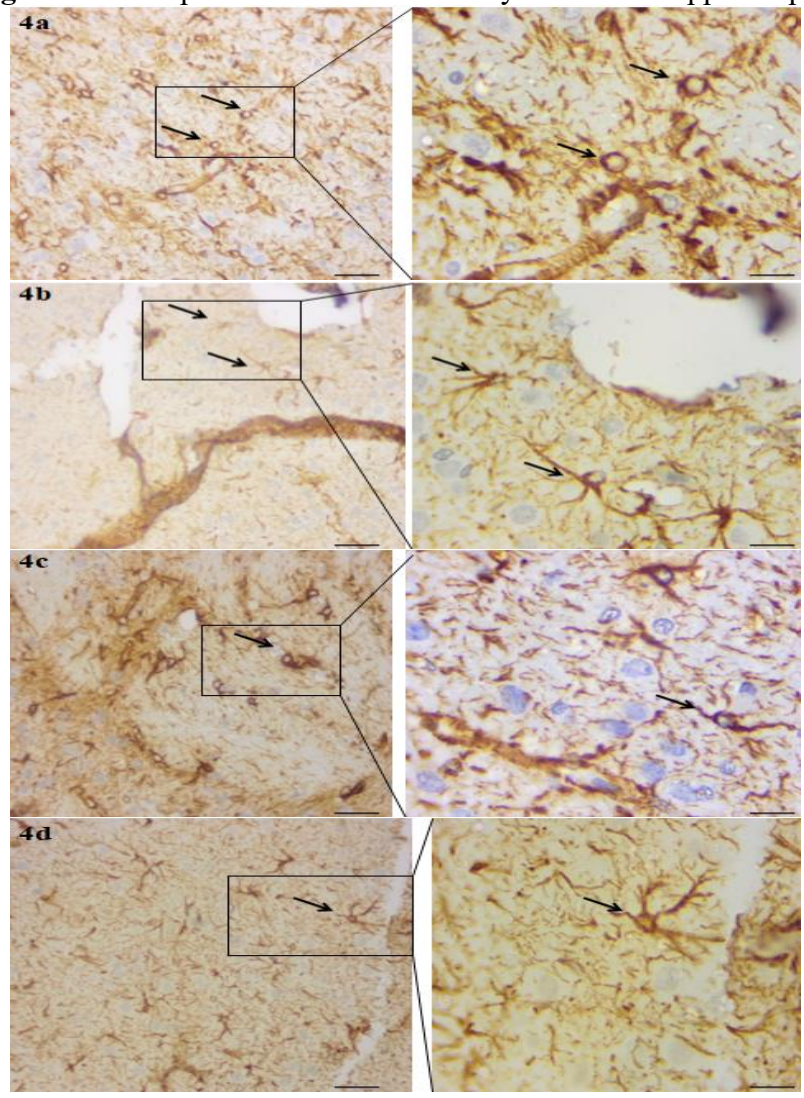


EEACL- Ethanolic extract of ACL, A-Asarone- Alpha Asarone
Values are expressed as mean ± SE(n= 6).
*-Significantly different from the control group.
#-Significantly different from stress group.

GFAP immunoreactivity

Newly proliferated astrocytes which appear as star-shaped cells bearing thin fibers having regular orderly course were two times increased in EE- ACL. when compared with A-Asarone group. In the stress group the glial fibres are twisted with an irregular course and were thickened with an increase in their staining intensity shown in Figure 4b. The effectiveness of drug on GFAP positive in the SGZ of DG neurogenesis was found to be EE-ACL >A-Asarone> stress

Figure 4 GFAP positive immunoreactivity in DG of hippocampus.



4a Control - slight positive brown glial Fibrillary acidic protein (GFAP) reaction small non branched dispersed astrocytes (arrows). (4b) Stress - dense positive brown expression of GFAP in large branched astrocytes (arrow). (4c) EE-ACL- Ethanolic extract of ACL treated rats positive brown expression of GFAP in small branched astrocytes (arrow). (4d) A-Asarone rats shows, positive brown expression of GFAP in branched astrocytes (arrows). Left (40x magnification) right is (100x magnification)

DISCUSSION

We investigated the effect of ACL in the restraint stress model in this study. Restraint stress caused neuronal apoptosis and suppressed granular cell proliferation in the dentate gyrus (DG) region of the hippocampus (Heine et al., 2004). Restraint for 21 days to induce stress resulted in CA3 and dendritic atrophy, as well as significant changes in the morphology of various brain regions (Gwendolyn 2003). The efficacies of EE-ACL and A-asarone were compared. The EE-ACL reduced the impairment in spatial memory and neuronal death caused by restraint stress. Furthermore, the efficacy of EE-ACL (100mg/kg/p.o/day) tested half an hour before stress reduced stress-induced neuronal damage significantly.The hippocampus is more vulnerable to stress and contains more GRr (De Kloet et al, 1998). Pyknotic nuclei were barely detected in the control group, whereas a greater number of pyknotic nuclei were observed in the stress group. Other stress-induced structural changes revealed by toluidine blue staining included condensed and dark neurons with nuclei extruded.

According to research on the cognitive functions of the hippocampus, selective hippocampus lesions result in a deficit in learning or exploration of a new environment (Cameron and McKay, 2001). Memory-related information passes through various regions of the hippocampus, including cornu ammonis (CA) and DG. CA1 then spread into the other major zones, CA2, and CA3. They are known as the hippocampus's three major synaptic loops. Central nervous system diseases, particularly neurodegenerative diseases, can affect the CA1, CA2, CA3, and DG hippocampal neurons, resulting in cognitive impairment (Broglio et al., 2015).

We also demonstrated that restraint stress significantly impairs learning and memory in rats in this study. Despite the fact that many researchers have studied the relationship between restraint stress and cognitive mechanisms, the inhibitory effect of restraint stress on learning and memory impairment remains unclear. To determine whether or not restraint stress causes cognitive impairment, we examined spontaneous alterations in spatial working memory using the Y- maze task. The results clearly show that restraint stress has an inhibitory effect on cognitive function in rats. When compared to the control group, the percentage of SAB was significantly lower in the rats subjected to restraint stress. After drug treatment, the performance of stress group rats showed a significant increase in SAB producing more triads in spatial memory.

Memory deficits caused by retraction of neuronal circuits in different regions of the hippocampus are likely to be the cause of this impairment in restraint rat performance. Lalonde and colleagues (2002). The rats were restrained for 6 hours for 21 days before being tested in the Y maze, and the deficit in SAB was attributed to neurochemical and neurobiological changes in different regions of the brain involved in spatial memory ability, rather than neophobia (Ryan et al., 2005).24. The neuronal basis of SAB is based on connections between limbic and non-limbic pathways, which include the hippocampus, thalamus, prefrontal cortex, basal forebrain, and dorsal striatum. Furthermore, neurotransmitters such as acetylcholine, gamma-aminobutyric acid, and dopamine interact linearly to produce SAB (Elsa et al, 2014). In one study, rats given vitamin E and piracetam before being subjected to restrained stress demonstrated greater willingness to perform new tasks in the Y-maze. Increased serum neurotrophin levels were linked to increased

Increased serum neurotrophin levels may have improved synaptic activity and cognitive function (Sangeetha et al., 2017).

Because the hippocampus contains glucocorticoid receptors (GCr), an increase in corticosterone levels would have caused hippocampal neurons to die. The hippocampus inhibits stress regulation via the Hypothalamo piyutary adrenal axis (HPA) pathway (De Kloet et al., 2005). Serum corticosterone levels in rats were found to be significantly higher immediately after a psychosocial stress episode (Rosanne et al., 2007)27, which is relevant to the current study. Increased CORT has been implicated as a direct mechanism in decreased neurogenesis (Gould et al., 1998). One explanation for neurodegeneration is the lack of or limited expression of glucocorticoid and mineralocorticoid receptors during CORT elevation, which may have an effect on neurogenesis (Garcia et al., 2004).

Immobilization stress increases the synthesis of corticosterone and catecholamine hormones, which also play a role in stress, and these hormones cause tissue injury by inducing oxidative stress in lipid, protein, and DNA damage in different areas of the rats' brain. (Liu et al., 2016).

Toluidine blue staining of the hippocampal region revealed the following observation in this study. The neurons in the control group were very normal in size, shape, and arrangement, with a dark blue coloured nucleus. The neuronal morphology projected larger cell bodies with a well-organized arrangement. There was no evidence of apoptosis or nuclear condensation. The histological findings revealed that the drug EE-ACL, A-Asarone supplementation reduced the damaging effect of stress, thereby ameliorating the stress-induced morphological changes in the hippocampus. The participation of the dendritic spine in the formation of new associative memories resulted in an increase in the density of neuronal cells in the hippocampal regions (Amin et al., 2013).

Previous research suggested that a temporary increase in spine density in the dentate gyrus is always associated with improved spatial learning (Malley, 2000). The dendritic spine has been identified as a potential source for improving contact with excitatory neurons and thus cognition (Vanderklish et al., 2000). Recent research has revealed that the hippocampus can generate new neurons. They can generate dendritic branches, which can play a role in neurogenesis by establishing new cell associations. It has been discovered that the subgranular zone of the DG contains progenitor cells capable of producing new granular cells (Cameron et al., 2001).

The biological effects of the study's test drug could be attributed to phytoconstituents in the plant. According to literature reviews, the presence of polyphenols, primarily flavonoids, in the rhizome of ACL has strong antioxidant effects (Devaki et al., 2016). The flavonoids' general bioavailability, particularly their ability to cross the blood brain barrier, may have played an important role in the expression of their effects in the central nervous system, lowering peripheral corticosterone levels (Liu et al., 2016).

Photochemical studies on the plant ACL have revealed the presence of polyphenolic compounds, glycosides, flavonoids, alkaloid, tannins, mucilage, volatile oil, and saponins (Dharini et al., 2012). A number of chemical constituents have been identified in the plant's rhizomes, leaves, and roots, including alpha asarone, beta asarone, elemicine, cisisoelemicine, and cis and trans isoeugenol (Kumar et al., 2013). Among the active compounds, alpha asarone is widely used to treat mental illnesses such as depression, anxiety, and dementia. It has also been shown to reduce the effects of chronic stress-related degenerative changes. According to new research, natural phytochemicals may protect against stress-induced injury by suppressing neuroinflammation and promoting cognitive performance via changes in synaptic plasticity (Spencer et al., 2007).

Because of their antioxidant or anti-depressant properties, EE-ACL may have shown neuroprotective effects, significantly lowering corticosterone levels and improving behavioral performance. The phytoconstituents present in the EE-ACL, which contains all constituents including the active principle, could explain why the rats outperformed the A- asarone. A number of scientific studies have found that triterpenoids have a relaxing effect on the central nervous system, and the rhizome of ACL is high in them (Pandy et al., 2009). ACL plays an important role in lowering corticosterone levels, which improves neurocognitive performance and makes it a good drug for neurocognitive disorders.

References

1. De Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci 2005;6:463-475.
2. Vachon-Presseau E. Effects of stress on the corticolimbic system: implications for chronic pain. Neuropsychopharmacology, biological psychiatry 2018;20;87:216-23.
3. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87: 873-904.
4. Joels, M, Karst, H, Krugers, H. J, and Lucassen P.J. Chronic stress: implications for neuronal morphology, function and neurogenesis. *Front. Neuroendocrinol* 2007;28, 72–96.

5. Elizabeth D, Sandra E, Muroy, Wayne G, Megan J, Laurel A et al. (2013). Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. Life sciences. 2013;1-23.
6. McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann N Y Acad Sci 2004;1032:1–7.
7. De Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. Endocr Rev 1998;19: 269–301.
8. McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Ann Rev Neurosci 2004; 27:1–28
9. Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. Physiol Rev 2005;85:523–69.
10. Gregus A, Amanda J. Wintink, Alicia C. Davis. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behav Brain Res 2005;156: 105-114.
11. Zhang, C. L, Zou, Y, Gage, F. H ,and Evans, R. M. A role for adult TLX-positive neural stem cells in learning and behaviour. Nature2008;451:1004–1007.
12. Shirwaikar A, Rajendran K, Punitha IS . In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. Biol. Pharm. Bull. 2006;29:1906-10.
13. Devaki R, Nirupama M, Nirupama H.N. Yajurve . Protective effect of rhizome extracts of the herb, vacha (Acorus calamus) against oxidative damage: An in vivo and in vitro study. Food Sci. Hum. Wellnes 2016; 5: 76–84
14. Singh C, Jamwal U, Singh P. *Acorus calamus* (sweet flag): An overview of oil composition, biological activity and usage. J of Med and Arom Plant Sci. 2001;23:687–708.
15. Acuna UM, Atha DE, Ma J, Nee MH, Kennelly EJ. Antioxidant capacities of ten edible North American plants Phytother Res 2002; 16:63-5.
16. Yanjie Wan, Jing Xu, Daqing Ma, Yinming Zeng, Mario Cibelli .Postoperative Impairment of Cognitive Function in Rats. A Possible Role for Cytokine-mediated Inflammation in the Hippocampus. Anesthesiology 2007;106:436–43.
17. Elly MH and Milos P (2015). Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Current Opinion in Cell Biology. 32:121–130.
18. Sangeetha A, Kumaresan M, Vijayaraghavan R, Senthilkumar S and Krishna Mohan S. Chronic Administration Of Vitamin E Elevates Brain Derived Neurotrophic Factor And Nerve Growth Factor In Chronic Stress Induced Wistar Rats. J Pharma Bio Sci 2017; 8: 1007-1016.
19. Wan Y, Xu J, Ma D, Zeng Y, Cibelli M and Maze M: Postoperative Impairment of cognitive function in rats. A Possible role for cytokine-mediated inflammation in the hippocampus. The American Society of Anesthesiologists 2007; 106: 436-43.
20. Heine, Vivi M; Maslam, Suharti; Joëls, Marian; Lucassen, Paul J. Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus–pituitary–adrenal axis activation. Neurobiol. Aging 2004;25(3): 361-375
21. Gwendolyn E, Wood L, Trevor Young, Lawrence Reagan, McEwen. Acute and chronic restraint stress alter the incidence of social conflict in male rats. Horm. Behav2003;43: 205–213.
22. Cameron HA and McKay RD . Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 2001; 435:406–17
23. Broglio C, Martín-Monzon I, Ocana F, Gómez A, Duran E. Hippocampal Pallium and Map-Like Memories through Vertebrate Evolution. Behav and Brain Sci 2015;5:109-120
24. Lalonde R. The neurobiological basis of spontaneous alternation. Neurosci Biobehav Rev 2002;26:91–104.

25. Ryan L. wright, Cheryl D. Conrad. Chronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. Stress 2005;8:151–154.
26. Elsa Y. Pioli, Brianna N. Gaskill, Gary Gilmour, Mark D. An automated maze task for assessing hippocampus-sensitive memory in mice. Behav. Brain Res 2014; 261: 249-257.
27. Rosanne T, Gregory H, and Daniel P. Acute psychosocial stress reduces cell survival in adult hippocampal neurogenesis without altering proliferation. The Jou of Neurosci 2007;27:2734 – 2743.
28. Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E . Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 1998;95:3168 –3171.
29. Garcia A, Steiner B, Kronenberg G, Bick-Sander A, Kempermann G. Age dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. Aging Cell 2004; 3:363–371
30. Liu S, Li T, Liu H, Wang X, Bo S, Xie Y et al. Resveratrol exerts anti-depressant properties in the chronic unpredictable mild stress model through the regulation of oxidative stress and mTOR pathway in the rat hippocampus and prefrontal cortex. Behav Brain Res 2016;302:191-9.
31. Pekny M, Pekna M. Astrocyte intermediate filaments in CNS pathologies and regeneration. J Pathol. 2004; 204: 428–437..
32. Malley AOC, Murphy KJ, Regan CM .Transient spine density increases in the mid-molecular layer of hippocampal dentate gyrus accompany consolidation of a spatial learning task in the Rodent. J. Neurosci 2000; 99:229–32.
33. Vanderklish PW, Krushel LA, Holst BH, Gally JA, Corssin KL Edelman. Marking synaptic activity in dendritic spines with a calpain substrate exhibiting fluorescence resonance energy transfer. Proc Natl Acad Sci USA 2000;97:2253–5.
34. Pourzaki M , Homayoun M, Sadeghi S , Seghatoleslam M , Hosseini M, Ebrahimzadeh B. Preventive effect of Coriandrum sativum on neuronal damages in pentylentetrazole- induced seizure in rats. Avicenna J Phytomed 2017;7: 116-128.
35. Dharini S, Gajalakshmi G, Sheela Devi R. Effect of acorus calamus linn and it's active principle on Noise stress-induced changes on immune system. Int Jour of Biomed and Adva Res 2012;3:494-501.
36. Spencer JPE, Rice-Evans C & Williams RJ. Modulation of pro-survival Akt/PKB and ERK1/2 signaling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. J Biol Chem 2003;278:34783–34793
37. Pandy V, Jose N, Subhash H, (2009). CNS activity of methanol and acetone extract of Acorous calamus linn leaves in mice. Journal of pharmacology and toxicology 4(2):79-86.
38. Kumar RS, Narayanan SN and Nayak S (2009). Ascorbic Acid protects against restraint stress-induced memory deficits in Wistar Rats. Clinics. 64(12):1211–1217