Original Article

Prevention of Neuronal Damage in Brains of Chronic Stress-induced Male Wistar Rats Administering *Centella asiatica* (L) Urban

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Abstract

Introduction: Physiological and psychological response of an organism to repetitive stimulus leads to chronic stress which results in depression. This affects the neuro-endocrine axis causing hypersecretion of glucocorticoids which damages the hippocampal neurons in brain through oxidative stress. The body responds by producing Catalase (CAT) an antioxidant found on peroxisomes, which splits the hydrogen peroxide produced by oxidative stress into water and oxygen which are nontoxic, thus offering a protective effect. The synaptic function of the hippocampal neurons is dependent on acetylcholinesterase (AChE) and oxidative stress affects the levels of AChE. The available anti-depressants have the late onset of action and increased toxicity. *Centella asiatica* (CA), an herb with neuroprotective properties, is known as neuro-tonic and has less toxicity and has been used in ancient traditional medicines. This study aims to examine the neuroprotective effects of crude extract of CA on hippocampal neurons using Nissls stain and levels of AChE and expression of mRNA CAT in the brain tissues of chronic unpredictable mild stress (CUMS)-induced male Wistar rats. **Materials and Methods:** Thirty-six Male Wistar rats aged 8–10 weeks were held in six groups. One group assigned as control, whereas the other groups were administered CUMS by various stressors, namely restrain, forced swimming in cold water, overnight food and water deprivation, wet bedding, cage tilt at 45°, tail pinching, overcrowding the cages, and change of cage mates randomly for a period of 64 days. One of the stress-induced groups was retained as model group and others were administered crude extracts of CA at the doses of 200, 400, 800, and fluoxetine (Flx) 10 mg/kg body weight. At the end of 64 days, the rats were euthanized and the brain tissue was collected for Nissls staining of the hippocampus, measure levels of AChE using ELISA and expression of mRNA CAT levels using RT-PCR. **Results:** The rats of the model group exhibited reduced number of viable neurons in the hippocampus as observed in Nissls stain, reduced levels of AChE, and reduced expression of mRNA CAT in the brain tissue while the rat groups receiving CA showed increase in the number of viable neurons, increase in level of AChE, and increase in the expression of mRNA CAT in the brain tissues. The results were comparable to that of Flx. **Conclusion:** CA effectively attenuates CUMS-induced neuronal loss in the hippocampus of the rat's brain, normalizes AChE levels, and also the expression of mRNA CAT antioxidant levels. CA could be used in the long-term prevention of chronic stress-induced depression.

Keywords: *Catalase, Centella asiatica, chronic stress, fluoxetine, hippocampus, neuronal damage*

Introduction

Depression is a pervasive disorder in humans that is linked to experiencing stressful life events. The American Psychiatric Association defines it as a condition marked by feelings of sadness, emptiness, or irritability, as well as physical and cognitive changes that greatly hinder a person's ability to function normally.[1] Depression is a prominent contributor to disability, impacting almost 280 million individuals globally.[2] This condition leads to significant emotional distress, substantial financial detriment, and notable societal strain. Stress is a phenomenon in which stressors, including physical and psychological demands on an individual, hinder the individual or organism's ability to cope with subsequent challenges effectively.[3] The numerous stressors can be categorized into three types: (a) acute and chronic, (b) significant and small, and (c) desirable and undesired. These categories help to highlight the varied components of stress in life. Chronic stress is characterized by the ongoing presence of stressors or the repeated occurrence of a stressful event.[4] Chronic stress is a significant factor in causing depression.

How to cite this article: Jagadeesan S, Gopalakrishna PK, Sura S, Karikalan B, Dandala KS, Ravindranadh G, *et al*. Prevention of neuronal damage in brains of chronic stress-induced male wistar rats administering *Centella asiatica* (L) urban. J Anat Soc India 2024;73:204-13.

Article Info

Received: 08 June 2024
Revised: 08 July 2024
Accepted: 11 August 2024
Available online: 25 September 2024

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Access this article online

Website: https://journals.lww.com/joai
DOI: 10.4103/jasi.jasi_80_24
Quick Response Code:

Researchers can subject the rats to chronic unpredictable mild stress (CUMS) to produce the animal models of depression.[5] Research conducted on both people and rodents has shown that the hippocampus has a role in the development and progression of depression. Given the hippocampus's role in learning and memory, impairments in these cognitive functions are a significant characteristic of depression.[6] Depression leads to hippocampal atrophy and a decrease in volume, as evidenced by experiments conducted on rat models of depression. These experiments have shown that depression causes neuron loss, neurogenesis inhibition, and retraction of dendritic processes in the hippocampus. Conversely, successful treatment of depression in rat models has been found to promote the growth of new neurons in the hippocampus, a process known as neurogenesis.[7,8] The degree of reduction in hippocampus volume is correlated with the early onset and duration of depression. Similar alterations have been reported in rats exposed to chronic stress, with more pronounced changes occurring in the dentate gyrus.[9]

In response to oxidative stress, the body produces enzymatic and nonenzymatic antioxidants to mitigate its effects. Superoxide dismutase, an antioxidant, transforms superoxide anions into hydrogen peroxide, thereby decreasing the interaction between the superoxide anion and nitric oxide, resulting in the formation of reactive peroxynitrite. Catalase (CAT) is an antioxidant enzyme present in the peroxisomes, and it acts by catalyzing the process of decomposing hydrogen peroxide into nontoxic molecules such as water and oxygen.[10] With its high oxygen demand and lipid-rich composition, the brain is exposed to significant reactive oxygen species (ROS) levels. The rise in ROS and the decrease in antioxidant levels seen in chronic stress render the brain extremely vulnerable to the effects of oxidative stress.[11]

Acetylcholinesterase (AChE) is an enzyme belonging to the serine hydrolase family and is highly involved in transmitting signals throughout the nervous system. It breaks down acetylcholine (ACh), a necessary cholinergic neurotransmitter involved in memory.[12] Prolonged stress causes changes in the levels of the AChE enzyme. This was shown in research conducted on Wistar rats housed in nonstimulating situations.[13] *Centella asiatica* (CA) belongs to the *Apiaceae* family in the plant kingdom and has been traditionally utilized in the Ayurvedic system of medicine for its therapeutic properties in treating a wide range of diseases and enhancing memory.[14] This plant has been widely utilized in traditional herbal treatment in Malaysia and Ayurveda in India and other regions of Asia. CA, sometimes called pegaga in Malaysia and gotu kola or pennywort in the Americas, is commonly consumed as a food and a beverage.[15] Ayurveda has used this herb to treat several chronic ailments, such as anxiety.[5] The extracts derived from pulverized leaves and roots of the CA plant are utilized to manage cognitive impairment, dyspepsia, rheumatism, and leprosy. In China, the CA plant treats scabies, measles, urinary complications, tuberculosis, jaundice, emesis, and dysentery.[15]

Although modern drugs have made a substantial impact on improving the quality of life for those with depression, they still have certain limits. Frequently, the reaction to the treatment is not entirely foreseeable, and occasionally, the reaction is only partial. Most medications require many weeks of use for their therapeutic benefits to become apparent.[16] The issues with current pharmaceutical agents are exacerbated by several drug interactions and the lack of clarity regarding their safety during pregnancy.[4] Therefore, there exists a diverse range of drugs, all of which lack both effectiveness and safety.

Materials and Methods

Plant extract preparation and chemicals

The CA extract, obtained using ethanol as a solvent, is procured from the Universiti Teknologi Mara in Malaysia. The reference number for this is AuRins-MIA-1-0. Using the ethanolic method, the CA extract was derived from the whole plant through dehydration and subsequent extraction into a brown powder.[17] Fluoxetine (Flx) was acquired from Cadila Pharmaceuticals Ltd, located in Bhat, Ahmedabad, India. The AChE ELISA kit was obtained from Elabscience USA.

Apparatus

The experimental apparatuses utilized in the study, consisting of an open field box, an elevated plus maze, a T-maze, and a transparent cylinder, were constructed at the Workshop in the Department of Human Anatomy, Faculty of Medicine and Health Sciences (FMHS), Universiti Putra Malaysia (UPM).

Animals

Thirty-six male albino Wistar rats, 8–10 weeks old and weighing 180 and 220 g, were acquired from Bistari Sdn Bhd, Selangor, Malaysia. They were housed at Animal House in the FMHS at UPM in a controlled laboratory environment, including a 12-h cycle of light and darkness. The lights were illuminated from 0700 to 19.00 h while maintaining a consistent temperature of 25°C ± 2°C. The rats were able to access food and water freely without any restrictions. The animals were acclimatized for 1 week. The Institutional Animal Care and Use Committee (IACUC) at UPM approved the number of rats utilized and the study procedures. The project was assigned the identification code UPM/IACUC/AUP-R078/2018.

Chronic unpredictable mild stress procedure

CUMS was administered to the experimental animals for 8 weeks through the application of psychosocial and environmental stressors, as reported in a previous study.[18] The rats that were not exposed to CUMS

were kept in their home cages except for routine handling and cleaning. The rats exposed to CUMS were exposed to nine different types of mild stressors (S), administering a minimum of two stressors per day for a continuous period of 8 weeks. The study involved several experimental conditions: S1: food deprivation for 24 h, S2: water deprivation for 24, S3: swimming in cold water (temperature of 5°C) for 5 min, S4: change of cage mates for 12 h, S5: 1 min of tail pinch maintained (pinch applied at 1 cm from the tip of the tail), S6: Keep cage tilted at an angle of 45° for 12 h of, S7: Overcrowding for 12 h, S8: Keep the bedding wet for 12 h, and S9: apply physical restraint for 4 h. The stressors were applied randomly during the 1st week, and the same schedule was maintained during the entire experimental period, with no stressor administered in succession to prevent adaptation [Table 1].

Design of the experiment

After acclimatization, the rats were assigned randomly to six groups, each with six rats (n = 6). Group 1 rats were not subjected to stress and were given normal saline and assigned as control. Group 2 rats were administered only CUMS and were given normal saline assigned as a model. Group 3 rats were subjected to CUMS and Flx (10 mg/kg orally). Groups 4, 5, and 6 were administered CUMS and different doses of CA (200 mg/kg, 400 mg/kg, and 800 mg/kg orally), respectively. The dosages were determined using the data from prior research.[19,20] Flx and CA were administered daily, 30 min before the start of CUMS administration, for 8 weeks, commencing from day 0 of the trial. Behavioral assessments were performed at midday, beginning in the 9th week of the investigation. The rats were sacrificed at the end of the experiments, and tissue samples were collected [Figure 1].

Sample collection and preparation

Following euthanization, the rat brains were promptly extracted and then preserved in 10% formalin for 7 days. Subsequently, the specimens underwent mechanized tissue processing and were incorporated into paraffin blocks. The brain tissues were stored at −80°C to facilitate biochemical analysis.

Nissl's staining

The tissue paraffin blocks were used to obtain the coronal sections with a microtome of 5–6 µm thickness. The portions were immersed in a water bath at 38°C to facilitate straightening and transferred onto individual glass slides. The brain tissue sections on the glass slides were subjected to deparaffinization at a temperature of 70°C for 1 h in an oven. Following paraffin removal, the slides were immersed in xylene for 5 min and subsequently underwent rehydration in a series of graded alcohol solutions, starting with 95% alcohol for 3 min, followed by 70% alcohol. The slides were immersed in distilled water for an additional 3 min. The tissues were stored with 0.1% cresyl violet acetate stain for 10 min in an oven at a temperature of 70°C. The slides were extracted and cleansed in distilled water for 3 min, followed by dehydration in ascending alcohol concentrations (70%, 95%, and 100%) for 3 min at each level. Subsequently, the slides were immersed in xylene for 5 min, affixed using mounting material known as dibutyl phthalate xylene, and shielded with a cover slip. The viability of cells in the tissue slides was assessed using the method outlined by Adele. Brain samples were obtained from three rats in each group. Five slices of the hippocampus were produced from each rat brain sample. Scoring was conducted on five sites within each of the hippocampal regions of cornu Ammonis 1 (CA1),

Table 1: Chronic unpredictable mild stress procedure[18]

Stages of stress	Type of stress	Description of the type of stress
S1	Food deprivation	The rats were subjected to 24 h food deprivation. Food was provided following the end of the deprivation period
S2	Water deprivation	The rats were subjected to 24 h water deprivation. Water was provided immediately following the end of the deprivation period
S3	Swimming in cold water	The rats were made to swim for 5 min in cylinders filled with cold water (4±1°C). At the end of this immediately after their swimming, the rats were removed, dried with a towel, and returned to their home cages
S4	Change of cage mates	The cage mates of the rats were changed for 12 h, after which the rats were returned immediately to their respective home cages
S5	Tail pinch	The tails of the rats were clamped at 1 cm from the tips of the tails for 1 min
S6	Cage tilt	The rat cages were kept tilted at 45° for 12 h
S7	Overcrowding of cage	6 rats were packed in a cage for a period of 12 h
S8	Wet bedding	200 mL of water was added to the beddings in the cages where the rats, where they retained for 12 h
S9	Physical restrain	The rats were individually restrained in plastic restrainers (5.5 cm diameter and 12 cm long) with proper ventilation for 4 h

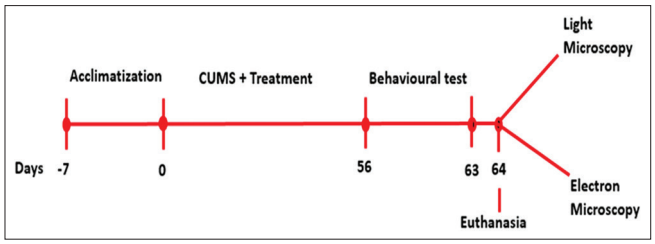

Figure 1: Experimental design

cornu Ammonis 2 (CA2), and cornu Ammonis 3 (CA3). The Olympus microscope, specifically the Olympus BX51TRF-CCD model, examined the tissue slices at a magnification ×400.[21]

The Enzyme-linked immunosorbent test

The AChE activity of the rat brain homogenates was assessed using the quantitative sandwich ELISA technique, following the instructions provided by the manufacturer (Elabscience USA). Subsequently, a Versamax microplate reader was used to determine the optical density of the samples, applying a wavelength of 450 ± 2 nm. The values obtained, which were directly proportionate to AChE quantities, were determined using the standard calibration curves.

Studies on the expression of genes

The RNA was extracted from the rats' brains using the Qiagen RNeasy mini kit, following the manufacturer's instructions manual. The RNA samples were assessed for purity using a Nanodrop spectrophotometer, and their integrity, as indicated by the 28S/18S ribosomal RNA ratio, was verified using agarose gel electrophoresis. The entire RNA sample (100 µg) was subjected to reverse transcription using a qPCRBIO cDNA synthesis kit according to the manufacturer's instructions manual.

The primers for the genes of interest (GOI) for CAT were generated by IDNA, along with one reference gene (RG), while glyceraldehydes-3-phosphate dehydrogenase was utilized to standardize the threshold cycle (CT) values for GOI. The real-time PCR was conducted using the Eppendorf Mastercycler ep realplex 4S instrument and the 2x qPCRBIO SyGreen Blue Mix Seoarate-Rox master mix. The amplification technique employed entailed heat activation at a temperature of 95°C for 2 min, succeeded by 40 cycles consisting of a denaturation phase for 15 s at 95°C, followed by an annealing phase for 30 s at 59°C, and finally an extension phase for 30 s at 72°C. The fluorescence signals were measured at a temperature of 59°C.

The Livak technique was employed to compute the fold change of gene expression based on the CT values acquired.[22] The mean CT values of each gene of interest (CT AVG GOI) were standardized using the mean CT values for the reference genes (ΔCT = CT AVG GOI-CT AVG RG). The ΔΔCT (ΔCT TREATMENT-ΔCT CONTROL) was computed, and each gene's relative change in expression level across the different rat groups was represented as 2-(ΔΔCT). After amplification, the specificity of the primers was determined using a melting curve study.

Quantitative analysis of data using statistical methods

The analysis of the data acquired was done using the one-way ANOVA with the GraphPad Prism version 6 software (ISI, USA). Tukey's *post hoc* comparison was employed, with the value of $P < 0.05$, to determine the statistical significance. The results were reported as the mean value plus or minus the standard deviation (mean ± SD).

Results

Centella asiatica protected the hippocampus Cornu Ammonis 1 pyramidal neurons from neurodegeneration in chronic unpredictable mild stress-induced rats' brains

The neuroprotective effects of CA on the CA1 area of the hippocampus in rats were evaluated by CV staining. Noticeable disparities in the quantity of functional neurons were noted in the hippocampus among the rats. The statistical analysis yielded a test statistic of F (5,444) =62.59, with a $P = 0.0001$. The hippocampus of rats that were administered with CUMS exhibited a significantly lower number of viable neurons (11.25 ± 1.875, $P = 0.0001$) compared to the control group of rats (15.47 ± 2.591). The quantity of functional neurons was significantly greater in rats induced with CUMS and treated with Flx (14.04 ± 1.202, $P = 0.0001$), CA 400 (14.95 ± 2.277, $P = 0.0001$), and CA 800 (14.93 ± 2.559, $P = 0.0001$), compared to the CUMS-induced model group (11.25 ± 1.875). There were no notable differences in the number of functional neurons among the control, Flx, CA 400, and CA 800 groups of rats [Figure 2].

Centella asiatica protected the hippocampus Cornu Ammonis 2 pyramidal neurons from neurodegeneration in chronic unpredictable mild stress-induced rats' brains

The neuroprotective effects of CA on the neurons in the CA2 area of the rats' hippocampus were assessed using CV stain. There were notable variations in the number of functional neurons in the CA2 area of the hippocampus across different groups of rats (F [5,444] = 52.77, $P = 0.0001$). The hippocampus of rats induced with CUMS exhibited a significantly lower number of viable neurons (11.40 ± 1.959, $P = 0.0001$) compared to the control group of rats (15.44 ± 2.688). The rats that were treated with CUMS and also given Flx (15.17 ± 2.286, $P = 0.0001$), CA 400 (14.87 ± 2.418, $P = 0.0001$), or CA 800 (15.00 ± 2.515, $P = 0.0001$) showed a significantly higher number of viable neurons compared to the rats that were only induced with CUMS (15.44 ± 2.688). There were no notable disparities in the number of functional neurons observed across the control, Flx, CA 400, and CA 800 groups of rats [Figure 3].

Centella asiatica protected the hippocampus and Cornu Ammonis 3 pyramidal neurons from neurodegeneration in chronic unpredictable mild stress induced rats' brains

The neuroprotective effects of CA on the neurons of the CA3 area in the rat's hippocampus were assessed using CV stain. A notable disparity was noted in the number of functional neurons in the CA3 area of the hippocampus across the different rats (F [5,444] = 93.98, P = 0.0001). The CUMS-induced model group of rats had a markedly reduced number of viable neurons (14.64 ± 2.769, P = 0.0001) compared to the control group (20.27 ± 3.202). A considerably higher number of viable neurons were found in the rats induced with CUMS and administered with Flx (19.81 ± 2.793, P = 0.0001), CA 400 (19.80 ± 1.366, P = 0.0001), and CA 800 (19.84 ± 1.452, P = 0.0001), compared to the CUMS-only model group (14.64 ± 2.769). There were no significant changes in the number of functional neurons among the control, Flx, CA 400, and CA 800 groups of rats [Figure 4].

The impact of *Centella asiatica* on the concentrations of acetylcholinesterase in rat brain tissues

The levels of AChE in the rats' brains were measured to determine the protective effects of CA on the cholinergic dysfunction caused by CUMS. The results of the one-way ANOVA showed statistically significant variations in the levels of AChE (F [5, 12] = 9.498, P = 0.0007) across the different groups of rats. Tukey's *post hoc* test comparison showed that AChE levels in the CUMS-induced rats (2.426 ± 0.46, P = 0.0013) were significantly higher than in the control group (1.27 ± 0.14). Significant reductions in AChE levels were statistically detected in rats induced with CUMS when supplied with

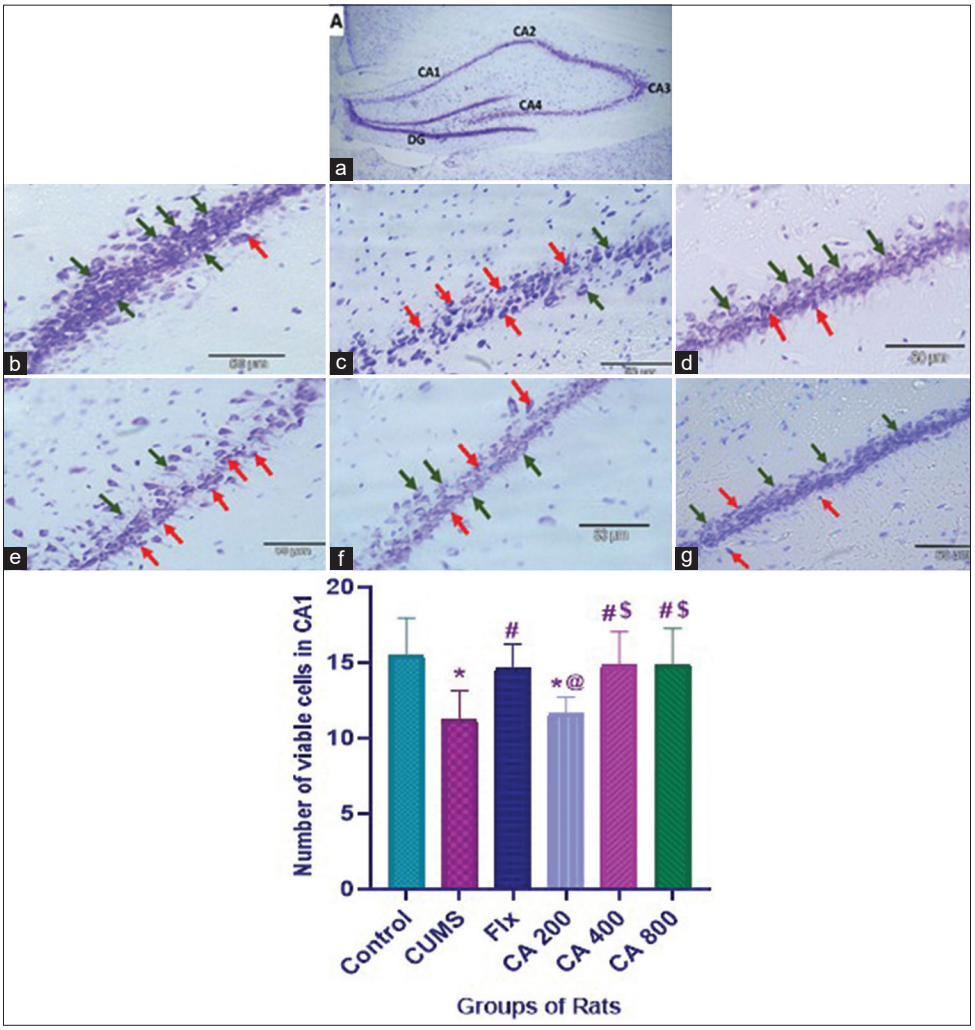

Figure 2: Neuro-protective effects of *Centella asiatica* (CA) on the hippocampal neurons of the CA1 region, as observed by cresyl violet stain of the chronic unpredictable mild stress (CUMS)-induced rats. Images of the cresyl violet staining of the hippocampus showing changes in number of viable cells (shown with green arrow) and degenerated cells (shown with red arrow) after CUMS induction and those co-administered with fluoxetine (Flx) and CA. (a) Hippocampal regions marked, (b) Control group, (c) CUMS-induced group, showing less viable cells (red arrow) (d) Flx group, shows more viable cells (green arrow) (e) CA 200 group shows less number of viable cells and (f and g) CA 400 and 800 groups of rats show more number of viable cells (green arrow). Data presented as mean ± SD, n = 6. *P < 0.05 versus control, #P < 0.05 versus CUMS, @P < 0.05 versus Flx, $$P$ < 0.05 versus 200

Flx (1.353 ± 0.233, P = 0.0024), CA 400 (1.503 ± 0.01, P = 0.0078), and CA 800 (1.38 ± 0.233, P = 0.0030), in comparison to rats exposed to CUMS alone (2.426 ± 0.46). No notable variations in the AChE levels were reported across the rats treated with Flx and CA (400 and 800) [Figure 5].

The impact of *Centella asiatica* on the catalase mRNA levels in brain tissues of chronic unpredictable mild stress-induced rats

The expression of CAT mRNA was done to determine the neuroprotective effects of CA in rats induced with CUMS. The findings revealed notable disparities among the groups of rats. The statistical analysis yielded a result of F (5, 12) = 25.50, with a P = 0.0001. The amount of CAT mRNA in the model group was significantly reduced (fold change of 0.42 ± 0.10, P = 0.0001) compared to the control group (fold change of 1 ± 0). However, in groups of rats induced with CUMS and administered Flx, CA 200, CA 400, or CA 800, there was a significant increase in CAT mRNA levels compared to the model group. The fold change for CAT mRNA levels was as follows: Flx (1.01 ± 0.05, P = 0.0001), CA 200 (0.61 ± 0.10, P = 0.0001), CA 400 (1.11 ± 0.16, P = 0.0001), and CA 800 (1.10 ± 0.10, P = 0.0001). The CAT mRNA levels for rats given CUMS alone were 0.42 ± 0.10. There were no noticeable variations in the levels of CAT mRNA between the Flx and CA (400 and 800) groups of rats, indicating no substantial increase or decrease in fold change [Figure 6].

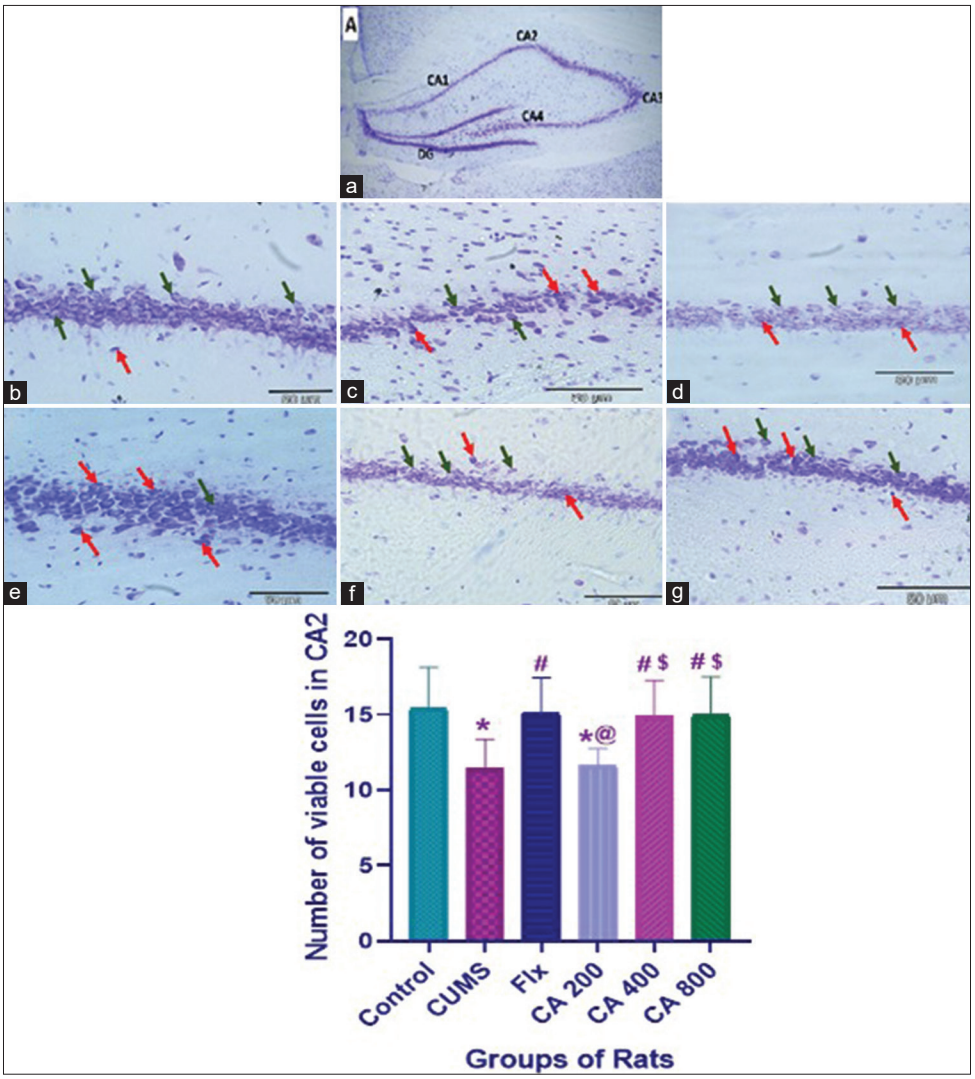

Figure 3: Neuro-protective effects of *Centella asiatica* (CA) on the CA2 region of the hippocampal neurons, observed by cresyl violet stain, on the chronic unpredictable mild stress (CUMS)-induced rats. Images of the cresyl violet staining of the hippocampus showing changes in number of viable cells (shown with green arrow) and degenerated cells (shown with red arrow) after CUMS induction and those co-administered with fluoxetine (Flx) and CA. (a) Hippocampal regions marked, (b) Control group, (c) CUMS-induced group, showing less viable cells (red arrow) (d) Flx group, showed more viable cells (green arrow) (e) CA 200 group shows less number of viable cells and (f and g) CA 400 and 800 groups of rats show a greater number of viable cells (green arrow) Data presented as mean ± SD, *n* = 6. **P* < 0.05 versus control, #*P* < 0.05 versus CUMS, @*P* < 0.05 versus Flx, $*P* < 0.05 versus 200

Discussion

The brain's hippocampus is a component of the limbic system associated with cognitive function and memory. The hippocampus also functions as a regulator of depressive mood, processing information, and can cause behavioral changes in depression.[23] Reduced hippocampus sizes have been seen in humans through postmortem examinations and MRI investigations conducted on individuals with severe depression (MD).[24] Studies have reported changes in the hippocampus brain in stress-induced depression models of rats, which include shrunken dendrites in CA3 and dentate gyrus neurons and loss of spines in CA1 neurons.[25] Multiple studies have reported observing hippocampal shrinkage in various animal models of depression.[26] The results of the present study also revealed structural alterations in the hippocampus of rats caused by CUMS, including reduced number of viable neurons in the pyramidal cell layers. The administration of Flx or CA at 400 and 800 mg/

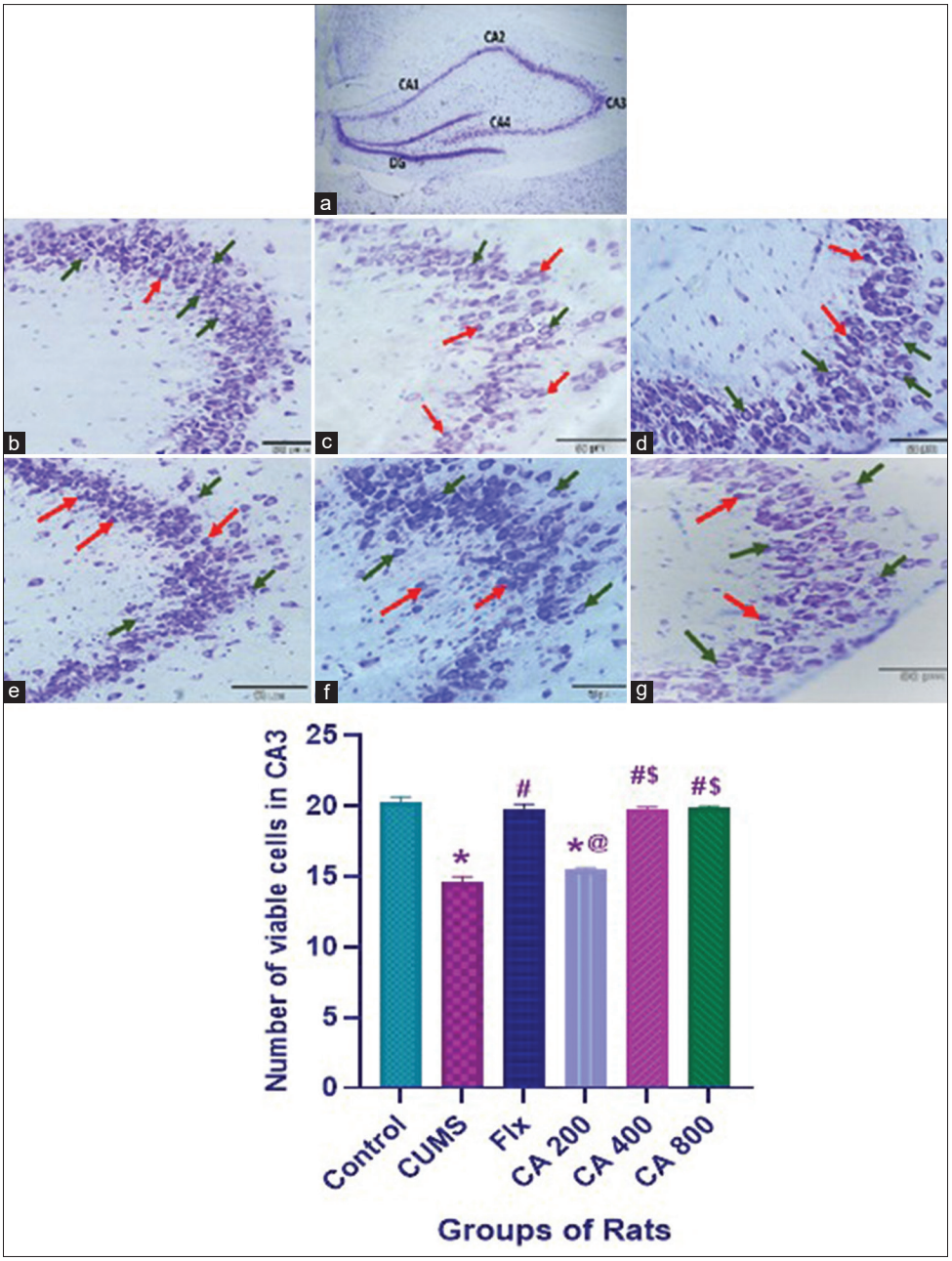

Figure 4: Neuroprotective effects of *Centella asiatica* (CA) on the neurons of the CA3 region of the hippocampus, as observed by cresyl violet stain, on the chronic unpredictable mild stress (CUMS)-induced rats. Images of the cresyl violet staining of the hippocampus showing changes in the number of viable cells (shown with green arrow) and degenerated cells (shown with red arrow) after CUMS induction and those co-administered with fluoxetine (Flx) and CA. (a) Hippocampal regions marked, (b) Control group, (c) CUMS-induced group, showing less viable cells (red arrow) (d) Flx group, showed more viable cells (green arrow) (e) CA 200 group shows less number of viable cells and (f and g) CA 400 and 800 groups of rats show more number of viable cells (green arrow). Data presented as mean ± SD, *n* = 6. **P* < 0.05 versus control, #*P* < 0.05 versus CUMS, @*P* < 0.05 versus Flx, $*P* < 0.05 versus 200

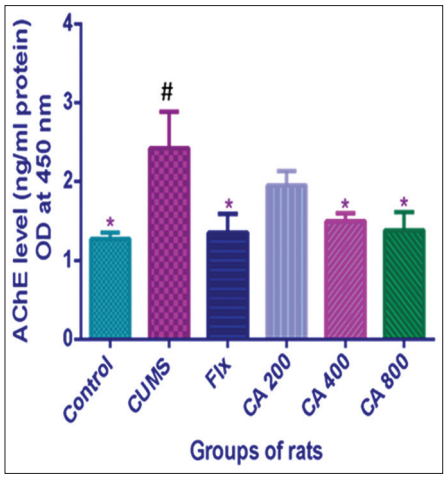

Figure 5: Effect of *Centella asiatica* on acetylcholinesterase levels in the rat's brains. Values are presented as mean ± SD, *n* = 6. #*P* < 0.05 versus control group, **P* < 0.05 versus chronic unpredictable mild stress group

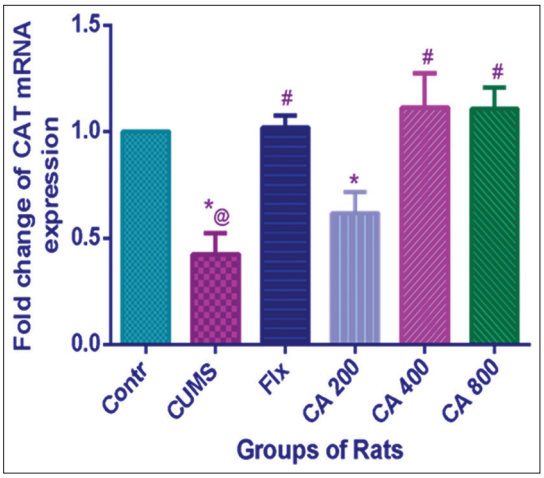

Figure 6: Effect of *Centella asiatica* on the levels of Catalase mRNA in rat's brain. Values are presented as mean ± SD, *n* = 6. @*P* < 0.05 versus control group, **P* < 0.05 versus control group, #*P* < 0.05 versus chronic unpredictable mild stress group

kg doses prevented the above-mentioned alterations. The neuroprotective effects of CA in this study were similar to those of Flx. Significantly, according to the existing literature, this study is the initial documentation of the neuroprotective effects of CA in the rat model of depression generated by CUMS. Prior research has documented the neuroprotective properties of CA in mice subjected to paracetamol toxicity, rats exposed to D-gal/AlCl3 toxicity, and rats with cerebral ischemia perfusion injury.[27-29]

AChE is an essential enzyme that plays a critical role in cholinergic transmission. Its primary function is to break down the neurotransmitter acetylcholine into acetate and choline. This process has a direct impact on memory. This phenomenon has been proven in Wistar rats exposed to long-term noise-induced stress.[30] Previous studies have documented similar findings in rats exposed to long-term stress, showing an elevation in AChE levels and impairments in memory.[31,32] The current investigation revealed that the model group of rats which were exposed only to CUMS had an increase in the levels of AChE in the hippocampus in comparison to the control group of rats. The elevation of AChE levels is believed to have a direct and causal impact on the development of memory impairments. The rat groups that received Flx and CA 400 and 800 also showed reduced AChE levels and enhanced memory performance. Therefore, it can be inferred that CA mitigates the alterations in AChE levels caused by CUMS, thereby improving memory performance. The CA at 400 and 800 mg/kg dosages exhibit nondose-dependent behavior, as they yield comparable benefits in reducing oxidative-antioxidative alterations and AChE levels. On the other hand, the effect of CA varies depending on the dosage. There is a noticeable distinction between CA at 200 mg/kg group and groups receiving CA at 400 and 800 mg/kg.

CAT plays a crucial role as an antioxidant in detoxifying hydrogen peroxide (H2O2).[33] The presence of oxidative stress in Wistar rats reduced the activity of CAT in the brain, leading to oxidative damage.[34] RT-PCR analysis revealed a notable decrease in mRNA expression of CAT in rats exposed to oxidative stress.[35] Rats exposed to long-term, unpredictable stress experienced a noteworthy reduction in their hippocampus's CAT levels.[36] Comparable findings were documented in Wistar rats exposed to stress by prolonged immobilization. The findings of the current investigation align with prior observations.

The current investigation confirms a decrease in mRNA CAT levels in the hippocampus of rats with depression produced by CUMS, compared to a control group of rats. The rats induced with CUMS and treated with Flx and CA at doses of 400 and 800 mg/kg, respectively, exhibited an elevation in mRNA CAT levels. There were no notable variations in mRNA CAT levels between the groups administered Flx and CA (400 and 800 mg/kg), respectively. The findings of this study showed that CA effectively protected against oxidative stress-related harm by elevating the levels of CAT in the brains of rat models induced with CUMS.

Conclusion

When an organism responds both physically and psychologically when exposed to chronic stress, resulting in depression. The changes in the body include alterations in the oxidative stress pathway; this affects its delicate balance by lowering the levels of CAT, which is an antioxidant, and AChE, which is essential for normal synaptic function, thus leading to the loss of hippocampal neurons. The CA, an herb with neuroprotective properties, has been shown to normalize the levels of CAT and AChE and protect the neurons in CUMS-induced rats with depression hence can be concluded that CA has the potential to treat depression.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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