**Centella asiatica** L. Urban protects against morphological aberrations induced by chronic unpredictable mild stress in rat’s hippocampus via attenuation of oxidative stress

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**ABSTRACT**

The present study evaluated the effect of **Centella asiatica** L. Urban (CA) extracts on ultrastructure and oxidative stress parameters on chronic unpredictable mild stress (CUMS) rat’s hippocampi. Rats were divided into six groups (n=6) and treated as follows: Group 1, Unstressed+ normal saline (control); Group 2, CUMS + normal saline (Model); Group 3, CUMS+ Flx (10 mg/kg) (FLX); Groups 4, CUMS + CA (200 mg/kg) (CA 200); Group 5, CUMS+ CA (400 mg/kg) (CA 400) and Group 6, CUMS + CA (800 mg/kg) (CA 800). Nineteen stressors were administered to the rats for 8 weeks consecutively, with concurrent administration of fluoxetine or CA. The hippocampi of the rats were evaluated by transmission electron microscopy (TEM) and oxidative stress biomarkers were also determined. Qualitative TEM results showed defective mitochondria, nucleus, myelin sheaths and reduced numbers of synapses in CUMS rats as well as increased oxidative stress biomarkers. Administration of CA prevented some of the structural and biochemical aberrations observed in response to stress in a manner comparable with fluoxetine, a selective serotonin-reuptake inhibitor. Conclusively, CA ameliorates structural defects induced by CUMS in the rat hippocampus, with a potential therapeutic importance in the treatment of stress and depression.

**Introduction**

Stress is a process where the physical and psychological demands of an individual, which are called the stressors, affect the ability of the individual or organism to adapt to the ensuing challenges [1]. There are different types of stressors which include acute and chronic, major and minor, and desirable and undesirable, which highlight the diversity of stresses that affect the life of an individual. Chronic stress occurs when a stressor persists or occurs as
a repetition of an initial stressful event [2]. Postmortem studies on patients diagnosed with chronic stress-induced depression and on animal models of depression have demonstrated various changes including a decrease in the size of parts of the limbic system of the brain such as the hippocampus and amygdala [3]. In a study carried-out on a chronic unpredictable mild stress (CUMS) rat model of depression, the CA1 region of the hippocampus showed irregularly arranged pyramidal neurons and different shapes of the mitochondria, including swollen mitochondria [4]. It has been demonstrated that when rats are subjected to chronic restraint stress, their neurons exhibited shrinkage of the nucleus, pyknosis of nuclei, with irregular and dispersed chromatin. In addition, the neuronal mitochondria appeared deformed, while the synaptic clefts were blurred [5].

The CUMS protocol involved successive application of different mild stressors such as 1-min tail pinch, 5-min cold swimming, overnight illumination, food and water deprivation, 15-min forced swimming etc on rats. These stressors are randomly arranged over a period of 1-week and repeated throughout the period of experiment 6-weeks, 8 = weeks or 10-weeks [6]. CUMS is a well-established protocol being used for the induction of depressive-like behaviors and cognitive impairments in rat models, it is also used for studying the underlying mechanisms [7]. The CUMS protocol is as classical as it could induce stress in an unpredictable routine which can be compared to the unpredictable stressors of human life with good predictive, face and construct validity [8–10]. Hence, CUMS is used widely for studying antidepressants since its highly reliable and it is also associated with several neuropsychiatric disorders, like anxiety and depression [11–13]. Meanwhile, the hippocampus is sensitive to CUMS, as a result it leads to the impairments of its structures and functions. Decreased expression of brain-derived neurotrophic factor (BDNF), reduced neurogenesis and altered synaptic morphology in the hippocampus has been reported in CUMS rats [14,15].

The current drug treatment for depression is based on selective serotonin reuptake inhibitors (SSRI’s), monoamine oxidase inhibitors (MAOI’s), and tricyclic antidepressants (TCA’s). These treatments have significantly contributed to enhancing the quality of life of individuals with depression, but they are not without their limitations. The current medications do not produce a uniform response among patients, it takes weeks for their effects to be observed and many treatments have significant side effects [16]. The concurrent use of multiple drugs complicates the problems through complex interactions and in particular gives rise to uncertainty regarding their safe use in pregnancy [17]. Fluoxetine is a commonly used antidepressant, and as an SSRI, it inhibits the serotonin transporters at the synaptic cleft. Though in wide use, fluoxetine has side effects including fatigue, weight gain, and sexual dysfunction [18,19]. Thus, though there is a wide range of medications available for the treatment of depression, none of them are universally effective or without side effects. Consequently, there is a need for new therapeutic agents with lesser side effects and broader efficacy [20]. In order to achieve this, it is necessary to consider the critical physiological processes that contribute to stress and depression.

One of the major mechanisms by which stress affects the human body is through the generation of reactive oxygen species (ROS) [21–23]. Malondialdehyde (MDA) is a product of degradation of polyunsaturated fatty acids and has a positive correlation with the severity of lipid peroxidation [24]. Wistar rats exposed to chronic restraint stress, demonstrated increased levels of MDA in the brain, highlighting the importance of lipid peroxidation induced by chronic stress [25]. In a study on patients with recurrence of depression,
elevated plasma levels of MDA were observed which correlated with the severity of working memory, short-term and delayed memory [26].

The ROS produced by mitochondrial oxidation within the cells in response to stress is neutralized by both non-enzymatic and enzymatic antioxidant defense systems [27]. For the enzymatic antioxidant mechanisms, the most studied markers are the levels of catalase (CAT) and superoxide dismutase (SOD) in Wistar rat models [23]. The principal marker for the non-enzymatic antioxidant defense mechanism is the glutathione (GSH) levels. SOD acts by converting superoxide anion radicals to hydrogen peroxide, thereby the process of superoxide anion interacting with nitric oxide to form reactive peroxynitrite is reduced [23].

Oxidative stress is triggered by the imbalance between the generation of ROS in the cells and the clearance of its intermediates or repair of resulting damage. It has been reviewed that post-mortem studies on oxidative stress in frontal lobes of the brain in patients with depression was increased compared to those of matched controls [28]. Studies on different animal models including rats have revealed that chronic stress could contribute to depression by damaging mitochondrial function, impairing neurogenesis and affecting cell survival [22,29].

Centella asiatica L. Urban (CA) is a green leafy herb that is valued for its medicinal properties and has been used in traditional medicines of many countries since ancient times [21]. The health benefits of CA have been described in Unani medicine in Sri Lanka, Ayurvedic medicine in India, Chinese traditional medicine, folk medicine in South Asian countries, and African traditional medicine [30]. Centella asiatica has been used for its neurological actions including memory-enhancing effects [31], and as a revitalizer for nerves and brain cells [32]. More broadly, CA has also been used for the treatment of headache and leprosy [33] wound healing, and memory enhancement [34]. Centella asiatica is most commonly used in many traditional medicinal practices for its neuroprotective effects [26,3536]. The ameliorative effects of CA on d-galactose and aluminum chloride-induced oxidative stress and cholinergic dysfunction causing neuronal degeneration, and cognitive impairment in male Wistar rats [37] and lead-induced toxicity in rats [38] have also been documented.

This present work explored the neuroprotective effects of CA in a CUMS rat model of depression, by studying the ultrastructure of the hippocampus and the expression of biomarkers for stress.

**Materials and methods**

**Animals**

Thirty-six male albino Wistar rats, aged 8–10 weeks and weighing 180–220 g, purchased from Bistari Ltd, Serdang, Selangor, Malaysia, were used in this study. The choice of males was made to exclude the influence of gender variability and the sex-dependent response to stress. The rats were kept in the Animal House, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia and maintained under standard laboratory conditions (in 12:12 light/dark cycle, with the light on from 07.00 hrs to 19.00 hrs, the ambient temperature was maintained at 25 ± 2°C, and the relative humidity 50 ± 10% with access to food and water ad libitum). The rats were acclimatized to the laboratory conditions for one week and were housed in pairs per home cage. The number of rats used and the protocols followed for the study were approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia, on 23 November 2018, with project reference number UPM/IACUC/AUP-R078/2018.

**Centella asiatica and fluoxetine**

The ethanolic extract of CA (Reference Number: AuRins-MIA-1-0, Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi Mara (UiTM), Puncak Alam, Selangor, Malaysia) [39] and fluoxetine (Flx) (Cadila Pharmaceuticals
Ltd, Ahmedabad, India), were acquired from the sources indicated. The CA and Flx were administered to the rats in the morning between 9:00 a.m and 11:00 a.m. Flx was administered orally at a dosage of 10 mg/kg body weight and CA was also administered orally in dosages of 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight. This dosage was chosen based on previous studies [40,41]. Oral administration was considered the most representative of how drugs would be administered during the treatment of psychiatric patients.

**Induction of CUMS in rats**

The rats were subjected to CUMS for 8 weeks by exposing them to both psychosocial and environmental stressors as previously described by Jagadeesan and Tong [6,20]. The rats which were not subjected to CUMS, were left in their home cages except for normal handling and cleaning. The rats that were subjected to CUMS experienced a course of nine types of mild stressors (S), with a minimum of two stressors per day for eight consecutive weeks (Table 1). These stressors were randomly administered for the first week and the same schedule was repeated throughout the rest of the experimental period. In order to avoid prophesy and adaptation, no stressor was applied consecutively.

**Experimental design**

Following one week of acclimatization, rats were randomly divided into six groups (n = 6): Group 1, Unstressed + normal saline (control); Group 2, CUMS + normal saline (CUMS); Group 3, CUMS + Flx (10 mg/kg) (Flx); Groups 4, CUMS + CA (200 mg/kg) (CA 200); Group 5, CUMS + CA (400 mg/kg) (CA 400) and Group 6, CUMS + CA (800 mg/kg) (CA 800). Flx and CA were administered starting from day zero of the study once a day for eight weeks. The rats were euthanized on day 64, and their brain tissues were harvested for morphological studies using electron microscopy and oxidative stress examination using photospectrometry (Figure 1).

**Transmission electron microscopy (TEM)**

The hippocampus was excised from the brains of decapitated rats and was cut into approximately 1 x 1 x 1 mm sections on a cold plate. The tissue sections were fixed in an electron microscopy (EM) grade primary fixative of 5% glutaraldehyde (Agar Scientific Ltd, UK) for 12 hours at 4°C for TEM studies. The tissue sections on the grids were stained first with uranyl acetate (Agar Scientific Ltd, UK), by placing the grid in 20 µl of uranyl acetate on parafilm for 10 minutes. The grids were then washed with filtered distilled water 3 times for 10 minutes each time. The grids were then stained with lead acetate (Agar Scientific Ltd, UK) for 10 minutes, then washed with filtered distilled water 3 times for 10 minutes each time. The grids were dried on filter papers and viewed with a TEM (LEO LIBRA-120, Japan).

**Biochemical assay of oxidative stress markers**

Hippocampal tissue from the study groups was weighed and homogenized in phosphate buffered saline (PBS) (tissue weight (g): PBS (mL) volume 1:9) using a tissue homogenizer. The homogenates were then subsequently centrifuged at 5000 x g for 5 minutes to get the supernatant. The total protein concentration of the rat brain tissues was measured using the bicinchoninic acid assay (BCA assay) kit, purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). Bovine serum albumin (BSA) (1 mg/ml) was used as the standard in the range of 0.01–0.1 mg/ml for its comparison with the total protein.

The oxidative stress markers (MDA and SOD) were quantified using their respective assay kits by colorimetric methods. Measurements of the MDA levels were done following the manufacturer’s guide, Cayman Chemical Company, Ann
Table 1. Chronic unpredictable mild stress procedures.

| Type of stress          | Details                                                                                      |
|------------------------|-----------------------------------------------------------------------------------------------|
| S1 Food deprivation    | The rats were subjected to 24 hours food deprivation. Food was provided following the end of the deprivation period. |
| S2 Water deprivation   | The rats were subjected to 24 hours 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 minutes 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 hours, 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 minute.          |
| S6 Cage tilt            | The rat cages were kept tilted at 45° for 12 hours.                                           |
| S7 Overcrowding of cage| 6 rats were packed in a cage for a period of 12 hours.                                        |
| S8 Wet bedding          | 200 ml of water was added to the beddings in the cages where the rats, were retained for 12 hours. |
| S9 Physical restrain    | The rats were individually restrained in plastic restrainers (5.5 cm diameter and 12 cm long) with proper ventilation for 4 hours. |

Arbor (Michigan, USA). The MDA levels were expressed as nmol per mg protein. The SOD activities were measured by the water soluble tetrazolium – 1 (WST-1) method. Measurements of SOD were also conducted according to the manufacturer's manual, (Elabscience, USA). The optical density (OD) readings were done using a microplate reader at 450 nm, and the SOD activities expressed as U/mg protein.

Statistical analysis

Using the GraphPad Prism version 6 (ISI, San Diego, CA, USA) software, the data obtained were analyzed using one way ANOVA. Tukey’s post hoc comparison was used where applicable, with p < 0.05 considered as being significant and the results presented as mean ± SD.

Results

CA attenuated mitochondrial aberrations in the brains of rats exposed to CUMS

Representative electron micrographs of mitochondria in the hippocampal sections of rats from all treatment groups were recorded (Figure 2). The hippocampi of the control group of rats exhibited healthy mitochondria which appeared round or ovoid-shaped with a dense matrix and parallel-arranged cristae (Figure 2(a)). Mitochondrial changes were observed in the brains of the CUMS rats in the form of elongated mitochondria, with some ruptured and others exhibiting fragmentation of the cristae (Figure 2(b)). The CUMS rats co-administered with Flx showed less mitochondrial aberrations, maintaining their parallel-arranged cristae and round or ovoid shapes (Figure 2(c)). Further, CUMS rats co-administered with CA 200, 400 and 800 mg/kg exhibited fewer damages to mitochondria of neuronal cells, although a few vacuolated mitochondria were observed in sections from rats administered with CA at the lowest dose, 200 mg/kg (Figure 2(d-f)).

Figure 1. Experimental design for the morphological and biochemical measures.
CA prevented nuclear abnormalities in the brains of rats exposed to CUMS

The effects of treatment with CA on CUMS-induced nuclear damage of the hippocampus of rats was evaluated through TEM (Figure 3). In the control hippocampus tissue, the nuclei appeared normal with prominent nucleoli, double-layered nuclear membrane, and a well-distributed chromatin (Figure 3(a)). The hippocampi of the CUMS rats showed pyknotic nuclei, degenerated chromatin and nucleoli (Figure 3(b)). Administration of fluoxetine to CUMS rats prevented some nuclear abnormalities as the sections showed normal nucleus with nucleoli in the hippocampi (Figure 3(c)). Administration of CA (200, 400, and 800 mg/kg) prevented some of the morphological changes seen in CUMS rats (Figure 3(d-f)). However, again, the hippocampal sections from the lowest dose CA treatment group (200 mg/kg) showed some distorted nuclear membrane and degenerated chromatin with less prominent nucleoli (Figure 3(d)).

CA treatment prevented myelin sheath defects in the brains of rats exposed to CUMS

The effects of treatment with CA on CUMS-induced myelination changes in rat hippocampal tissue were investigated (Figure 4). The myelin sheaths of neurons in the sections from the untreated control group of rats were highly electron-dense, thick, continuous, and tightly wrapped around the axonal cytoplasmic membranes. The CUMS rats model exhibited myelin defects which included the loose wrapping of the myelin sheath around the cytoplasmic membranes of axons, bulging of the sheaths,

Figure 2. TEM images of rat’s brains showing mitochondrial aberrations in CUMS rats and those co-administered with fluoxetine and CA. a) Control group of rats showing normal mitochondria (blue arrow) with dense matrix, b) CUMS rats showing elongated and burst mitochondria (yellow arrow), c) fluoxetine group of rats showing normal mitochondria like those of the control group of rats, d) CA 200 group of rats showing normal mitochondria (blue arrow) and vacuolated mitochondria (red arrow), and e-f) CA 400 and 800 groups of rats, showing normal mitochondria.
Figure 3. TEM micrographs of rat’s brains showing nuclear abnormalities in CUMS rats and those co-administered with Fluoxetine and CA. a) Control group of rats showing nucleoli (red arrow), evenly distributed chromatin and double nuclear membrane (blue arrow), b) CUMS rats showing pyknotic nucleus and crescent formation, c) fluoxetine group of rats showing normal nucleus with double nuclear membrane (blue arrow), d) CA 200 group of rats showing nucleus with degenerated chromatin and gaps in their nuclear membrane (purple arrow), e-f) CA 400 and 800 groups of rats with normal nucleus showing intact nuclear membrane (blue arrow) and nucleoli (red arrow).

Figure 4. TEM micrographs of rat brains showing myelin sheath defects in CUMS rats and those co-administered with fluoxetine and CA. a) Control group of rats showing normal myelin sheaths appeared dense, thick and tightly wrapped around their axons (blue arrow). b) The CUMS rats showing discontinuous myelin (purple arrow) and onion-like bulging (red arrow) of their myelin sheaths. c) fluoxetine group of rats showed normal myelin sheaths. d) CA 200 group of rats showing distorted myelin sheaths (yellow arrow). e) CA 400 group of rats showing normal myelin sheath tightly wrapped around an axon (blue arrow) and f) CA 800 group of rats showing normal myelin sheath.
and discontinuous myelination of axons (Figure 4(b)). The administration of Flx to the CUMS rats prevented some of the morphological aberrations caused by CUMS. The administration of CA (400 and 800 mg/kg) protected the rats from the myelin sheath defects (Figure 4(e-f)).

CA treatment prevented synaptic irregularities in the brains of rats exposed to CUMS

The synaptic morphology in the hippocampal sections of the rats was evaluated to determine the protective effects of CA in the CUMS rats (Figure 5). Sections from the untreated control group of rats (Figure 5(a)) exhibited an abundance of normal synapses with intact pre and postsynaptic vesicles. However, the sections from CUMS rats showed a marked reduction in the number of synapses, which also had blurred synaptic clefts and irregular synaptic connections (Figure 5(b)). Administration of Flx to the CUMS rats prevented these morphological aberrations (Figure 5(c)). While the hippocampal sections from CUMS rats that were treated with CA (400 and 800 mg/kg) had higher numbers of normal synapses compared to those of the CUMS rats model (Figure 5(e-f)).

Figure 5. TEM micrographs of rat’s brains showing synaptic abnormalities in CUMS rats and those co-administered with fluoxetine and CA. a) Control group of rats showing abundant synapses (red arrows). b) CUMS rats showing few synapse. c) fluoxetine group of rats showing many normal looking synapses. d) CA 200 group of rats showing less numbers of synapses. e-f) CA 400 and 800 groups of rats showing abundant normal synapses.

CA treatments prevented upsurge of MDA levels in CUMS rats brain tissues

The probable antioxidant capacity of CA in CUMS rats was evaluated by measuring the levels of MDA in their brains. There were significant differences in the levels of MDA among the various groups of rats \[F(5, 12) = 38.94, p < 0.001\] (Figure 6(a)). Significant increases in MDA levels were observed in the CUMS rats model \((9.42 \pm 0.456, p < 0.001)\) when compared to the control group of rats \((5.24 \pm 0.35)\). Significant decreases were observed in the
levels of MDA in the CUMS rats administered with Flx (5.37 ± 0.35, p < 0.001), CA 200 (7.37 ± 0.701, p < 0.001), CA 400 (5.96 ± 0.503, p < 0.001) and CA 800 (5.36 ± 0.25, p = 0.0001), when compared to CUMS model group (9.42 ± 0.456). Significant increases of MDA levels in the CUMS model group (9.42 ± 0.456, p < 0.001) and CA 200 (7.37 ± 0.701, p < 0.001) groups of rats, when compared to the Flx (5.37 ± 0.35, p < 0.001) group of rats. No statistically significant differences were observed between Flx and CA (400 and 800) groups of rats.
CA treatment increased the levels of SOD in CUMS rats brain tissues

The levels of SOD in the rat brains were evaluated to check the protective effects of CA on CUMS rats. There were significant differences in the level of SOD among the various groups of rats \( F(5, 12) = 9.792, p < 0.001 \) (Figure 6(b)). Significant decreases of the levels of SOD in the CUMS model group of rats \( (7.6 \pm 1.07, p < 0.001) \) when compared to the control group of rats \( (14.67 \pm 1.145) \). Conversely, significant increases in SOD levels were observed in the CUMS rats which were administered with Flx \( (11.97 \pm 1.89, p = 0.016) \), CA 400 \( (12.3 \pm 1.15, p = 0.009) \) and CA 800 \( (13.27 \pm 1.51, p = 0.002) \), when compared to CUMS model group \( (7.6 \pm 1.07) \). No significant differences were observed between Flx and CA (400 and 800) groups of rats.

Discussion

The hippocampus as a part of the limbic system has a role in the modulation of depressive mood, processing of information, and behavioral changes in depression [42]. Decreased hippocampal volumes have been detected in humans based on postmortem studies and MRI studies on patients suffering from major depression (MD) [43,44]. Shrinkage of the dendrites of CA3 and dentate gyrus neurons and the loss of spines of CA1 neurons have also been reported in the hippocampus of the rat brains subjected to stress-induced depression [45]. Further, studies have reported ultrastructure aberrations in CUMS rodents, including mitochondrial atrophy, enlarged endoplasmic reticulum, condensation of the chromatin material, distortion of mitochondrial structure, and damages of other structures in their hippocampus [46]. Decreased synaptic density in mice hippocampus [47] and decreased dendritic spine and synaptic density in medial prefrontal neurons [48] were also observed in stress-induced rat models. Decreased postsynaptic density in the CA1 region of the hippocampus was also observed in CUMS mice models of depression [49]. In conformity with the preceding reports, marked ultrastructural aberrations were observed in the brain of the CUMS rats model in the present study. Based on the available literature to date, the present study is the first to report on the protective effects of CA on the neuronal ultrastructural aberrations observed in the mitochondria, myelin sheaths, nucleus, and synapses of the hippocampus of CUMS-induced depressive-like rats.

The structural integrity of mitochondria is of paramount importance for effective energy generation and cell survival. The CUMS rats have been reported to exhibit mitochondrial alterations like enlargement, fragmentation, and disappearance of cristae which resulted in empty intermembrane space [50,51]. Similarly, TEM images in the present study also revealed some unique mitochondrial alterations, including elongation of mitochondria, fragmentation, and loss of cristae, vacuolation, and breach of the outer membrane of mitochondria in the hippocampus of CUMS rats models. The aforementioned changes could disturb the normal mitochondrial functions such as the electron transport chain [49]. Of the particular significance in the present study is that the administration of CA prevented the mitochondrial aberrations which could be the reason for the reversal of the depressive-like behaviors in the rats as previously reported [6].

Structural damages to the nucleus in CUMS exposed rats model of depression, including chromatin aggregation, condensation, margination, and nuclear karyopyknosis, have been reported and were considered as indicators of apoptotic changes [44]. In the present study, the CUMS rats also exhibited nuclear structural changes like pyknosis, degeneration of chromatin, and disappearance of nucleoli. The administration of fluoxetine or CA (400 and 800 mg/kg) to CUMS rats prevented some of the nuclear changes. This finding is similar to
those of earlier reports demonstrating that CA prevented nuclear abnormalities in the prefrontal cortex of the D-gal/AlCl₃ mediated model of AD-rats [41].

The myelin sheath plays a vital role in the central nervous system because of its role in the trophic support of axons and rapid signal conduction [52]. The myelin sheath also has a role in the protection and insulation of axons. Hence, damage to the myelin sheaths could result in severe neurological deficits affecting the normal signaling, and leading therefore to neurological dysfunction. An increase in the number of demyelinated axons and destruction of the myelin sheath around axons were reported in rats exposed to either physiological or physical stressors [53]. It has been reviewed that demyelination is associated with anxiety- and depression-related behaviors in rodents [54]. In this study, CUMS rats also exhibited defects of the myelin sheaths which include detachment from the axons, discontinuous, and bulging of myelin sheath. These structural anomalies of the myelin sheath could be attributed to the depression-like behaviors and cognitive deficits observed in the CUMS rats [6]. Prevention of the structural alterations was seen in fluoxetine and CA (400 and 800 mg/kg) treated groups of rats in the current study. Rao and his team have also reported that CA extract significantly increased the dendritic arborization of hippocampal CA3 neurons in vivo studies on rats [55]. In another study on chronic mild stress-induced rat models of depression, it was observed that fluoxetine delayed the development of white matter demyelination [56].

Cumulative evidence has shown that biological mechanisms of depression lie in the dysfunction of synaptic plasticity, particularly in the hippocampus and prefrontal cortex [5,42]. In a previous study, a significant loss of postsynaptic density was reported in CUMS rats [39]. Similarly, CUMS rats from the present study revealed synaptic morphological alterations such as less number of synapses with fewer pre and post-synaptic vesicles, blurry synapses, and irregular synaptic clefts which were all prevented by the administration of CA.

To further understand the mechanisms of ROS in the pathogenesis of depression, researchers have subjected rats to CUMS, as an appropriate model of depression, widely accepted as a clinically relevant model for depression [57,58]. It has been revealed that in humans and rat models of depression there was a significant decrease in plasma concentrations of antioxidants such as SOD with an increase in the levels of MDA [28]. In conformity with previous research, marked changes in the levels of the oxidative stress marker MDA and antioxidant SOD were observed in the brain of CUMS rat models of depression in the current study. The present study is the first to report the neuroprotective effect of CA on the oxidative stress in CUMS rat models of depression. MDA is highly reactive and produced as an end result of lipid peroxidation of polyunsaturated fatty acids by ROS, which causes toxic neuronal damage [59]. It has also been observed that chronic stress increases level of MDA in various parts of the brain including hippocampus [60,61]. In a study, rats subjected to chronic unpredictable stress showed increases in the level of MDA in the prefrontal cortex and hippocampus [62]. In the current study, increase in the levels of MDA in the hippocampus of CUMS rat models of depression were demonstrated in comparison to the control group of rats. It was also observed that the levels of MDA in the hippocampus were significantly lower in the rat groups which were administered with Flx and CA (400 and 800 mg/kg) in comparison to CUMS rat models. There were no significant difference in the levels of MDA between the Flx and CA (400 and 800 mg/kg) treated groups, respectively.

Superoxide dismutase is an important enzyme involved in the first line of defense against the ROS (Hajipour et al., 2016) [63]. Lower levels of SOD could be responsible for
an increase in lipid peroxidation of the brain of rats. A study on Wistar rats subjected to stress with chronic restraint revealed a reduction in activity of SOD in their brains, thereby enhancing an oxidative stress \[64\]. Depressive-like behavioral changes and decreased levels of SOD were reported in rats subjected to unpredictable chronic stress \[65,66\]. The result of the current study confirmed that the levels of SOD in the hippocampus were significantly reduced in comparison with the control group. The findings of this study agree with those of the previous studies \[58,59\]. The present study revealed an increase in the levels of SOD in the hippocampus among rats administered Flx and CA (400 and 800 mg/kg) and prevented the manifestations of stress. No significant differences were observed between the rats administered Flx and those administered CA (400 and 800 mg/kg).

**Conclusion**

The present study indicated that CA prevented neuronal damage in the hippocampus of CUMS rat models. Therefore, treatment with CA could be a promising approach for the corrections of neuronal defects in CUMS rats. The study supports the hypothesis that CA prevents oxidative stress-induced neuronal damage in the hippocampus of CUMS rat models (Figure 7).

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**Disclosure statement**

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Ethical Issues

The study was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia, on 23 November 2018, with a project reference number UPM/IACUC/AUP-R078/2018.

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