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Original Article

Azadirachta indica A. Juss flower extract attenuates memory deficit induced by restraint stress in male rats

Thaneeya Hawiset [1*], Napatr Sriraksa [2,3], Ratchaniporn Kongsui [2], Utcharaporn Kamsrijai [1], Keerati Wanchai [1], Prachak Inkaew [4]

1. School of Medicine, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand
2. School of Medical Sciences, University of Phayao, Muang, Phayao, 56000, Thailand
3. Unit of Excellence in The Pulmonary and Cardiovascular Health Care, University of Phayao, Muang, Phayao, 56000, Thailand
4. School of Science, Mae Fah Luang University, Muang, Chiang Rai, 57100, Thailand

ABSTRACT

Introduction: Chronic stress is related to cognitive impairment. *Azadirachta indica* A. Juss. (*A. indica*) leaf extract possesses antioxidant and cognitive enhancement effects. Therefore, this study was set up to evaluate the cognitive-enhancing effects of *A. indica* flower extract in stressed rats.

Methods: Male Wistar rats were randomly divided into control and stress groups. Restraint stress was induced to stress groups 3 h daily. The stressed rats were given vehicles, donepezil (a positive control meditation used to improve cognition), and *A. indica* at 250, 500, and 1000 mg/kg BW for 30 days. The novel object recognition test (NORT) was used to assess cognitive function, and the open field test (OFT) was utilized to assess spontaneous locomotor activity. Their brains and blood were taken to measure levels of brain-derived neurotrophic factor (BDNF), blood cortisol levels, and the density of survival neurons.

Results: The discrimination index ratio of the stressed rats treated with either donepezil or *A. indica* flower extract at all doses was significantly improved as measured by NORT. Moreover, there was no significant difference between the control and stress groups in the locomotor behaviors of rearing and number of crossing. The stressed rats treated with donepezil and *A. indica* flower extract had significantly higher BDNF levels and also survival neuron density in the brain. However, their blood cortisol levels were lower than the stressed rats given the vehicle.

Conclusion: *A. indica* flower extract helps improve cognitive function in stressed rats by boosting BDNF and protecting against neuronal loss in the brain.

Keywords:
Azadirachta indica A. Juss.
Memory
Restraint stress
BDNF
Survival neuron density

Introduction

People experience stress in various situations such as continual study, work pressures, financial difficulties, and health issues (Salleh, 2008). Stress impacts all physiological systems, including the neurological system (Dayi et al., 2015). Chronic stress affects cognitive

* Corresponding author: Thaneeya Hawiset, thaneeya.haw@mfu.ac.th
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performance, structure, and function of various brain areas, including the cerebral cortex and the hippocampus (Wang et al., 2014). Nowadays, the prevalence of cognitive disorders is on the rise worldwide leading to lower quality of life, more disability, and higher healthcare costs (Kumar et al., 2013). The regulation of cognitive function is linked to the release of hormones and neurotransmitters by the controlling of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system involved in memory consolidation, storage, and retrieval (Sandi and Pinelo-Nava, 2007). Chronic restraint stress (CRS) has been widely used as a model of physical and psychological stress to induce learning and memory deficit in animals (Sahin and Gümüslü, 2007). CRS induces cognitive impairment by interrupting neurogenesis, synaptic plasticity, BDNF signaling pathway, and HPA axis regulation (Woo et al., 2018; Kim and Diamond, 2002; Ortiz et al., 2014). CRS also induces neurotoxicity, neuronal damage, oxidative stress, and mitochondria dysfunction (Woo et al., 2018; Stier et al., 2019). As a result, CRS might affect the density of survival neurons, the brain BDNF levels, and blood cortisol levels. Cognitive medications including donepezil, rivastigmine, and memantine are used for relieving the symptoms of memory deficit (Husain and Mehta, 2011). However, these medications have several negative side effects such as nausea, vomiting, diarrhea, sleeplessness, and exhaustion (Husain and Mehta, 2011). Therefore, for the development of cognitive enhancers from medicinal plants, a quest for novel therapeutic drugs with minimal side effects is necessary.

Azadirachta indica A. Juss. (*A. indica*), also known as Neem tree, is a plant that belongs to Meliaceae family. *A. indica* is one of the most famous traditional plants in Asia and Africa (Eid et al., 2017). The flower and leaf of Neem are commonly utilized in Ayurvedic, Homoeopathic, Chinese, and Unani medicine to treat a variety of ailments in the world (Eid et al., 2017). *A. indica* is widely used in a variety of traditional treatments, including relieving stress, anxiety, and sleeplessness (Eid et al., 2017; Alzohairy, 2016). Investigations on the pharmacological activity and protective properties of *A. indica* have been conducted based on these traditional treatments. The leaf extract of *A. indica* shows anti-stress (Sen et al., 1992), anti-anxiety (Jaiswal et al., 1994), and cognitive boosting properties in Alzheimer's disease model in rats (Raghavedra et al., 2013). The flower extract of *A. indica* scavenges free radicals by reducing lipid peroxidation in cancer cells (Chaisawangwong and Grisanapan, 2009). The flower extract *A. indica* also promotes nerve recovery in diabetic rats (Sriraksa et al., 2019), and protects the rat's kidney from oxidative stress by increasing the activity of renal scavenging enzyme (Wanchai et al., 2021). The extract also helps reduce anxiety and depression in stressed rats by improving brain serotonin (5-HT) and dopamine (DA) levels (Hawiset et al., 2022). However, the cognitive benefits of *A. indica* flower extract in stress conditions are limited. An investigation of the cognitive enhancing impact of *A. indica* flower extract in stressed rats is necessary to better understand these issues.

Materials and methods

Plant material and preparation of the aqueous extract

Fresh *A. indica* flowers were collected from Maetumboonyong, Muang, Phayao, Thailand. The number of voucher specimens was 003805, found in the herbarium of the Faculty of Biology, Naresuan University, Thailand. Dr. Pranee Nangngam of the Faculty of Biology at Naresuan University in Thailand verified the plant extract. The flower was washed 3 times with distilled water, and then blended with distilled water for 10 min (the ratio of flower to water was 1:3). The blended flower was filtered with a Whatman No.1 filter paper, and dried at 60 °C for 10 h in a hot air oven. The blended flower was kept at -20 °C until use.

Animals

Young adult male Wistar rats (age approximately 8 weeks and weight between 200 and 220 g), were obtained from Nomura Siam International Co., Ltd. (Bangkok, Thailand). All experiments were approved by the Ethics Committee of the Laboratory Animal Research Center, Mae Fah Luang University (Approval no. AR 03/62). The animals were kept in a room with a consistent temperature (25°C), and a 12-hour light/dark cycle during the experiment. The animals were given free access to sterilized food and water. The animals were randomly divided into six experimental groups (n = 6 per group).

Drugs and administration

The animals were assigned to six experimental groups including (1) control group, (2) a stress + vehicle group,

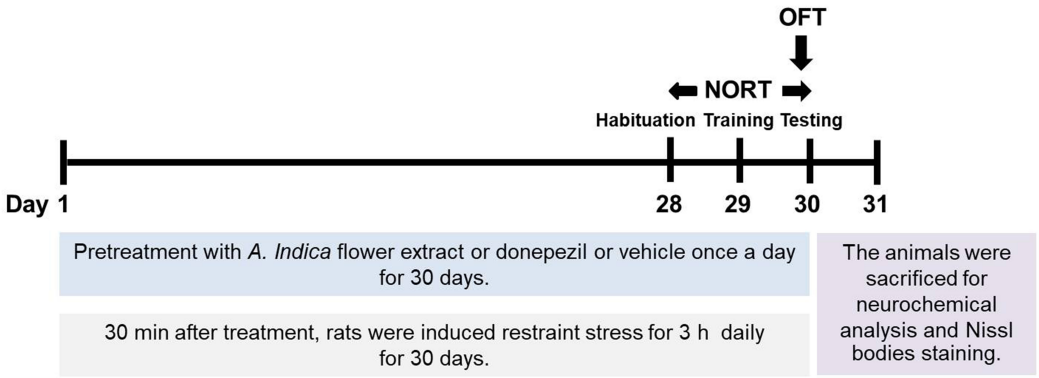

FIGURE 1. The experimental design and the schedule of behavior tests. NORT: Novel Object Recognition Test; OFT: Open Field Test.

(3) a stress + donepezil 3 mg/kg BW group, (4) stress + *A. indica* 250 mg/kg BW group, (5) stress + *A. indica* 500 mg/kg BW group, and (6) stress + *A. indica* 1000 mg/kg BW group. The animals were administered only once daily with distilled water used as a vehicle or donepezil HCl (a cognitive enhancer drug, manufactured and distributed by Eisai Co., Ltd. Tokyo, Japan) or *A. indica* flower extract via oral gavage (0.5 mL/300 g BW) between 9.00 and 10.00 AM for 30 consecutive days. 30 minutes after treatment, the animals were immobilized to induce restraint stress for 3 h daily. The behavioral tests were evaluated on day 30 after 30 min restraint stress. At the end of the experiment, all animals were sacrificed, and their brains were used to determine the brain BDNF levels and the density of neurons. Their blood was used to determine cortisol levels.

The behavioral test of the rats

On day 30, behavioral tests including the novel object recognition test (NORT) and the open field test (OFT) were administered. Figure 1 shows the experimental design and schematic diagram for the animal test.

Novel object recognition test (NORT)

The NORT was used to determine cognitive function. The experiment was carried out in a clear plexiglass box (length 40 cm, width 40 cm, height 40 cm) with a continuous light condition (40 lux). The NORT was divided into three parts: habituation, training, and testing (Lin et al., 2016). The animals were allowed to adjust to the empty box for 5 min on habituation day before being returned to their home cage.

The animals were placed in the box for 5 min on the training day to examine two familiar objects. The animals were placed to study a familiar object (Object A) and an unfamiliar object (Object B) on the test day (24 hours after the training). The exploration time was the duration that rats pointed their noses to the objects at a distance of less than 2 cm. After each rat explored the objects, the box and the objects were cleansed with a 70% ethanol solution. The following equation was used to determine the discrimination index (DI):

$$DI = \frac{Time\ spent\ exploring\ object\ B - Time\ spent\ exploring\ object\ A}{Time\ spent\ exploring\ object\ B + Time\ spent\ exploring\ object\ A}\ x\ 100$$

Open field test (OFT)

The OFT is a test commonly used to assess an animal's exploratory behavior and general activity (Hall, 1934). The OFT was modified and described by Thippeswamy et al., 2011. A clear plexiglass box (length 40 cm, width 40 cm, height 40 cm) was used for the test. The floor was divided into 16 equal squares. Each rat was placed in the center and given 5 min to freely explore the field. The number of crossings was calculated by counting the number of squares rats crossed with four paws. The number of rats standing on their hind legs indicated the number of rearing.

Measurement of BDNF levels

The rats were anesthetized with thiopental sodium (40 mg/kg BW) via the intraperitoneal route at the end of the experiment. The right hemisphere of the brain was removed, and the cerebral cortex and the hippocampus were immediately removed and stored at -80 °C for BDNF measurements. The left hemisphere was retained in 12.5% sucrose for further brain sectioning. Brain tissues of the right hemisphere were weighed and then homogenized in PBS with a glass homogenizer on ice

(tissue weight (g): PBS volume (mL) = 1:9). The homogenates were then centrifuged at 5000 xg for 5 min at 4 °C to obtain the supernatant. The levels of BDNF were measured by ELISA (Elabscience Biotechnology Co. ltd, Catalog No. E-EL-0046, Houston, USA). The intra-assay coefficient of variation of BDNF was less than 10%. The sensitivity of assays for BDNF was 18.75 pg/mL.

Tissue processing and cresyl violet staining

The left brain hemisphere was kept in 12.5% sucrose for cryoprotection following the rat sacrification. The brains were sliced into coronal sections (brain slice thickness was 30 μm) using a cryostat microtome (AST500, Amos Scientific, Australia). The brain sections were stained with 0.2% cresyl violet stain solution (Sigma-Aldrich, St. Louis, MO, USA) for 5 min, then rinsed 2 times with distilled water and dehydrated in ethanol series (70%, 95%, and 100%) for 5 min each. The slides were rinsed with xylene 3 times and covered with dibutylphthalate polystyrene xylene (DPX).

Cell count analysis

The frontal cortex and hippocampal images (-3.14 mm from Bregma) were captured at 20x magnification using a Carl fluorescence microscope (AxioScope, Rushmore Precision Co., Ltd.) with ZEN 2.3 program (blue edition). The density of survival neurons was evaluated using ImageJ software. The images of the hippocampus were cropped into 4 subregions including Cornu Ammonis 1 (CA1), Cornu Ammonis 2 (CA2), Cornu Ammonis 3 (CA3), and Dentate Gyrus (DG). The data were presented as the neuronal density (cells/200 μm^2). Ten sections were used for the quantification of cresyl violet staining for each rat.

Measurement of blood cortisol levels

The animals were sacrificed and their blood samples were collected from the heart and centrifuged at 1500 xg for 15 min. The supernatant was obtained and kept at -80 °C until use. The Dissociation Reagent (DR) was taken from a refrigerator and left at room temperature for 60 min before use. 5 μL of DR was pipetted into a 1.5 mL centrifuge tube. Then, 5 μL of serum was added into the DR tube and shaked gently for 1 min. The mixture was then incubated at room temperature for 5 min, and diluted with 490 μL of supplied Assay Buffer in a 1:100 ratio. The cortisol ELISA kit (Catalog No. EK7119, Boster Biological Technology, Pleasanton, USA) was used for the analysis. The cortisol test had a sensitivity limit of 17.3 pg/mL. The intra-assay and inter-assay coefficients of variation were 14.7% and 10.9%, respectively.

Statistical analysis

The mean and standard error of the mean (S.E.M.) were used to express the data. One-way analysis of variance (ANOVA) was used in the statistical analysis, followed by the LSD Post Hoc test. The difference between groups was considered statistically significant when the probability level was less than 0.05 ($P<0.05$).

Results

Effect of A. indica flower extract on recognition memory

The NORT was utilized to determine if *A. indica* flower extract could help to protect the rats from stress-related cognitive impairment. The increasing discrimination index ratio in this behavioral test was considered as the rats' ability to memorize the tested objects. One-way analysis of variance was used for data analysis. When compared to the control rats, the stressed rats showed memory impairment indicated by having a lower discrimination index ratio ($F_{(5, 30)} = 1.973$, $P<0.05$). The stressed rats treated with donepezil and *A. indica* flower extract at doses of 250, 500, and 1000 mg/kg BW improved recognition memory indicated by increasing discrimination index ratio as compared to untreated stressed rats ($F_{(5, 30)} =1.973$, $P< 0.05$ for all groups). The results were demonstrated in Figure 2.

Effect of A. indica flower extract on spontaneous locomotor activity

Spontaneous locomotor activity was determined by OFT to indicate the rats' physical and mental states. The results were shown in Figure 3. When compared among the control group and the stressed rat groups, the stressed rats treated with donepezil and *A. indica* flower extract at all doses exhibited no significant change in the number of crossings and rearing.

Effect of A. indica flower extract on BDNF levels in rat's brain

Figure 4 depicted the results of BDNF levels. The

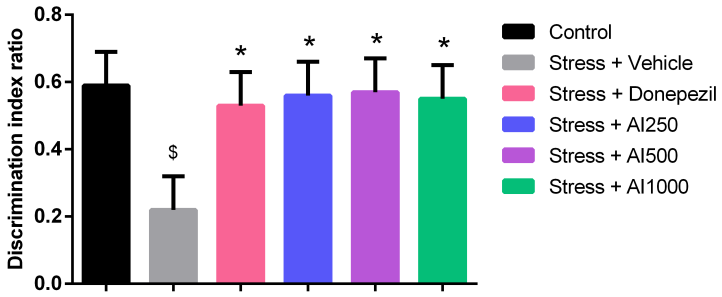

FIGURE 2. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW) on recognition memory in the novel object recognition test in rats. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n = 6 per group). $^{\$}P<0.05$ as compared with the control group; $^{*}P<0.05$, as compared to the stress + vehicle group.

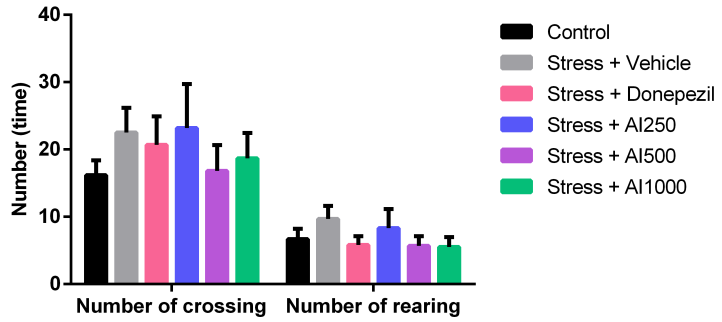

FIGURE 3. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW) on spontaneous locomotor activity in the open field test in rats. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n = 6 per group).

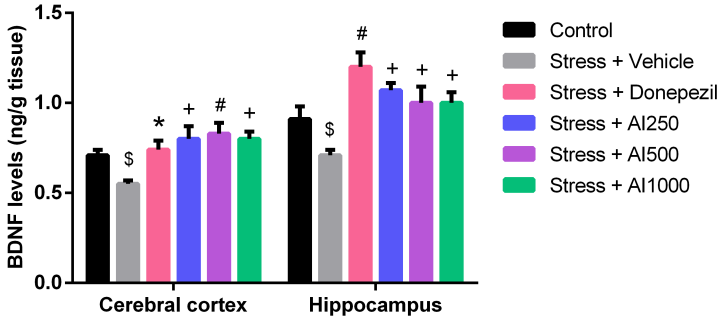

FIGURE 4. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW) on the brain-derived neurotrophic factor (BDNF) in rat's brain. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n= 6 per group). $^{\$}P< 0.05$ as compared with the control group; $^{*}P < 0.05$, $^{+}P < 0.01$, $^{\#}P < 0.001$ as compared to the stress + vehicle group.

stressed rats treated with vehicle had significantly lower level of BDNF in the cerebral cortex ($F_{(5,30)} = 4.337$, $P<0.05$) and the hippocampus ($F_{(5,30)} = 6.316$, $P<0.05$) compared to the control rats. When compared to the stressed rats administered with vehicle, the stressed rats treated with donepezil and *A. indica* flower extract at doses of 250, 500, and 1000 mg/kg BW had significantly higher BDNF levels in the cerebral cortex ($F_{(5, 30)} = 4.337$, $P<0.05$, $P<0.01$, $P<0.001$, $P<0.01$, respectively) and the hippocampus ($F_{(5, 30)} = 6.316$, $P<0.001$, $P< 0.01$, $P<0.01$, $P<0.01$, respectively).

Effect of A. indica flower extract on neuronal density in the cerebral cortex and the hippocampus

The effect of *A. indica* flower extract on neuronal density was investigated using cresyl violet staining in the

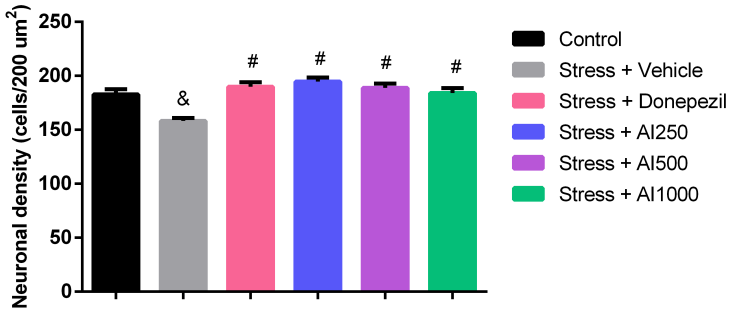

FIGURE 5. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW) on neuronal density in the cerebral cortex. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n = 6 per group). $^{\&}P < 0.001$ as compared with the control group; $^{\#}P < 0.001$ as compared to the stress + vehicle group.

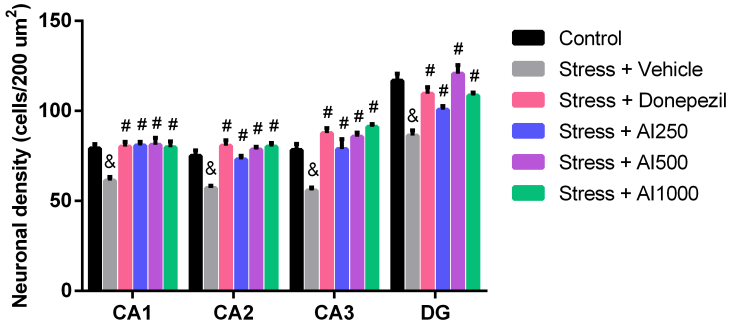

FIGURE 6. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW on neuronal density in the hippocampus. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n = 6 per group). $^{\&}P < 0.001$ as compared with the control group; $^{\#}P < 0.001$ as compared to the stress + vehicle group.

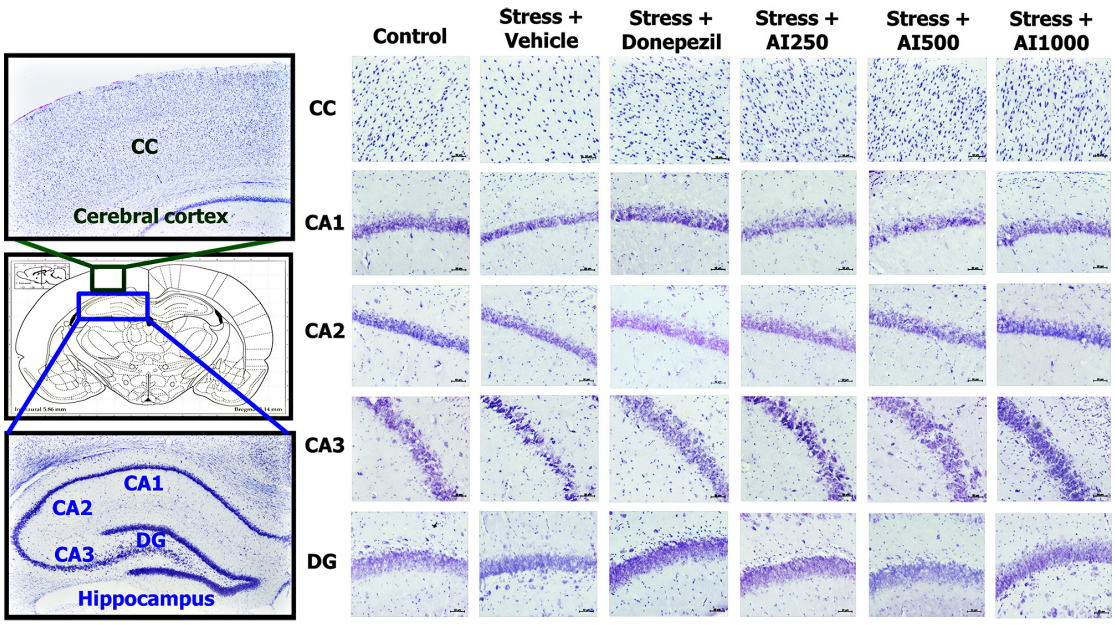

FIGURE 7. Images of the cerebral cortex (CC), Cornu Ammonis 1 (CA1), Cornu Ammonis 2 (CA2), Cornu Ammonis 3 (CA3), and Dentate Gyrus (DG) of the hippocampus histologically stained with cresyl violet at 4x and 20x magnifications. Scale bar: 50 µm.

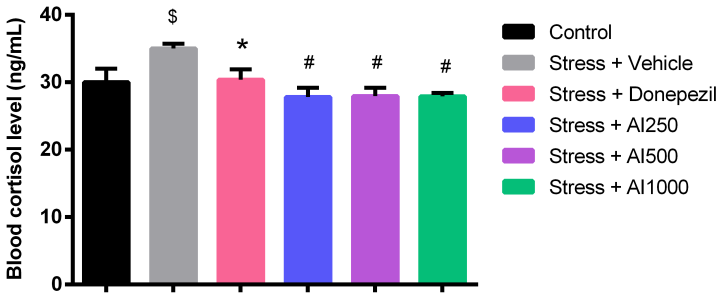

FIGURE 8. Effects of donepezil and *A. indica* (AI) flower extract (250, 500, and 1000 mg/kg BW) on blood cortisol levels. One-way analysis of variance was used for data analysis. Data are presented as mean ± S.E.M. (n = 6 per group). $^{\$}P < 0.05$ as compared with the control group; $^{*}P < 0.05$ and $^{\#}P < 0.001$ as compared to the stress + vehicle group.

cerebral cortex and the hippocampus. The results were shown in Figures 5 and 6, respectively. When compared to the control rats, the stressed rats had significantly lower neuronal density in the cerebral cortex ($F_{(5, 30)}$ = 10.10, P<0.001) and the hippocampus (CA1: $F_{(5, 30)}$ = 7.722, P<0.001; CA2: $F_{(5, 30)}$ = 15.11; P<0.001; CA3: $F_{(5, 30)}$ = 16.10, P<0.001; DG: 13.96, P<0.001). The stressed rats administered with donepezil and *A. indica* flower extract at doses of 250, 500, and 1000 mg/kg BW had higher neuronal density in both the cerebral cortex ($F_{(5, 30)}$ = 10.10, P<0.001 for all groups) and the hippocampus (CA1: $F_{(5, 30)}$ = 7.722, P<0.001 for all groups; CA2: $F_{(5, 30)}$ = 15.11; P<0.001 for all groups; CA3: $F_{(5, 30)}$ = 16.10, P<0.001 for all groups; DG: 13.96, P<0.001 for all groups) compared to the stressed rats treated with the vehicle. Figure 7 is representative images of the cerebral cortex, CA1, CA2, CA3, and DG histologically stained with cresyl violet at 4x and 20x magnifications. The scale bars of these images were 50 µm. The image of rat's brain was adapted from Paxinos and Watson atlas (Paxinos and Watson, 1997).

Effect of A. indica flower extract on blood cortisol levels

The results demonstrated that the stressed rats administered with vehicle had significantly higher blood cortisol levels ($F_{(5,30)}$ = 5.259, P<0.05) than the control group. In addition, the stressed rats administered with either donepezil or *A. indica* flower extract at doses of 250, 500, and 1000 mg/kg BW had significantly lower blood cortisol levels ($F_{(5,30)}$ = 5.259, P<0.05, P<0.001, P<0.001, and P<0.001, respectively) than the stressed rats administered with vehicle. Blood cortisol levels were presented in Figure 8.

Discussion

The cognitive-enhancing properties of *A. indica* flower extract against neuronal damage caused by persistent restraint stress were investigated in this study. The stressed rats were given *A. indica* flower extract once a day. After 30 min of treatment, they were put under restraint stress for 3 h daily for 30 days. CRS was found to impair cognitive hippocampus-dependent processes of learning and memory in animals (McLaughlin et al., 2007). Our results reported that the stressed rats had a considerably low discrimination index ratio indicating that restraint stress induced cognitive deficit in NORT (Sun et al., 2020). CRS altered the structures and functions of the cerebral cortex and the hippocampus by reducing neurogenesis, neuronal density, and synaptic plasticity (Woo et al., 2018; Kim and Diamond, 2002; Ortiz et al., 2014). CRS also disrupted BDNF signaling and HPA axis pathways (Numakawa et al., 2017).

In the central nervous system (CNS), BDNF is extensively expressed in the hippocampus and cortical regions and plays an important role in the maintenance of neurons (Martinowich and Lu, 2008). BDNF is mediatd by Tropomyosin receptor kinase B (TrkB), a high-affinity receptor for BDNF, and regulates neuronal survival (Huang and Reichardt LF, 2001). The BDNF/TrkB pathway is important for neurogenesis and synaptic plasticity in the CNS (Numakawa et al., 2017). Recent research suggested that BDNF plays a role in cognitive function, including memory acquisition and consolidation (Miranda et al., 2019).

The HPA axis is activated in response to stressful events and regulates the secretion of glucocorticoid hormones. Glucocorticoids (GCs), particularly cortisol, known as the stress hormone, are hormones that mediate

the stress response and regulate metabolism. The receptors of GCs (GRs) are distributed in the hippocampus and the prefrontal cortex (Lupien and McEwen, 1997; de Kloet et al., 1999). Therefore, the body's response to prolonged stress is involved with the brain's neuronal activity (McEwen et al., 2016). Chronic stress activated the HPA axis results in excessively high blood GCs levels, which might play a role in cognitive disorders (Khan and Khan, 2017; Ávila-Villanueva et al., 2020). It was well recognized that excessive cortisol accelerates neuronal toxicity leading to neuronal cell death (Behl et al., 1997).

GCs and BDNF affect neuronal growth and function. As a result, neurogenesis is influenced by both the BDNF/TrkB and the GCs/GRs systems (Numakawa et al., 2017). Many investigations found that injection of GCs has a negative effect on BDNF mRNA expression in the hippocampus and cortical regions (Smith et al., 1995; Vellucci et al., 2001). In neuron-like cells derived from mouse hippocampal cells, GCs exposure inhibited BDNF mRNA expression by binding GRs to regulate the BDNF gene (Chen et al., 2017). Recent research showed that BDNF/TrkB-related intracellular signaling modulates GRs activity, and intense GCs downregulate BDNF expression, resulting in suppression of neurogenesis (Numakawa et al., 2017). Our results indicated that the stressed rats given *A. indica* flower extract showed lower blood cortisol levels, but higher brain BDNF levels and neuronal density than the stressed rats given vehicle. Moreover, there were no significant differences in locomotor activity in any rat groups observed by OFT. However, administration of *A. indica* flower extract produced the same results in all assessments because the lowest concentration of the flower extract could be enough to produce therapeutic benefits and cognitive ability of the rats. As a result, a low-dose treatment was found to be both safe and effective.

According to previous studies, nimbin, azadirachtin, salannin, flavonoids, and quercetin were the antioxidant compounds found in *A. indica* extract (Eid et al., 2017; Alzohairy, 2016). Recent studies suggested that quercetin was the main active compound in *A. indica* flower extract (Duangjai et al., 2019; Hawiset et al., 2022). The amount of quercetin in the *A. indica* flower extract of our work was 7.654 ppm. According to in vitro experiments using Blood Brain Barrier (BBB) models, approximately 65.54% of quercetin permeated across the BBB (Ren et al., 2010). However, in vivo studies, low levels of quercetin were found in the brain tissue after the administration (de Boer et al., 2005). After ingestion, most of the quercetin was absorbed as metabolites in the forms of glucuronidated, methylated, and sulfated quercetin. Nevertheless, quercetin and its metabolites had been shown to have neuroprotective effects (Li et al., 2015; Shirai et al., 2006; Yeh et al., 2011). As a result, quercetin found in *A. indica* flower extract might protect against neuronal damage induced by chronic stress. The results of our work agreed with an in vitro investigation by Nakagawa and Ohta which reported that quercetin inhibits beta-amyloid synthesis in the stress conditions of amino acid deficiency, viral infection, endoplasmic reticulum stress, and heme deprivation (Nakagawa and Ohta, 2019). Quercetin could protect mice with Alzheimer's disease from cognitive deterioration (Wang et al., 2014). The rats with chronic stress for 6 h daily for 21 days exhibited impaired spatial learning and memory as assessed by the Morris water maze test. However, the administration of quercetin in the stressed rats improved recognition memory and hippocampal antioxidant enzyme activity (Mohammadi et al., 2014). Samed and coworkers reported that quercetin could protect mice against immobilization and stress-induced cognitive impairment by boosting antioxidant enzymes and 5-HT levels in the rats' brains (Samed et al., 2018). The serotonergic and dopaminergic projections innervate various cortical brain regions to regulate behaviors, as well as mood and cognition (Jenkins et al., 2016; Borwicka et al., 2020). DA promotes the proliferation and differentiation of neural stem cells and progenitor cells in adult brains by enhancing the formation of new neurons in the hippocampus (Ohira, 2017). Furthermore, 5-HT through 5-HT receptor subtypes increases adult cell proliferation and neurogenesis in the dentate gyrus (Djavadian, 2004). Recently, it was demonstrated that the administration of *A. indica* flower extract to stressed rats helps in reducing the symptoms of anxiety and depression by improving brain 5-HT and DA levels (Hawiset et al., 2022). Hence, quercetin found in *A. indica* flower extract could be the principal active compound in *A. indica* flower extract that improves cognitive performance in stressed rats.

The limitations of the current study were as follows: (1) The study focused on BDNF levels in the brain, but not TrkB receptor levels. Therefore, this study was unable to give information on the role of BDNF–TrkB

signaling in the regulation of neurogenesis and synaptic plasticity in the brain; (2) Our research did not investigate whether orally administered *A. indica* flower extract could cross the BBB. However, oral administration allowed the extract to be digested via the digestive system and produce quercetin metabolites and other compounds. When circulating in the blood vessels, some substances might cross through the BBB and be delivered to target cells, resulting in enhanced cognitive abilities.

Conclusion

Chronic stress impaired cognitive function by reducing survival neuron and nerve growth factors. Treating stressed rats with *A. indica* flower extract improved their memory by increasing BDNF levels, which governed neuronal density in the cerebral cortex and the hippocampus. Therefore, *A. indica* flower extract might operate as a neuroprotective agent, preventing neuronal loss caused by prolonged stress.

Acknowledgments

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

The Ethics Committee of the Laboratory Animal Research Center, Mae Fah Luang University approved this project on September 20, 2019 (Approval no. AR 03/62).

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