

Jobelyn[®], a Sorghum-Based Nutritional Supplement Attenuates Unpredictable Chronic Mild Stress-Induced Memory Deficits in Mice

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Abstract

The ability of an organism to adapt to aversive stressful situations or life challenging circumstances is very crucial to its state of health and survival. However, breakdown in adaptation due to persistent uncontrollable stress, leads to impairment of bodily functions and onset of a variety of pathological disorders especially memory decline. This study was designed to evaluate the effect of Jobelyn[®] (JB), a potent antioxidant sorghum-based food supplement on unpredictable chronic mild stress (UCMS)-induced memory impairment in mice. Male Swiss mice were given JB (5 - 50 mg/kg, p.o) 30 min prior to exposure to UCMS for 14 consecutive days before testing for memory. Thereafter, the serum corticosterone level was estimated by using ELISA kits. The levels of malondialdehyde (MDA) and glutathione (GSH) as well as acetylcholinesterase activity were estimated in the brain homogenate using spectrophotometer. Histology of the brain tissues and estimation of the populations of viable neurons in the hippocampal region were done after staining with hematoxyline and eosin. Our results showed that JB reversed memory impairment and suppressed corticosterone concentrations induced by UCMS. Moreover, JB reduced oxidative stress in the brain of UCMS-mice as shown by decreased MDA levels and elevated GSH concentrations. It also decreased

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brain acetylcholinesterase activity when compared with chronic stress group ($p < 0.05$). Furthermore, JB (5 - 10 mg/kg, p.o) offered significant protection against UCMS-induced degeneration and death of neuronal cells of the cornu ammonis 3 (CA3) of the hippocampal region of the brain indicating neuroprotection. Taken together, these findings suggest that JB attenuates memory deficits induced by UCMS in mice and may be useful therapeutically for stress-related cognitive dysfunctions. The reduction in the levels of serum corticosterone, antioxidation, neuroprotection and inhibition of cholinesterase enzyme might be contributing significantly to the positive effect of JB on memory in mice exposed to unpredictable chronic mild stress.

Keywords

Jobelyn[®], Unpredictable Chronic Mild Stress, Memory Performance, Oxidative Stress, Neuroprotection

1. Introduction

Stress is an integral component of human life, and is considered to be any condition which results in perturbation of the body's homeostasis [1]. Response to stress includes adaptation, but when stress persists over a long period, the homeostatic mechanisms of the organism become deficient resulting in the pathogenesis of a variety of diseases [2]-[4]. Stress affects almost every organs of the body and hastens the ageing process. The brain is the major regulator of stress response as it plays a critical role in the body's perception of stress [3] [4]. The effects of stress on memory include interference with the individual's ability to encode memory and retrieve vital information [2] [5].

The mechanism involved in stress-induced memory impairment is related to over secretion of cortisol by adrenal glands during stress response. The cortisol causes excess amounts of calcium to enter brain cells, which eventually leads to over production of free radicals that initiates the cascade for neuronal degeneration [6] [7]. Long-term exposure to cortisol has been reported to damage cells in the hippocampus, the brain region that plays a critical role in memory functions [6] [7]. This damage results in impaired learning and also inhibits memory retrieval of already stored information [7]-[9]. Consistent with the findings in humans, rats that are exposed to stress or given corticosterone have deficits in spatial memory [10]-[12]. Furthermore, chronic stress has been shown to impair hippocampus-dependent object recognition memory in both humans and rodents [13]. Moreover, exposure to UCMS produced permanent loss of neurons and atrophy of hippocampal dendrites over the course of weeks in rodents [6]-[8]. The stressors in the UCMS paradigm were applied in random order and at varying times in order to maximize unpredictability. Unlike other stress models, the UCMS mimics the ways humans encounter stressors on daily basis and thus more suitable for elucidation of pathological impacts of stress.

Although chronic stress has been shown to produce memory deficits for many centuries, drugs which could be used to mitigate stress-related cognitive decline are yet to be discovered. Thus, this study was designed to evaluate the effect of JB, a potent antioxidant food supplement, with stress relieving property, on memory impairment induced by UCMS in mice. JB is an African based preparation obtained from the leaf sheath of *Sorghum bicolor*, a plant widely cultivated for its nutritional and medicinal qualities [14] [15]. JB contains several biologically active constituents with antioxidation, anti-neuroinflammation and neuroprotection [14]-[17]. Moreover, JB has gained international recognition as a remedy for treatment of anaemia, arthritic pains and relief of stress [17]. We have previously reported that JB exhibited anti-depressant and anti-amnesic properties in rodents [18] [19]. In this present study, we report on the effect of Jobelyn[®] on unpredictable chronic mild stress-induced memory impairment in mice.

2. Materials and Methods

2.1. Experimental Animals

Male Swiss mice (20 - 22 g) used in the study, were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature and were allowed free access to commercial

food pellets and water *ad libitum*. They were acclimatized for 2 weeks before use for experimentations. The animals were handled in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and Preparation

Jobelyn[®]—JB (Health Forever Products Ltd., Lagos, Nigeria) and donepezil-DP (Pfizer, USA) were dissolved in distilled water immediately before use. The doses of JB and DP used in this experiment were chosen based on the results obtained from preliminary investigations.

2.3. Unpredictable Chronic Mild Stress (UCMS) Paradigm

The UCMS protocol was carried out in accordance with the method previously described by Yalcin *et al.*, [20] with slight modifications. After 2 weeks of acclimatization, mice were randomly distributed into 7 groups (6 per group): 1) non stress control group, 2) DP-treated group (1 mg/kg); 3) JB-treated group (5 mg/kg); 4) JB-treated group (10 mg/kg); 5) JB-treated group (25 mg/kg); 6) JB-treated group (50 mg/kg) and 7) Stress control group. Treatments were performed orally by gastric gavage 30 min before exposure to two of the following stressors in the UCMS protocol: damp sawdust (3 h), forced swim test (5 min), hypoxia (15 min), social defeat (30 s), no beddings in cage (overnight), starvation (overnight), tail suspension (6 min), predator (30 s), water deprivation (overnight) and noise (30 min). This procedure was repeated daily for 14 consecutive days before testing for memory performance. The stressors were applied in a random order and at varying times to maximize unpredictability. Mice in non-stress control group received distilled water (10 ml/kg) but were not subjected to UCMS. On the other hand, mice in the stress control group also received distilled water (10 ml/kg) but were subjected to UCMS.

2.4. Test for Memory Performance

The effect of JB on memory impairment induced by UCMS in mice was assessed using the Y maze paradigm according to the procedure earlier described by Casadessus *et al.* [21]. Briefly, stressed mice and non-stress counterparts were placed individually at arm A of the Y-maze and allowed to explore all the three arms freely for 5 min. The number and sequence of arm entries were recorded and the apparatus was cleaned after each test. An entry was scored when the four paws of the animals were completely in the arm of the Y-maze. The percentage alternation, which gives a measure of working memory, was calculated by dividing the total number of alternations by the total number of arm entries, minus two and multiplied by 100 [21]. An alternation behavior was defined as consecutive entries into all three arms (*i.e.* ABC, CAB or BCA but not BAB).

2.5. Biochemical Assays

After testing for memory, mice were decapitated under ether anaesthesia and the brains were immediately removed and kept in the refrigerator with ice block for 30 min. Thereafter, the whole brain was weighed and divided into two with half being used for biochemical assays and the other half for histo-morphology. The brain portion for biochemical assays were homogenized with 10% w/v phosphate buffer (0.1 M, pH 7.4). Each brain tissue homogenate was separated into various portions and used for the different biochemical assays.

2.5.1. Determination of Brain Glutathione (GSH) Concentration

The concentration of total reduced glutathione GSH in the mouse brain homogenate was determined using the method of Moron *et al.* [22]. Briefly, equal volume (0.4 ml) of the tissue homogenate and 20% trichloroacetic acid (TCA) (0.4 ml) were mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 ml) was added to 2 ml of 0.6 mM DTNB and the final volume was made up to 3 ml with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against blank reagent using spectrophotometer. The concentration of GSH in the brain tissues were expressed as micromoles per gram tissue ($\mu\text{mol/g}$ tissue).

2.5.2. Determination of Brain Levels of Malondialdehyde (MDA)

The brain MDA levels of mice subjected to UCMS was estimated according to the method of Okhawa *et al.* [23].

Briefly, 0.5 ml of distilled water and 1.0 ml 10% TCA were added to 0.5 ml of each of brain tissue homogenate and centrifuged at 3000 rpm for 10 min. Then 0.1 ml thiobarbituric acid (0.375%) was added to the supernatant. The mixture was placed in a water bath at 80°C for 40 min and then cooled to room temperature. Upon cooling, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ m}^{-1}\cdot\text{cm}^{-1}$ (Buege and Aust, 1978) and values were expressed as μmoles of MDA per gramme tissue.

2.5.3. Determination of Acetylcholinesterase (AChE) Activity in Mouse Brain

Acetylcholinesterase activity, an indicator for cholinergic nervous system activity, was measured according to the procedure previously described by Ellman *et al.* [24]. Briefly, aliquot of the brain homogenate (0.4 ml) was added to 2.6 ml of phosphate buffer (0.1 M, pH 7.4) and 0.1 ml of 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB). Then, 0.1 ml of acetylthiocholine iodide was added to the reaction mixture. The absorbance was read using a spectrophotometer at wavelength of 412 nm and change in absorbance for 10 min at 2 min interval was recorded. The rate of acetylcholinesterase activity was measured by following the increase of colour produced from thiocholine when it reacts with DTNB. The change in absorbance per min was determined and the rate of acetylcholinesterase activity was calculated and expressed as $\mu\text{moles}/\text{min}/\text{g}$ tissue.

2.5.4. Estimation of Serum Corticosterone Levels

After behavioral testing, 1 ml of blood sample was obtained through cardiac puncture from both chronic stressed mice and non-stress counterparts under ether anesthesia for the determination of serum corticosterone levels. The serum corticosterone (ng/ml) level was estimated using ELISA kit (Oxford Biomedical Research, USA) according to the manufacturer's instructions. Briefly, blood sample was centrifuged at 3000 rpm for 15 min and serum was collected for estimation of corticosterone levels. Samples, standards, controls and Cortisol-HRP conjugate were added to a micro-plate coated with mAb to cortisol and incubated at room temperature for 1 hr. The bound cortisol-HRP was measured using tetramethylbenzidine (TMB) substrate. The TMB (150 μl) substrate was added to each well and incubated at room temperature for 30 min and the reading was taken at 650nm using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional plate reader equipped with Softmax Pro v 5.4 (SMP 5.4), and a 5-parameter sigmoid minus curve fit determined unknown concentrations.

2.6. Histological Studies of the Hippocampal Region of UCMS-Mice

The brain tissues were fixed for 7 days in 10% formaldehyde. Thereafter, the tissues were trimmed by sharp sagittal cutting of the anterior one-third section. They were further processed by passing through the process of dehydration, clearing, infiltration, embedding, sectioning and staining with H & E. The slides were then viewed with an Olympus CH (Japan) binocular microscope for histomorphology of the cornu ammonis (CA3) of the hippocampal region of mice brains. Photomicrographs were acquired with a Sony DSC-W 3 digital camera (Japan).

2.7. Determination of Neuronal Density of the Hippocampal Region of UCMS-Mice

The density of neuronal cells was estimated by counting the number of surviving cells in the pyramidal layer of the CA3 of the hippocampal region of the brain. The surviving neuronal cells were counted within a given square area of the circular view in a section utilizing the eyepiece of an Olympus CH (Japan) binocular microscope at x 400 magnification and graticule as described by Sugihara *et al.* [25]. Six separate measurements were made on each section from all the experimental and control groups, and the mean of the sum of each of the dimensions obtained was then calculated. The surviving neuronal cells were defined as round-shaped, cytoplasmic membrane-intact cells, without any nuclear condensation or distorted aspect.

2.8. Statistical Analysis

Data were analyzed using Graph Pad Prism software version 4.0 and expressed as mean \pm S.E.M. Statistical analysis was done using one-way ANOVA, followed by Newman-Keuls post-hoc test. *P* values <0.05 were considered statistically significant.

3. Results

3.1. Effect of Jobelyn® on Memory Impairment Induced By Unpredictable Chronic Mild Stress

There was a significant ($p < 0.05$) reduction in alternation behaviors in mice subjected to UCMS when compared with non-stress control, which suggest impairment of memory performance (Figure 1). One-way ANOVA showed that there were significant differences between treatment groups: memory [$F(7, 40) = 6.699$; $p < 0.0001$]. However, JB (5 - 20 mg/kg, p.o) significantly ($p < 0.05$) improved memory performance when compared with stressed group, suggesting positive effect on cognition. As shown in Figure 1, DP; standard anti-AD drug also produced significant ($p < 0.05$) improvement in memory in mice exposed to UCMS.

3.2. Jobelyn® Reduces Oxidative Stress in the Brain of Mice Exposed to Unpredictable Chronic Mild Stress

The effects of JB on MDA and glutathione concentrations in the brain of mice subjected to UCMS are shown in Table 1. One-way ANOVA showed that there were significant differences between treatment groups; MDA [$F(7, 40) = 5.514$; $p = 0.0002$] and GSH [$F(7, 40) = 14.12$; $p < 0.0001$]. There was a significant ($p < 0.05$) increase in MDA concentrations accompanied by depletion of GSH in mice subjected to chronic stress, which indicate increased oxidative stress in the brain. However, the increased oxidative stress in the brain was reduced by JB (5, 10, 25 and 50 mg/kg), as shown by a significant ($p < 0.05$) suppression of MDA and elevation of GSH levels in the brain of mice subjected to UCMS indicating antioxidant activity (Table 1).

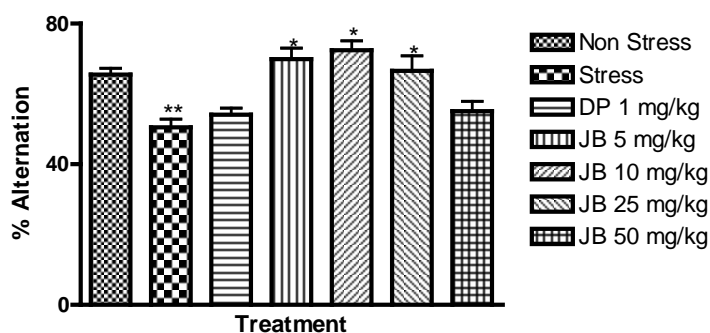


Figure 1. Effect of Jobelyn® on unpredictable chronic mild stress (UCMS)-induced memory impairment in mice. Values represent the mean \pm S.E.M for 6 animals per group. * $p < 0.05$ compared to stress control group, ** $p < 0.05$ compared to non-stress control group (ANOVA followed by Newman Keuls test).

Table 1. Effect of Jobelyn® on malondialdehyde (MDA) and glutathione (GSH) concentrations in the brain of mice exposed to unpredictable chronic mild stress.

Treatment Group	Dose (mg/kg)	(MDA $\mu\text{mol/g}$ tissue)	(GSH $\mu\text{mol/g}$ tissue)
Non stress control	-	20.68 \pm 2.86	30.18 \pm 1.61
Stress control	-	32.82 \pm 2.76**	22.33 \pm 1.35**
JB	5	20.86 \pm 2.52*	33.68 \pm 1.51*
JB	10	18.35 \pm 1.87*	40.73 \pm 2.91*
JB	25	16.52 \pm 1.95*	46.52 \pm 2.01*
JB	50	23.96 \pm 2.65*	38.22 \pm 2.14*
DP	1	28.75 \pm 1.53	26.85 \pm 2.27

Values represent the mean \pm S.E.M for 6 animals per group. * $p < 0.05$ compared with chronic stress group, ** $p < 0.05$ compared with non-stress group (ANOVA followed by Newman Keuls test).

3.3. Effect of Jobelyn® on Brain Acetylcholinesterase Activity

The effect of Jobelyn® on brain acetylcholinesterase activity in mice subjected to UCMS is presented in **Table 2**. One-way ANOVA showed that there were significant differences between treatment groups: [F (7, 40) = 4.136; p = 0.0017]. As in **Table 2**, there was a significant increase in the activity of AChE in the brain of mice subjected to UCMS, which suggests reduced brain levels of acetylcholine. However, JB (5 - 50 mg/kg, p.o) given daily for 14 days produced significant (p < 0.05) inhibition of AChE activity in the brain of UCMS-mice (**Table 2**). DP (1 mg/kg, p.o) also significantly (p < 0.05) reduced the activity of the enzyme in comparison with chronic stress-mice.

3.4. Jobelyn® Reduces Serum Corticosterone Level in UCMS-Mice

The effect of Jobelyn® on serum corticosterone level in mice exposed to UCMS is shown in **Table 3**. One-way ANOVA revealed that there were significant differences between treatment groups: [F (7, 40) = 3.429, p = 0.0142]. Post-hoc analysis by Newman Keuls test showed that there was a significant (p < 0.05) increase in the concentration of serum corticosterone in chronic stressed group when compared with non stress group. However, the increase in the concentration of serum corticosterone produced by UCMS was reduced by JB in a significant manner (**Table 3**).

3.5. Effect of Jobelyn® on Neuronal Density of the Hippocampus Region of UCMS-Mice

The effect of JB on the population of neuronal cells of the hippocampal regions of UCMS-mice is shown in **Figure 2**. One-way ANOVA revealed that there were significant differences between treatment groups: [F (5, 30) = 21.24; p < 0.0001]. Post-hoc analysis by Newman Keuls test showed that chronic stress significantly (p < 0.05) decreased the population of viable neuronal cells in the pyramidal layer of the CA3 when compared with non-stress control, which suggests neurodegeneration (**Figure 2**). However, as shown in **Figure 2**, JB (5 - 10 mg/kg, p.o)

Table 2. Effect of Jobelyn® on acetylcholinesterase (AChE) level in mice exposed to unpredictable chronic mild stress.

Treatment	Dose (mg/kg)	AChE (UCMS) $\mu\text{mol}/\text{min}/\text{g}$ tissue
Non stress control	-	354.00 \pm 8.27
Stress control	-	388.20 \pm 8.45**
JB	5	337.80 \pm 12.52*
JB	10	328.70 \pm 7.45*
JB	25	346.80 \pm 9.93*
JB	50	375.2 \pm 10.28
DP	1	344.50 \pm 12.33*

Values represent the mean \pm S.E.M for 6 animals per group. *p < 0.05 compared to stress control group, **p < 0.05 compared to non-stress control group (ANOVA followed by Newman Keuls test).

Table 3. Effect of Jobelyn® on corticosterone level in the brain of mice exposed to unpredictable chronic mild stress.

Treatment Group	Dose (mg/kg)	Serum Corticosterone Levels (ng/ml)
Non stress control		4.70 \pm 0.11
Stress control		7.24 \pm 0.11**
JB	5	4.27 \pm 0.17*
JB	10	4.62 \pm 0.27*
JB	25	4.85 \pm 0.17*
JB	50	4.87 \pm 0.26*
DP	1	4.17 \pm 0.72*

Each value represents Mean \pm S.E.M for 6 animals per group. **p < 0.05 compared with non-stress control (ANOVA followed by Newman Keuls test), *p < 0.05 compared with chronic stress (ANOVA followed by Newman Keuls test).

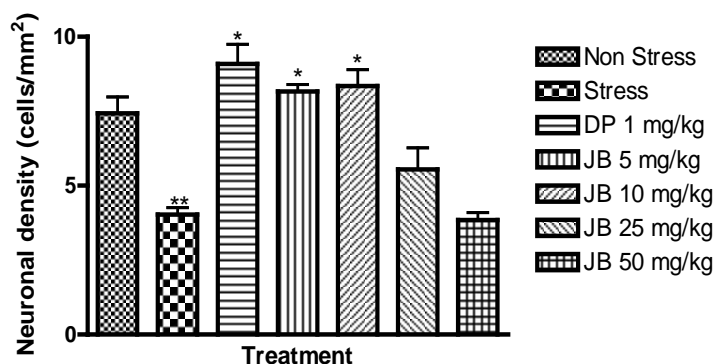


Figure 2. Effect of Jobelyn® on the density of neuronal cells in pyramidal layer of the CA3 of the hippocampus of mice exposed to unpredictable chronic mild stress. Each column represents mean \pm S.E.M for 6 animals per group. ** $p < 0.05$ compared with non-stress control (ANOVA followed by Newman Keuls test), * $p < 0.05$ compared with chronic stress group (ANOVA followed by Newman Keuls test).

significantly ($p < 0.05$) attenuated the loss of neuronal cells of the hippocampal region of the brain of mice exposed to UCMS, which further indicates neuroprotection.

3.6. Effects of JB on Histological Alterations of the Hippocampus of Mice Brains Subjected to UCMS

Figure 3 showed the photomicrographs of the effects of JB on UCMS-induced histological changes in the CA3 of the hippocampal region of the brain of mice. As shown in **Figure 3**, there were normal histological patterns of the cellular layers and pyramidal neurons of the hippocampal region with scattered multiple pyknotic neurons of the brain of mice in non-stress group. However, UCMS produced histological changes of the cellular layers and pyramidal neurons characterized by distorted cytoarchitecture in hippocampal CA3 region (**Figure 3**). As shown in **Figure 3**, JB (5 - 10 mg/kg, p.o) offered significant protection against UCMS-induced hippocampal injury suggesting neuroprotective effect.

4. Discussion

The ability of an organism to adapt to aversive stressful situations or life challenging circumstances is very crucial to its state of health and survival. However, breakdown in adaptation due to persistent stress, leads to impairment of bodily functions and onset of a variety of pathological disorders including memory decline [4]. Both preclinical and clinical studies have shown that prolonged stress plays a prominent role in memory deteriorations and represents the major risk factor that triggers AD pathology [4]. In this study, the effect of JB on stress-induced memory impairment was investigated in mice exposed to UCMS using Y maze paradigm. The Y maze test is routinely used to measure memory function based on the ability of rodents to remember the sequence of arms entry known as alternations [26]. The results of this study showed that UCMS impaired memory performance, as measured by decreased alternation behaviors of mice on the Y-maze task. Our results further confirmed earlier studies, which showed that chronic stress impairs spatial memory performance in rodents [10] [11] [13]. Thus, the finding that JB significantly increased the degree of alternation behaviors suggests improvement of memory in mice exposed to UCMS. This finding further supports previous studies that JB has memory enhancing effect and reversed cognitive deficits induced by scopolamine [19].

UCMS was found to increase brain AChE activity, which is an enzyme responsible for degradation of acetylcholine (ACh), the transmitter substance responsible for cognitive function [27]. Previous studies have shown that prolonged stress depletes brain ACh stores and impairs intellectual capability, an effect that may be related to induction of AChE activity [28]-[30]. Also, it is well established that central cholinergic system plays an important role in learning and memory, and thus maintaining brain ACh level is critical for cognitive function [31]. Moreover, memory deficits associated with AD is known to be related to the degeneration of cholinergic pathways in the brain [31]. Thus, current therapeutic approach for treating AD is the use of AChE inhibitors such as

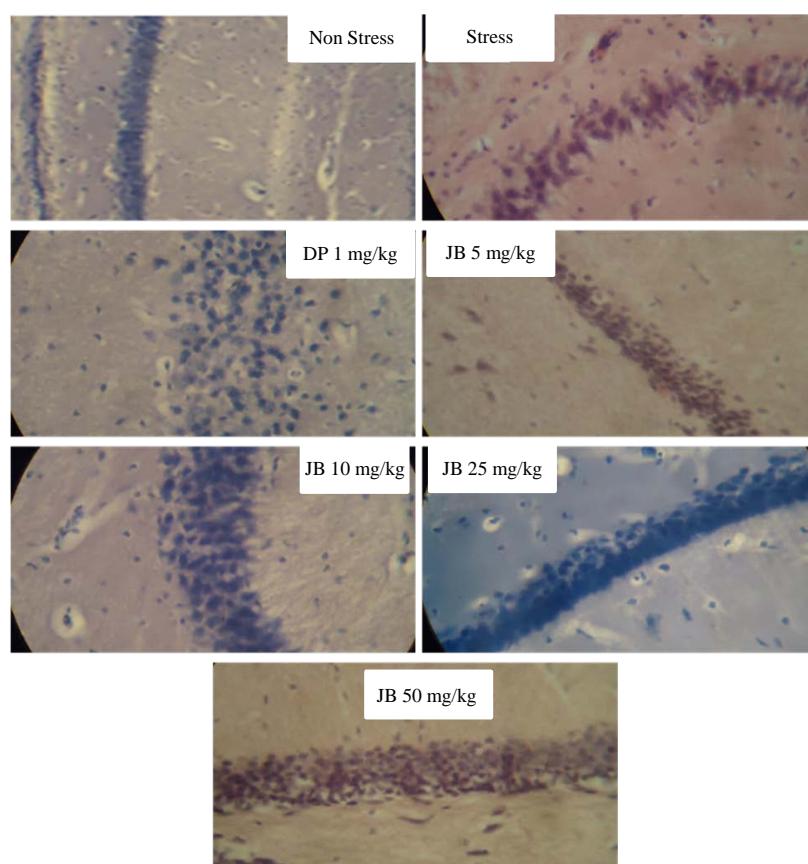


Figure 3. Effect of Jobelyn[®] (JB) and donepezil (DP) on the representative stained sections of cornu ammonis 3 (CA3) of mice brain exposed to unpredictable chronic mild stress H & E x 400.

donepezil, rivastigmine and galantamine to increase the availability of ACh in the central cholinergic synapses [31]. The antagonism of chronic stress-induced spatial memory impairment in rats on Y-maze task by tianeptine further confirms the potential benefits of AChE inhibitors in the prevention of persistent stress-induced cognitive decline [32]. Thus, the positive effect of JB on memory performance in stressed animals may also be related to enhancement of central cholinergic neurotransmission through inhibition of AChE activity.

Both preclinical and clinical studies have shown that memory loss due to prolonged stress is due to excess secretion of cortisol by the adrenal glands [4] [6] [7]. Excess amount of cortisol is the major mediator of memory impairment induced by chronic stress. Excess cortisol is known to divert blood glucose to exercising muscles and thus reduces the amount of energy that reaches the hippocampus. This creates an energy crisis in the hippocampus, which makes it unable to create new memories and explain why short-term memory is usually the first casualty of age-related memory loss resulting from a lifetime of stress [7]. Cortisol also interferes with the function of neurotransmitters, especially acetylcholine by causing loss of cholinergic neurons [7]. Cortisol also causes excess production of free radicals, the reactive molecules that initiate the cascade for neuronal degeneration and atrophy of the hippocampal cells accompanied by cognitive decline [4] [7]. The hippocampus is very sensitive to cortisol because of its high expression of glucocorticoid receptors and very susceptible to stress-induced damage. Hippocampus plays a prominent role in the feedback mechanism that regulates cortisol production; thus a damaged hippocampus causes cortisol levels to get out of control, which further compromise memory and cognitive function [6] [11] [33]. Consistent with the findings in humans, rats that were exposed to stress or administered corticosterone have deficits in spatial memory [10]-[12]. Previous studies have shown that the exposure of rodents to unpredictable chronic mild stress (UCMS) caused permanent loss of neurons and atrophy of hippocampal dendrites over the course of weeks in rodents [8] [33] [34]. The results of this study collaborated previous investigations, as UCMS was found to produce an increase in oxidative stress accompanied by dege-

neration and death of neuronal cells of the hippocampal region of the brain.

There are several evidences that support the use of antioxidant supplementation in the prevention or treatment of age-related disorders [35] [36]. Antioxidants could produce improvement in cognitive function by protecting neurons against the injurious effects of reactive oxygen species [35] [36]. Oxidative stress occurs in brain tissues; whenever there is increased generation of reactive oxygen species and impaired antioxidant defense mechanisms [35] [37]. Brain tissue is more susceptible to the damaging effects of ROS because it has small amounts of protective antioxidant defense systems [35] [37]. The findings that JB reduced brain levels of MDA and elevated GSH; major biomarkers of oxidative stress might also be contributing to its memory promoting effect. By virtue of its antioxidant and corticosterone suppressing effects, JB might protect neurons against oxidative stress-mediated injury and prevent stress-induced memory loss. This speculation is supported by the histological findings that JB attenuated UCMS-induced degeneration and death of neuronal cells of the hippocampal region of the brain, which indicates neuroprotection. Moreover, JB increases the number of surviving neurons of the hippocampal region of the brain of mice exposed to UCMS, which further suggest neuroprotective effect.

The current results provide evidences, which suggest that memory improvement produced by JB in mice exposed to UCMS may be related to its anti-cholinesterase, antioxidant and neuroprotective effects. Moreover, the finding that JB suppressed the levels of serum corticosterone; a major indicator of stress response in UCMS-mice further supports its potential utility for the treatment of conditions associated with stress-related disorders like AD. In addition, JB have been reported to contain various bioactive compounds such as naringenin, apigenin and luteolin which have been reported to exhibit antidepressant, anti-neuroinflammation, anti-oxidant and cytoprotective effects [14] [15] [17] [38]. Apigenin and naringenin were also reported to exhibit analgesic activity in various acute and chronic animal models of pain [39] [40]. Also, naringenin and luteolin were shown to increase memory performance and to reverse scopolamine-induced amnesia in rodents through inhibition of AChE activity and oxidative stress-related processes [38] [41]. Furthermore, compounds with antioxidant and neuroprotection are currently being investigated as potential therapeutic agents for the treatment of age-related disorders like AD [35] [42] [43]. However, it remains to be established which of these chemical constituents might be playing a prominent role in the ability of JB to ameliorate UCMS-induced memory impairment in mice.

5. Conclusion

The results of this study provide evidence suggesting that JB promotes adaptability to stress and reversed memory impairment induced by chronic stress in mice. The antioxidant, anti-cholinesterase and neuroprotective activities demonstrated by JB may be playing a significant role in its positive effect on memory performance in mice exposed to chronic stress. Furthermore, the finding that Jobelyn[®] suppressed the levels of corticosterone, a major indicator of stress response, in mice subjected to unpredictable chronic mild stress, further supports its use as energizer and in conditions associated with stress-related disorders.

Competing Interests

Authors declare that they have no competing interests.

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