

# Therapeutic Effects of *Spirulina platensis* Against Adolescent Stress-Induced Oxidative Stress, Brain-Derived Neurotrophic Factor Alterations and Morphological Remodeling in the Amygdala of Adult Female Rats

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**Objective:** The amygdala structural and functional abnormalities have been implicated in numerous neuropsychiatric and neurodevelopmental disorders. Given the important role of the amygdala in stress responses and the susceptibility of the females to adolescent stress, the present study investigated the beneficial effects of *Spirulina platensis* microalgae (SP) as a neuroprotective supplement against adolescent stress-induced oxidative stress, brain-derived neurotrophic factor (BDNF) alterations, molecular and morphological remodeling in the basolateral amygdala (BLA) of adult female rats.

**Methods:** During the adolescent period (PNDs 30–40) rats were subjected to restraint stress (2 h/day for 10 days). Then, the animals were subjected to 15 days treatment (PNDs 41–55) with SP (200 mg/kg/day) followed by biochemical (BDNF and stress oxidative markers), molecular (BDNF and its receptor tropomyosin receptor kinase B [TrkB] mRNA expression), and morphological (dendritic length and spines) assessments in the BLA.

**Results:** The study revealed that adolescent stress decreased BDNF levels and reduced apical dendritic length and branch points of pyramidal neurons in the BLA. In addition, chronic stress significantly increased oxidative stress parameters and decreased BDNF and TrkB mRNA expression in the BLA. Treatment with SP alleviated both biochemical, molecular, and neuroanatomical deficits that induced by adolescent stress.

**Conclusion:** Our findings provide important evidence that SP as a non-pharmacological intervention during adolescent period can protect against chronic stress-induced neuroanatomical biochemical, and molecular deficits in adulthood, and thus, reduce stress-related disorders.

**Keywords:** adolescent stress, amygdala, BDNF, dendritic remodeling, *Spirulina*, oxidative markers

## Introduction

Studies have demonstrated that stressful experiences, which occur during critical periods of brain development (pre-pubertal period) persistently change the responses to stressors as well as associate with increasing the vulnerability to stress-related disorders later in adulthood.<sup>1,2</sup> Pre-pubertal period is a time of continued brain maturation, particularly in limbic and cortical regions that characterized by

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changes in brain structure and function.<sup>3,4</sup> During this period substantial remodeling occurs in brain areas such as the prefrontal cortex (PFC), hippocampus, and amygdala which are involved in emotional and learning processes.<sup>5</sup> It has been suggested that the HPA axis in adolescent rat is fully developed, while other key brain areas (limbic and cortical regions) are still undergoing significant maturation processes.<sup>6,7</sup>

Rodent models have indicated more information about amygdala function and its role in emotional stress regulation. The amygdala facilitates the encoding of emotional memories by coordination with other brain areas particularly the prefrontal cortex and hippocampus.<sup>8</sup> The amygdala through interacting with other brain structures obviously regulates stress response and plays a key role in emotional and stress-related behavior.<sup>9</sup> Exposure to acute or chronic stress can induce morphological and functional changes in the amygdala and these changes play an important role in stress-induced neuropsychiatric disorders.<sup>10,11</sup> Animal studies have revealed that stress influences synaptic plasticity, morphological remodeling, neurotoxicity, and neurogenesis in the brain and all these stress-derived physiological effects can potentially influence cognitive functions.<sup>12,13</sup>

Adolescent stress increases anxiety behavior,<sup>14</sup> remodels corticolimbic structure,<sup>15</sup> and alters neural gene expression in adulthood.<sup>16,17</sup> It has been reported that depression-related behaviors are associated with reducing the expression of BDNF and its receptor TrkB.<sup>18</sup> BDNF is the primary neurotrophin family that has emerged as a major synaptogenesis regulator directly involved in synaptic plasticity underlying cause the learning and memory in the brain.<sup>19</sup> In a previous study, we showed that exposure of female rats to restraint stress during adolescence decreased hippocampal BDNF and reduced apical dendritic length and branch points of CA3 pyramidal neurons in adulthood.<sup>20</sup> Several lines of studies suggest that adolescent stress alters the morphology of pyramidal neurons in the prelimbic region of the mPFC.<sup>1,21,22</sup> It has been reported that chronic immobilization stress induced dendritic atrophy and debranching in CA3 pyramidal neurons of the hippocampus but these events were opposite in the amygdala.<sup>15</sup> Several lines of evidence indicated that females typically are more susceptible to stress effects than the male; it seems that the risk of depressive disorders in females is higher than the males.<sup>23–25</sup>

In the present research, we are looking to find effective and non-pharmacological therapies for alleviating the

detrimental effects of adolescent stress. Plants and natural products have been used as therapeutic agents worldwide. On the search for non-pharmacological compounds, *Spirulina platensis* (SP) is considered as a novel and natural agent with potent antioxidant properties. SP is a marine microalgae which belongs to the class of cyanobacteria widely used as traditional intervention, known to contain various antioxidants, especially phycocyanin and basic necessary nutrients has been used as a natural food supplement.<sup>26,27</sup> SP exerts various effects including anti-inflammatory, antioxidant, antimicrobial, antitumoral, and immunomodulatory properties.<sup>28–31</sup> Studies suggest that *Spirulina* has protective effects in the treatment of neurodegenerative disorders.<sup>32,33</sup> It has been reported that *Spirulina* reduces oxidative stress in the hippocampus and protects against damaging neurobehavioral effects of systemic kainic acid.<sup>34</sup> SP enhanced the beneficial effect of exercise on oxidative stress and lipid profiles in rats.<sup>35</sup> The antioxidant property of *Spirulina* is ascribed to phycocyanin component, which is a potent antioxidant. It has been shown that administration of SP alleviates neuropathic pain induced by chronic constriction injury (CCI) through increasing FRAP levels in male rats.<sup>36</sup> Previous findings from our group indicate that SP has protective effects against adolescent stress-induced deficits in learning and memory, hippocampal BDNF and morphological remodeling in adult female rats.<sup>20</sup> In another study, we showed that SP prevents scopolamine-induced memory impairment in young female rats.<sup>37</sup>

To date, the effects of SP on biochemical, molecular, and morphological changes in basolateral (BLA) amygdala in the adult brain have not been investigated. Therefore, the present study investigated the effects of SP against adolescent stress-induced oxidative stress, BDNF alterations, molecular and morphological remodeling in the BLA of female rats in adulthood.

## Materials and Methods

### Animals

Wistar female adolescent rats (30 days old, 60–70 g) obtained from the breeding colony of the Semnan University of Medical Sciences (SUMS), Semnan, Iran. Animals were maintained at 12-h light/dark cycle and in a controlled room temperature ( $22 \pm 2^\circ\text{C}$ ) and, housed 5 to 6 per a cage ( $27 \times 21 \times 14$  cm) given free access to food and water ad libitum. All experiments were approved by the Ethics Committee of Semnan University of Medical Sciences

(IR.SEMUMS.REC.1394.208). The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

## Experimental Groups

Rats randomly were distributed into 4 following groups (10 animals in each group). Control group: rats received only oral physiological saline using gavage tube; SP group: rats received 200 mg/kg SP per day for 15 days using gavage tube (PNDs 41–55); Stress group: rats exposed to 10 days restraint stress (PNDs 30–40) and Stress + SP group: rats exposed to stress and then received SP for 15 days (PNDs 41–55). Biochemical assessments began at the PND 61 after the termination of all intervention procedure. In each group, half of the animals were used for histology assessment and half of them were used for measurement of oxidative stress markers (left BLA) and BDNF levels, and BDNF and TrkB mRNA expression (right BLA) (Figure 1, Timeline of experiments).

## Spirulina platensis

*Spirulina platensis* (SP) was purchased as pure powder from Setendorf Company, Jask, Bandar Abbas, Iran (Product No: SPM-12462016). *Spirulina* administrated at a dose of 200 mg/kg per day by oral gavage for 15 days (PNDs 41–55). The volume of gavage solution was 1 mL per rat. The control and stress rats received distilled water alone and solutions were refreshed daily. The dose of SP was chosen based on dose-response effects of SP in our previous study.<sup>20</sup>

## Stress Induction

Rats were exposed to restraint stress (2 h/day for 10 days, PNDs 30–40) between 10:00 Am and 12:00 Am.<sup>15,20</sup> During the restraint, each rat was restrained in a clear polyethylene cylinder. The cylinder size was adjusted

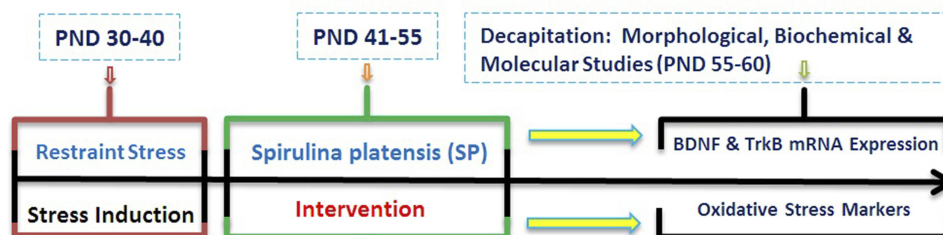
according to the size of the rats. A hole in the front of the cylinder was set to allow the animals to breathe freely.

## BDNF Measurements

The rats were decapitated, and the BLA was dissected and then immediately frozen at  $-70^{\circ}\text{C}$  until used for preparation of homogenates. The tissues were homogenate in cold lysis buffer and the supernatants, which were obtained after centrifugation at 12,000g for 20 min at  $-4^{\circ}\text{C}$ , were used for BDNF assay. The BDNF protein levels were assessed using Rat BDNF ELISA kits (Hangzhou Eastbiopharm Co., LTP) according to the manufacturer's recommendations.

## Molecular Study (RT-PCR)

The mRNA expression of BDNF and its receptor TrkB was determined by using RT-PCR method. The animals were decapitated after anesthesia and their brains were removed and the BLA rapidly dissected out on ice and flash frozen at  $-80^{\circ}\text{C}$ . RNA was reverse transcribed (RT) into cDNA by using a commercial cDNA synthesis kit (Bioneer Inc., Seoul, South Korea) and stored at  $-20^{\circ}\text{C}$  until further use. The primers were synthesized by Shenggong Biotech Limited Company (Shanghai, China). The sequences of the primers are presented in Table 1. Accuracy of the primers was confirmed by direct sequencing of target sequences. PCR was performed using a thermal cycler (ABI 7900HT, 96 wells, SYBR Green) consisting of denaturation at  $95^{\circ}\text{C}$  for 2 min, 45 additional cycles at  $94^{\circ}\text{C}$  for 30 s, and then 45 s of primer annealing at  $58^{\circ}\text{C}$  (for BDNF),  $56.5^{\circ}\text{C}$  (for TrkB), and  $53^{\circ}\text{C}$  (for GAPDH), followed by a final extension at  $72^{\circ}\text{C}$  for 8 min. All amplification was performed on Applied Biosystems RT-PCR (ABI 7900HT Fast Real-Time PCR System, US). Gene expression was normalized to the levels of GAPDH as a reference gene in all reactions and gene expression



**Figure 1** Timeline of Experiments. Rats were exposed to restraint stress (2 h/day for 10 days, PNDs 30–40). Then, the animals were subjected to SP treatment between PNDs 41 and 55 of age. Following the interventions, dendritic remodeling, BDNF levels, Oxidative stress markers and mRNA expression of BDNF and TrkB in amygdala were examined.

**Abbreviations:** PND, postnatal day; SP, *Spirulina platensis*; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B.

**Table 1** Primer Sequences Used in Quantitative Reverse Transcriptase Polymerase Chain Reaction

Primer Name	Primer Sequence (5'-3')	Product Size (bp)
BDNF	Forward: GAC CCT GAG TTC CAC CAG G Reverse: CCA GAG TCC CAT GGG TCC	165
TrkB	Forward: GAG TTG ACT ATG AGA CAA ACC C Reverse: GCA GCA TCA CCA GCA GGC	231
GAPDH	Forward: CTT CCA GGA GCG AGA TCC C Reverse: GTG GTT CAC ACC CAT CAC AAA C	267

was calculated by using the  $2^{-\Delta\Delta Ct}$  method. All primers were designed using AlleleID<sup>®</sup> version 7.5. Primer Select program (PREMIER Biosoft, USA) and synthesized by Bioneer Inc. (Seoul, South Korea). Accuracy of the primers was confirmed by direct sequencing of target sequences. All tests were conducted in duplicate for each sample.

## Histology and Dendritic Analysis

After completion of stress protocol and SP intervention, the animals were decapitated after anesthesia and their brains were removed quickly. The blocks of tissue containing the amygdala were dissected and processed for rapid Golgi staining technique as described previously.<sup>20</sup> Analysis of apical dendritic length and branch points of pyramidal neurons in the BLA was performed using an image J (1.48 version) software. For each animal, an average apical dendritic length and branch points within a 120- $\mu$ m thick section of each dendritic tree of 6–8 selected pyramidal neurons was calculated. From each experimental group, five animals randomly were selected for morphology analysis.

## Measurements of Oxidative Stress

### Markers

#### FRAP and MDA Activity Assay

Animals were decapitated under anesthesia and their brains were quickly removed, cleaned with ice-cold saline and stored at  $-80^{\circ}\text{C}$ . For ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) assays, a fraction of tissue was homogenized (1:10 w/v) in cold 1.15% KCl. Homogenates were prepared in a ratio of 100 mg tissue to 1 mL phosphate buffer (50 mmol/l; pH 7.5) containing 1 mM EDTA. The supernatants obtained after centrifugation at  $20,000\times g$  for 15 min at  $4^{\circ}\text{C}$  were used for biochemical analysis. The level of total protein in supernatants was determined by the Bradford method using bovine serum albumin as a standard.<sup>38</sup>

## Enzymes Activity Assay

SOD and GPx activities were measured in the supernatant by using ZellBio kits (Zellbio GmbH, Ulm, Germany). After adding reagents, samples, and standards into the wells, absorbance was measured at 0 and 2 mins with an ELISA microplate reader (AD Touch Reader, apDia, Belgium) at 412 nm. The concentration of SOD and GPx was expressed as IU/mg protein. The sensitivity of the assay for SOD and GPx was 1IU/mL and 5IU/mL, respectively.

## Statistical Analysis

Results are expressed as mean  $\pm$  S.E.M. The data were analyzed two-way ANOVA using SPSS software which followed by Tukey post-hoc test for multiple comparisons. The accepted level of significance for all tests was  $p < 0.05$ .

## Results

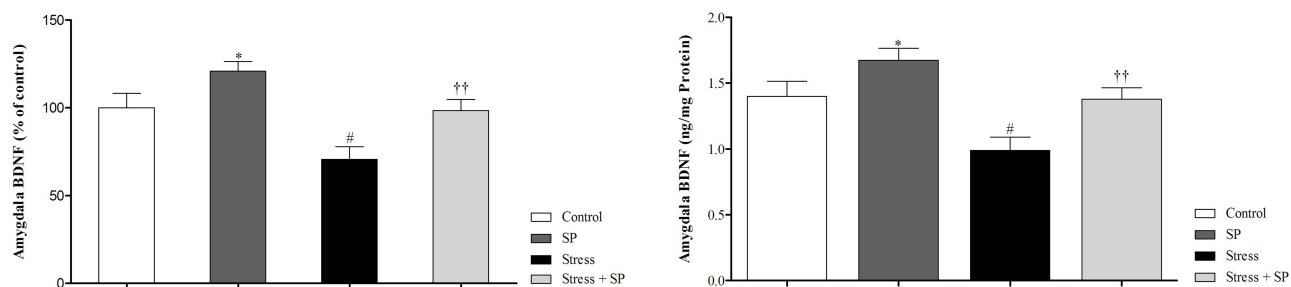
### Amygdala BDNF Levels

As shown in Figure 2, a two-way ANOVA analysis revealed significant effects of stress ( $F_{1, 16} = 14.260$ ,  $P = 0.002$ ), significant effect of treatment ( $F_{1, 16} = 12.584$ ,  $p = 0.003$ ) and no significant interaction between two factors ( $F_{1, 16} = 0.260$ ,  $p = 0.617$ ). Between-group comparisons indicated that the BDNF levels in the SP treated groups were significantly higher than the stress group ( $P = 0.001$ ). BDNF levels in the stress group were also significantly lower than those of the control group ( $P = 0.036$ ).

### BDNF and TrkB mRNA Expression in BLA

#### BDNF mRNA Expression

A two-way ANOVA on amygdala BDNF mRNA expression (Figure 3A) revealed no significant effect of stress ( $F_{1, 11} = 0.651$ ,  $p = 0.437$ ), a significant effect of treatment ( $F_{1, 11} = 21.597$ ,  $p = 0.001$ ), and no significant interaction between two factors ( $F_{1, 11} = 0.116$ ,  $p = 0.740$ ). Paired comparisons showed that amygdala BDNF mRNA



**Figure 2** Amygdala BDNF levels (% of control in left side, and actual data in right side) in stressed or non-stressed rats subjected to SP treatment. Chronic stress reduced amygdala BDNF, and treatment with SP restored this deficit. Data are expressed as mean±S.E.M. #P = 0.036 than the corresponding control group; ††P = 0.001 than the stressed group; \*P = 0.047 than the control group. Five animals were used per each group.

expression in the stress group was significantly lower than control group ( $p = 0.01$ ). Also, BDNF mRNA expression in the stressed treated group with SP were significantly higher than the stress group ( $p = 0.001$ ).

### TrkB mRNA Expression

Two-way ANOVA on amygdala TrkB mRNA expression (Figure 3B) revealed significant effect of stress ( $F_{1, 11} = 6.001, p = 0.032$ ), significant effect of treatment ( $F_{1, 11} = 16.156, p = 0.002$ ) and no significant interaction between two factors ( $F_{1, 11} = 0.003, p = 0.955$ ). Paired comparisons showed that TrkB mRNA expression in the stress group was significantly lower than the SP group ( $p=0.01$ ). Also, TrkB mRNA expression in the stressed treated group with SP was significantly higher than the stress group ( $p=0.001$ ).

### Morphology of BLA Neurons

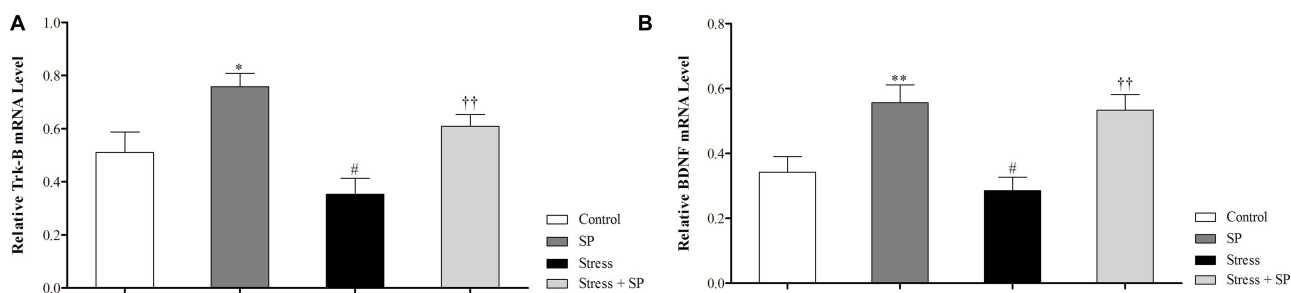
#### Apical Dendritic Length

A two-way ANOVA on apical dendritic length (Figure 4A) revealed a significant effect of stress ( $F_{1, 16} = 23.455, p = 0.0001$ ), a significant effect of treatment ( $F_{1, 16} = 372.015, p = 0.0001$ ) and a significant

interaction between two factors ( $F_{1, 16} = 92.875, p = 0.0001$ ). Paired comparisons showed that dendritic length in the stress group was significantly lower than control group ( $p = 0.0001$ ). Also dendritic length in the SP treated groups was significantly higher than the stress group ( $p = 0.0001$ ).

### Number of Dendritic Spines

A two-way ANOVA on number of dendritic spines (Figure 4B) revealed a significant effect of stress ( $F_{1, 16} = 0.542, p = 0.472$ ), a significant effect of treatment ( $F_{1, 16} = 372.015, p = 0.0001$ ) and a significant interaction between two factors ( $F_{1, 16} = 6.644, p = 0.020$ ). Paired comparisons showed that number of dendritic spines in the stress group was significantly lower than SP group ( $p = 0.0001$ ). Also dendritic spines in the SP treated groups were significantly higher than the stress group ( $p = 0.0001$ ). A photograph of computer-assisted and Golgi stained BLA neurons are presented in Figure 4C and D, respectively. These neurons were selected because they are representative of dendritic lengths near their respective group means.



**Figure 3** Amygdala BDNF (A) and TrkB (B) mRNA expression in stressed or non-stressed rats subjected to SP treatment. BDNF mRNA expression in the stress group was significantly lower than control group (A). Stressed rats showed decreased in TrkB mRNA expression than control group (B). Data are expressed as mean±S.E.M. In (A and B) #P = 0.01 than the corresponding control group; ††P = 0.001 than the stressed group; \*P = 0.049; \*\*P = 0.001 than the control group. Five animals were used per each group for molecular analysis.

## Activity of Antioxidant Enzymes

### SOD Activity

The data for the SOD level are illustrated in Figure 5A. A two-way ANOVA on SOD level revealed significant effects of stress ( $F_{1, 16} = 86.443, p = 0.0001$ ), of treatment ( $F_{1, 16} = 64.149, p = 0.0001$ ), but no significant interaction between two factors ( $F_{1, 16} = 0.520, p = 0.481$ ). Between-group comparisons indicated that SOD levels of the stress group were significantly higher than those the control, SP, and stress + SP groups ( $p = 0.0001$ ).

### GP<sub>x</sub> Activity

The data for the GP<sub>x</sub> level are illustrated in Figure 5B. Two-way ANOVA on GP<sub>x</sub> level revealed significant effects of stress ( $F_{1, 16} = 23.890, p = 0.0001$ ) of treatment ( $F_{1, 16} = 22.289, p = 0.0001$ ) and a significant interaction between two factors ( $F_{1, 16} = 4.573, p = 0.048$ ). Post-hoc comparison revealed that the GP<sub>x</sub> levels of the stress group were significantly higher than the control, SP, and stress + SP groups ( $P = 0.001, p = 0.0001$ ).

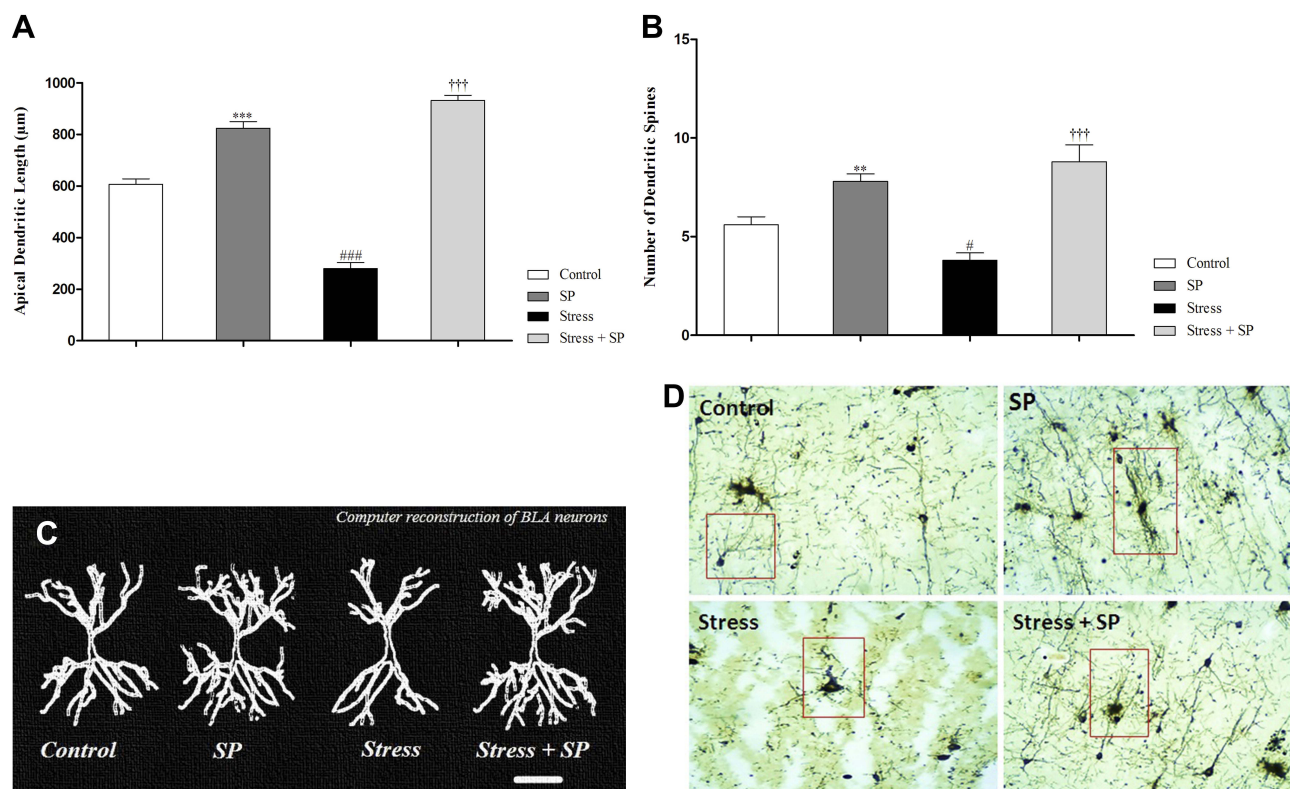
## Oxidative Stress Markers

### FRAP Levels

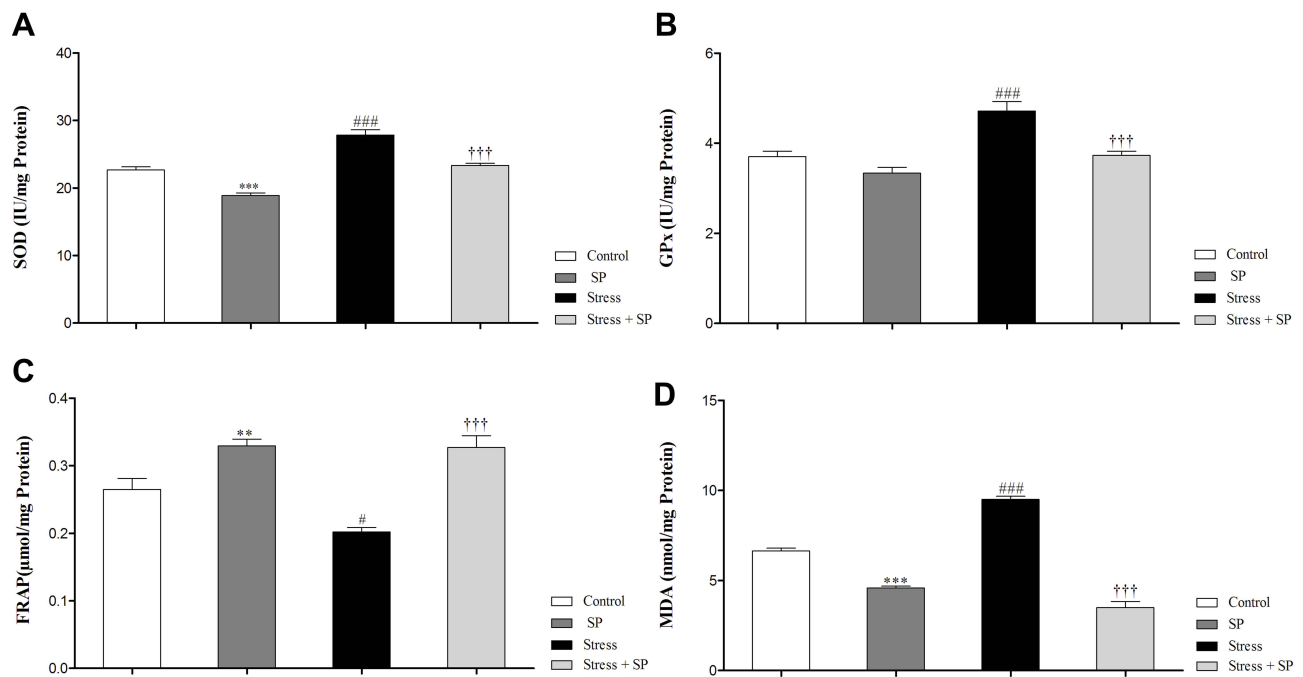
The data for the FRAP level are illustrated in Figure 5C. Two-way ANOVA on FRAP level revealed significant effects of stress ( $F_{1, 16} = 5.975, p = 0.026$ ), of treatment ( $F_{1, 16} = 50.529, p = 0.0001$ ) and a significant interaction between two factors ( $F_{1, 16} = 5.060, p = 0.039$ ). Post-hoc comparison showed that the FRAP levels of the stress group were significantly lower than the control, SP, and stress + SP groups ( $P = 0.01, p = 0.0001$ ).

### MDA Levels

The data for the MDA level are illustrated in Figure 5D. Two-way ANOVA on MDA level revealed significant effects of stress ( $F_{1, 16} = 17.610, p = 0.001$ ), of treatment ( $F_{1, 16} = 363.342, p = 0.0001$ ) and a significant interaction between two factors ( $F_{1, 16} = 87.308, p = 0.0001$ ). Post-hoc comparison showed that the MDA levels of the stress group were significantly higher than the control, SP, and stress + SP groups ( $p = 0.0001$ ).



**Figure 4** Apical dendritic remodeling of the BLA pyramidal neurons in stressed or non-stressed rats subjected to SP treatment. Stressed rats showed decreased dendritic length (A) and dendritic spines (B) in the BLA and treatment with SP alleviate these deficits. Computer-assisted reconstruction of BLA neurons (C). These neurons were selected because they are representative of dendritic lengths near their respective group means. (D) A photomicrograph of Golgi stained BLA neurons. Scale bar: 50 µm. Data are expressed as mean±S.E.M. In (A and B) #P = 0.01; ###P = 0.0001 than the corresponding control group; †††P = 0.0001 than the stressed group; \*\*P = 0.001; \*\*\*P = 0.001 than the control group. Five animals were used per each group for morphological analysis.



**Figure 5** Oxidative stress markers and antioxidant enzymes activity in the amygdala of stressed or non-stressed rats subjected to SP treatment. Stressed rats showed increased SOD (A) and GPx (B) activities. FRAP in the stress group was significantly lower (C) and MDA was significantly higher than control group (D). Data are expressed as mean±S.E.M. In (A, B, C, and D) #P = 0.01; ###P = 0.0001 than the corresponding control group; †††P = 0.0001 than the stressed group; \*\*P = 0.001; \*\*\*P = 0.0001 than the control group. Five animals were used per each group.

## Discussion

The present study for the first time investigated the effects of *Spirulina platensis* against adolescent stress induced biochemical, molecular, and neuroanatomical deficits in adulthood. Findings showed that adolescent stress decreased BDNF levels and reduced apical dendritic length and branch points of BLA neurons in adult female rats. In addition, adolescent stress induced oxidative stress and reduces the BDNF and TrkB mRNA expression in the amygdala. Treatment with *Spirulina platensis* alleviated these deficits and even exerted the positive effects on BDNF and TrkB mRNA expression and apical dendritic lengths in the amygdala of non-stressed animals.

In this study we found that chronic stress reduces BDNF levels and its mRNA expression in the amygdala, suggesting that downregulation of BDNF and its receptor in amygdala could be a risk factor for anxiety of depressive-like behaviors. The amygdala is one of the limbic structures involved in emotion and changes its structure and function following chronic stress may contribute to mood disorders like depression and anxiety. Recent studies indicated that BDNF may create an important link between stress and mental disorders.<sup>19,39</sup> It has been established that stress is a risk factor of mental diseases and has

a potent effect on signaling via BDNF.<sup>40</sup> Stress is a major risk factor in depression, and modulates BDNF expression. Studies have shown that acute or chronic stress disrupted BDNF signaling in the brain, mainly via decreasing its expression or release.<sup>41–43</sup> BDNF is one of the major neurotrophic factors in the brain that promotes neuronal viability during development and in adulthood. The effects of BDNF are mediated by the activation of intracellular signaling pathways upon high affinity binding to the TrkB receptor, a main member of tyrosine kinase receptors.<sup>44</sup> Our findings indicated that adolescent stress decreased BDNF and TrkB mRNA expression in the BLA reflecting the potential involvement of BDNF and its receptors in stress-induced depressive disorders. Treatment with *Spirulina platensis* normalized decreased mRNA expression of BDNF and its content in the BLA. In a recent study, we indicated that the combined treatment of *Spirulina platensis* with either environmental enrichment or physical exercise significantly increased hippocampal BDNF levels in female rats during adulthood.<sup>20</sup> Based on these findings, we assumed that BDNF and its receptor may be a target for adverse effects of stress on the amygdala, resulting that the impairment of amygdala induced by adolescent stress may be involved in the development of depression-like behaviors in the adulthood.

A growing body of research indicated that stress has profound effects on the morphology of corticolimbic structures including PFC, hippocampus, and amygdala.<sup>45–47</sup> Studies revealed that chronic stress alters hippocampal structure including modification of dendritic length and remodeling of dendritic spines in the CA3 region.<sup>46,48</sup> Ample pieces of evidence have demonstrated that chronic stress has powerful effects on the branching patterns of apical dendrites in mPFC and amygdala pyramidal cells, causing dendritic retraction in the PL and IL regions of the mPFC<sup>21,49</sup> but hypertrophy in the amygdala.<sup>15,45</sup> These morphological modifications involve increased corticosterone levels as a recent study showed that chronic treatment with highly physiological levels of cortisone for 10 days induced dendritic hypertrophy in the BLA.<sup>50</sup> These findings are opposite with our morphologic results showing that chronic restraint stress reduced apical dendrites length and dendritic spines in the BLA. This apparent discrepancy between these studies may be due to different factors such as duration of stress, sex, and age of animals (for example, female and juvenile rats in our study and male and adult rats in the study of Vyas et al 2002<sup>15</sup>), time of histological assessment, and experimental intervention following the stress. In our study, animals received stress between PNDs 30–40 and then morphological assessment was done between PNDs 55–60. While in the study of Vyas 2002,<sup>15</sup> morphological changes of BLA neurons were studied after completion of stress protocols. Clearly, more work needs to be done to elucidate these discrepancies.

The present study showed that treatment with *Spirulina platensis* alleviated adolescent stress induced dendritic retraction in the BLA pyramidal neurons. To date, there is little information about the effects of *Spirulina platensis* against stress-induced dendritic remodeling in the amygdala. The positive effects of *Spirulina platensis* on dendritic remodeling might be mediated through increasing BDNF levels. As we previously showed that *Spirulina platensis* combined with physical activity and enriched environment could reverse the cognitive-behavioral deficits induced by adolescent stress and increases the hippocampal BDNF in female rats during adulthood.<sup>20</sup> It was previously reported that *Spirulina platensis* through enhancing total antioxidant capacity improves scopolamine induced-memory deficit.<sup>36</sup> Recent studies have shown *Spirulina platensis* reduces oxidative stress in the hippocampus and protects CA3 neurons against damaging of systemic kainic acid.<sup>34</sup> *Spirulina platensis* has different

roles, such as anti-inflammatory, anti-oxidative, neuroprotective, and hepatoprotective properties.<sup>20,31,32,35</sup> Because of its neuroprotective and antioxidant properties, the findings of the present study suggest that the application of *Spirulina platensis* as a natural food supplement is useful to prevention the cognitive-behavioral impairment induced by stress.

Our findings indicated that adolescent stress induces oxidative stress in the BLA. Adolescent stress significantly decreased total antioxidant power, increased MDA production and enhanced the activity of antioxidant enzymes in the BLA. Stress through impairment of antioxidant defenses leads to oxidative damage in the central nervous system. Oxidative stress caused by the imbalance between the generation and detoxification of reactive oxygen and nitrogen species.<sup>51</sup> A growing body of evidence has demonstrated that the corticolimbic structures such as the PFC, hippocampus, and amygdala are highly vulnerable to oxidative stress.<sup>52,53</sup> It has been reported that chronic restraint stress increased plasma levels of corticosterone, induced oxidative stress in the hippocampus and impaired spatial learning and memory.<sup>54</sup> *Spirulina platensis* significantly modulates the levels of oxidative markers in the amygdala. We found that chronic stress increased lipid oxidation in the BLA, and this effect was blocked by *Spirulina platensis* treatment. We also found a significant reduction in total antioxidant power in the stressed animals, which was prevented by *Spirulina platensis* intervention. In line with earlier studies, we demonstrated a significant increase in the activities of antioxidant defense enzymes (SOD and GPx) in the BLA following chronic stress. The results of the present study can be interpreted in terms of two mechanisms: an elevation of glucocorticoid levels following chronic stress and a compensatory response to chronic stress. Therefore, the increased SOD and GPx activities observed in the present study are possibly adaptations to chronic stress. We have shown that *Spirulina platensis* decreases the activities of these enzymes in the BLA. This could be due to the fact that *Spirulina platensis* leads to reductions of reactive oxygen species, which in turn decreases the need for antioxidant enzymes. Another possibility is that *Spirulina platensis* directly modulates synthesis of the SOD and GPx, which needs further work to test this assumption. *Spirulina platensis* contains many carotenoids with powerful antioxidant effects and may protect amygdala neurons from oxidative damage.

*Spirulina platensis* has protective effects against oxidative stress and this effect is related to C-phycocyanin, an active ingredient of *Spirulina*, which has significant antioxidant properties.<sup>55,56</sup> There have been many reports on



the antioxidant properties of *Spirulina platensis* in different animal models.<sup>31,36,57</sup> It has been reported that *Spirulina* has protective effect against apoptotic cell death induced by free radicals DPPH and ABTS.<sup>57</sup> A study has shown *Spirulina platensis* through its antioxidant properties suppressed vascular ischemic injury in rats.<sup>58</sup> Given the wide variety of effects, it is suggested potential application of incorporating *Spirulina* into food products during pre-pubertal is useful to prevention stress-induced deficits in adulthood.

Our study has a few limitations. One limitation is the lack of sex hormone measurements during estrus cycle. It has been proved hormonal changes that occur with the estrus cycle may be correlated with alterations in both neuronal and behavioral indices in females.<sup>59</sup> However, due to the use of animals of the same age kept in similar conditions, the estrous cycle can be largely synchronized. Another limitation is the lack of an intact group (receiving no stress and gavage) as control. Although we used “no stress + gavage group” as a control for “stress + gavage group”, but the former group might display some of the stress effects and thus, the SP effects might be reduced.

## Conclusion

In summary, our study is the first to demonstrate the beneficial effects of *Spirulina platensis* against adolescent stress-induced oxidative stress, BDNF alterations, molecular and morphological remodeling in the amygdala of female rats. Our findings revealed that chronic stress decreased BDNF levels and reduced apical dendritic length and branch points of pyramidal neurons in BLA. In addition to biochemical and morphological changes, we observed that chronic stress induced oxidative stress and altered BDNF and TrkB mRNA expression in the amygdala. Based on our current and previous findings, it can be concluded that adolescent stress through reducing important neuronal growth factors and remodeling the limbic structures causes neuropsychiatric disorders in adulthood. So given the criticality of the adolescence period and sensitivity of the females to stress, our findings provide important evidence that neuroprotective herbal supplement such as *Spirulina platensis* during pre-pubertal period can be used as a non-pharmacological therapy to alleviate the harmful effects of stress in adulthood.

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## Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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