Original Article

Ginkgo biloba modulates hippocampal BDNF expression in a rat model of chronic restraint stress-induced depression

Seyed Abdolmajid Ayatollahi[1,2#], Shahrokh Khoshsirat[3#], Ali Asghar Peyvandi[3], Omidvar Rezaei[4], Fatemeh Zare Mehrjardi[5], Arezo Nahavandi[6], Somayeh Niknazar[3, 4*] iD

1. Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Hearing Disorders Research Center, Loghman Hakim Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Skull Base Research Center, Loghman Hakim Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Neurobiomedical Research Center, School of Medicine, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran
6. Neuroscience Research Center, Department of Physiology, Iran University of Medical Sciences, Tehran, Iran

Abstract

Introduction: Mood disorders such as depression and anxiety disorders have been affecting a relatively high proportion of the world's population. Neuroplasticity hypothesis of depression proposes that lack of brain-derived neurotrophic factor (BDNF) can cause structural changes in the brain. The extract of *Ginkgo biloba* (Gb) leaves can restore much of the damage in the nervous system. We examined the antidepressant role of Gb extract (EGb 761) on BDNF expression modulation in the hippocampus of rats subjected to repeated restraint stress (RRS).

Methods: Adult male rats were randomly divided into 10 groups: control, control-vehicle treated, stress, stress-vehicle treated, as well as three control and three experimental groups pretreated with EGb (15, 30, 60mg/kg, IP daily) for 21 days. They underwent restraint stress on a daily basis, 6 hours for 21 consecutive days. Weight changes, locomotor activity and forced swim test (FST) were employed to assess depressive-like symptoms. The serum corticosterone level was also measured by ELISA. Hippocampal BDNF DNA methylation and protein expression were assayed by methylation sensitive restriction enzymes (Real Time PCR) and Western-blotting respectively in all groups.

Results: Pre-treatment with 30 and 60 mg/kg/day of Gb extract significantly attenuated depressive-like effects in the body weight, FST and serum corticosterone level in RSS rats compared to control groups. Further, it inhibited chronic stress-induced alterations in the hippocampal BDNF DNA methylation and protein expression.

Conclusion: These findings suggest that Gb can induce an antidepressant role through its modulation effect on the hippocampal BDNF expression.

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Introduction

Mood disorders such as depression and anxiety disorders affect a relatively high percentage of the world population (Bakhtiarzadeh et al., 2018; Belmaker and Agam, 2003; Noori-Daloii et al., 2015; Mineka et al., 1998). Major depression disorder (MDD) is the third most common mental illness of the modern world. Evidence suggests that the prevalence of MDD is growing across the population (Hidaka, 2012). Depression is one of the most common mental disorders worldwide. Several hypotheses have been proposed for the mechanisms and pathophysiology of depression. However, despite extensive research, the pathophysiology and treatment of this disease are not fully understood. Post-mortem studies of brain structure in depressed patients have identified abnormalities such as hippocampal volume reduction, apoptosis and molecular changes (Malykhin and Coupland, 2015).

Brain-derived neurotrophic factor (BDNF) has been identified as a stress-responsive gene. The role of BDNF in stress-related disorders has been studied for several years. Evidence suggests that BDNF protects neurons under chemical stress conditions (Spina et al., 1992). In 1995, Smith et al. reported that BDNF expression in the brain was regulated by stress. Others have also determined the role of BDNF in recurrent depression, aging and post-traumatic stress disorder (Smith et al., 1995a; Smith et al., 1995b). The neuroplasticity hypothesis of depression suggests that altered synthesis and/or release of neurotrophins such as BDNF can contribute to the depression pathogenesis (Hashimoto et al., 2004). Environmental factors can also contribute to epigenetic changes in DNA that could influence gene activity, protein expression and organism function (Jaenisch and Bird, 2003). Epigenetic modifications are usually associated with DNA methylation or histone modifications that can contribute to increase or suppress gene transcription (Vaissière et al., 2008). Recent studies have indicated the epigenetic changes of the BDNF gene in response to external stimuli such as social stress and electroconvulsive seizures (Tsankova et al., 2006; Tsankova et al., 2004). Roth et al. (2011) indicated that chronic exposure to psychosocial stress induces hypermethylation of the promoter IV region of BDNF and in turn diminished BDNF expression in the rat hippocampus. In addition, increased DNA methylation exon IV in the hippocampus of mice was also associated with depression-related behaviour (Onishchenko et al., 2008). Today, medicinal herbs as one of the conventional approaches are applied to prevent and treat depression and anxiety disorders. The *Ginkgo biloba* (Gb) leaves and seeds were used in Chinese herbal medicine for centuries (Howes and Houghton, 2003; Sarris et al., 2011). The extract of Gb leaves can restore much of the damage to the nervous system (Chandrasekaran et al., 2001; Woelk et al., 2007). Although its role in improving depression in animals has been proposed, with respect to the complexity of chemical composition and multiple mechanisms of Gb, studies continue to determine the physiological basis of the Gb effect on depression. Based on the above-mentioned data, we examined the antidepressant effect of Gb through modulation of BDNF DNA methylation and protein expression in the hippocampus of rats.

Materials and methods

Animals

For this study, adult male Wistar rats (n=60, 230-250g, 3 months) were obtained from Shahid Beheshti Medical University, Animal Experiment Centre. They were allowed to adapt to the laboratory environment 1 week before the experiment was started. They were held in Type T IV cages (n=6) at constant temperatures (21±1°C) and 12:12 h light / dark cycle with free access to standard diet and water. Male rats were randomly assigned into 10 equal groups (n=6): control, control group treated with vehicle (saline, IP), stress, stressed group treated with vehicle (saline, IP), as well as three control and three experimental groups treated with EGb 15, 30, 60 mg/kg (dissolved in sterile saline, administered IP 30min before each stress episode). This study was confirmed by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.REC.1396.95) based on National Institutes of Health Publication guide for the care and use of laboratory. Experimental samples of ginkgo plant were prepared in accordance with the US Pharmacopoeia method (United State Pharmacopeias) USP 28, NF23. The 1.0g of *ginkgo biloba* powdered was passed to a 250ml Erlenmeyer flask and add 50ml ethanol, 20ml water and 8ml hydrochloric acid. The mixture solution was refluxed for 135min. Once the sample was cooled to room temperature, it was filtered into 100ml volumetric

flask, then diluted with water to volume and well mixed by inversion. A sample aliquot was filtered and prepared for analysis with HPLC. Standardized Gb extract (EGb 761) utilized in the present research contained 24% flavanol glycosides, 6% terpene lactones and trace amounts of other substances such as proanthocyanidins and organic acids (DeFeudis, 2003; Yoshitake et al., 2010).

Stress procedure

Stress session involved 6 hours (8am to 2pm) of immobilization period inside a cylindrical tube (with holes for ventilation) at room temperature. Each restraint session was conducted for 21 consecutive days. Control animals were habituated by daily handling and sacrificed through decapitation at the same time.

Body weight

The weight data of all rats were collected on day 0 (before) and day 21 (last day) of the repeated restraint stress (RRS).

Locomotion assay

A square-shaped box (Infrared Locomotor Cage, 40×40×40 cm) made of transparent Plexiglas with a wire mesh floor was used for the locomotor activity. When a rat was placed in the centre of the chamber, the movement of animal was recorded through breaking invisible infrared by an analyser in 5min.

Forced swim test (FST)

FST was performed in accordance with the method of Porsolt et al. (1979). It was performed in two sessions with animals placed in a cylindrical tank. In the first session (pre-test), they were trained for 15min in water. One day after the experiment, the rats were tested through FST for 5min. In summary, the rats were forced to swim in a glass. Initially, the rats swam around vigorously. A few minutes later, their activity decreased and finally they started to turn immobile and just floated in the water in an upright position. FST was employed after completion of 21 days of the stress phase. Immobility time was measured during the 5min period.

Serum corticosterone assay

Forty-eight hours after the final restraint stress, blood samples were collected via cardiac puncture between 9:30 and 10:30am. The samples were collected from rats under deep terminal anaesthesia immediately prior to decapitation to guarantee correct measurements of corticosterone level while remaining unaffected by blood withdrawal. Intracardiac puncture — a terminal blood collection method — is a suitable technique to obtain a single, large and good quality sample from a euthanized rat. At the end of the experiment, the animals were sacrificed (Parasuraman et al., 2010). The serum was isolated from the blood through centrifugation at 3000rpm, 4°C for 15min. The ELISA kit (AlpcoDiagnostics, USA) was used to check the serum corticosterone levels.

Hippocampal samples' preparation

Under deep anaesthesia, animals were sacrificed. Briefly, after removing the skull, the brain was extracted and placed in an ice-cold phosphate-buffered saline solution. The brain was cut along the longitudinal fissure or interhemispheric fissure of the cerebrum using a surgical knife. The mid brain was discarded and the hippocampus was isolated from each brain hemisphere. The entire hippocampus tissues were dissected and frozen in liquid nitrogen and then stored at -80°C until conducting molecular assay.

Methylation procedure and Real Time PCR

Hippocampal genomic DNA was prepared using KoGeneBioTech kit. HpaII and MspI (New England Biolabs, MA) restriction enzymes were used for digestion of DNA samples. Both enzymes recognize the same site (CCGG). Methylated cytosine residue in DNA can be detected by HpaII but not MspI. Genomic DNA (1μg) was diluted by NEBuffer1 (50μl) (New England Biolabs, MA) and then subdivided into three aliquots. Two separate aliquots of DNA were digested with the HpaII or MspI, while the third aliquot was undigested and considered as the background control. The reaction mix was incubated for 2h at 37°C and then stored at -20°C. PCR amplifications were carried out in 20μl reaction mixtures by Step One™ Plus system (PE, Applied Biosystems, CA). Also, methylated DNA associated with BDNF exons was detected using the following primers: forward (5′_AAG ACT GCA GTG GAC ATG TCC_3′) and reverse (5′_CCT TCG TGT AAC CCA TGG GAT_3′).

Table 1: Effect of EGb treatment on body weight and weight gain of stressed rats (mean±SEM).

Group	BW(g)		WG
	Day 0	Day 21	
Control	241.3 ± 0.6	317.5± 0.7$^{\alpha}$	76.1 ± .6
Control- Vehicle	242.5 ± 0.7	318.0 ± 1$^{\alpha}$	75.6 ± 1.2
Stress	244. 5 ± 1.3	280.7 ± 1.5$^{\alpha}$	36 ± 2.$^{\alpha}$
Stress – Vehicle	244. ± 1.29	281.7 ± 1.4$^{\alpha}$	37.6 ± 2.2 $^{\alpha}$
Control- EGB 15 mg/kg	241.7 ± 2	313± 1.39$^{\alpha}$	71.3 ± 3.2
Control- EGB 30 mg/kg	242.7 ± 1.8	312 ± 2.9 $^{\alpha}$	70.1 ±2.2
Control- EGB 60 mg/kg	244 ±1.59	311 ± 3.83 $^{\alpha}$	67 ± 3.8
Stress- EGB 15 mg/kg	239.5 ± 1.2	279.7 ± 2.3$^{\alpha}$	40.1 ± 1.8 $^{\alpha}$
Stress- EGB	241.5 ± 2.7	306± 4.9$^{\alpha}$	64.6± 6.3$^{\$ \Phi}$
Stress- EGB	243.5 ± 1.4	313 ± 1.1$^{\alpha}$	69.5 ± 1.6 $^{\Phi}$

Body weight of male rats on day 21 (last day) compared to day 0 (before) of chronic stress phase (Student's t test). Effect of Gb treatment on of stressed rats. EGb: *Ginkgo biloba* extract, BW: body weight, WG: weight gain. $^{\alpha}P$<0.001 compared to day 0, control, control – vehicle and control-EGB treated (15, 30 and 60mg/kg) groups; $^{\$}P$<0.05 compared to control and control –vehicle groups; $^{\Phi}P$<0.001 compared to stress, stress- vehicle and stress- EGB treated (15mg/kg) groups. Data were analyzed by One-way ANOVA.

PCR thermo-cycling steps were as follows: 40 cycles of 94°C for 15s, then 63°C for 30s and 72°C for 30s followed by 72°C for 10min. All SYBR Green (PR081A, TaKaRa) PCR assays were performed in 96-well optical plates in duplicate for every DNA sample. The ΔΔCT for each sample was determined by the following equation: ΔΔCT = ΔCT (MSPI-UD) - ΔCT (HpaII-UD) (Niknazar et al., 2016).

Western blot assay

Western blot assay was performed on the hippocampus of rats as previously described (Niknazar et al., 2017b). In summary, the frozen hippocampus was homogenized with ice-cold lysis buffer system (Santa Cruz Biotechnology, sc-24948, USA). Total proteins were separated on SDS - PAGE and transferred onto nitrocellulose membranes. After the blocking step, the membrane was probed with primary antibodies for BDNF *(*AB, 6201, 1-1000; Abcam, Cambridge, UK) and β Actin (AB, ab8227*,*1-2000; Abcam , Cambridge, UK) (as an internal control). Then washed with TBST three times for 10min, and eventually incubated with HRP-conjugated anti-rabbit IgG secondary antibody (AB, 6721*,*1-2000; Abcam, Cambridge, UK) for 2h at room temperature. The blots were visualized using an ECL-advanced chemiluminescence detection kit (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA) after which the film was exposed to x-rays. The scanned film signals were analysed by TotalLab software (Nonlinear Dynamics Ltd, USA).

Data analysis

Data have been represented as mean±SEM. Statistical significance was analysed by Graph Pad Prism version 5.04 Windows (Graph Pad Software, San Diego, CA, USA). Statistical significance was detected by the Student's t test and One-way ANOVA followed by post-hoc Tukey's test to compare the differences between groups. P<0.05 was considered significant.

Results

Effects of Gb extract on body weight changes in the stressed rat

Following the course of 21 days of stress phase, the body weight was significantly elevated in control (n=6), stressed (n=6) and experimental (n=6) groups compared to day 0 (P<0.001, Table 1). However, the weight gain was significantly reduced in stressed (P<0.001) and experimental groups treated with EGb 15 (P<0.001) and 30mg/kg (P<0.05) compared to the non-stressed groups. On the other hand, weight gain was significantly intensified in groups administered
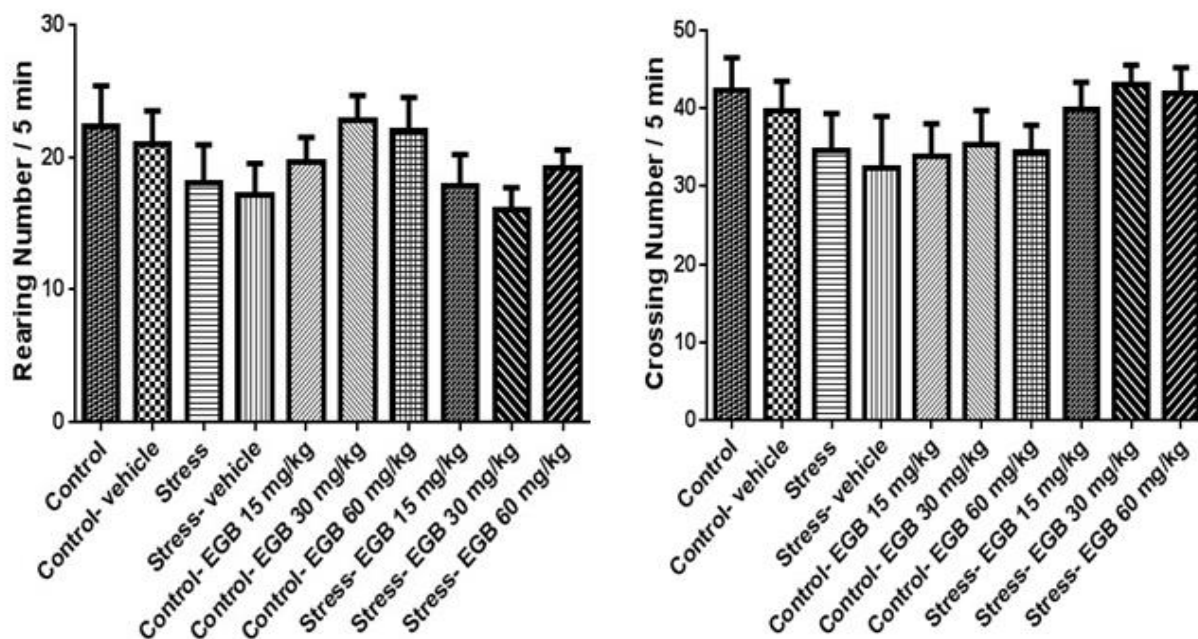
Fig.1. Effect of EGb treatment on locomotor activity of stressed rats (mean±SEM), EGb: *Ginkgo biloba* extract. Data were analyzed by One-way ANOVA.

Table 2: Effect of EGb treatment on immobility time of stressed rats in forced swimming test (mean±SEM).

Group	Control	Control - Vehicle	Stress	Stress- Vehicle	Control- EGb 15 mg/kg	Control - EGb 30 g/kg	Control- EGb 60 mg/kg	Stress- EGb 15 mg/kg	Stress- EGb 30 mg/kg	Stress- EGb 60 mg/kg
Immobility Behaviour (Sec)	86.8 ± 8.3	91.33 ± 11. 1	218 ± 9 $^\alpha$	224.8 ± 11.4$^\alpha$	87.17± 6.98	89.83± 7.5	93.33± 9.2	182.7± 15.7 $^\alpha$	131 ± 8.1 $^\Phi$	93.83 ± 9.2$^\Phi$

EGb: *Ginkgo biloba* extract. $^\alpha P<0.001$ compared to control, control – vehicle and control-EGB treated (15, 30 and 60mg/kg) groups; $^\Phi P<0.001$ compared to stress, stress- vehicle and stress- EGB treated (15mg/kg) groups. Data were analyzed by One-way ANOVA.

EGb 30 and 60mg/kg ($P<0.001$) compared to the stressed groups ($F_{(9,50)}$= 29. 47, the overall effect size f=2.8; Table 1).

Effects of Gb extract on locomotor activity in the stressed rat

Here, 24h after the last session of stress phase, the locomotor activity was analysed during 5min in all groups (n=6). No significant Gb treatment effect was detected in the number of rearing $F_{(9,50)}$=1.06, (the overall effect size f=0.39) and crossing $F_{(9,50)}$= 0.9, (the overall effect size f=0.37) of stressed rats compared to control groups (Fig. 1).

Effects of Gb extract on immobility time of rats in

the FST

One-way ANOVA analysis revealed that 30 and 60mg/kg of Gb extract reduced the immobility time of rats in the FST ($P<0.001$) compared to stressed rats. Immobility time significantly increased in stressed and experimental groups treated with 15mg/kg of Gb extract ($P<0.001$), as compared to control rats (n=6 in each group, $F_{(9,50)}$= 32.88, the overall effect size f=2.2; Table 2).

Effects of Gb extract on serum corticosterone response in the stressed rat

According to One-way ANOVA analysis, chronic stress induced a dramatic rise in the level of serum corticosterone in the stressed and experimental
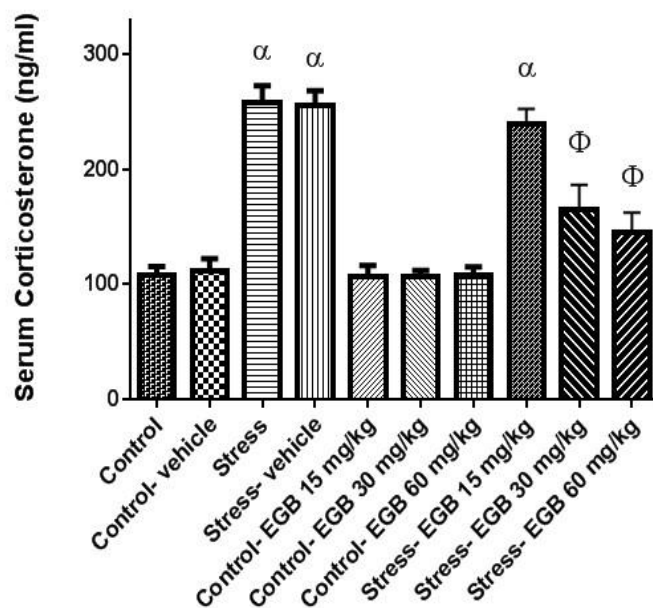
Fig.2. Effect of EGb treatment on corticosterone response of stressed rats (mean±SEM). EGb: *Ginkgo biloba* extract. $^{\alpha}P<0.001$ compared to control, control – vehicle and control-EGB treated (15, 30 and 60mg/kg) groups; $^{\Phi}$ $P<0.001$ compared to stress, stress- vehicle and stress- EGB treated (15mg/kg) groups. Data were analyzed by One-way ANOVA.

group treated with EGb 15mg/kg ($P<0.001$) compared to the control groups. On the other hand, the serum corticosterone response was not significantly different between the experimental groups treated with EGb 30 as well as 60mg/kg and control groups. Further, pre-treatment with EGb 30 and 60mg/kg attenuated ($P<0.001$) the stress-induced growth in the serum corticosterone concentration (n=6 in each group, $F(9,50)= 25.94$, the overall effect size f=1.9 compared to control groups; Fig. 2).

Effects of Gb extract on hippocampal BDNF DNA methylation in the stressed rat
ANOVA analysis of DNA methylation indicated that chronic stress daily for 21 days resulted in a significant increment in methylation status of the exon IV of BDNF ($P< 0.001$) gene in the hippocampus of adult male rats compared to the non-stressed male rats (Fig. 3). Hypermethylation of the hippocampal BDNF promoter was observed in stressed group ($P<0.001$) and in those administered a vehicle or EGb 15 ($P<0.001$) compared to the control groups. DNA hypermethylation induced by chronic stress was markedly attenuated ($P<0.001$) with 30 and 60mg/kg of Gb extract pretreatment (n=6 in each group, $F(9,50)= 51.33$, the overall effect size f=2.7; Fig. 3).

Effects of Gb extract on hippocampal BDNF protein expression in the stressed rat

Hippocampal protein samples were evaluated for changes in BDNF expression. The antibody specifically recognizes the band for BDNF on the blot at about 28kDa. Densitometric analysis indicated that following the chronic stress induction phase, hippocampal BDNF protein expression of male rat decreased in stress, stress- vehicle and stress- EGb 15mg/kg treated groups (n=6, $P<0.001$) in comparison with the control groups (n=6). However, hippocampal BDNF protein expression was significantly enhanced in experimental groups treated with EGb 30 (n=6, $P<0.001$) and 60mg/kg (n=6, $P<0.001$) compared to the stressed groups (n=6, $F(9,50)= 15.15$, the overall effect size f=1.5; Fig. 4).

Discussion
This study investigated the antidepressant effect of Gb extract through modulation of hippocampal BDNF expression induced by RRS in adult male rats. For this purpose, we studied the Gb effect on depressive-like behaviour, endocrine responses, as well as hippocampal BDNF gene methylation and protein expression in RRS rats. Our findings revealed that pre-treatment with 30 and 60mg/kg of Gb extract significantly attenuated the chronic stress-induced alterations including increased immobility time in the FST, serum corticosterone and BDNF DNA methylation level, as well as reduced protein expression of this gene in the hippocampus of the
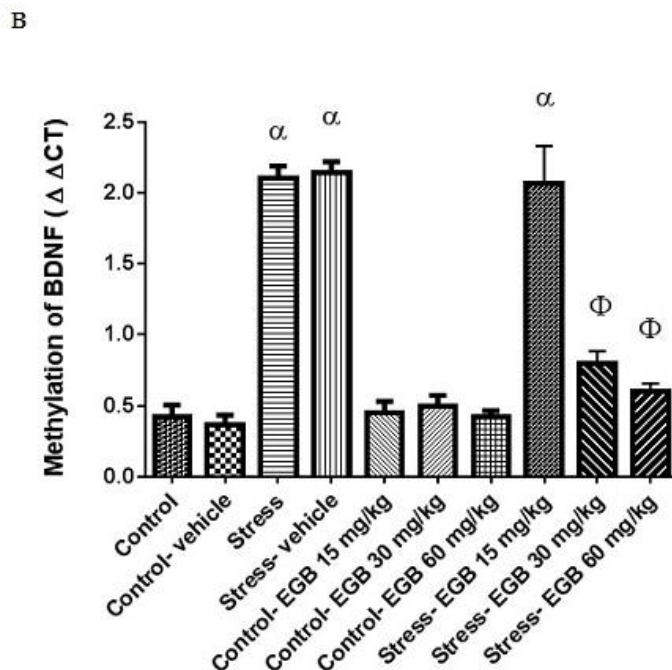
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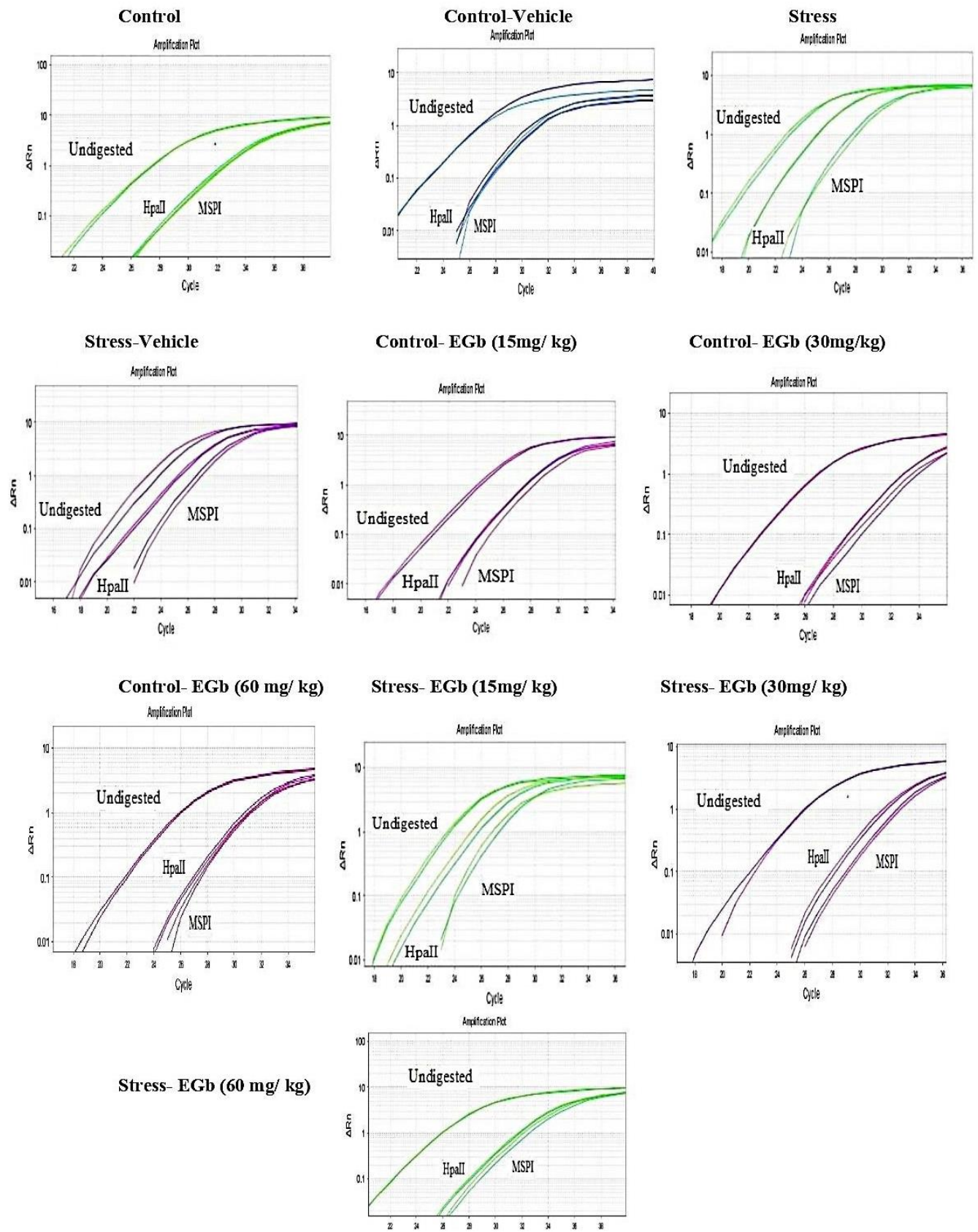
Fig.3. Effect of EGb treatment on the hippocampal BDNF DNA Methylation of stressed rats (mean±SEM). EGb: *Ginkgo biloba* extract. (A) The DNA methylation level of hippocampal BDNF was assayed by Real Time PCR (B) $^{\alpha}P$<0.001 compared to control, control – vehicle and control-EGB treated (15, 30 and 60mg/kg) groups; $^{\Phi}P$<0.001 compared to stress, stress- vehicle and stress- EGB treated (15mg/kg) groups. Data were analyzed by One-way ANOVA.
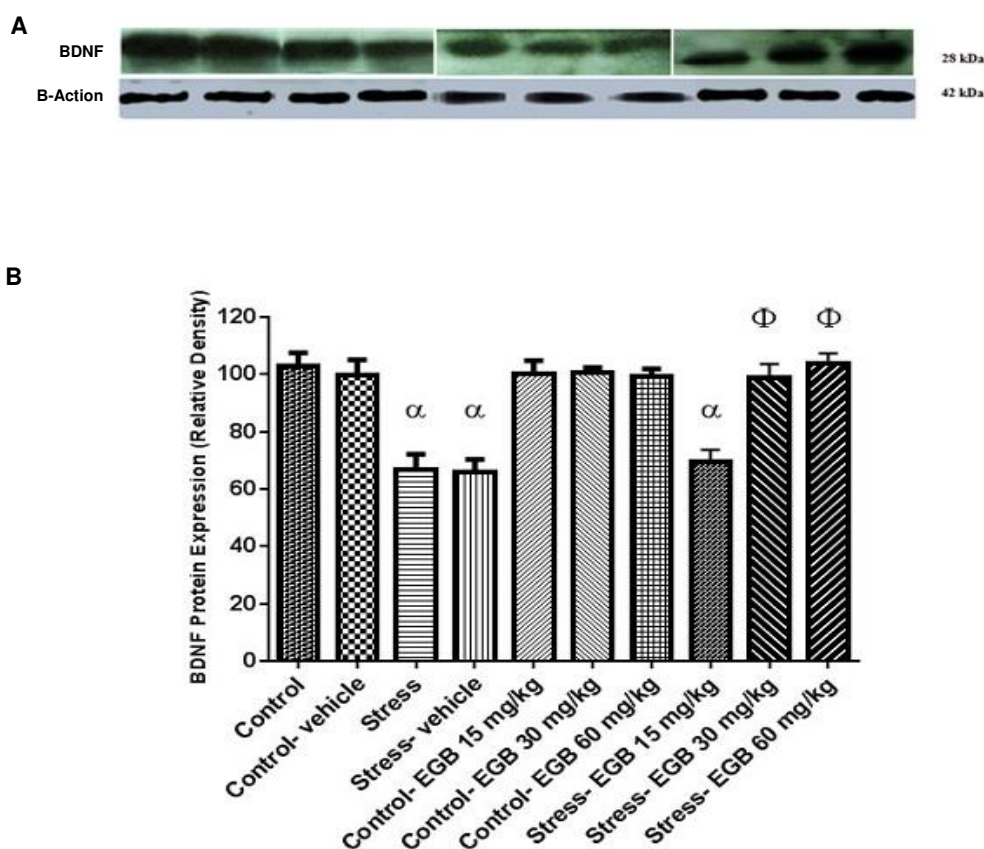

Fig.4. Effect of EGb treatment on the hippocampal BDNF protein expression of stressed rats (mean ±SEM). EGb: *Ginkgo biloba* extract. (A) The protein level of BDNF was assayed by Western blot. (B) $^{\alpha}P$<0.001 compared to control, control – vehicle and control-EGB treated (15, 30 and 60mg/kg) groups; $^{\Phi}P$< 0.001 compared to stress, stress- vehicle and stress- EGB (15mg/kg) groups. Data were analyzed by One-way ANOVA.

animals. However, the locomotor activity of rats was not significantly altered. Indeed, locomotor activity was performed before FST to show that despite the normal locomotion of animals they were immobile in the FST test.

False-positive may result from some drugs such as sedatives, stimulants or motor-impairing compounds which could influence the immobility time even if they are free of antidepressant action (Slattery and Cryan, 2012). Our results suggested that the anti-immobility effect of Gb extract was not associated with motor deficits, as the animals treated with EGB 761 did not experience impaired locomotor activity. Thus, reduced immobility time of treated rats in the FST is not due to the effect on locomotor activity. Although FST is mostly used to assess the antidepressant effectiveness of novel drugs, the FST has also been used by a large number of studies to test depressive behaviours in animal models of mood disorders (Amiri et al., 2015; Andre et al., 2014; Brenes et al., 2008; Zheng et al., 2016). In addition, increased serum corticosterone concentration has been consistent with previous studies suggesting that physiological responses to repeated stress are associated with activation of the hypothalamic–pituitary–adrenal axis (Niknazar et al., 2013; Niknazar et al., 2016; Ottenweller et al., 1992). Other results of the current study showed decreased expression of hippocampal BDNF and increased methylation of this gene in the stressed groups as compared to control. BDNF expression in the brain is regulated by stress. The role of BDNF has been found in the pathogenesis of depression and post-traumatic stress disorder (Bus et al., 2015; Li et al., 2009). A previous study noted that treatment with BDNF reversed the depression-like behaviour in an animal model of depression (Shirayama et al., 2002). Human studies have also provided findings supporting the role of BDNF in mood disorders. Chen et al. (2001) determined that BDNF levels are elevated in the hippocampus of individuals treated with antidepressant drugs in comparison to those not treated with anti-depressant medications at the time of death. Also, Karge et al. (2002) reported that serum BDNF levels decreased in 30 patients with major depression. Another key finding according to Sklar et al. (2002) was supporting the role of BDNF in the biology of mood disorders, suggesting that the BDNF gene was a predisposing factor in depression.

In addition, depression is associated with a reduction in BDNF- tyrosine kinase receptor (TrkB) signalling pathway. In rats, decreased BDNF-TrkB signalling could enhance their resistance to anti-depressant treatment in FST (Saarelainen et al., 2003). Several studies have concluded that changes in BDNF DNA methylation can be considered as a biomarker of neuropsychiatric disorders such as major depression and schizophrenia (Zheleznyakova et al., 2016). Epigenetic alteration in gene transcription has recently been proposed as a regulator of the essential gene expression changes in neural plasticity, which may also be affected by stress. DNA methylation and histone acetylation are epigenetic modifications that may lead to increase or suppress transcription of the gene (Franklin and Mansuy, 2010; Sabunciyan et al., 2012; Suzuki and Bird, 2008). Several studies have suggested that chronic restraint stress induces a depressive-like state. Indeed, this model, as a type of chronic stress, is a well-established source of stress which causes depressive behaviour in rats (Chiba et al., 2012; Lee et al., 2013; Naert et al., 2011; Wang et al., 2017). It has been reported that immobilization stress for one or several days (2 hours/ day) considerably reduces BDNF mRNA level in the hippocampus of rats (Smith et al., 1995b). In addition, Ueyama et al. (1997) revealed that male rats subjected to prolonged immobilization stress exhibited decreased levels of neurotrophins (BDNF, nerve growth factor and Neurotrophin-3) of the brain especially in the hippocampus. Increases in DNA methylation level of BDNF exon IV and simultaneous reductions in BDNF mRNA and protein expression were reported in rats experiencing chronic and traumatic stress (Niknazar et al., 2016; Niknazar et al., 2017a; Roth et al., 2011). In addition, studies of human post-mortem brain tissue sample obtained from suicide victims revealed significant BDNF IV region hypermethylation (Kang et al., 2013) and a simultaneous decline in BDNF mRNA in the Wernicke area compared to no-suicidal subjects (Keller et al., 2010). Today, medicinal plants, as a conventional treatment, are used for preventing and treating depression and anxiety symptoms (Fajemiroye et al., 2016). The results of a previous study suggested that Gb restored brain catecholamines, serotonin and plasma corticosterone responses to stress in rats exposed to forced immobilization (Shah et al., 2003). Flavonoids (glycosides) and terpenoids (ginkgolide)

are primary active constituents of Ginkgo's leaves' extract (Biber, 2003; Deng and Zito, 2003; Diamond et al., 2000; Sticher, 1993; Victoire et al., 1988). Standardized Ginkgo preparation, EGb 761, has 24% ginkgo flavone glycosides and 6% terpenoids (Chan et al., 2007; DeFeudis, 2003; DeFeudis and Drieu, 2000; Yoshitake et al., 2010). This heterogeneous chemical constituent of EGb 761 and synergistic action of its components generate its neuroprotective effects against psychiatric disorders (Montes et al., 2015). In addition, several studies have indicated mild side effects of EGb 761. Gavrilova et al. (2014) demonstrated that EGb 761 improves neuropsychiatric symptoms and cognitive performance in patients with mild cognitive impairment. Their findings suggested that EGb 761 is safe and well tolerated in patients. In addition, a study by Carlson et al. (2007) concluded that *Ginkgo biloba* product is safe and improve quality of life in cognitively intact older people. Other studies have indicated that the components of *Ginkgo biloba* leaf extracts (GB), mainly the flavonoid, possess antidepressant behavioural effects and improve cognitive function through several mechanisms. They include changes in the level of neurotransmitters such as norepinephrine, dopamine, serotonin and acetylcholine as well as in neurotrophins (Hou et al., 2010; Kehr et al., 2012; Malireddy et al., 2012; Yoshitake et al., 2010). Epigenetic modifications have recently been proposed as a significant gene expression regulator and may also be affected by stress-related disorders. Epigenetic changes usually influence DNA methylation and acetylation of histones which are associated with augmented or repressed gene transcription (Gräff and Mansuy, 2008; Miranda and Jones, 2007; Vaissière et al., 2008). Methylation of cytosine residues in mammalian genomic DNA is catalysed by DNA methyltransferases (DNMTs) enzyme. Epigenetic studies have shown that subclasses of flavonoids such as flavones reduce DNMT enzyme activity and reverse DNA hypermethylation of cytosine bases in CPG-rich region, which can lead to its anti-cancer effect (Kanwal et al., 2016). Researchers have concluded that the dietary flavones can act as DNMT inhibitors. Flavonoids can also function as DNMT enzyme inhibitor both directly through interaction with the active site of the enzymes and through indirect mechanisms (Venturelli et al., 2016).

Conclusion

Overall, this study revealed that repeated administration of the extract of Ginkgo's leaves alongside with the stress paradigm may prevent the effects of chronic stress. The main finding of the study was the drug-mediated prevention of a decrease in BDNF protein expression which supported by increased methylation patterns in the BDNF promoter. Although the current work showed one of the molecular mechanisms behind the Gb antidepressant effect, future research is required to fully explain the mechanisms that can mediate the antidepressant action of Gb.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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