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Venlafaxine protects against stress-induced oxidative DNA damage in hippocampus during antidepressant testing in mice

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ABSTRACT

Venlafaxine (VLF) is an approved antidepressant that is claimed to have superior clinical efficacy to comparable drugs. Recently, many studies showed the relationship between depression and increased oxidative stress. This study investigated the relationship between the antidepressant effect of VLF and its ability to protect animals against stress-induced oxidative lipid peroxidation and DNA damage induced during antidepressant testing. *Methods*: The antidepressant effect of long-term treatment (21 days) of VLF in doses 5, 10 and 20 mg/kg/day, i.p. was tested using forced swimming test (FST) and tail suspension test (TST). The effects of VLF on hippocampal lipid peroxidation (MDA), nitric oxide (NO), glutathione (GSH), total antioxidant (TAC) levels and glutathione-S-transferase (GST) activity were tested. Furthermore, the corresponding changes in serum and hippocampal 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured. *Results*: Long-term VLF treatment showed a significant, antidepressant effect in both FST and TST. VLF could decrease the hippocampal MDA and NO and to increase hippocampal GSH and TAC levels and GST activity in the tested animals. Only GSH and TAC levels were increased by VLF in the non-tested animals. In addition, both serum and hippocampal 8-OHdG levels were significantly reduced by VLF in animals exposed to antidepressant tests. *Conclusion*: Long-term VLF treatment in the effective antidepressant doses can protect against stress-induced oxidative cellular and DNA damage. This action may be through antagonizing the oxidative stress and enhancing the antioxidant defense mechanisms. Consequently, pharmacological modulation of stress-induced oxidative DNA damage as a possible stress-management approach should be an important avenue of further research.

1. Introduction

Depression is a considerable medical problem as patients with major depressive disorder are at increased risk for serious illnesses. Recently, studies showed the relationship between depression and oxidative DNA damage (Mustak et al., 2010). Depression is characterized by activation of the inflammatory response system with increased production of proinflammatory cytokines. These cytokines and cytokine-induced reactive oxygen and nitrogen species, ROS & RNS (i.e. singlet oxygen, superoxide anion radical, perhydroxyl radical, hydroxyl radical and nitroso radicals) increase tissue injury and lipid peroxidation (Dantzer et al., 2011). ROS & RNS contribute to tissue injury and DNA-damage by reacting with biomolecules such as lipids (Gehrmann et al., 2010), proteins and nucleic acids (van Berlo et al., 2010). Moreover, psychological stress, which accompanies severe depression, may increase lipid peroxidation (Yager et al., 2010).

There are various antioxidant mechanisms in the brain that neutralize the harmful effects of ROS & RNS. However, with depression, the loss of efficiency of antioxidant mechanisms and the alterations in the proinflammatory cytokine system result in increasing the free radical formation due to activation of phagocytic cells (Kim and Kim, 2010). ROS & RNS can induce neuronal damage via depletion of non-enzymatic antioxidants in the brain (Lesgards et al., 2011). Also, it may decrease the activity of antioxidant enzymes as glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), catalase and superoxide dismutase (Lesgards et al., 2011).

ROS & RNS can interact with DNA to produce damage including single and double-stranded DNA breaks, deletions and nucleoside modifications. 8-hydroxy-2′-deoxyguanosine (8-OHdG), the oxidized form of the nucleoside 2′-deoxyguanosine present in DNA, is one of the most reliable and abundant markers for free radical-induced oxidative lesions and DNA damage because it reflects extremely low levels of oxidative damage (Evans et al., 2004). Hence, urinary 8-OHdG has been widely used as a biomarker for oxidative stress and carcinogenesis (Valavanidis et al., 2009). Recently, serum 8-OHdG was started to be

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used as a useful biomarker for oxidative DNA damage. Also, the relationship between serum 8-OHdG and depression was reported (Forlenza and Miller, 2006).

Venlafaxine (VLF) is an approved antidepressant that is an inhibitor of both serotonin and norepinephrine transporters. It has a higher affinity for serotonin reuptake than for noradrenaline (Gould et al., 2006). VLF is claimed to have superior clinical efficacy to comparable drugs due to its faster onset of action (Deakin and Dursun, 2002). VLF was found to modulate depression-induced oxidative stress in brain and medulla of tested rats (Eren et al., 2007b) and possible involvement of its effect on nitric oxide (NO) and RNS production in its antidepressant effect was also reported (Dhir and Kulkarni, 2007).

The aim of this work was to study the ability of VLF to protect against stress-induced neuronal lipid peroxidation, changes in the antioxidant defenses and oxidative DNA damage, induced by stress during antidepressant testing using forced swimming test (FST) and tail suspension test (TST), and to correlate these effects with VLF antidepressant activity.

2. Materials and methods

2.1. Chemicals

Venlafaxine, reduced glutathione, thiobarbituric acid, serum albumin, Folin-Phenol reagent and Griess reagent were purchased from Fluca Co., (Germany). Ellman's reagent [(5,5-Dithiobis (2-nitrobenzoic acid), DTNB] was purchased from Uptima Co. (France). Total Antioxidant Assay kit, glutathione-S-transferase assay kit and 8-hydroxy-2′-deoxyguanosine (8-OHdG) assay kit were purchased from Cayman's Chemical Co., (USA). All other chemicals were of analytical grade.

2.2. Animals

Adult male Swiss-Webster mice, weighing 22–25 g that were obtained from the Animal House of Assiut University were used in all experiments. Animals were maintained at 22–27 °C with free access to water and food ad libitum, under a 12:12 h light:dark cycle (lights on at 7:00 h). All experiments were carried out between 9:00 and 16:00 h. The experiments were performed in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. The animal-testing protocol used in the present investigation was approved by the Institutional Animal Ethics Committee. All efforts were made to minimize animal suffering.

2.3. Experimental protocol

Animals were randomly divided into 8 groups, 10 mice each. The first group used as a non-tested (naïve) group was not exposed to antidepressant testing, treated with saline and was left undisturbed in the home cage except for general handling (i.e. regular cage cleaning and measuring body weight). Group 2 was used as a depressive stress (control) group, treated with saline i.p. for 21 days and exposed to antidepressant testing. Groups 3, 4 and 5 were treated with VLF in doses 5, 10 and 20 mg/kg/day, i.p. for 21 days, each to one group and were not exposed to antidepressant testing. Groups 6, 7 and 8 were treated by the same way but exposed to antidepressant testing at the end of treatment protocol. VLF solution was prepared freshly directly before injection by dissolving VLF in 2 ml physiological saline and injected intraperitoneally. Twenty four hours after the last treatment, animals were exposed to antidepressant testing; FST followed 1 h by TST. Directly, after performing TST, animals were killed by decapitation and blood and hippocampus were obtained for biochemical analysis.

2.4. Antidepressant testing

2.4.1. Forced swimming test (FST)

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C. The total test duration was 6 min and the immobility of animal during the last 4 min of the test was measured. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1997).

2.4.2. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period.

2.5. Biochemical measurements

Blood samples were kept at 4 °C for 30 min for clotting. Clear serum was obtained by centrifugation of the blood samples after clotting at 3000 rpm for 15 min and kept frozen until used for 8-OHdG measurement. The brain was rinsed in ice-cold saline; the whole hippocampus was separated, washed with ice-cold saline, blotted carefully, weighed and then homogenized in phosphate buffer (pH 7.4). The homogenate was divided into two parts. The first part was centrifuged at 10,000 × *g* at 4 °C for 15 min and the supernatant was collected for determination of LP, NO, TAC levels and GST activity. The second part was mixed with equal volume of perchloric acid (1 mol/l) and mixed by vortexing. The mixture was allowed to stand for 5 min at 25 °C. After centrifugation at 10,000 × *g* at 4 °C for 5 min the supernatant was collected and used for determination of GSH and 8-OHdG levels.

2.5.1. Determination of lipid peroxidation

Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels. It is an end product of lipid peroxidation and its level was determined spectrophotometrically by use of thiobarbituric acid reactive substances method previously described by Ohkawa et al. (1979).

2.5.2. Determination of nitrite level

Nitric oxide (NO) formation was measured by assaying nitrite level in samples, one of the stable end products of NO oxidation. Nitrite concentration was measured spectrophotometrically using the Griess reagent [1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution) and 0.1% N-1-naphthylethylenediamine dihydrochloride in bidistilled water (NED solution)] as described by Green et al. (1982). Standard curve was blotted for calculating sample concentrations.

2.5.3. Determination of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) was measured by using Total Antioxidant Assay kit purchased from Cayman's Chemical Company, (USA). Aqueous and lipid soluble antioxidants are not separated, thus the combined antioxidant activities of all constituents including vitamins, proteins, lipids, glutathione, uric acid are assessed. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS®$^{\bullet-}$ by metmyoglobin. All procedures were carried out as described in the manufacturer manual.

2.5.4. Determination of intracellular GSH

The GSH content of the neutralized supernatant of hippocampal homogenate was assayed using Ellman's reagent [5,5-dithiobis-2-

nitrobenzoic acid (DTNB solution)] according to the method of Ellman (1959).

2.5.5. Determination of GST activity

Glutathione S-transferase (GST) a family of enzymes that catalyse the conjugation of reduced glutathione via a sulfhydryl group to electrophilic centers on a wide variety of substrates (Douglas, 1987). GST activity in samples was measured using Cayman's glutathione S-transferase assay kit purchased from Cayman's Chemical Co., (USA). It measures the total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. All procedures were carried out as described in the manufacturer manual.

2.5.6. Determination of 8-hydroxy-2′-deoxyguanosine (8-OHdG) level

Cayman's 8-hydroxy-2′-deoxyguanosine (8-OHdG) assay kit purchased from Cayman's Chemical Co., (USA) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and tissue homogenate. It recognizes both free 8-OHdG and DNA-incorporated 8-OH-dG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OHdGTracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with the provider manual.

2.5.7. Determination of protein content

The protein content in the supernatant of hippocampal homogenate was measured by method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.6. Statistical analysis

All experimental results are expressed as the mean ± SEM and the data were analyzed using One-Way or Two-Way Analysis of Variance (ANOVA) where appropriate. If any statistically significant change was found, post-hoc comparisons were performed using Bonferroni test. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Effect of VLF on the immobility time in the FST and TST

Results shown in Fig. 1 show the effect of VLF on the immobility time of FST. These results revealed a significant effect [$F(3, 36)=6.292$, $p=0.0015$] of long-term administration of VLF for 21 days in doses 5, 10 and 20 mg/kg/day, i.p. on the immobility time in the FST. Post hoc analysis indicated a significant decrease in the immobility time elicited by the administration of VLF ($p<0.05$) at doses 5 and 10 mg/kg/day and ($p<0.01$) at dose 20 mg/kg/day indicating that VLF in the selected doses has effective antidepressant effect in this model.

Similarly, Fig. 2 shows the effect of VLF on the immobility time of TST. The results showed a significant change [$F(3, 36)=6.662$, $p=0.0011$] in the immobility time of TST in VLF-treated animals. Post hoc analysis indicated a significant decrease at level $p<0.05$ in the immobility time of TST elicited by the administration of VLF in a dose 5 mg/kg and at level $p<0.01$ with doses 10 and 20 mg/kg indicating that VLF in the selected doses shows effective antidepressant effect in TST. However, this effect was not dose dependent.

3.2. Effect of VLF on lipid peroxidation and NO levels

The effects of long-term treatment of VLF on hippocampal levels of lipid peroxidation measured as MDA and NO measured as nitrite are shown in Table 1. Results revealed a significant effect of long-term VLF treatment on hippocampal MDA [$F(3, 72)=3.000$, $p=0.0361$] and NO [$F(3, 72)=2.793$, $p=0.0464$] levels respectively. Post hoc analysis indicated that exposure of animals to stress during antidepressant testing (FST and TST) significantly increased hippocampal MDA ($p<0.05$) and nitrite ($p<0.01$) levels compared with naïve animals. In groups exposed to stress through antidepressant tests after treatment with VLF, there was a significant decrease in hippocampal MDA ($p<0.01$) with doses 5 and 10 mg/kg/day and ($p<0.001$) with 20 mg/kg/day. Similar results were obtained from the effect of VLF on hippocampal NO concentration. No significant changes were observed in hippocampal MDA or NO levels in animals that were treated with VLF without exposure to antidepressant testing.

3.3. Effect of VLF on TAC, GSH levels and GST activity

Results of changes in antioxidant defense system, TAC, GSH levels and GST activity in hippocampus of tested animals after long-term treatment with VLF are shown in Table 1.

The results revealed a significant effect of long-term VLF treatment on hippocampal TAC level [$F(3, 72)=13.43$, $p<0.001$]. Post hoc analysis indicated that exposure to stress during testing antidepressant activity significantly ($p<0.01$) decrease hippocampal TAC level relative to naïve animals. Also, there was an increase in hippocampal TAC level in animals that were not exposed to antidepressant testing after treatment with VLF in doses 5, 10 mg/kg/day and 20 mg/kg/day at significant levels $p<0.01$ and $p<0.001$ respectively relative to naïve animals. VLF pretreatment in the tested doses antagonized the decrease in TAC levels induced by depressive stress during FST and TST at significant level $p<0.01$ with dose 5 mg/kg and at level $p<0.001$ with doses 10 and 20 mg/kg of VLF.

In addition, results showed a significant change in hippocampal GSH level of tested animals after long-term treatment with VLF [$F(3, 72)=12.76$; $p<0.001$]. Post hoc analysis showed a significant decrease ($p<0.01$) in hippocampal GSH after exposure of animals to depressive stress. There was a significant increase ($p<0.01$) in hippocampal GSH level in animals that were not exposed to

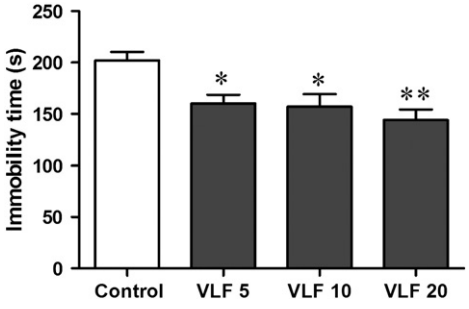

Fig. 1. Effect of venlafaxine (VLF) in doses 5, 10 and 20 mg/kg/day, i.p for 21 days, on the immobility time of forced swimming test (FST) in mice. Results represent mean ± SEM ($n=10$). [$F(0.3458)=6.343$, $p=0.0014$]. *$p<0.05$ vs stress control. **$p<0.01$ vs stress control.

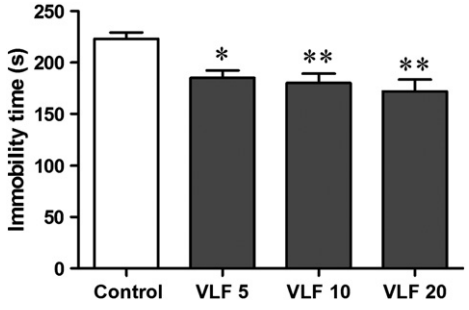

Fig. 2. Effect of venlafaxine (VLF) in doses 5, 10 and 20 mg/kg/day, i.p for 21 days, on the immobility time of tail suspension test (TST) in mice. Results represent mean ± SEM ($n=10$). [$F(0.3436)=6.281$, $p=0.0015$]. *$p<0.05$ vs stress control. **$p<0.01$ vs stress control.

Table 1
Effect of venlafaxine (VLF) in doses 5, 10 and 20 mg/kg/day, i.p for 21 days, on hippocampal levels of malondialdehyde (MDA), nitrite, total antioxidants (TAC) and reduced glutathione (GSH) levels and glutathione-S-transferase (GST) activity in mice.

Treatment (mg/kg/day)	MDA (nM/mg Pr)	Nitrite (μg/ml)	TAC (nM/mg Pr)	GSH (nM/mg Pr)	GST (nM/mg Pr)
Naïve	0.11 ± 0.02	245.5 ± 22.32	0.32 ± 0.03	0.096 ± 0.002	0.526 ± 0.009
VLF (5)	0.11 ± 0.05	246.7 ± 33.53	0.53 ± 0.06[a]	0.116 ± 0.006[a]	0.527 ± 0.011
VLF (10)	0.12 ± 0.04	248.7 ± 32.44	0.55 ± 0.06[a]	0.117 ± 0.006[a]	0.531 ± 0.011
VLF (20)	0.14 ± 0.05	252.6 ± 17.82	0.61 ± 0.03[b]	0.119 ± 0.003[a]	0.533 ± 0.014
Stress (control)	0.32 ± 0.04[c]	383.5 ± 35.63[a]	0.08 ± 0.05[a]	0.067 ± 0.004[a]	0.473 ± 0.008[c]
Stress + VLF (5)	0.11 ± 0.03[d]	268.5 ± 18.43[d]	0.31 ± 0.05[d]	0.092 ± 0.005[b]	0.527 ± 0.013[d]
Stress + VLF (10)	0.10 ± 0.06[d]	264.5 ± 18.43[d]	0.35 ± 0.06[e]	0.094 ± 0.005[e]	0.531 ± 0.013[d]
Stress + VLF (20)	0.08 ± 0.04[e]	245.7 ± 17.43[e]	0.37 ± 0.05[e]	0.097 ± 0.007[e]	0.536 ± 0.012[e]

Results represent mean ± SEM. (n = 10).
[a] $p<0.01$ vs naïve.
[b] $p<0.001$ vs naïve.
[c] $p<0.05$ vs naïve.
[d] $p<0.01$ vs stress control.
[e] $p<0.001$ vs stress control.

antidepressant testing after treatment with VLF in the tested dose levels. Moreover, VLF significantly decreased the reduction in hippocampal GSH level induced by stress at a significant level $p<0.01$ with a dose 5 mg/kg and at $p<0.001$ with the doses 10 and 20 mg/kg.

Furthermore, results showed a significant change in hippocampal GST activity [$F(3, 72) = 3.828$; $p = 0.0133$] by VLF treatment. Post hoc analysis showed a decrease in hippocampal GST activity in animals exposed to FST and TST. VLF in the selected doses did not significantly change the GST activity in hippocampi of animals that were not exposed to antidepressant testing. On the other hand, it significantly increased hippocampal GST activity in animals exposed to antidepressant testing at significant level $p<0.01$ with doses 5 and 10 mg/kg and at level $p<0.001$ with the dose 20 mg/kg of VLF relative to stress control.

3.4. Effect of VLF on 8-OHdG levels

Results of the measurement of serum and hippocampal levels of 8-OHdG are shown in Figs. 3 and 4 respectively. These results revealed significant changes in serum [$F(3, 72) = 3.694$; $p = 0.0156$] and hippocampal [$F(3, 72) = 7.680$; $p = 0.001$] 8-OHdG levels after long term treatment of animals with VLF and exposure to antidepressant testing. Post hoc analysis showed that VLF did not significantly change 8-OHdG levels in both serum (Fig. 3) and hippocampus (Fig. 4) of animals that were not exposed to FST and TST.

Exposure of animals to stress during antidepressant testing increased both serum and hippocampal levels of 8-OHdG reflecting the increased neuronal DNA damage induced by depressive stress.

Long-term treatment with VLF significantly decreased the stress-induced increase in serum 8-OHdG levels in dose 5 mg/kg ($p<0.05$) and doses 10 and 20 mg/kg/day ($p<0.01$) as shown in Fig. 3. Moreover, VLF decreased the stress-induced increase in hippocampal 8-OHdG levels in doses 5 mg/kg ($p<0.05$), 10 mg/kg ($p<0.01$) and 20 mg/kg ($p<0.001$) reflecting the protective effect of VLF against neuronal DNA damage induced by stress during the antidepressant tests FST and TST as shown in Fig. 4.

4. Discussion

The present work studied the ability of long-term VLF treatment for 21 days to protect animals, exposed to stress during testing antidepressant activity by FST and TST, from stress-induced oxidative stress. Changes in the cellular lipid peroxidation, NO levels and changes in the antioxidant defense system, including GSH and TAC levels and GST activity in the hippocampus of the tested animals, were studied. Moreover, the ability of VLF to protect hippocampal neurons from stress-induced DNA damage was tested. Long-term VLF treatment was selected in this study depending on previously published data showed that short-term antidepressant treatment does not alter oxidative–antioxidative systems in depression (Sarandol et al., 2007).

The antidepressant effect of VLF was evaluated using FST and TST, two animal models based on behavioral despair, which are widely

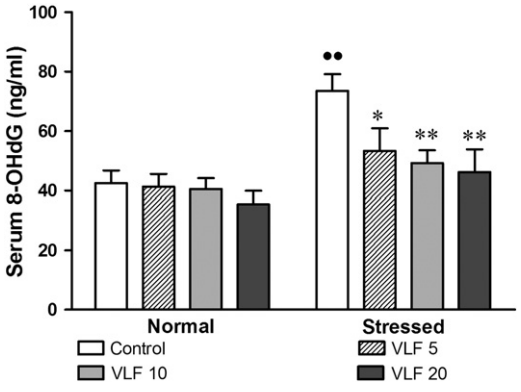

Fig. 3. Effect of venlafaxine (VLF) in doses 5, 10 and 20 mg/kg/day, i.p for 21 days, on serum level of 8-OHdG in non-tested and antidepressant testing-exposed (stressed) mice. Results represent mean ± SEM (n = 10). [$F(0.3131) = 4.688$, $p = 0.0002$]. ●●$p<0.01$ vs naïve, *$p<0.05$ vs stress control and **$p<0.01$ vs stress control.

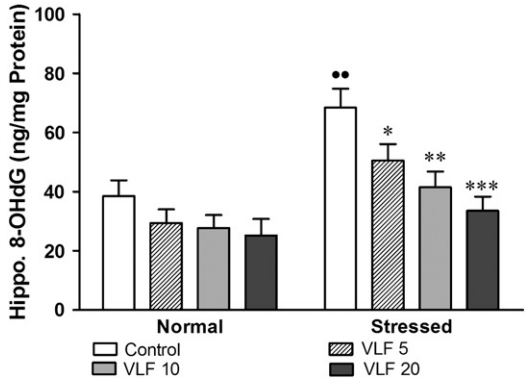

Fig. 4. Effect of venlafaxine (VLF) in doses 5, 10 and 20 mg/kg/day, i.p for 21 days, on hippocampal level of 8-OHdG in non-tested and antidepressant testing-exposed (stressed) mice. Results represent mean ± SEM (n = 10). [$F(0.3273) = 5.003$, $p = 0.0001$]. ●●$p<0.01$ vs naïve, *$p<0.05$ vs stress control, **$p<0.01$ vs stress control and ***$p<0.001$ vs stress control.

used to access the antidepressant properties (McArthur and Borsini, 2006). The forced swimming-induced state of immobility in animals claimed to represent a condition similar to human depression and amenable to reversal by antidepressant drugs (Borsini and Meli, 1988). Both FST and TST are sensitive to all major classes of antidepressant drugs and are important tools to study the neuropharmacological mechanisms involved in the antidepressant responses (Renard et al., 2003).

In the present study, exposure of animals to depressive stress in FST and TST increased the MDA level in the hippocampus of the tested animals. Combined dysregulation of lipid metabolism and antioxidant defenses as an integral component of stress and depression was reported (Yager et al., 2010). Assay of MDA, a major oxidative degradation product of membrane unsaturated fatty acids and arachidonic acid peroxidation, is employed as a measure for lipid peroxidation and thus also for oxidative stress. It has been shown to be biologically active with ROS properties (Gałecki et al., 2009). Furthermore, it may modify lipids and proteins to generate advanced lipoxidation end (ALE) products. ALEs have detrimental effects as they are pro-inflammatory, weaken the antioxidant defenses and impair DNA repair (Aldini et al., 2007).

Depression is characterized by activation of the inflammatory response system with increased production of procytokines that activate the immune cells leading to overproduction of ROS, which leads to increase in the MDA levels (Kubera et al., 2011). However, the assumption of involvement of proinflammatory cytokines in the elevation of MDA in our results is not strong. This is because of the fact that, exposure to acute stress increases plasma hydrocortisone concentration as adrenal steroids are considered stress hormones and are secreted during stress (Reddy, 2006). Corticosteroids are powerful anti-inflammatory agents and inhibit the expression and production of proinflammatory cytokines (Elenkov and Chrousos, 2002). Furthermore, the short period of stress during FST and TST (6 min) is unlikely to activate the inflammatory response (Bourin et al., 2005).

On the other hand, exposure to stress leads to increased mobilization of free fatty acids and stimulation of the immune system. Activation of immune cells leads to overproduction of ROS. Increased ROS production may cause the destruction of phospholipids and altered viscosity of neuronal membranes, leads to increase in the level of lipid peroxidation products as MDA. (Howland and Parikh, 2010).

In the present study, hippocampal NO significantly increased in animals exposed to FST and TST. The relationship between the increase in NO levels and the increase in ROS, RNS, and signs of damage caused by ROS & RNS to fatty acids and DNA was reported (Caimi et al., 2010). NO is synthesized from L-arginine by NOS, a family of enzymes consisting of constitutive (cNOS) and inducible (iNOS) forms. The latter is induced by cytokines and generated the inflammatory effects of NO. Production of NO may cause tissue toxicity after reacting with superoxide anions, which generates peroxynitrite anions and peroxynitrous acid (Bauer and Sotníková, 2010). Studies indicated presence of an increase in NO production and signaling by depressive stress (Wegener et al., 2010). Moreover, increased expression of hippocampal iNOS by stress was reported (Harvey et al., 2006).

Exposure to acute stress does not only increase ROS & RNS production but also attenuate the antioxidant defenses. Results of the present study showed that, exposure of animals to stress during antidepressant testing significantly reduced the levels of both GSH and TAC and activity of GST in hippocampal neurons.

Glutathione is an important antioxidant formed in the liver from three aminoacids namely glycine, glutamine and cysteine. Cysteine is the rate limiting step in the synthesis of reduced glutathione (GSH), the active form of glutathione. GSH is a strong antioxidant that protects cells against damage caused by free radicals, and it recycles vitamin C and E, so that they again become active as antioxidants after having been used in antioxidant processes (Maes et al., 2000). GSH and GSH-related enzymes play a key role in protecting the cells against the damaging effects of reactive oxygen species. GSH acts as a reductant, reduces hydrogen peroxide and lipid hydroperoxides directly to H_2O. Depletion of intracellular GSH, under conditions of continuous oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species (Du et al., 2008). Moreover, previous studies showed that, chronic mild stress is accompanied by a significant decrease in GSH levels in the prefrontal cortex of rats (Eren et al., 2007a). Additionally, N-acetyl cysteine, a GSH precursor, attenuates the depressive symptoms in patients evaluated by the Montgomery–Asberg Depression rating scale (Berk et al., 2008). The exact mechanisms of stress-induced changes in brain GSH concentrations are not completely elucidated.

The observed decrease in the total antioxidant (TAC) defense in hippocampi of animals exposed to acute stress during FST and TST is in agreement with previous studies. Stress is characterized by a decreased antioxidant status, as evidenced by lowered tryptophan, tyrosine, albumin, zinc and vitamin E (Owen et al., 2005). Furthermore, TAC is lower in patients with major depression than in healthy volunteers (Cumurcu et al., 2009).

Exposure to stress induces marked disturbances in oxidative parameters and decrease in the activity of antioxidant enzymes (Lucca et al., 2009). GST is one of the most important GSH-dependent enzymes involved in detoxifying processes. It is responsible for the removal of hydrogen and organic peroxides and formation of oxidized glutathione. The latter can be reduced back to its thiol form (GSH) by glutathione reductase enzyme, utilizing NADPH produced in the pentose phosphate pathway (Berk et al., 2008). Moreover, genetic polymorphisms in GST and its altered expression and activity are associated with oxidative DNA damage. Antioxidant enzymes, including GST may represent an important target in the actions of antidepressant drugs (Balk Rde et al., 2010).

In the results of the present study, VLF antagonized the stress-induced elevation in hippocampal MDA and NO. Moreover, it increased the activity of the antioxidant defense system, including TAC, GSH levels in the naïve animals, the fact that reflected its ability to stimulate the antioxidant defenses. In addition it significantly antagonized the deterioration in GSH and TAC levels and GST activity in the hippocampus of tested animals in doses that showed antidepressant activity in FST and TST. These results reflected its ability to replenish the stress-induced depletion in GSH and TAC cellular stores and reactivate GST enzyme inhibited by exposure to stress. This may indicate the link between antioxidant response and the antidepressant effect of VLF.

Moreover, several studies have provided evidence for the antioxidant effects of antidepressant drugs (Zafir and Banu, 2007; Schmidt et al., 2008; Kirkova et al., 2010). These results were obtained by demonstrating the reversal of oxidative disturbances induced by depression after antidepressant treatments (Khanzode et al., 2003), suggesting that antioxidant properties may contribute to their clinical effects. Furthermore, studies showed that VLF, in its antidepressant doses, can protect brain of animals from lipid peroxidation in experimental models of depression (Eren et al., 2007b; Dhir and Kulkarni, 2007; Kumar et al., 2009).

Studies have revealed that serotonin (5-HT) has relevant antioxidant properties. The molecule has been demonstrated to have powerful free radical scavenging properties generated in vitro by its chemical system (Andron and Pappolla, 2001; Herraiz and Galisteo, 2004). In addition, tryptophan and 5-HT have also been used to prevent in vitro chemically-induced free radical generation (Candenas et al., 1989). Reduction of 5-HT content in the brain of animals induced dose-dependent lipid peroxidation and antioxidant status depletion in the brain. In consequence, brain tissue is especially susceptible to the induction of oxidative-dependent tissue damage in situations accompanied by a

reduction of 5-HT synthesis. Hence, VLF may exert its protective effect against oxidative lipid peroxidation through inhibiting 5-HT reuptake and noradrenaline mechanisms because it is a dual serotonin and noradrenaline reuptake inhibitor leading to accumulation of 5-HT which by his role protects against oxidative stress and lipid peroxidation (Zafir et al., 2009).

Moreover, VLF decreased the hippocampal NO levels in its antidepressant doses. This indicated the ability of VLF to inhibit the formation of nitrosoradicals (RNS) in addition to ROS. This may represent another mechanism by which VLF protects against oxidative damage and lipid peroxidation. These results are in agreement with previous results that showed the involvement of L-arginine–NO–cyclic guanosine monophosphate pathway in the antidepressant effect of VLF in mice (Dhir and Kulkarni, 2007). In addition, Kumar et al. (2009) indicated the involvement of the NO mechanism in the protective effect of VLF against acute-immobilization stress-induced lipid peroxidation and attenuation in the antioxidant defenses.

On the other hand, this work investigated the effect of VLF on the GSH antioxidant defense system in the hippocampus of tested animals, an important structure involved in the regulation of mood and cognition. VLF showed the ability to reactivate the antioxidant defenses including GSH, TAC and GST that were depressed by exposure to the acute stress. These results are in agreement with previous studies. Studies have reported disturbances in GSH levels and in the GSH metabolizing enzymes in patients with major depression (Ozcan et al., 2004). Eren et al. (2007b) indicated that long-term treatment of rats with VLF increased GSH, vitamin A and C concentrations and GSH-Px activity in brain cortex and erythrocytes. In addition, VLF can reactivate the antioxidant enzymes as catalase and glutathione peroxidase inhibited by oxidative stress (Kumar et al., 2009).

Increased oxidative stress-induced by stress may increase the incidence of neuronal and DNA damage. This was clear in our results that showed a significant increase in both serum and hippocampal levels of 8-OHdG, a biomarker for oxidative DNA damage (Valavanidis et al., 2009) after exposure to acute stress during FST and TST. In normal conditions, ROS & RNS attack nuclear and mitochondrial DNA causing oxidized nucleosides and consequently, mutagenic DNA lesions. One of these lesions is 8-OHdG, the end product of the hydroxylation of guanine. The DNA lesions are consequently removed by the base excision repair (BER) pathway, which prevents replication of DNA lesions. Moreover, ROS & RNS inhibit the BER system through direct interactions with cellular repair proteins (Feng et al., 2006). Since the BER pathway removes the mutagenic 8-OHdG lesions, the inhibitory effects of ROS & RNS pathways on BER activity may potentiate mutagenesis and DNA damage. Once eliminated, the 8-OHdG lesions may be found in the plasma and are excreted in the urine (Wu et al., 2004). Furthermore, serum levels of 8-OHdG are significantly higher in depressed patients and are also higher in patients with recurrent depressive episodes (Forlenza and Miller, 2006). In addition, 8-OHdG was found to be significantly higher in peripheral leucocytes of depressed patients (Irie et al., 2005). A recent study indicated that, oxidative damage to RNA, rather than to DNA, occurs in vulnerable neurons of the brain in patients with major mental illness and may contribute to the pathology of these disorders (Che et al., 2010).

VLF significantly antagonized the increase in 8-OHdG level in both serum and hippocampus in doses that showed antidepressant activity in both FST and TST. The decrease in hippocampal 8-OHdG reflects the inhibition of central DNA-damage that can be related to the ability of VLF to protect hippocampal neurons from oxidative stress and its ability to stimulate the antioxidant defenses as mentioned above. Moreover, it can be related to the ability of VLF to stimulate hippocampal expression of brain-derived neurotrophic factor (BDNF). BDNF is a key neurotrophic factor in the brain. It plays an important role in the etiopathogenesis and pharmacotherapy of mental disorders, such as depression or schizophrenia (Czubak et al., 2009). In recent years, studies have shown that exposure to depressive stress, significantly changes BDNF levels in the brain. Larsen et al. (2010) reported that, hippocampal BDNF expression decreases by exposure to chronic unpredictable stress and increases by chronic treatment with antidepressants particularly VLF. Similar results were reported by Cooke et al. (2009) and Zhang et al. (2010). Also, synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in BDNF expression in rat hippocampus were reported (Xu et al., 2006). These results indicate the involvement of the effect on BDNF in the mechanisms of the neuroprotective and antidepressant effects of VLF.

The decrease in serum 8-OHdG in this study may be related to both central and systemic protection against oxidative DNA damage. Hence, our results showed that, in addition to its antidepressant properties, VLF has antioxidant and protective effects against stress-induced oxidative DNA-damage. These effects may refer to the ability of VLF to protect not only against depression but also against neuronal damage and the systemic effects of oxidative stress.

5. Conclusions

From the results of this study, we can conclude that, in addition to the antidepressant activity of VLF mediated by non-selective inhibition of serotonin and norepinephrine, it has also antioxidant properties exerted by enhancing the antioxidant defenses and attenuation of oxidative stress and lipid peroxidation. In addition, VLF has a neuroprotective effect exerted through protection of neurons against DNA damage and cell death. Protection against DNA damage may represent an important target for treatment of depression in the future.

Conflicts of interest statement

Authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.pbb.2011.07.015.

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