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Full Length Article

Possible antidepressant effects of vanillin against experimentally induced chronic mild stress in rats

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ABSTRACT

Vanillin is a flavoring agent widely used in food and beverages such as chocolates and dairy products and it is also used to mask unpleasant tastes in medicine. It has been reported to have antioxidant, anti-inflammatory and antiapoptotic properties. The current study was designed to investigate the protective effects of vanillin against experimentally induced stress in rats. Briefly rats were subdivided into four groups. Three groups were subjected to chronic mild stress and the fourth group served as normal control group. One week before induction of stress drugs or saline was administered daily and continued for another nine weeks. At the end of the experimental period behavioral tests including sucrose preference test, forced swim test and elevated plus maze test were assessed. In addition, brain biochemical parameters including MDA, GSH, NO and serotonin were determined. Vanillin succeeded to restore the behavioral and biochemical changes associated with stress. It significantly increased sucrose consumption in sucrose preference test and time spent in open arm in elevated plus maze test as compared to stress control group. It also reduced immobility time in forced swim test and time spent in closed arm in elevated plus maze test. Additionally, it significantly decreased brain MDA and NO levels and significantly increased brain GSH and Serotonin levels compared to stress control group. It could be concluded that vanillin showed beneficial protective effects against experimentally induced stress in rats.

1. Introduction

Depression is a disabling and widely distributed disorder that is associated with exposure to stressful life events. Studies of chronic stress in animal models and postmortem tissues from depressed patients demonstrated reduced size of limbic brain regions that regulate mood and cognition, and decreased neuronal synapses in these brain areas may play a major role in the pathogenesis of depression (Masi and Brovedani, 2011).

So far, the specific mechanism of depression is not well defined; yet, theories mainly focus on the involvement of the

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neurotransmitter serotonin. Compounds that suppress the elevated concentration of serotonin in the synaptic cleft have been authenticated to possess antidepressant effects (Belmaker and Agam, 2008). Recently, oxidative stress has shown important role regarding psychiatric diseases such as depression and has been suggested as an important factor in the pathogenesis of depression (Ng et al., 2008). Maes et al. (2011) demonstrated the entanglement of oxidative and nitrosative stress in the pathophysiology of depression.

Treatments for depression include tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin–noradrenergic reuptake inhibitors (SNRIs), and other atypical antidepressant drugs such as monoamine oxidase inhibitors (MAOIs) (Nemeroff, 2007). However, the efficiency of these antidepressants is variable, and most of them possess serious adverse effects including sleep disturbance, sedation, agitation and tiredness, thus, there is a crucial demand for brand new, efficient, and well tolerated antidepressants (Nestler et al., 2002).

Venlafaxine is an SSNRIs mainly used in the treatment of depression and obsessive diseases (Golden and Nicholas, 2000). Pharmacokinetic data demonstrated venlafaxine as a sole treatment in many clinical trials (Mbaya, 2002). It restrains the serotonin transporter by binding the receptor at a site other than active binding site for serotonin (Murphy et al., 2004). Venlafaxine was selected in the present study as a standard drug because it is widely prescribed for treating major depression and it is well tolerated with fewer side effects (Dubovicky et al., 2014).

Vanillin (4-hydroxy-3-methoxybenzylaldehyde) is the main component of natural vanilla. Vanillin has been widely used as a flavoring agent and preservative in food and cosmetics. Vanillin has a beneficial protective role against oxidative stress such as protein oxidation and lipid peroxidation in hepatic mitochondria (Liu and Mori, 1993). Moreover, Vanillin has also demonstrated an ability to inhibit the lipopolysaccharide (LPS)-stimulated nuclear factor kappa-B activation and cyclooxygenase-2 gene expression in murine macrophages. It also reduces the expression of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, interferon-γ, and tumor necrosis factor-alpha (TNF-α) (Murakami et al., 2007).

The present research was constructed to elucidate the beneficial role of vanillin in chronic mild stress induced in rats and to explicate the possible underlying mechanisms.

2. Materials and methods

2.1. Animals

Male Wistar Albino rats (230–250 g) supplied from the National Cancer Institute, Cairo, Egypt were used in all the experimental procedures. Rats were left to acclimatize in the animal facility of the Faculty of Pharmacy, Beni Suef University, for one week. All animals were kept under a 12 h light–dark cycle, with controlled humidity (60–80%) and constant temperature (22 °C ± 1 °C). Food and water were supplied ad libitum except when rats were submitted to chronic mild stress (CMS). All experimental procedures were controlled and approved by the Ethics Committee of Faculty of Pharmacy, Beni Suef University.

2.2. Drugs and chemicals

Venlafaxine and vanillin were obtained from Sigma-Aldrich, USA. All other chemicals were of the highest category available in the market. Venlafaxine and vanillin were freshly prepared just before administration to the rats by dissolving them in normal saline.

2.3. Experimental design

After an accommodation period of one week, rats were randomly distributed into four groups ($n = 8$ rats per group) as follows: Group I, normal control group. In this group rats received regular diet and water ad libitum only. Rats did not receive any treatment. Group II (untreated stress group): Animals were subjected to CMS regime and received 1 ml of saline orally for 9 weeks. Groups III and IV: Rats received venlafaxine, 40 mg/kg and vanillin 100 mg/kg respectively as a single oral daily dose one week before induction of CMS and continued for another 9 weeks.

2.4. Chronic mild stress (CMS)

For consecutive twenty eight days, rats were randomly subjected to one of the following external stimulus one each day: food restriction for 24 h, switching of day and night, unclean cages (150 ml of water per cage) for 22 h, cage inclining (45 degree) for 22 h, overcrowded housing (8 animals per cage) for 12 h, introduction of an unusual odor (air freshener) for 12 h, administration of restraint stressor for 20 min, cold stress 4–8 °C and heat stress 38–39 °C for 20 min and intermittent noise for 5 h for 3 periods (Nirmal et al., 2008).

2.5. Behavioral tests

2.5.1. Sucrose preference test (SPT)

Before starting sucrose preference test, rats were kept singly and accustomed for 48 h of forced sucrose solution (1%) drinking using two bottles on each side. Soon after, rats were subjected to 16 h water deprivation and then, two preweighted bottles were put for each rat. The first one has 1% sucrose solution and the second has water. To escape bias, the side of the two bottles was randomly allocated. After one hour, the bottles were reweighted and the alteration in weight difference was calculated. Sucrose preference was calculated as a percentage of the consumed 1% sucrose solution in relation to the total amount of liquid intake (Jiang et al., 2013).

2.5.2. Forced swim test (FST)

Each rat was forced to swim in a cylindrical box. For each rat the total time of immobility during 5 minutes was calculated. Rat was considered immobile when it stopped struggling and floated without movement in the water, doing only moves that make its head above water. The drug is said to have antidepressant like effect if there is a significant decrease in the time of immobility (Porsolt et al., 1979).

2.5.3. *Elevated plus maze*

In the elevated plus maze times spent in open and closed arms were recorded. This depends on the rat's susceptibility concerning darkness, enclosed areas (approach) and an unconditioned fear of heights/open areas (avoidance) (Walf and Frye, 2007).

2.6. *Biochemical analysis*

Brain homogenate from each rat was used for the assessment of biochemical parameters.

2.6.1. *Brain homogenate preparation*

Twenty four hours after behavioral assessment, rats were killed by decapitation and the brains were quickly removed and washed with ice-cold sterile saline (0.9%). Then, brains were homogenized using ice-cold 0.1 M phosphate buffer (pH 7.4). This step was repeated five times. The homogenate was centrifuged at 2500 × *g* (4 °C) for 15 min to remove any cellular debris. After centrifugation supernatants were separated and used for the estimation of biochemical parameters.

2.6.2. *Determination of lipid peroxidation level*

Brain lipid peroxides content was estimated by determination of the level of thiobarbituric acid reactive substances (TBARS) measured as MDA according to the method of Wills (1966). Briefly, 0.5 ml of tris hydrochloric acid (0.1 M, pH 7.4) was added to 0.1 ml of the supernatant and the solution was incubated for 2 h. One ml of trichloroacetic acid (10% w/v) was added to the solution and centrifuged at 1000 × *g* for 10 min. Then, 1 ml (0.67% w/v) of thiobarbituric acid (TBA) was added to 1 ml of supernatant, and kept in the boiling water bath for 10 min, cooled and then 1 ml of distilled water was added. Absorbance was measured at 532 nm using a spectrophotometer (UV-1700 Shimadzu, Japan).

2.6.3. *Determination of reduced glutathione level*

Brain reduced glutathione content was determined in accordance with the method of Ellman (1959). 1 ml of the supernatant was precipitated using 1 ml of 4% sulfosalicylic acid and cooled at 4 °C for 1 h. The samples were centrifuged at 1200 × *g* for 15 min at 4 °C. To 1 ml of the supernatant, 2.7 ml of phosphate buffer (0.1 mol/l, pH 8) and 0.2 ml of 5, 5-dithio-bis (2-nitrobenzoic acid) were added. Absorbance was measured immediately at 412 nm (UV-1700 Spectrophotometer, Shimadzu, Japan).

2.6.4. *Determination of nitric oxide level*

The assay determines total nitrite/nitrate level based on the reduction of any nitrate to nitrite followed by the estimation of total nitrite (intrinsic nitrite obtained from reduction of nitrate) by Griess reagent according to the method by Green et al. (1982). Absorbance was determined at 540 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). The concentration of nitrite in the brain was expressed as micromole per milligram of protein.

2.6.5. *Determination of serotonin level*

Serotonin level in the brain was determined according to the method of Hou et al. (2006) using rat Enzyme Linked Immunoassay kit (Abcam, Cambridge, UK).

2.6.6. *Statistical analysis*

Data were presented as mean ± SD. Statistical analysis of the data was carried out using one way analysis of variance (ANOVA) followed by Tukey–Karmer multiple comparisons test for post hoc analysis. Statistical significance was acceptable to a level of $p < 0.05$.

3. Results

3.1. *Sucrose preference percentage*

The mean value for sucrose consumption in normal control rats was 61.20 ± 8.70. Sucrose consumption was significantly reduced in stress control group as compared to normal control group. On the other hand, stress rats treated with venlafaxine or vanillin showed significant increase in sucrose consumption as compared to non-treated stress group (66.67 ± 11.29, 58.02 ± 12.80 respectively) (Fig. 1).

3.2. *Immobility time in forced swim test (FST)*

As shown in Fig. 2, the mean time of immobility in normal control group was 132.20 ± 12.50 seconds. Time of immobility was significantly increased in control non-treated stress group to 176.9 ± 23.51 as compared to normal control group. Treatment of stress rats with venlafaxine or vanillin showed a significant decrease of immobility time as compared to non-treated stress group (140.90 ± 16.82, 138.60 ± 14.99, respectively).

3.3. *The time spent in closed and in open arms in elevated plus maze test*

In normal control group the mean values for time spent in closed arm and open arm (seconds) were 139.90 ± 21.11 and 156.80 ± 18.61 respectively. In stress non-treated control group, a significant increase in time spent in closed arm and a significant decrease in time spent in open arm were detected as compared to normal control group (180.30 ± 24.16 and 132.50 ± 11.95 respectively). Rats treated venlafaxine or vanil-

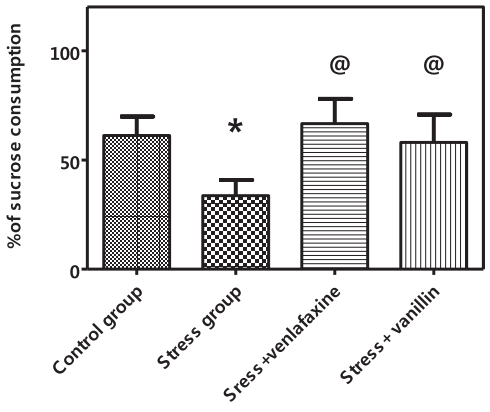

Fig. 1 – Effects of venlafaxine and vanillin on consumption of sucrose in sucrose preference test. Data are given as mean ± SD (n = 8). *Compared to normal control group at P < 0.05. @Compared to the stress-control group at P < 0.05.

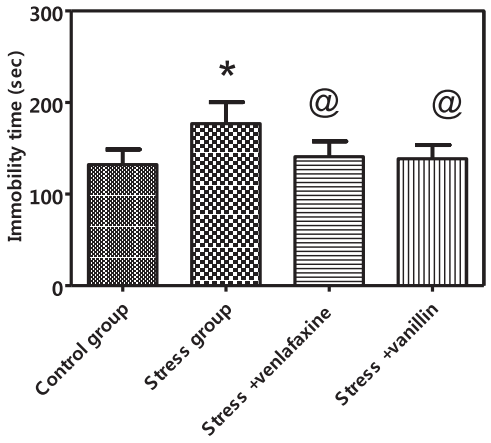

Fig. 2 – Effects of venlafaxine and vanillin on immobility time in forced swim test. Data are given as mean ± SD (n = 8). *Compared to normal control group at P < 0.05. @Compared to the stress-control group at P < 0.05.

lin showed a significant lower time spent in closed arm as compared to stress control group (143.50 ± 17.13,138.2 ± 2.78 respectively) and a significant longer time spent in open arm (139.40 ± 14.25, 155.50 ± 18.01, respectively) (Fig. 3A,B).

3.4. *Effect of treatments on brain MDA level*

Brain MDA level in normal control rats was 35.12 ± 4.44 (nmol/g tissue). CMS significantly elevated brain MDA level as compared to normal control group (84.90 ± 6.08). On the contrary, administration of venlafaxine or vanillin to stress rats partially reduced brain MDA level as compared to stress control group (Table 1).

3.5. *Effect of treatments on GSH brain level*

As shown in Table 1, brain GSH level in normal control rats was 0.16 ± 0.03 (ng/mg tissue). Brain GSH level was notably decreased in stress non-treated rats. Treatment of stress rats with venlafaxine or vanillin significantly elevated brain GSH level as compared to stress control group.

3.6. *Effect of treatments on nitric oxide (NO) brain level*

A significant increase in brain NO level was expressed in stress non-treated control rats in comparison with normal control rats. Treatment of stress rats with venlafaxine or vanillin significantly reduced brain nitric oxide level in stress rats.

3.7. *Effect of treatments on brain serotonin brain level*

The mean value for serotonin brain level in normal control group was 17.08 ± 2.27. Brain serotonin level was significantly reduced in non-treated stress group as compared to normal control group. On the other hand, treatment of rats with venlafaxine or vanillin significantly increased brain serotonin level to 15.22 ± 1.37 and 14.86 ± 2.10 as compared to stress control rats (Table 1).

4. Discussion

The present investigation aimed at elucidating the possible protective effects of vanillin in chronic mild stress induced in rats.

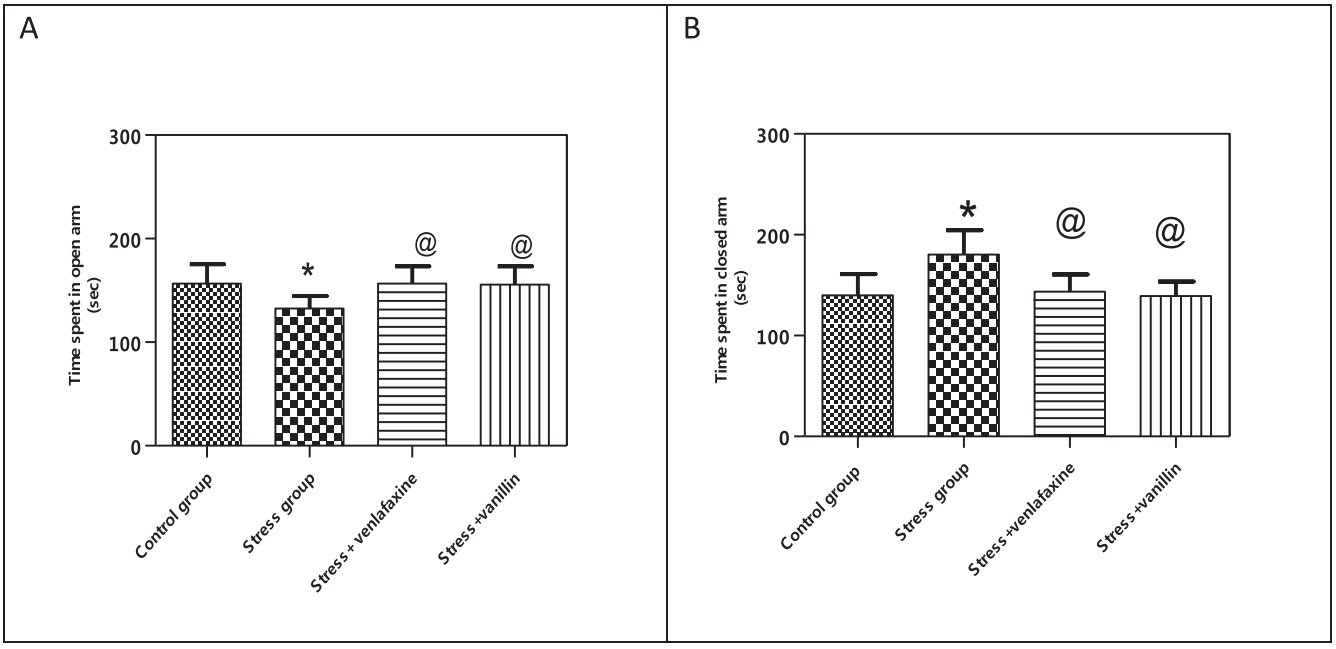

Fig. 3 – Effects of venlafaxine and vanillin on the time spent in (A) closed arm and (B) open arm in elevated plus maze test. Data are given as mean ± SD (n = 8). *Compared to normal control group at P < 0.05. @Compared to the stress-control group at P < 0.05.

Table 1 – Effects of venlafaxine and vanillin on brain MDA, GSH, NO and serotonin levels.

	Normal control (n = 8)	Stress-groups (n = 8)		
		Control	Venlafaxine (40 mg/kg)	Vanillin (100 mg/kg)
MDA level (nmole/g tissue)	35.12 ±1.57	84.90 ±2.15[a]	49.72 ±2.66[a,b]	66.91 ±1.60[a,b]
GSH (ng/mg tissue)	0.16 ±0.010	0.056 ±0.009[a]	0.15 ±0.011[b]	0.16 ±0.014[b]
NO level (μmol/gm tissue)	15.06 ±0.41	26.66 ±1.09[a]	17.28 ±0.71[b]	15.32 ±0.77[b]
Serotonin level (ng/ml)	17.08 ±0.80	7.84 ±0.53[a]	15.22 ±0.48[b]	14.86 ±0.74[b]

Data are given as mean ± SE (n = 8).
[a] Compared to normal control group at $P < 0.05$.
[b] Compared to the stress-control group at $P < 0.05$.

To achieve this goal, two sets of experiments were conducted: a behavioral study to reveal depression incidence, and a biochemical study to investigate the underlying mechanisms behind anti depression test agent effects.

The CMS method is a representative method for the induction of depression in rodents and is similar to the development of stress in humans (Xu et al., 2015).

In the present study, CMS significantly decreased sucrose consumption in sucrose preference test, increased immobility time in the FST, increased in time spent in closed arm and a decreased in time spent in open arm in elevated plus maze test. Immobility time in the FST is an indicator of behavioral despair (Cryan et al., 2002; Dalvi and Lucki, 1999). Moreover, the low level of sucrose consumption in stressed rats has represented an important marker of anhedonia, which is a very important symptom of depression. Anhedonia happens when there is a loss of interest in pleasurable and rewarding experiences (Muscat et al., 1990; Willner et al., 1992). Bekris et al. (2005) suggested that chronic stress is responsible for the damage of nerve cells in the neural reward system and this impairment could be correlated to the serotonergic (5-HT) and dopaminergic (DA) systems.

In the present investigation, vanillin significantly increased sucrose consumption, significantly reduced the time of immobility in the FST, decreased in time spent in closed arm and an increased in time spent in open arm. This suggests that vanillin could have an antidepressive effect. This antidepressant effect could be attributed to vanillin's action on serotonin and dopamine activity (Komiya et al., 2006).

Oxidative stress is characterized by loss of balance in oxidation-reduction reactions. In oxidative stress, the ability of the antioxidant defense system to remove the excess of reactive oxygen species is reduced. It is to be noted that increased oxidative stress was observed in patients with depression. (Kumar et al., 2004; Sarandol et al., 2007). The present study showed that chronic mild stress significantly increased oxidative stress as demonstrated by increased brain MDA, nitric oxide levels and decreased brain GSH level. Oxidative stress has been suggested as an important contributive factor in the pathogenesis of depression (Ng et al., 2008).

Previous studies demonstrated the role of oxidative and nitrosative stress in the pathophysiology of depression (Maes, 2011; Maes et al., 2011). Elevated levels of ROS (Maes et al., 2011) and NO (Dhir and Kulkarni, 2011; Suzuki and Colasanti, 2001) and inconsistent levels of the antioxidant GSH in the postmortem depression brain were observed (Gawryluk et al., 2011). Consequently, oxidative and nitrosative mechanisms have been suggested as targets for new antidepressant drugs (Lee et al., 2013).

Vanillin attenuated oxidative stress as marked by reduced MDA, nitric oxide levels. It also restored glutathione level as compared to stress control group. Vanillin is known to be a potent antioxidant. It could inhibit singlet oxygen-induced protein and lipid oxidation. It traps superoxide and hydroxyl radicals. These indicate its potential role in prevention of oxidative damages in tissues. The antioxidant property of vanillin could contribute to its antidepressant activity (Kamat et al., 2000; Santosh Kumar et al., 2002).

The present study revealed that chronic mild stress significantly reduced serotonin level in brain homogenate. Previous theories suggested the involvement of traditional signal transduction mechanisms including abnormalities in the gamma amino butyric acid (GABA) and serotonin receptor systems in the pathophysiology of depression (Kessler et al., 2005).

Vanillin significantly increased serotonin level in brain homogenate. 5-HT is closely associated with depression (Cowen, 2008) and previous results showed that vanillin could relieve symptoms of depression in the rat model of chronic depression via increment of serotonin level in the brain (Xu et al., 2015).

To conclude, vanillin showed antidepressant activity in rats. This antidepressant activity could be due to its antioxidant and serotonin agonistic actions.

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