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Evidence for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice

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ABSTRACT

Dietary flavonoids possess a multiplicity of neuroprotective actions in various central nervous pathophysiological conditions including depression. Ellagic acid is a polyphenolic compound that occurs in plants such as raspberries, nuts and eucalyptus species. The present study was designed to investigate the antidepressant-like effect of ellagic acid in mice using forced swimming test (FST) and tail suspension test (TST). The involvement of the monoaminergic and opioid systems in the antidepressant-like activity of ellagic acid was also studied. Our results showed that ellagic acid when administered acutely or chronically to mice (25, 50 and 100 mg/kg, p.o.), produced a significant reduction in the duration of immobility, with a profile comparable to that of fluoxetine (20 mg/kg, p.o.). However, ellagic acid treatment had no effect on the locomotor activity of mice when tested in actophotometer. The reduction in immobility time observed with ellagic acid treatment (50 mg/kg, p.o.) was prevented by pretreatment with *p*-chlorophenylalanine (100 mg/kg, i.p., a serotonin synthesis inhibitor), pindolol (10 mg/kg, i.p., a β-adrenoceptors blocker/$5HT_{1A/1B}$ receptor antagonist), ketanserin (5 mg/kg, i.p., a $5HT_{2A/2B}$ receptor antagonist), ondansetron (1 mg/kg, i.p., a $5HT_3$ receptor antagonist), prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist) and yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), but not with naloxone (1 mg/kg, i.p., an opioid receptor antagonist). Our results suggest that ellagic acid produced an antidepressant-like effect which was unrelated to its locomotor activity. Furthermore, this anti-immobility effect seems most likely to be mediated through an interaction with the monoaminergic system (serotonergic and noradrenergic systems) and not through the opioid system.

1. Introduction

Major depression is one of the most common psychiatric disorders and is characterized by changed mood, lack of interest in the surroundings and psychosocial and physical impairment (Aldous and Mann, 1963). In modern medicine, a large number of antidepressant drugs are available for the treatment of depression. Although these drugs provide improvement in the clinical condition of patient, their slow onset of action and adverse effects are major concerns. This is further complicated by the fact that, approximately 30% of patients may not respond to drug therapy. Therefore, the search for newer drugs for the treatment of major depression is urgently needed (Kulkarni and Dhir, 2009; Montgomery, 2006).

Monoamines are thought to play important roles in the development of symptoms of depression. The causes of depression have been, in part, attributed to the irregularities of one or all of the monoamine neurotransmitters at the synapse, principally serotonin (5-HT) (Nutt, 2008; Prange, 1974). Many antidepressant drugs work by modulating these neurotransmission systems. In fact, most of the prescribed antidepressants directly affect serotonin turnover in the brain (Kreiss and Lucki, 1995), inhibit serotonin reuptake and also interact with 5-HT_1 and 5-HT_2 receptors (Bruning et al., 2011; Cryan et al., 2005). There is also substantial evidence of the role of noradrenaline in the behavioral effects of several drugs having anti depressant-like activity (Jesse et al., 2010). Monoamine oxidase (MAO) is the key enzyme that is associated with metabolism of monoamines thus, regulating their intracellular concentrations in the brain. Therefore, the abnormal function of this enzyme is thought to be involved in several psychiatric disorders, such as depression (Wang et al., 2007). Recently, accumulating studies have demonstrated that neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), are required for the behavioral effects of antidepressants. Mounting evidence indicates that chronic stress results in down-regulation of BDNF, and the decreased level of BDNF could be reversed by chronic antidepressant treatment (Li et al., 2012; Xiong et al., 2011). In addition to the monoaminergic system, many classical antidepressants activate the opioid system (Brocardo et al., 2009). So it is important to study the possible role of monoamines (serotonin and noradrenaline) along with opioid system in the anti-depressant like effect of investigational drugs.

Flavonoids are low molecular weight bioactive polyphenols which possess a multiplicity of neuroprotective actions in various central nervous pathophysiological conditions including depression (Ullah

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and Khan, 2008). Ellagic acid is a polyphenolic compound that occurs largely as ellagitannins in plants such as raspberries, the stem and bark of eucalyptus species and nuts. This bioflavonoid has been reported to have antioxidant, antifibrotic, anti-inflammatory, cardioprotective and anticancer properties (Girish and Pradhan, 2008). Some of the polyphenolic compounds obtained from plants have been investigated for their antidepressant-like activity in various behavioral models. Curcumin, green tea and cocoa flavonoids are examples of a few such compounds which showed an antidepressant-like effect in animal models (Messaudi et al., 2008; Singal et al., 2004; Wang et al., 2007). The antidepressant-like effect of curcumin has been shown to be related to serotonergic system and may be mediated by an interaction with 5-$HT_{1A/1B}$ and 5-HT_{2C} receptors (Wang et al., 2007). Yi et al. (2010) was reported that naringenin, a dietary flavonoid, possess potent antidepressant-like property via central serotonergic and noradrenergic system. The total flavonoids isolated from the extract of Xiaobuxin-Tang reversed the depressive-like behaviors in chronically mildly stressed rats and normalized the neurotransmitter changes, including the decreased serotonin and its metabolite 5-hydroxyindoleacetic acid levels in hippocampus and prefrontal cortex (An et al., 2008). These results further suggest that dietary flavonoids possess a therapeutic potential in disorders especially where monoaminergic system is involved (An et al., 2008). Therefore we were curious, like other flavonoids mentioned above, whether ellagic acid can also produce an anti-depressant-like activity in behavioral despair models and its influence on the monoaminergic system.

Therefore, the present study was designed to evaluate the antidepressant-like activity of ellagic acid in mice forced swimming test (FST) and tail suspension test (TST), which are predictive models of antidepressant activity. For understanding the mechanism, the possible role of the monoaminergic system and the opioid system in its antidepressant-like effect was also investigated.

2. Materials and methods

2.1. Experimental animals

Adult female albino mice of 2–3 months old (weighing 25–30 g) were maintained at 22–25 °C, under a 12:12 h light/dark cycle. They were housed six per cage with free access to food and water. All experiments were performed on separate groups of animals (N = 6 animals per group) between 9 am and 4 pm, and each animal was used only once in each test. The study protocol was approved by the Institutional Animal Ethics Committee and the procedures in this study were performed according to the guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals, India. All efforts were made to minimize the suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

All the drugs used were obtained from standard commercial suppliers. Ellagic acid (4,4′,5,5′,6,6′-Hexahydroxydiphenic acid 2,6,2′,6′-dilactone), fluoxetine ((±)-N-Methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine hydrochloride), *p*-chlorophenylalanine (PCPA), pindolol (1-(1*H*-Indol-4-yloxy)-3-(isopropylamino)-2-propanol), ketanserin (3-(2-[4-(4-Fluorobenzoyl)-1-piperidinyl]ethyl)-2,4(1H,3H)-quinazolinedione (+)-tartrate salt), ondansetron (1,2,3,9-Tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one hydrochloride), prazosin (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine hydrochloride) and yohimbine (17-Hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride) were obtained from Sigma Chemical Co, USA. All the receptor antagonists were dissolved in saline or 1% Tween 80 and administered to mice by gavage, in a constant volume of 10 mg/ml body weight. Appropriate vehicle treated groups were also assessed simultaneously.

2.3. Experimental procedure

2.3.1. Evaluation of the antidepressant-like effect after acute treatment with ellagic aid

Animals received a single oral dose of saline, ellagic acid or fluoxetine and underwent FST and TST after 30 min. Ellagic acid was made as a suspension in saline and given at three different dose ranges of 25, 50 and 100 mg/kg. The dose selection of ellagic acid was based on a pilot study conducted in our laboratory. The minimum dose that elicited a reduction in immobility period in FST was found to be 25 mg/kg. So we have decided to increase the doses in a logarithmic fashion, i.e. 50 and 100 mg/kg, in order to find any dose response relationship. Moreover, in rodents, the peak plasma concentration of ellagic acid was reported to be achieved about 30 min after a single oral dose (Lei et al., 2003; Teel and Martin, 1988). Therefore, we have evaluated the antidepressant-like activity 30 min after ellagic acid administration. Fluoxetine (20 mg/kg, p.o., single dose), a selective serotonin reuptake inhibitor, was also administered 30 min prior to the tests and served as a positive control (Wang et al., 2007; Yi et al., 2011).

2.3.2. Evaluation of the antidepressant-like effect after chronic treatment with ellagic aid

For the chronic study, saline, fluoxetine or ellagic acid (25, 50, 100 mg/kg) was administered to mice orally for 14 days. On the 14th day, 30 min after the last dose, the animals were evaluated for antidepressant effect in TST and FST.

2.3.3. Evaluation of ellgic acid's possible mechanism of antidepressant-like action using FST

Mice were pretreated with the different receptor antagonists or their respective vehicles and after 30 min; ellagic acid was administered (50 mg/kg, p.o.). FST was conducted 30 min after ellagic acid treatment. The dose and pretreatment period of all the antagonists were decided based on earlier reports (Bruning et al., 2011; Capra et al., 2010; Wang et al., 2007).

2.3.3.1. Role of the serotonergic system in the antidepressant-like effect of ellagic acid in FST. Mice received the injection of PCPA (100 mg/kg i.p., a serotonin synthesis inhibitor) once daily for four consecutive days as pretreatment. 30 min after the last injection of PCPA, mice were treated with either a vehicle or ellagic acid and tested by FST (Wang et al., 2007). To investigate the possible involvement of 5-$HT_{1A/1B}$, 5-$HT_{2A/2C}$ receptors and 5-HT_3 receptors in the antidepressant-like effect of ellagic acid, animals were pretreated with pindolol (10 mg/kg, i.p., a 5-$HT_{1A/1B}$ receptor antagonist), ketanserin (5 mg/kg i.p., a $5HT_{2A/2C}$ receptor antagonist) or ondansetron (1 mg/kg i.p., $5HT_3$ receptor antagonist). After 30 min, they received ellagic acid or vehicle and were tested with the FST 30 min later (Bruning et al., 2011; Wang et al., 2007).

2.3.3.2. Role of the noradrenergic system in the antidepressant-like action of ellagic acid in FST. Animals were pretreated with prazosin (1 mg/kg, i.p., a α_1-adrenoceptor antagonist) and yohimbine (1 mg/kg, i.p., a α_2-adrenoceptor antagonist). After 30 min, they received ellagic acid or vehicle and were tested in FST (Capra et al., 2010).

2.3.3.3. Role of opioid receptors in the antidepressant action of ellagic acid in FST. Animals were pretreated with naloxone (1 mg/kg, i.p., a non-selective opioid receptor antagonist). After 30 min, they received ellagic acid or vehicle and were tested in the FST after an additional 30 min (Bruning et al., 2011).

2.4. Behavioral analysis

2.4.1. Forced swimming test (FST)

The test was conducted using a slightly modified method described by Porsolt et al. (1977). Briefly, each mouse was placed individually in a five liter glass cylinder which was filled with 15 cm of water and was observed for a duration of 6 min. The duration of immobility was recorded manually (Kaster et al., 2007). The mouse was considered immobile when it floated motionless or made only those movements necessary to keep its head above the water surface (Porsolt et al., 1977). They were removed and dried with a towel after the procedure. The water was changed after each test.

2.4.2. Tail suspension test (TST)

The method described by Steru et al. (1985) which is based on the fact that mice will develop an immobile posture when suspended by their tail, an inescapable, short-term stressor. For that, the mice, which were acoustically and visually isolated, were suspended by the tail on a thin horizontal steel rod, 50 cm above the surface with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered to be immobile only when they hung passively and were completely motionless (Steru et al., 1985; Capra et al., 2010).

2.4.3. Locomotor activity of mice using actophotometer

The method described by Boissier and Simon (1965) with slight modification was used. All the mice were assessed in this test to rule out any change induced by the test drug in locomotor activity of the animals. The dose of receptor antagonists used in our study was chosen on the basis of previous studies and was reported not to influence the locomotor activity of mice (Bruning et al., 2011; Dias Elpo Zomkowski et al., 2004; Guilloux et al., 2006; Jesse et al., 2010; Kaster et al., 2005; Redrobe and Bourin, 1997; Wang et al., 2007; Yalcin et al., 2005). The actophotometer contains a square arena (30×30 cm) with walls that are fitted with photocells just above the floor level. The photocells were checked before the beginning of the experiment. The number of times each animal crossed the light beam was recorded automatically. The drug/vehicle treated mice were then individually placed in the arena. After a two minute acclimatization period, the digital locomotor scores were recorded for the next 6 min (Boissier and Simon, 1965; Devadoss et al., 2010).

2.5. Statistical analysis

The data were represented as mean ± S.D. The difference between groups was calculated by one way ANOVA or two way ANOVA followed by Newman–Keuls test as *post hoc* comparison when appropriate. Probability values less than 0.05 (P value<0.05) were considered as statistically significant.

3. Results

3.1. The effect of acute treatment with ellagic acid on the immobility time in FST of mice

Fig. 1 depicts the effect of acute administration of ellagic acid at the doses of 25, 50, and 100 mg/kg p.o. on the immobility time in the FST. *Post hoc* analysis showed that doses of 25, 50 and 100 mg/kg of ellagic acid significantly decreased the immobility time of mice in FST [F (4,25) = 11.46, P<0.0001]. The dose 50 mg/kg of ellagic acid was chosen for all further studies carried out for possible mechanism of action. The positive control fluoxetine (20 mg/kg, p.o.) significantly decreased (P<0.01) the immobility time in FST.

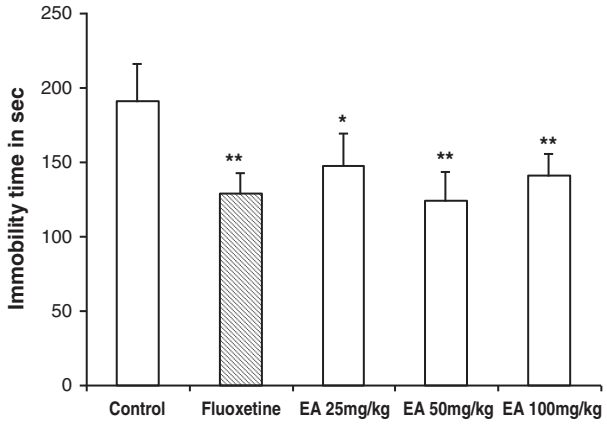

Fig. 1. Effect of acute administration of ellagic acid and fluoxetine in mouse forced swimming test. Ellagic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) were administered p.o., 30 min before the test. Each column represents mean ± S.D. from 6 animals per group. * P<0.01, ** P<0.001 when compared with the control group. EA — ellagic acid.

3.2. The effect of acute treatment with ellagic acid on the immobility time in TST of mice

The effect of acute administration of ellagic acid in three different doses was also evaluated by TST in mice. Single dose administration of 25, 50 or 100 mg/kg of ellagic acid produced a statistically significant reduction in the immobility time as compared to the saline treated group [F (4,25) = 4.67, P<0.007] (Fig. 2). The reduction in duration of immobility time was comparable to that of the standard drug, fluoxetine.

3.3. The effect of chronic treatment with ellagic acid on the immobility time in FST of mice

Fig. 3 shows the effect of chronic administration of ellagic acid at the doses of 25, 50 and 100 mg/kg, p.o. on the immobility time in the FST. Ellagic acid significantly reduced the immobility time in FST at all three doses studied as compared to control group in a dose dependent fashion [F (4,25) = 26.332, P<0.0001]. Also, fluoxetine (20 mg/kg) produced a significant reduction in the immobility time in the FST.

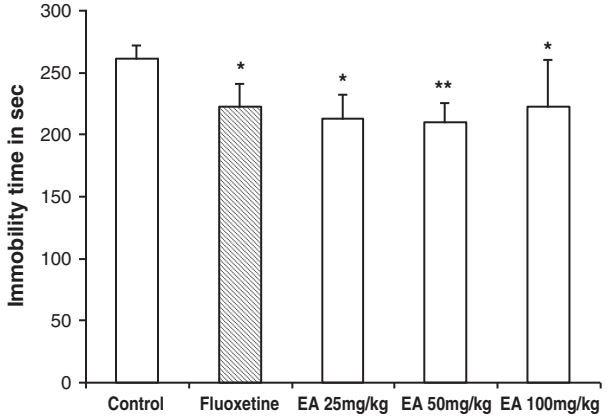

Fig. 2. Effect of acute administration of ellagic acid and fluoxetine in mouse tail suspension test. Ellagic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) were administered p.o., 30 min before the test. Each column represents mean ± S.D. from 6 animals per group. * P<0.05, ** P<0.01 when compared with the control group. EA — ellagic acid.

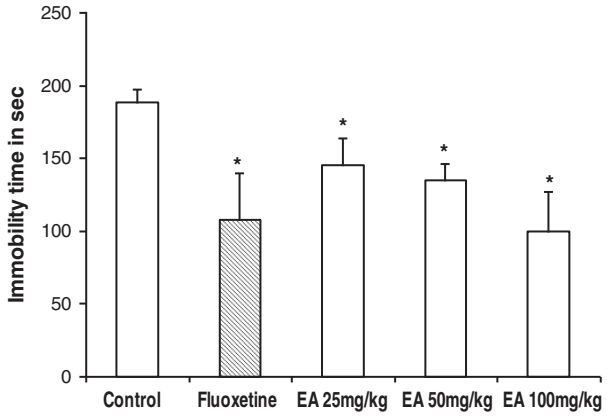

Fig. 3. Effect of chronic administration of ellagic acid and fluoxetine in mouse forced swimming test. Ellagic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) were administered p.o. for 14 days and forced swimming test was performed 30 min after the last dose. Each column represents mean ± S.D. from 6 animals per group. * $P<0.001$ when compared with the control group. EA — ellagic acid.

3.4. The effect of chronic treatment with ellagic acid on the immobility time in TST of mice

The chronic administration of ellagic acid for 14 days produced a statistically significant reduction in the immobility period at 25 and 50 mg/kg doses (Fig. 4). This was comparable to the positive control drug, fluoxetine (20 mg/kg), which also produced significant reduction in the immobility period as compared to control group [$F(4,25)=11.25$, $P=0.0618$]. However, 100 mg/kg of ellagic acid did not produce any statistically significant change in immobility time in the chronic study.

3.5. Role of the serotonergic system in the antidepressant action of ellagic acid in FST

For this, PCPA, pindolol, ketanserin or ondansetron was administered 30 min before ellagic acid and the forced swimming test was performed 30 min after ellagic acid administration. The results in Fig. 5A show that PCPA alone (100 mg/kg, once a day for 4 consecutive days) did not modify the immobility time, while pretreatment of mice with PCPA significantly blocked the reduction in the immobility time elicited by ellagic acid (50 mg/kg, p.o.) in the FST. Two way ANOVA revealed a significant effect of ellagic acid treatment [$F(1,20)=8.20$, $P=0.0096$], PCPA pretreatment [$F(1,20)=15.99$, $P=0.0007$] and ellagic acid × PCPA interaction [$F(1,20)=13.94$, $P=0.0013$]. Further, the antidepressant-like effect of ellagic acid (50 mg/kg, p.o.) was also prevented by pre-treatment of mice with pindolol (10 mg/kg, i.p.) (Fig. 5B). There was a significant effect of ellagic acid [$F(1,20)=15.84$, $P=0.0007$], pindolol [$F(1,20)=33.80$, $P=0.0001$], and ellgic acid × pindolol interaction [$F(1,20)=14.72$, $P=0.001$]. The reduction in immobility time produced by ellagic acid was also blocked by pretreatment with ketanserin (5 mg/kg, i.p.) (Fig. 5C) [ketanserin pretreatment: $F(1,20)=24.15$, $P=0.0001$, ellagic acid treatment: $F(1,20)=8.369$, $P=0.0090$, ellagic acid × ketanserin interaction: $F(1,20)=19.59$, $P=0.0003$] and ondansetron (1 mg/kg, i.p.) (Fig. 5D) [ondansetron pretreatment: $F(1,20)=28.58$, $P<0.0001$, ellagic acid treatment: $F(1,20)=3.211$, $P=0.0883$, ellagic acid × ondansetron interaction: $F(1,20)=18.34$, $P=0.0004$].

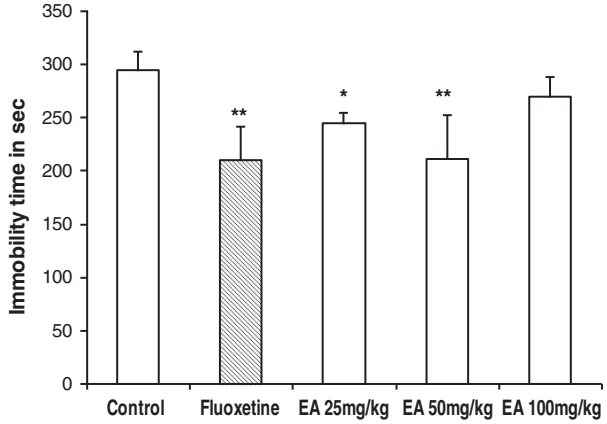

Fig. 4. Effect of chronic administration of ellagic acid and fluoxetine in mouse tail suspension test. Ellagic acid (25, 50 and 100 mg/kg) and fluoxetine (20 mg/kg) were administered p.o. for 14 days and tail suspension test was performed 30 min after the last dose. Each column represents mean ± S.D. from 6 animals per group. *$P<0.05$, **$P<0.01$ when compared with the control group.

3.6. Role of the noradrenergic system in the antidepressant-like action of ellagic acid in FST

Prazosin or yohimbine was administered 30 min before ellagic acid and the force swimming test was performed 30 min after ellagic acid administration. The anti-immobility effect caused by ellagic acid (50 mg/kg, p.o.) was significantly prevented by pretreatment of mice with prazosin (1 mg/kg, i.p.) (Fig. 6A) or yohimbine (1 mg/kg, i.p.) (Fig. 6B). A two way ANOVA revealed a significant effect of ellagic acid [$F(1,20)=12.35$, $P=0.0022$], prazosin [$F(1,20)=13.56$, $P=0.0015$], and ellagic acid × prazosin interaction [$F(1,20)=22.72$, $P=0.0001$]. Similar response was also observed with yohimbine pretreatment as well [yohimbine pretreatment: $F(1, 20)=20.48$, $P<0.0002$, ellagic acid treatment: $F(1,20)=8.20$, $P=0.0096$, ellagic acid × yohimbine interaction: $F(1,20)=12.42$, $P=0.0021$].

3.7. Role of opioid receptors in the antidepressant-like action of ellagic acid in FST

Naloxone was administered 30 min before ellagic acid and the forced swimming test was performed 30 min after ellagic acid administration. The results depicted in Fig. 7 show that pretreatment of mice with naloxone (1 mg/kg, i.p.), an opioid receptor antagonist, was unable to reverse the antidepressant like effect of ellagic acid (50 mg/kg, p.o.) in the FST. The two way ANOVA did not show significant differences of ellagic acid [$F(1, 20)=0.02470$, $P=0.8767$], naloxone pretreatment [$F(1,20)=55.19$, $P=0.0001$] and ellagic acid × naloxone interaction [$F(1,20)=2.72$, $P=0.1148$].

3.8. Effect caused by ellagic acid on the locomotor activity of mice in actophotometer

Neither acute [$F(4, 25)=2.157$, $P=0.1033$] nor chronic [$F(4,25)=0.9228$, $P=0.4664$] treatment of ellagic acid at any of the three doses (25, 50 and 100 mg/kg, p.o.) caused a change in the locomotor activity of mice (Table 1). Fluoxetine, the positive control used, also had no stimulatory or inhibitory effect on locomotor activity when tested in actophotometer.

4. Discussion

In the present study, the antidepressant-like effect was evaluated after acute and chronic treatment with ellagic acid. An effort was made to explore the possible mechanism of action of ellagic acid. To the best of our knowledge, this is the first study which demonstrated the antidepressant-like activity of ellagic acid in FST and TST. In addition, this work provides evidence that the antidepressant-like effect of ellagic acid in FST is mediated by the serotonergic and noradrenergic systems but is not dependent on the opioid system.

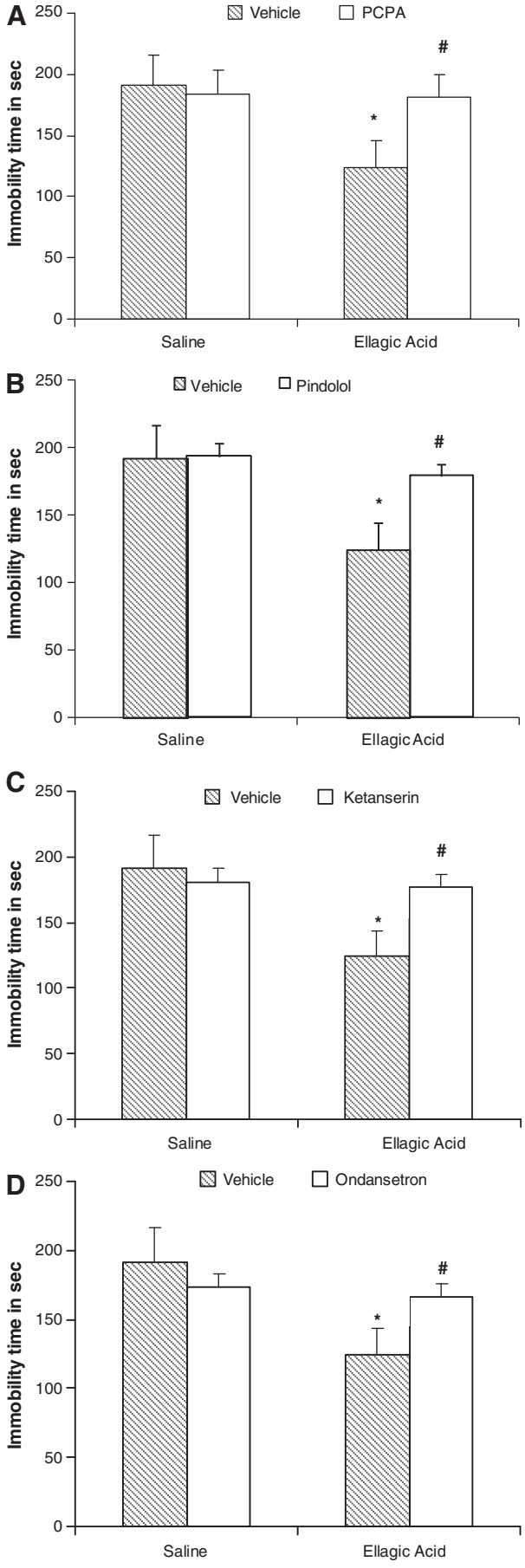

Fig. 5. Effect of pretreatment of mice with PCPA (100 mg/kg, i.p., panel A), pindolol (10 mg/kg, i.p., panel B) ketanserin (5 mg/kg i.p., panel C) and ondansetron (1 mg/kg i.p., panel D) on the immobility time of ellagic acid (50 mg/kg p.o.) in the forced swimming test. Each column represents the mean ± S.D. of 6 animals. $^{*}P<0.01$ when compared with the vehicle treated control. $^{\#}P<0.01$ as compared with ellagic acid alone.

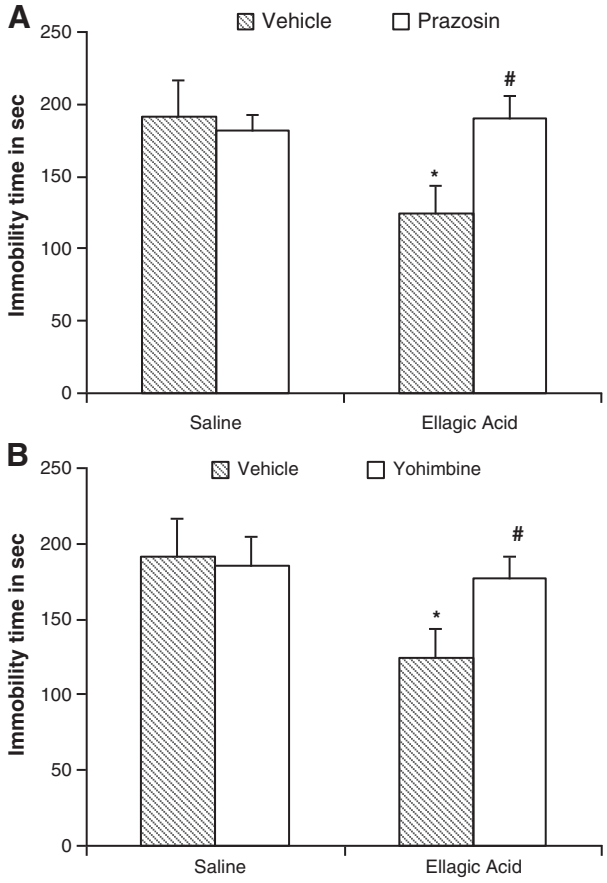

Fig. 6. Effect of pretreatment of mice with prazosin (1 mg/kg i.p., panel A) and yohimbine (1 mg/kg i.p., panel B) in the forced swimming test. Each column represents the mean ± S.D. of 6 animals. $^{*}P<0.01$ when compared with the vehicle treated control. $^{\#}P<0.01$ as compared with ellagic acid alone.

Presently, the actions of flavonoids on the central nervous system have attracted much attention. Flavonoids are shown to have neuroprotective, anxiolytic, sedative and anticonvulsant activities (Spencer, 2009; Spencer et al., 2009). The neuroprotective effects of flavonoids, including ellagic acid, may be mediated through direct actions on enzymes, receptors and signaling pathways (Williams et al., 2004). The other biological effects, such as anti-inflammatory, antioxidant and metal chelating properties, have been shown to augment their neuroprotective effects. They can also protect against neuronal apoptosis through selective actions within stress activated cellular responses including protein kinase signaling cascade (Schroeter et al., 2006). Ellagic acid, a dietary bioflavonoid, also exhibited some of the bioactivities related to central nervous system improvement (Pavlica and Gebhardt, 2005; Yang et al., 2008). Many of these flavonoid compounds which have the ability to improve memory may serve as potential lead compounds for drug development for neurodegenerative disorders including depression (Hanrahan et al., 2011; Ullah and Khan, 2008).

The FST and TST are widely used animal models for screening potential antidepressants. These test models are based on the observation that rats or mice when forced to swim or suspended in a restricted space, eventually ceases to struggle, surrendering themselves to the experimental conditions. This state is considered to be

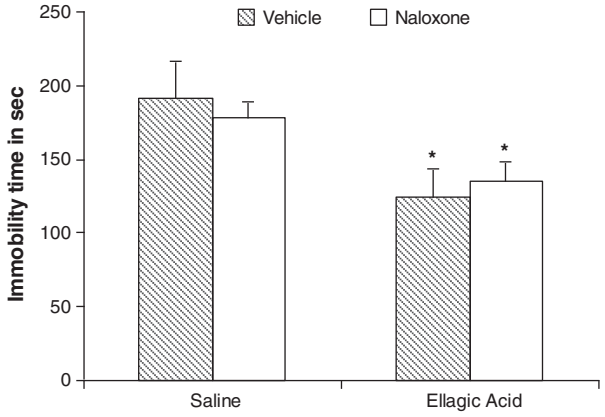

Fig. 7. Effect of pretreatment of mice with naloxone (1 mg/kg i.p.) in the forced swimming test. Each column represents the mean ± S.D. of 6 animals. *$P<0.01$ when compared with the vehicle treated control.

the state of depression and is used to evaluate various antidepressant drugs (Porsolt et al., 1977; Steru et al., 1985). Considering that depression is more prevalent in women than men, and to minimize the variations which can arise due to gender difference, we used only female mice for all behavioral tests (Capra et al., 2010; Dalla et al., 2011). In the present study, we provide convincing evidence that ellagic acid, when administered orally, produced an antidepressant-like effect in FST and TST. In the acute study, ellagic acid reduced the duration of immobility time, which was comparable to that observed with fluoxetine, a standard antidepressant drug. The antidepressant-like activity was found at all the three doses of 25, 50 and 100 mg/kg. There was no clear dose response trend observed in the mice treated with the three acute doses of ellagic acid in the behavioral tests. Many antidepressant compounds including the conventional antidepressant drugs are reported to produce a U-shaped trend when subjected to behavioral tests (Detke et al., 1995; Posser et al., 2009; Yi et al. 2011). In our study, ellagic acid treatment showed a similar trend when subjected to FST, but the present sample size does not allow us to make any such assumptions.

It is reported that the acute increase in the levels of monoamines at the synapse may be only an early step in the potentially complex cascade of events that ultimately results in antidepressant activity. Moreover, clinical antidepressant effects often needs chronic treatment with the drugs. Therefore, we have also evaluated the long term effects of treatment of mice with ellagic acid in FST and TST (Pineyro and Blier, 1999; Xu et al., 2005). We demonstrated that ellagic acid administered for 14 days in mice produced antidepressant-like activity in these animal models. Here, the treatment with ellagic acid showed a dose dependent reduction in the immobility time in the FST. However, ellagic acid at 100 mg/kg, p.o dose, which produced an antidepressant-like effect after acute treatment, failed to produce such an effect when administered for 14 days in the mice TST. This difference in the response of ellagic acid may be related to different sensitivity and variability factors that are associated with these models (Cryan et al., 2005; Kulkarni and Dhir, 2007; Thierry et al., 1986). Although the underlying principle measuring the lack of active coping behavior is identical in the FST and TST, their variability in response to certain antidepressants indicates potentially different substrates and neurochemical pathways mediating performance (Bai et al., 2001; Yi et al., 2011). A plausible pharmacokinetic explanation for the absence of anti-immobility effect at higher dose of ellagic acid in TST may be also considered. The antidepressant effect of certain drugs may be exerted only in certain plasma concentration, i.e. they have a therapeutic window for their antidepressant action (Lavergne and Jay, 2010). We speculate that chronic administration at 100 mg/kg would have resulted in plasma concentrations of ellagic acid higher than that of its therapeutic range (Lavergne and Jay, 2010).

Table 1
Effect of acute and chronic treatment with ellagic acid on the locomotor activity in the actophotometer performance of mice.

Drug	Number of crossings	
	Acute treatment	Chronic treatment
Saline	249.17 ± 8.19	255.14 ± 6.27
Fluoxetine, 20 mg/kg	260 ± 8.69	251 ± 7.96
Ellagic acid, 25 mg/kg	276.6 ± 7.15	248.6 ± 8.22
Ellagic acid, 50 mg/kg	246.2 ± 7.07	268.2 ± 7.21
Ellagic acid, 100 mg/kg	262 ± 9.61	259 ± 9.98

Results are expressed as mean ± S.D. of 6 animals. A) In acute study, mice received single dose of saline or one of the above drugs, [$F(4,25)=2.157$, $P=0.1033$] and in chronic study, mice received saline or one of the above drugs for 14 days [$F(4,25)=0.9228$, $P=0.4664$] before being tested in actophotometer.

Some of the classical antidepressant drugs are also central nervous system stimulants, leading to false positive antidepressant-like effects. This was eliminated in this study by showing that ellagic acid, at doses that produced an antidepressant-like effect, had no effect on the locomotor function (Devadoss et al., 2010; Yi et al., 2011).

Considering the importance of the monoaminergic systems in the pathophysiology of major depression, the present study aimed at investigating the influence of ellagic acid on the monoaminergic system in the FST (Naughton et al., 2000; Xu et al., 2005). According to previous reports, PCPA at the present dose for four consecutive days was able to deplete the endogenous store of serotonin successfully without affecting the noradrenergic or dopaminergic levels (Eckeli et al., 2000; Wang et al., 2007). In the present study, pretreatment with PCPA prevented the anti-immobility effect induced by ellagic acid in FST, suggesting the involvement of the serotonergic system in the antidepressant-like effect of this bioflavonoid.

5-HT_{1A} receptors, which are abundantly expressed in the brain, are thought to be essential to the antidepressant like response. 5-HT_{1A} receptors are located presynaptically in the raphae nuclei and post synaptically in limbic and cortical regions (Blier and Ward, 2003). These receptors act by inhibiting the firing rate of 5-HT neurons and particularly the antidepressant responses (Bruning et al., 2011). It is also reported that 5-HT_{1A} receptor knockout mice cannot be rescued with antidepressant drug administration in the TST (Mayorga et al., 2001; Yi et al., 2011). The 5-HT_2 receptors are also widely distributed throughout the brain (Capra et al., 2010; Celada et al., 2004). There was a significant hypersensitivity of 5-HT_2 receptors in the brain of depressed suicide victims. The elevated densities of 5-HT_{2A} receptors in depressed patients showed a significant reduction with a clinical recovery (Maj et al., 1985). The role of 5-HT_3 receptors in depressive disorders is also reported (Bruning et al., 2011). In our study, the anti-immobility effect elicited by ellagic acid in the FST was blocked by the pretreatment of mice with both pindolol and ketanserin. Similarly, pretreatment with ondansetron, a 5-HT_3 receptor antagonist also abolished the reduction in immobility time induced by ellagic acid in FST. These results indicate that the antidepressant-like action of ellagic acid is mediated, at least in part, by an interaction with the 5-$HT_{1A/1B}$, 5-$HT_{2A/2B}$ and 5-HT_3 receptors. A similar modulatory effect of flavonoids from Xiaobuxin-Tang and naringenin on the monoaminergic system was reported by other researchers, indicating a similar mode of action of these compounds (An et al., 2008; Yi et al., 2010).

In parallel with the serotonergic system, noradrenaline also plays a major role in behavioral effects of several drugs (Yi et al., 2011). Repeated administration of antidepressants increased the number and responsiveness of α-$_1$ receptors in the rat brain, thus increasing the release of noradrenaline at certain synapses (Maj et al., 1985, 2000; Yi et al., 2011). Moreover, α-$_1$ and α-$_2$ adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioral models of antidepressant activity (Kaster et al.,

2007). In the present study, the decrease in immobility time elicited by ellagic acid was reversed by pretreatment of mice with an α-$_1$ adrenoceptors blocker, prazosin and an α-$_2$ adrenoceptors blocker, yohimbine indicating the involvement of these receptors in the antidepressant-like action of ellagic acid in the FST (Capra et al., 2010). These results are in agreement with the fact that the antidepressant effect of several compounds and plant extracts in animal models were shown to be reversed by prazosin and yohimbine (Freitas et al., 2010; Machado et al., 2007; Nishizawa et al., 2007).

Recent evidence supports that the activation of the opioid system is implicated in the mechanisms underlying the effect of antidepressants and in the response to stressors and emotionally salient stimuli (Amir, 1982; Brocardo et al., 2009; Schreiber et al., 2002). Furthermore, it has been shown that there is a pronounced reduction in μ-opioid receptor availability in the posterior thalamus and anterior cortex of patients with major depressive disorder (Bruning et al., 2011; Kennedy et al., 2006). In our study, naloxone was ineffective in reversing the decrease in immobility time induced by ellagic acid in the FST, indicating that the antidepressant-like activity of ellagic acid was not mediated through opioid receptors.

The exact mechanism by which ellagic acid modulates the monoaminergic system is rather unclear. It may inhibit the monoamine oxidase enzymes and cause an increase in the amount of monoamines stored and released from the nerve terminals, thus increasing monoaminergic activity (Xu et al., 2005). Studies have been shown that curcumin can inhibit both types A and B monoamine oxidase enzymes in mouse brain (Kulkarni et al., 2008; Wang et al., 2007; Bruning et al., 2011). Moreover, tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of noradrenaline and dopamine and its activity can also be modulated in the antidepressant-like effect of ellagic acid (Kaster et al., 2007).

The focus of current research is moving toward molecular mechanisms that underlie long-lasting downstream changes in the brain after chronic antidepressant treatment. Serotonergic receptors take their physiologic effects by affecting adenylyl cyclase catalytic activity and cyclic adenosine monophosphate (cAMP) concentration. Adenylyl cyclase–cAMP second messenger pathway has been recently suggested to play an important role in depression (Li et al., 2009). Curcumin has been reported to enhance adenylyl cyclase activity and cAMP levels in platelet and various brain regions, and up-regulated mRNA expressions of adenylyl cyclase subtypes adenylyl cyclase 2, adenylyl cyclase 8 and cAMP response element binding protein in the hippocampus, cortex and hypothalamus of the chronic unpredictable mild stress rats (Li et al., 2009). Therefore it is possible that the compounds like ellagic acid may regulate the adenylyl cyclase–cAMP signal pathway which needs to be studied. Evidence also shows that increased BDNF can induce neurogenesis in the hippocampus and has potent neurotrophic effects on a wide range of neuronal populations (noradrenergic, serotonergic, dopaminergic) (Li et al., 2012; Xiong et al., 2011). Apart from this, BNDF is a downstream effect of increased serotonin/norepinephrine neurotransmission. So, it could be hypothesized that ellagic acid might exert antidepressant-like activity by regulating the interaction between the serotonergic system and BDNF, thereby modulating the neurotrophic effect and neurogenesis (Li et al., 2012; Xiong et al., 2011).

In conclusion, this is probably the first study which reports the antidepressant-like activity of ellagic acid in animal behavior despair models. The acute treatment with this bioflavonoid produced anti-immobility effect in FST and TST, which are widely used tests in the screening of antidepressant activity of drugs. In addition, this study provides evidence that the antidepressant-like effect of ellagic acid in FST is dependent on the interaction with the serotonergic (5-HT$_1$, 5-HT$_2$, 5-HT$_3$ receptors) and noradrenergic (α-$_1$ and α-$_2$ adrenoceptors) systems without influencing the opioid system. The antidepressant-like effect was further confirmed after chronic administration of ellagic acid to mice in FST and TST, which more closely resembles the clinically produced antidepressant effect of drugs. However, future experimental and clinical trials may be needed to determine whether ellagic acid will produce a similar therapeutic effect in depressed patients.

Conflict of interest

None.

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