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Antidepressant-like activity of ellagic acid in unstressed and acute immobilization-induced stressed mice

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Abstract:
Background: The aim of present study was to evaluate antidepressant-like activity of ellagic acid in Swiss young male albino mice; and to explore the possible underlying mechanisms for this activity.
Methods: Mice were immobilized for 150 min once only for induction of stress. Ellagic acid (8.75, 17.5, 35 mg/kg, *po*) and fluoxetine (20 mg/kg, *ip*) *per se* were administered to unstressed and stressed mice; and immobility periods were recorded using tail suspension test and forced swim test. The plasma nitrite levels were also estimated in unstressed and stressed mice. Effects of 7-nitroindazole (nNOS inhibitor), aminoguanidine (iNOS inhibitor), prazosin (α_1-adrenoceptor antagonist), sulpiride (selective D_2-receptor antagonist), and p-chlorophenylalanine (p-CPA – tryptophan hydroxylase inhibitor) on antidepressant-like activity of ellagic acid were also evaluated.
Results: Ellagic acid (17.5 and 35 mg/kg, *po*) and fluoxetine *per se* significantly decreased immobility periods of unstressed and stressed mice, indicating significant antidepressant-like activity. There was no significant effect on locomotor activity of the mice. Ellagic acid significantly decreased the plasma nitrite levels in stressed mice only. Aminoguanidine significantly potentiated antidepressant-like activity and plasma nitrite decreasing effect of ellagic acid (35 mg/kg) in stressed mice. 7-Nitroindazole did not enhance antidepressant-like activity and plasma nitrite decreasing effect of ellagic acid in unstressed mice. Prazosin and p-CPA significantly attenuated antidepressant-like effect of ellagic acid (35 mg/kg) in unstressed mice only.
Conclusion: Thus, ellagic acid showed antidepressant-like activity in unstressed mice probably by interaction through adrenergic and serotonergic systems. On the other hand, antidepressant-like activity of ellagic acid in stressed mice might be through inhibition of inducible NOS.

Key words:
antidepressant, ellagic acid, immobilization stress, inducible NOS, neuronal NOS, monoamines

Abbreviations: 5-HT – 5-Hydroxy tryptamine, 7-NI – 7-nitroindazole, AG – aminoguanidine, eNOS – endothelial nitric oxide synthase, FST – forced swim test, iNOS – inducible nitric oxide synthase, nNOS – neuronal nitric oxide synthase, NO – nitric oxide, p-CPA – p-chlorophenylalanine, TST – tail suspension test

Introduction

Depression is one of the major mental disorders. It affects up to 25% of women and 12% of men and is a highly chronic disorder [8]. Antidepressant drugs

used in the treatment of major depressive disorders are believed to act on the central monoaminergic systems mainly 5-HT and nor-adrenergic synaptic neurotransmissions. Selective serotonin reuptake inhibitors and noradrenaline reuptake inhibitors are effective in treating most depressive episodes, but about one third of these patients show only partial or no response to the treatment [40]. Therefore, research for new antidepressants with greater effectiveness is still desirable.

Stress has been observed to play a key role in the etiology of neurodegenerative diseases and mental disorders [6]. Restraint stress for 120 min has been reported to enhance depression-like behavior in mice [22]. Acute exposure to 2 h of restraint stress exhibited a decrease in the concentration of 5-HT and its metabolite – 5-hydroxyindoleacetic acid, in the hippocampus, leading to stress-induced behavioral depression [12]. NO is a short lived, lipophilic molecule generated from L-arginine by nitric oxide synthase. There are three NOS isoforms – iNOS, nNOS and eNOS [31]. NO production is increased in depression [29]. A differential role is played by neuronal and inducible isoforms of NOS in depression in mice under unstressed and stressed conditions. Acute restraint stress has been observed to significantly increase expression of iNOS and plasma nitrite levels in rodents [18, 20, 33]. Aminoguanidine, an inhibitor of inducible NOS, reversed stress-induced depression-like behavior in rats [38]. The antidepressant-like activity of 7-nitroindazole and 1-(2-trifluoromethylphenyl)imidazole, inhibitors of neuronal NOS, have been reported in unstressed mice [37, 41].

Ellagic acid is a naturally occurring polyphenolic compound which has been reported to possess a wide spectrum of pharmacological activities such as antioxidant [13], anticancer [15], antiallergic [25], antimalarial [27], antiwrinkle [2], antiglycative and anti-inflammatory [5]. Further, ellagic acid showed neuroprotective activity against oxidative damage [36], inhibited Aβ 42-induced neurotoxicity *in vitro* [7] and is also a β-secretase inhibitor [16]. Ellagic acid has also been reported to inhibit iNOS [35]. The no-observed-adverse-effect level (NOAEL) of ellagic acid was estimated to be 778 mg/kg b.w./day for rats in 90 days subchronic toxicity study [30]. So, ellagic acid is safe to use.

As mentioned above, ellagic acid has antioxidant and neuroprotective activities, but its antidepressant-like activity and the possible modulation of monoaminergic and nitriergic systems in this activity have not been explored till date. Therefore, the present study was designed to explore antidepressant-like activity of ellagic acid in unstressed and acute immobilization-induced stressed mice; and also to study the possible underlying mechanisms for this activity.

Materials and Methods

Animals

Swiss male albino mice (3 months old, weighing around 20–25 g) were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Since estrogens have been found to have antidepressant effect, so we excluded female mice and used only male mice for the study [19]. Animals were housed separately in groups of 10 per cage (polycarbonate cage size: 29 × 22 × 14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The animals were acclimatized for at least five days before behavioral experiments which were carried out between 9:00 and 17:00 h. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) vide letter number IAEC/69-72, dated 04th January, 2011. The animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Registration no. 0436).

Drugs and chemicals

Prazosin hydrochloride, (±)-sulpiride, 7-nitroindazole, aminoguanidine (all from Sigma-Aldrich, St. Louis, USA); ellagic acid, DL-p-chlorophenylalanine, Tween 80, sodium hydroxide pellets (all from Hi-Media Laboratories Pvt. Ltd., Mumbai, India); fluoxetine hydrochloride (Gnosis Pharmaceuticals Pvt. Ltd., Sirmour, H.P., India); hydrochloric acid (Qualigens fine chemicals, Mumbai); acetic acid glacial (Central Drug House Pvt. Ltd., New Delhi, India) were used in the present study. Ellagic acid was suspended in 0.1% gum acacia. Fluoxetine hydrochloride, prazosin hy-

drochloride and aminoguanidine were dissolved separately in normal saline (0.9% w/v sodium chloride). Sulpiride was dissolved in normal saline followed by addition of one drop of glacial acetic acid. p-Chlorophenylalanine was dissolved in minimum quantity of 0.1 M sodium hydroxide and pH was adjusted to 7 with 0.1 M hydrochloric acid. 7-Nitroindazole was dissolved in normal saline with few drops of Tween 80. Volume of *ip* injection was 1 ml/100 g of mouse.

Selection of doses

Doses of various drugs were selected on the basis of literature, that is, 20 mg/kg for fluoxetine [17], 35 mg/kg of ellagic acid [34], 62.5 μg/kg for prazosin, 50 mg/kg for sulpiride, 100 mg/kg for p-CPA [4, 24]; 50 mg/kg for aminoguanidine [9] and 20 mg/kg for 7-nitroindazole [10].

Immobilization-induced stress in mice

Stress was produced in mice by immobilizing them for 150 min (10 a.m. – 12:30 p.m.) by placing them on their back and taping all its four limbs and trunk on a wooden board [26]. Mice subjected to immobilization were called as stressed mice. Behavioral tests were performed in independent groups of unstressed and stressed mice. Drugs were administered 45 min before immobilization in case of stressed group. Behavioral testing was started 10 min after setting the animals free from immobilization [10].

Behavioral models

Tail suspension test (TST)

It is commonly employed behavioral model for screening antidepressant-like activity in mice [28]. For the test, the mouse was individually suspended on the edge of a table, 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under the test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. The test was conducted in quiet room to avoid disturbances to animals.

Forced swim test (FST)

This test was carried out in mice according to the method of Porsolt et al. [23]. Briefly, mouse was forced to swim individually for 6 min, in open glass chamber (25 × 15 × 25 cm), containing fresh water up to a height of 15 cm and maintained at 25 ± 1°C. After 6 min, they were removed and dried with a towel. Water in the chamber was changed after subjecting each animal to FST because "used water" has been shown to alter the behavior [1]. Mice placed in the chamber for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. They were again forced to swim 24 h later in a similar environment for a period of 6 min [42]. After the first 2 min, activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating in water, making only those movements necessary to keep their head above water. Following the swimming session, mice were towel dried and returned to their housing conditions.

Estimation of plasma nitrite

For nitrite estimation, blood was withdrawn from tail vein of immobilized mice immediately before setting the animal free and subjecting it to behavioral tests in all the groups. The sampling procedure was completed during immobilization to avoid the extra stress incurred upon mice during mouse immobilization for handling the tail of mice. Plasma was separated at 4°C using refrigerated centrifuge (Remi, Mumbai, India) at 2,500 rpm for 10 min. It was stored in a refrigerator and processed for nitrite estimation within 24 h [9, 10]. Plasma nitrite was measured by spectrophotometric assay based on Griess reaction [11]. Briefly, plasma was mixed with equal volume of Griess reagent (1% sulfanilamide + 0.1% naphthylenediamine dihydrochloride + 2.5% phosphoric acid) and incubated at room temperature for 10 min to yield a chromophore. Absorbance was read at 543 nm using UV-VIS-NIR spectrophotometer [Varian Cary-5000 (Christ, Netherlands)]. The nitrite concentration was calculated from standard curve using sodium nitrite as standard and expressed as micromoles of nitrite.

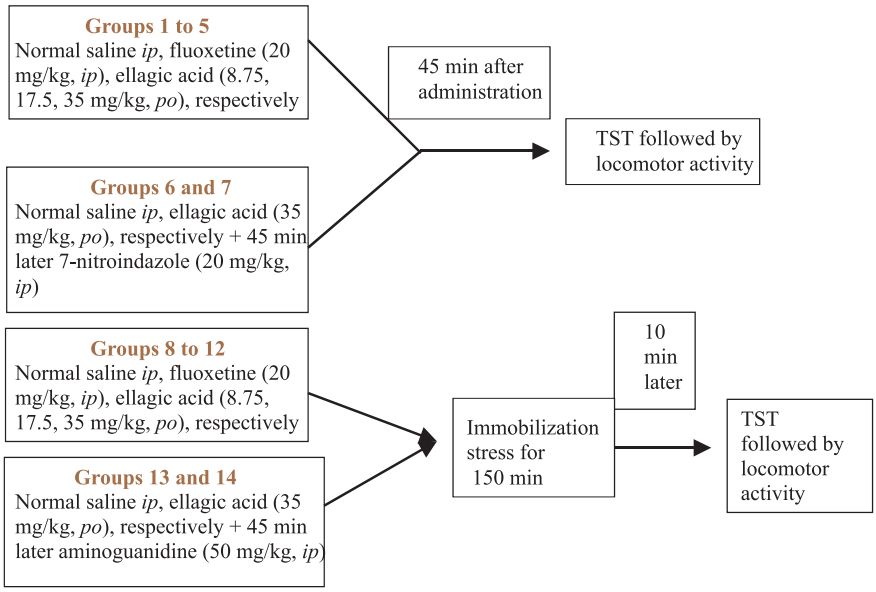

Fig. 1a. Experimental protocol for evaluating antidepressant-like activity and nitriergic mechanism of ellagic acid in unstressed and stressed mice employing TST

Locomotor activity

To rule out the effects of various drug treatments on immobility period, total locomotor activities of control and test animals were recorded for a period of 5 min using photoactometer (INCO, Ambala, India).

Experimental protocol

The animals were divided in the following groups having 10 mice in each group:

Groups for TST (Fig. 1a)

Groups 1 to 5: Normal saline *ip*, fluoxetine (20 mg/kg, *ip*) and ellagic acid (8.75, 17.5, 35 mg/kg, *po*), respectively, were administered and 45 min after the administration, immobility periods were recorded.

Groups 6 and 7: Normal saline *ip* and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, 7-nitroindazole (20 mg/kg, *ip*) was injected and 45 min after the injection, the animals were subjected to TST.

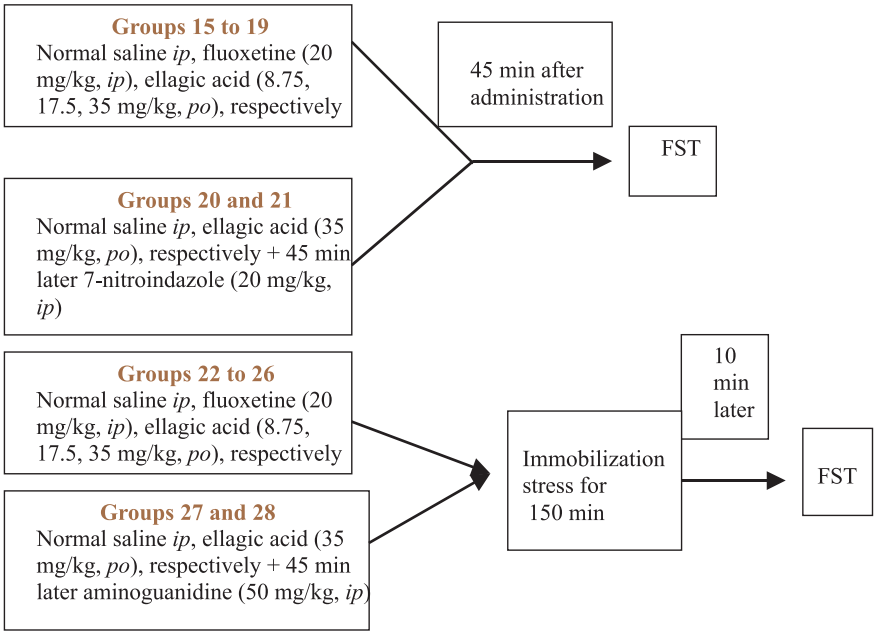

Fig. 1b. Experimental protocol for evaluating antidepressant-like activity and nitriergic mechanism of ellagic acid in unstressed and stressed mice employing FST

Groups 8 to 12: Normal saline *ip*, fluoxetine (20 mg/kg, *ip*) and ellagic acid (8.75, 17.5, 35 mg/kg, *po*), respectively, were administered and 45 min after the administration, immobilization stress was produced according to procedure mentioned above. TST was performed 10 min after setting the animals free from immobilization

Groups 13 and 14: Normal saline *ip* and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, aminoguanidine (50 mg/kg, *ip*) was injected and 45 min after the injection, immobilization stress was produced. TST was performed 10 min after setting the animals free from immobilization.

Groups for FST (Fig. 1b)

Groups 15 to 28: These groups were the same as groups 1 to 14, except the immobility periods were recorded in separate groups of animals using FST.

Groups for locomotor activity (Fig. 1a)

These were the same as groups 1 to 14, except the locomotor activity was measured using photoactometer.

Groups for investigating monoaminergic mechanisms of antidepressant-like activity of ellagic acid in TST (Fig. 2)

Groups 29 and 30: Normal saline and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, sulpiride (50 mg/kg, *ip*) was injected. After 45 min of the injection, the animals were subjected to TST.

Groups 31 and 32: Normal saline and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, prazosin (62.5 µg/kg, *ip*) was injected and 45 min after the injection, the animals were subjected to TST.

Groups 33 and 34: p-CPA (100 mg/kg, *ip*) was injected from 1st day to 4th day. On 4th day, 45 min after the injection of p-CPA, normal saline and ellagic acid

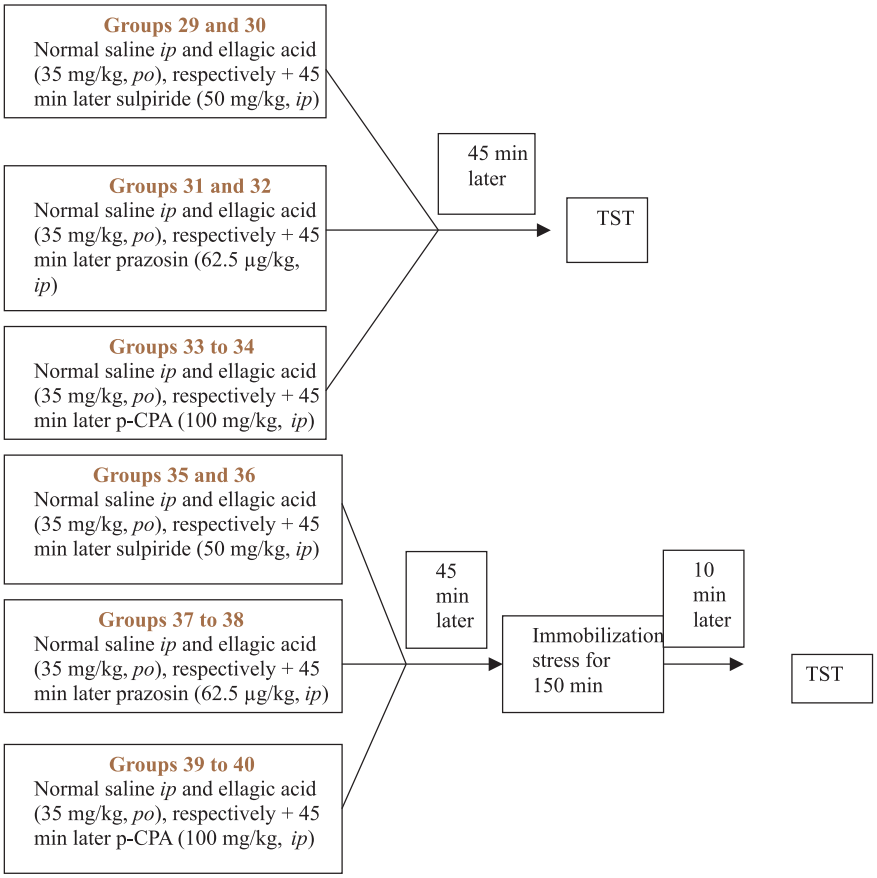

Fig. 2. Experimental protocol for evaluating the monoaminergic mechanisms of antidepressant-like activity of ellagic acid in unstressed and stressed mice

(35 mg/kg, *po*), respectively, were administered. Then, 45 min after administration of normal saline or ellagic acid, the animals were subjected to TST.

Groups 35 and 36: Normal saline and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, sulpiride (50 mg/kg, *ip*) was injected. After 45 min of the injection, immobilization stress was produced. TST was performed 10 min after setting the animals free from immobilization.

Groups 37 and 38: Normal saline and ellagic acid (35 mg/kg, *po*), respectively, were administered and after 45 min of administration, prazosin (62.5 µg/kg, *ip*) was injected. After 45 min of the injection, immobilization stress was produced. TST was performed 10 min after setting the animals free from immobilization.

Groups 39 and 40: p-CPA (100 mg/kg, *ip*) was injected from 1st day to 4th day. On 4th day, 45 min after the injection of p-CPA, normal saline and ellagic acid (35 mg/kg, po), respectively were administered. Then, 45 min after administration of normal saline or ellagic acid, immobilization stress was produced. TST was performed 10 min after setting the animals free from immobilization.

Statistical analysis

All the results are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control groups which were analyzed by using Student's unpaired *t*-test, in Graph Pad Instat, version 3.05; $p < 0.05$ was considered as statistically significant.

Results

Effect of ellagic acid, its combination with aminoguanidine and 7-nitroindazole on immobility periods of stressed and unstressed mice in TST and FST

Mice previously submitted to immobility stress once for 150 min increased the immobility time in both TST and FST, indicating depressant-like behavior. The lowest dose (8.75 mg/kg, *po*) of ellagic acid did not significantly decrease immobility time of unstressed and stressed mice as compared to their respective control groups. But higher doses (17.5 mg/kg and 35 mg/kg, *po*) of ellagic acid and fluoxetine (20 mg/kg, *ip*) *per se* significantly decreased immobility time of both unstressed and stressed mice as compared to their respective control groups, indicating their significant antidepressant-like activity. 7-Nitroindazole (20 mg/kg) significantly decreased immobility period of unstressed mice as compared to vehicle treated unstressed mice. Aminoguanidine (50 mg/kg) decreased the immobility time in stressed mice as compared to vehicle treated stressed mice. Aminoguanidine significantly enhanced antidepressant-like effect of stressed mice pre-treated with ellagic acid (35 mg/kg), as indicated by greater decrease in immobility time as compared to aminoguanidine and ellagic acid *per se*. But 7-nitroindazole did not significantly potentiate antidepressant-like effect of unstressed mice pre-treated with ellagic acid (Tabs. 1 and 2).

Effect of ellagic acid on locomotor activity of unstressed and stressed mice

Immobilization significantly decreased locomotor activity as compared to vehicle treated unstressed mice. Ellagic acid, aminoguanidine, 7-nitroindazole and their combinations used in the present study did not significantly affect the spontaneous locomotor activity of unstressed and stressed mice as compared to their respective control groups (Tab. 3).

Effect of combination of ellagic acid with sulpiride, prazosin and p-CPA on immobility periods of unstressed and stressed mice in TST

Sulpiride (50 mg/kg, *ip*), prazosin (62.5 µg/kg, *ip*) and p-CPA (100 mg/kg, *ip*) significantly increased the immobility period as compared to respective control groups in both unstressed and stressed mice. Pretreatment of animals with prazosin or p-CPA significantly reversed the decrease in immobility time produced by ellagic acid (35 mg/kg) in unstressed mice only (Tab. 4).

Effect of ellagic acid, its combination with aminoguanidine and 7-nitroindazole on plasma nitrite levels

Immobilization stress significantly increased plasma nitrite levels as compared to vehicle treated unstressed mice. Ellagic acid (8.75 mg/kg) and fluoxet-

Tab. 1. Effect of ellagic acid and its combinations with 7-nitroindazole and aminoguanidine on immobility periods of unstressed and stressed mice in TST

Treatment	Dose (kg^{-1})	Immobility period (s) Unstressed mice	Immobility period (s) Stressed mice
Vehicle	10 ml	168.1 ± 5.33	195.9 ± 2.57[a]
Ellagic acid	8.75 mg	163.4 ± 4.23	188.5 ± 2.19
Ellagic acid	17.5 mg	138.3 ± 4.18[a]	171.7 ± 3.17[b]
Ellagic acid	35 mg	122.8 ± 4.38[a]	136.1 ± 2.32[b]
Fluoxetine	20 mg	101.8 ± 3.74[a]	87.2 ± 1.75[b]
Vehicle + 7-NI (U)	10 ml + 20 mg	114.3 ± 3.54[a]	–
Ellagic acid + 7-NI (U)	35 mg + 20 mg	109.3 ± 4.18	–
Vehicle + AG (S)	10 ml + 50 mg	–	101.0 ± 3.62[b]
Ellagic acid + AG (S)	35 mg + 20 mg	–	56.4 ± 1.87[c,d]

n = 10 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control mice, which were analyzed by Student's unpaired *t*-test. F (6, 63) = 38.13; $p < 0.0001$; (for unstressed mice); F (6, 63) = 439.82; $p < 0.0001$ (for immobilization-induced stressed mice). [a] $p < 0.05$, significant difference from vehicle-treated unstressed mice); [b] $p < 0.05$, significant difference from immobilization-induced stressed mice; [c] $p < 0.05$, significant difference from ellagic acid (35 mg/kg)-treated stressed mice; [d] $p < 0.05$, significant difference from aminoguanidine-treated stressed mice; AG(S): aminoguanidine (stressed mice); 7-NI(U): 7-NI (unstressed mice)

Tab. 2. Effect of ellagic acid and its combinations with 7-nitroindazole and aminoguanidine on immobility periods of unstressed and stressed mice in FST

Treatment	Dose (kg^{-1})	Immobility period (s) Unstressed mice	Immobility period (s) Stressed mice
Vehicle	10 ml	137.1 ± 4.70	172.7 ± 3.17[a]
Ellagic acid	8.75 mg	129.4 ± 2.76	171.8 ± 3.64
Ellagic acid	17.5 mg	121.2 ± 2.95[a]	155.1 ± 3.07[b]
Ellagic acid	35 mg	109.5 ± 3.71[a]	136.2 ± 2.34[b]
Fluoxetine	20 mg	93.8 ± 3.16[a]	97.1 ± 2.48[b]
Vehicle + 7-NI (U)	10 ml + 20 mg	117.7 ± 2.86[a]	–
Ellagic acid + 7-NI (U)	35 mg + 20 mg	108.4 ± 3.11	–
Vehicle + AG (S)	10 ml + 50 mg	–	145.4 ± 2.77[b]
Ellagic acid + AG (S)	35 mg + 20 mg	–	110.6 ± 1.69[c,d]

n = 10 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control mice, which were analyzed by Student's unpaired *t*-test. F (6, 63) = 18.14; $p < 0.0001$; (for unstressed mice); F (6, 63) = 107.26; $p < 0.0001$; (for immobilization-induced stressed mice). [a] $p < 0.05$, significant difference from vehicle-treated unstressed mice; [b] $p < 0.05$, significant difference from immobilization-induced stressed mice; [c] $p < 0.05$, significant difference from ellagic acid (35 mg/kg)-treated stressed mice; [d] $p < 0.05$, significant difference from aminoguanidine-treated stressed mice. AG(S): aminoguanidine (stressed mice); 7-NI(U): 7-NI (unstressed mice)

Tab. 3. Effect of ellagic acid and its combinations with 7-nitroindazole and aminoguanidine on locomotor activity of unstressed and stressed mice

Treatment	Dose (kg^{-1})	Locomotor activity counts (Unstressed mice)	Locomotor activity counts (Stressed mice)
Vehicle	10 ml	356.1 ± 15.09	309.5 ± 5.35[a]
Ellagic acid	8.75 mg	364.3 ± 12.87	314.4 ± 3.39
Ellagic acid	17.5 mg	349.1 ± 18.12	308.1 ± 9.81
Ellagic acid	35 mg	338.4 ± 10.07	312.6 ± 6.69
Fluoxetine	20 mg	357.8 ± 7.42	320.1 ± 2.28
Vehicle + 7-NI (U)	10 ml + 20 mg	356.3 ± 6.54	–
Ellagic acid + 7-NI (U)	35 mg + 20 mg	367.9 ± 14.68	–
Vehicle + AG (S)	10 ml + 50 mg	–	306.2 ± 10.28
Ellagic acid + AG (S)	35 mg + 20 mg	–	305.9 ± 8.91

n = 10 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control mice, which were analyzed by Student's unpaired *t*-test. F (6, 63) = 0.586; $p > 0.05$; (for unstressed mice); F (6, 63) = 0.492; $p > 0.05$ (for immobilization-induced stressed mice). [a] $p < 0.05$, significant difference from vehicle-treated unstressed mice; AG(S): aminoguanidine (stressed mice); 7-NI(U): 7-NI (unstressed mice)

Tab. 4. Effect of pCPA, prazosin and sulpiride *per se* and their combination with ellagic acid on immobility periods of unstressed and stressed mice in TST

Treatment	Dose (kg^{-1})	Immobility period (s) Unstressed mice	Immobility period (s) Stressed mice
Vehicle	10 ml	168.1 ± 5.33	195.9 ± 2.57
Ellagic acid	35 mg	122.8 ± 4.38[a]	136.1 ± 2.32[b]
Vehicle + pCPA	10 ml + 100 mg	198.2 ± 1.33[a]	218.9 ± 2.78[b]
Ellagic acid + pCPA	35 mg + 100 mg	158.3 ± 2.39[c]	145.0 ± 2.11
Vehicle + prazosin	10 ml + 62.5 µg	206.0 ± 2.54[a]	209.2 ± 3.22[b]
Ellagic acid + prazosin	35 mg + 62.5 µg	161.6 ± 4.80[c]	143.2 ± 3.90
Vehicle + sulpiride	10 ml + 50 mg	192.5 ± 3.01[a]	210.4 ± 3.45[b]
Ellagic acid + sulpiride	35 mg + 50 mg	129.5 ± 2.55	138.4 ± 3.08

n = 10 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control mice, which were analyzed by Student's unpaired *t*-test. F (7, 72) = 75.45; $p < 0.0001$ (for unstressed mice); F (7, 72) = 153.43; $p < 0.0001$ (for immobilization-induced stressed mice). [a] $p < 0.05$, significant difference from vehicle treated unstressed mice; [b] $p < 0.05$, significant difference from immobilization-induced stressed mice; [c] $p < 0.05$, significant difference from ellagic acid (35 mg/kg)-treated unstressed mice

ine did not produce any significant change in plasma nitrite levels in both unstressed and stressed mice as compared to respective vehicle treated groups. The higher doses of ellagic acid (17.5 and 35 mg/kg) significantly decreased the plasma nitrite levels in stressed mice only. 7-Nitroindazole and aminoguanidine *per se* significantly decreased plasma nitrite levels in unstressed mice and stressed mice, respectively, as compared to the respective vehicle treated controls. Aminoguanidine significantly potentiated the plasma nitrite

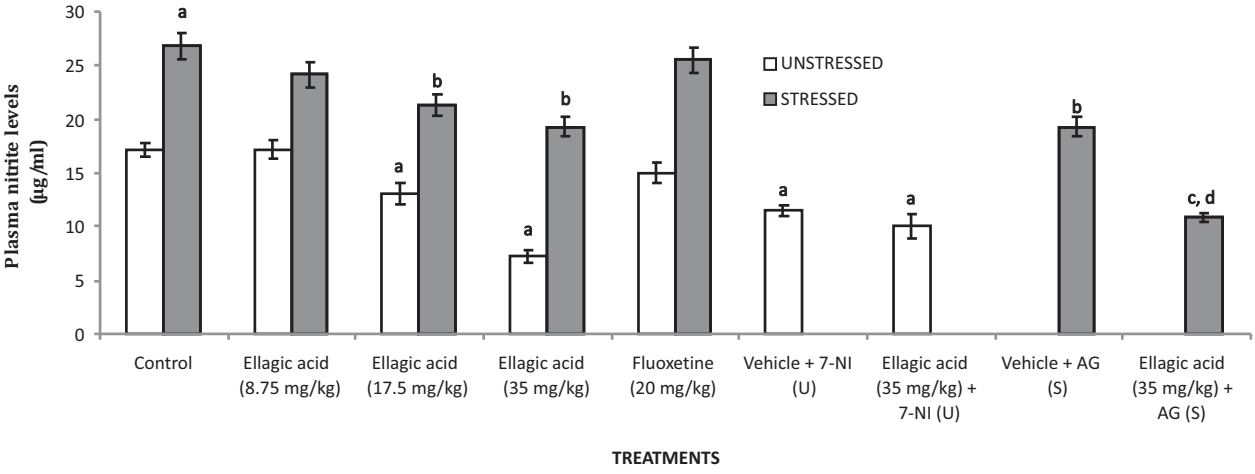

Fig. 3. Effect of ellagic acid and its combinations with 7-nitroindazole and aminoguanidine on plasma nitrite levels of unstressed and stressed mice. n= 6 in each group. Values are expressed as the mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, except data for unstressed and stressed control mice which were analyzed by using Student's unpaired *t*-test. F (6, 35) = 20.83; $p < 0.0001$; (for unstressed mice); F (6, 35) = 48.36; $p < 0.0001$; (for immobilization-induced stressed mice). [a] $p < 0.05$, significant difference from unstressed control mice; [b] $p < 0.05$, significant difference from immobilization-induced stressed control mice; [c] $p < 0.05$, significant difference from ellagic acid (35 mg/kg)-treated stressed mice; [d] $p < 0.05$, significant difference from aminoguanidine-treated stressed mice. AG(S): aminoguanidine (stressed mice); 7-NI(U): 7-NI (unstressed mice)

decreasing effect of ellagic acid (35 mg/kg) in stressed mice, when compared to ellagic acid and aminoguanidine *per se* treated groups. But 7-nitroindazole did not significantly potentiate plasma nitrite decreasing effect of ellagic acid in unstressed mice as compared to ellagic acid and 7-nitroindazole *per se* (Fig. 3).

Discussion

In the present study, ellagic acid (17.5 mg/kg and 35 mg/kg, *po*) showed antidepressant-like activity in unstressed and immobilization-induced stressed mice. This is the first study showing antidepressant-like activity of ellagic acid. FST and TST were used for evaluation of antidepressant-like activity. These behavioral despair models are widely employed in rodents to predict antidepressant potential by measuring the decrease in immobility periods [24, 29]. In FST, mice are forced to swim in a restricted space from which they cannot escape, and are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in FST. This immobility, referred as behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [28, 31, 39]. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity [31].

Out of the three doses of ellagic acid employed, the highest dose of 35 mg/kg, *po* produced most significant antidepressant-like effect in both unstressed and stressed mice, hence this dose was employed for elucidating the probable mechanisms of antidepressant-like activity. Ellagic acid did not show any significant change in locomotor functions of unstressed and stressed mice as compared to their respective controls, so it did not produce any overt motor effects. This supports the hypothesis that the antidepressant-like effect of the ellagic acid is specific and not a false positive. According to our results, the antidepressant-like effect of ellagic acid (35 mg/kg) was significantly reversed by pretreatment with prazosin (an α_1-adrenoceptor antagonist) and p-CPA in unstressed mice tested in TST. p-CPA is serotonin synthesis inhibitor and it has to be administered for 4 consecutive days for depleting serotonin [4]. Thus, ellagic acid (35 mg/kg) might produce antidepressant-like effect in unstressed mice by interaction with α_1-adrenoceptors and serotonergic receptors, hence increasing the levels of norepinephrine and serotonin. Levels of

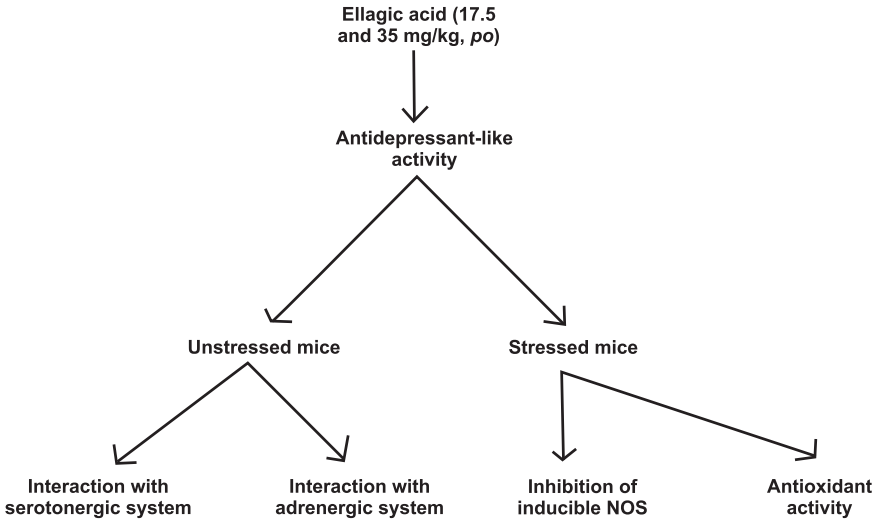

Fig. 4. Possible mechanisms of antidepressant-like activity of ellagic acid in unstressed and stressed mice

monoamines like norepinephrine and serotonin are decreased in depression, so drugs like tricyclic antidepressants and monoamine oxidase inhibitors which enhance the levels of these monoamines have been used as antidepressant drugs [23]. 7-Nitroindazole (nNOS inhibitor) produced antidepressant-like activity in unstressed mice, which is also supported by the literature [41]. Administration of 7-nitroindazole to unstressed mice pre-treated with ellagic acid (35 mg/kg), did not significantly produce greater decrease in immobility period as compared to that of ellagic acid and 7-nitroindazole *per se*, indicating that antidepressant-like activity of ellagic acid in unstressed mice might not be through inhibition of neuronal NOS. 7-Nitroindazole alone significantly decreased plasma nitrite levels in unstressed mice as compared to its vehicle treated control. But, 7-nitroindazole did not significantly potentiate plasma nitrite decreasing effect of ellagic acid in unstressed mice as compared to ellagic acid and 7-nitroindazole *per se*, which further supports the non-involvement of nNOS for antidepressant-like activity of ellagic acid.

In stressed mice, antidepressant-like effect of ellagic acid (35 mg/kg) was not significantly reversed by pretreatment with prazosin or p-CPA or sulpiride, thus indicating that antidepressant-like activity of ellagic acid in stressed mice might not by through interaction of monoaminergic system. So, there might be some other mechanisms involved in antidepressant-like activity of ellagic acid in stressed mice. Acute restraint stress has also been observed to significantly increase expression of inducible NOS in rodents [33]. Administration of aminoguanidine (iNOS inhibitor) to stressed mice pre-treated with ellagic acid (35 mg/kg), significantly produced greater decrease in immobility time as compared to ellagic acid and aminoguanidine *per se*, indicating that ellagic acid might produce antidepressant-like activity in stressed mice by inhibition of inducible NOS. Ellagic acid and aminoguanidine *per se* significantly decreased the plasma nitrite levels in stressed mice. Enhancement of plasma nitrite levels decreasing effect of ellagic acid by aminoguanidine in stressed mice further supports the involvement of inducible NOS inhibition for antidepressant-like activity of ellagic acid in stressed mice. Moreover, ellagic acid has been reported to have antioxidant activity [13], which might also contribute to its antidepressant-like activity in stressed mice. The restraint stress reduced the levels of superoxide dismutase, catalase and enhanced lipid peroxidation [43]. A series of studies performed in humans correlate depressive disorders with oxidative stress either in the brain and blood [3, 21]. Further, a decrease in antioxidant enzyme activities in patients diagnosed with major depression has been demonstrated, while the antidepressant treatment ameliorated this effect [14].

In conclusion, ellagic acid showed antidepressant-like activity in unstressed mice probably by interaction through adrenergic and serotonergic systems. On the other hand, ellagic acid showed antidepressant-like activity in stressed mice probably by inhibition of inducible NOS and also due to its antioxidant activity (Fig. 4). Therefore, ellagic acid may be explored further for its potential in the management of clinical depression.

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