Research Article

Evaluation of Antidepressant-Like Activity of *Eriobotrya japonica* in the Immobilization induced Stressed Mice

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ABSTRACT

The aim of present study was to evaluate the antidepressant-like activity of *Eriobotrya japonica* in Swiss albino mice, and to explore the possible underlying mechanisms of action. Mice were immobilized for 2h daily for 7 days for induction of stress. *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) and fluoxetine (20 mg/kg, *ip*) *per se* were administered to stressed mice; and immobility periods were recorded using tail suspension test and forced swim test. The brain nitrite levels were also estimated in unstressed and stressed mice. Effects of aminoguanidine, an inducible nitric oxide synthases (iNOS) inhibitor and methylene blue, a neuronal nitric oxide synthases (nNOS) inhibitor and soluble guanylyl cyclase (sGC) inhibitor on the antidepressant-like activity of *Eriobotrya japonica* were also evaluated. *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) and fluoxetine (20 mg/kg, *ip*) *per se* significantly decreased immobility periods of stressed mice compared to their respective stressed control, indicating significant antidepressant-like activity. Out of the three doses of *Eriobotrya japonica* in use, 200 mg/kg, *ip.* was found to produce most significant antidepressant-like effect in stressed mice, hence this dose was further used for elucidating the probable mechanisms of antidepressant-like activity. *Eriobotrya japonica* had no significant effect on the locomotor activity of the mice. *Eriobotrya japonica* leaves extract significantly decreased the brain nitrite levels in stressed mice only. Aminoguanidine and methylene blue significantly potentiated antidepressant-like activity and brain nitrite decreasing the effect of *Eriobotrya japonica* leaves extract (200 mg/kg) in stressed mice. Thus, *Eriobotrya japonica* leaves extract showed antidepressant-like activity in stressed mice probably due to its iNOS, nNOS and sGS inhibitory action.

Keywords: Antidepressant, Methylene blue, Inducible NOS, Neuronal NOS.

INTRODUCTION

Depression is one of the leading causes of disease throughout the world. Current diagnostic measurement of depression is based on presence, descriptions, and magnitude of symptoms over time.[1] Depression has a high rate of morbidity, recurrence, and mortality.[2] Antidepressant medications mainly used for the treatment of biologically based depression are thought to increase the availability of brain neurotransmitters or increase the sensitivity of receptors in the brain.[3] Although there is a great advancement in the understanding of the pathophysiology and neurotransmitter systems of major depression and several newer antidepressants have been introduced, only 60%–70% of patients respond to antidepressant therapy.[4] Therefore, research for evaluating further new therapeutic options with greater effectiveness is still desirable. Stress plays a provocative role in the etiology depressive illness[2] by disturbing the physiological homeostasis [5] and thus precipitates the mood disorders.[6] Immobilization stress evokes glutamate release and enhances proinflammatory cytokines mainly Interleukin-1β (IL-1β), Interleukin-6 (IL-1 β), and Tumor necrosis factor-α (TNF-α) expression in the brain.[7] Glutamate and IL-6 activates the NMDA receptors [8], which causes the activation of neuronal nitric oxide synthases (nNOS)[9], further enhances nitric oxide (NO) production. Enhanced expression of proinflammatory cytokines IL-1 and TNF-α increase the expression of nuclear factor-κB (NF-κβ) pathway [10], which increase expression of inducible nitric oxide synthases (iNOS)[11] and continuous NO production. NF-κβ activation also reduces monoamines; neurotrophic factors production and thus increases excitotoxicity. Nitric oxide (NO) is a unique neurotransmitter, synthesized from L-arginine by nitric oxide synthases (NOS).[12] Nitric oxide (NO) modulates the extracellular levels of serotonin (5-HT) [13] by inactivating the rate limiting enzyme involved in the synthesis of 5-HT [14] and therefore NO influences the release, reuptake, and function of 5-HT.[15] Serotonin (5-HT) is a neurotransmitter, which is responsible for the regulation of mood and behavior[16], and therefore its deficiency and reduced transmission contribute to depression .[17] In laboratory animal depression like behavioral alterations is caused by immobilization stress.[18] Immobilization stress has been observed to significantly increase expression of NF-κβ pathway and brain nitrite levels rodents.[11] Aminoguanidine, an inhibitor of inducible NOS, reversed stress induced depression-like behavior in rats.[19] Methylene blue, a nNOS and soluble sGC inhibitor and is used in depressive illness.[20] *Eriobotrya japonica* belonging to family Rosaceae, commonly known as ''loquat,'' with narrow leaves dark green on the upper and lighter color underneath the surface. It has been used as a medicinal plant in Japan and China from a long period of time. Compounds isolated from the leaves of *Eriobotrya japonica* are glycosides[21], Polyphenols [22], Polyhydroxylated triterpenes.[23] As per the literature

report, *Eriobotrya japonica* is known to possess the numerous pharmacological activities such as anti-inflammatory[24], antitumor[25], antinociceptive[26], hypoglycemic[21], antioxidant[27], and also have free radical scavenging activity.[28] Leaves of *Eriobotrya japonica* also inhibit iNOS expression.[26] Although *Eriobotrya japonica* showed antioxidant and oxidative-stress inhibitory effect it has not been explored yet for antidepressant activity and its possible mechanism of action. The present study was carried out to investigate *Eriobotrya japonica* for its antidepressant-like activity in the immobilization induced stressed mice; and also to investigate the possible involvement of nitriergic mechanism in this activity.

MATERIAL AND METHODS

Animals and housing

Swiss male albino mice (4 months old, weighing around 20–25 g) were procured from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Animals were housed separately in groups (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 during the light period (08.00-16.00 h). The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC). 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.1095).

Drugs and chemicals

Fluoxetine (Cadila Pharmaceuticals, Ahmedabad, India), Aminoguanidine (Himedia, India) ; Methylene Blue; 0.1% of N-1-napththyl ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% o-phosphoric acid (CDH, India) were used in the present study. All drugs and chemicals were diluted separately in normal saline (0.9% w/v sodium chloride). Volume of *ip.* injection was 1 ml/100 g of mouse.

Selection of doses

The doses of various drugs were selected on the basis of the literature, and the doses are 20 mg/kg for fluoxetine (Walia, 2016), 50 mg/ kg for aminoguanidine[19] and 20 mg/kg, *ip.*for methylene blue. [20]

Immobilization Stress (IMS)

Mice were immobilized for 2h daily for 7 days by placing the mice in a 50 ml syringe which was used as a mice restrainer, making a circular big hole on the nozzle end of it so that mice may breathe properly and closing the open end with cotton plug. [29]

Behavioral models

Tail suspension test (TST)

It is most commonly used behavioral test for the screening of antidepressant- like activity in mice. For the test, each mouse was hanged individually at a height of 50 cm from the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. The test was conducted in quiet room to avoid disturbances to animals. The total immobility period was manually recorded for 6 min duration. Mouse was considered to be immobile when it showed no body movements, hung passively and completely immobile.[19]

Forced swim test (FST)

FST is the most frequently used behavioral test for the assessment of antidepressant- like activity in rodents. Mouse was individually forced to swim in the open glass chamber (25 cm x 15 cm x 25 cm) containing fresh water to a height of 15 cm and maintained at 26±1°C. Each mouse showed vigorous movements during the initial 2 min period of the test. The duration of immobility was 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. Used water in the chamber should be changed after FST of each animal to avoid any behavioral change [19, 30].

Estimation of brain nitrite

Mice were sacrificed by decapitation and the brains were removed. Brains were then rinsed with the isotonic saline (0.9%) and weighed. A 10% (w/v) of brain homogenates were prepared by using 0.1 M phosphate buffer (pH 7.4). The homogenates were then subjected to refrigerated centrifuge for 15 min at 10000 rpm and the supernatants were collected. Nitrite was measured in cell free supernatants from brain homogenates by spectrophotometeric assay with Greiss reagent (0.1% of N-1-napththyl ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% o-phosphoric acid). Equal volume of the brain homogenate and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature and absorbance was measured at 540 nm (Konstandi et al., 2000). The nitrite concentration in supernatant was determined by using the sodium nitrite standard curve and expressed in µM of nitrite. [31]

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 6 mice in each group:

Group for TST:

Group 1: Normal saline *ip.* was administered and on 7th day 45 min after the ad-ministration, immobility periods were recorded.

Group 2: Normal saline *ip.* was administered and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 3 to 6: Fluoxetine (20 mg/kg; *ip.*), *Eriobotrya japonica* leaves extract (100 mg/kg; *ip.*), *Eriobotrya japonica* leaves extract (200 mg/kg; i.p.) and *Eriobotrya japonica* leaves extract (400 mg/kg; i.p.) were administered and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 7: Fluoxetine (20 mg/kg; *ip.*) administration was followed by *Eriobotrya japonica* leaves extract (200 mg/kg; *ip.*) and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 8: Aminoguanidine (50 mg/kg; *ip.*) was administered and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 9: Aminoguanidine (50 mg/kg; *ip.*) administration was followed by *Eriobotrya japonica* leaves extract (200 mg/kg; *ip.*) and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 10: Methylene blue (20 mg/kg, *ip.*) was administered and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group 11: Methylene blue (20 mg/kg, *ip.*) administration was followed by *Eriobotrya japonica* leaves extract (200 mg/kg; *ip.*) and animals were subjected to immobilization stress of 2h daily for 7 days and on 7th day 45 min after the administration, immobility periods were recorded.

Group for FST:

Groups 12 to 24: These groups were the same as groups 1 to 11, except the immobility periods were re-corded in separate groups of animals using FST.

Group for Locomotor Activity:

Groups 25 to 36: These were the same as groups 1 to 11, except the locomotor activity was measured using photoactometer.

Group for estimation of Brain Nitrite Levels:

Groups 37 to 48: These were the same as groups 1 to 11, except the animals were sacrificed and brain nitrite levels were estimated

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, in Graph Pad Instat, version 3.05; $p < 0.01$ was considered as statistically significant.

RESULT

Effect of Eriobotrya japonica and its combinations with aminoguanidine and methylene blue on TST in stressed mice compared to respective stressed control group.

Vehicle treated mice previously subjected to immobilization stress showed significant enhancement in immobility time in TST as compared to unstressed control group, indicating depression-like behavior. *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) significantly decreased the immobility period of stressed mice as compared to their respective stressed control group. From the three different doses of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) was found to be the most effective. Fluoxetine (20 mg/kg, ip.) treatment in stressed mice showed significant decrease in the immobility period as compared to their respective stressed control group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) significantly reduced the duration of immobility period in stressed mice as compared to their respective vehicle treated stressed group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) followed by the administration of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*), significantly produced greater decrease in immobility time as compared to aminoguanidine, methylene blue and *Eriobotrya japonica per se*. (Figure 1).

Effect of Eriobotrya japonica and its combinations with aminoguanidine and methylene blue on FST in stressed mice compared to respective stressed control group.

Vehicle treated stressed control mice showed significant enhancement in immobility time in FST as compared to unstressed control group, indicating depression-like behavior. *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) significantly decreased the immobility period of stressed mice as compared to their respective stressed control group. From the three different doses of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) was found to be the most effective. Fluoxetine (20 mg/kg, *ip.*) treatment in stressed mice showed significant decrease in the immobility period as compared to their respective stressed control group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) significantly reduced the duration of immobility period in stressed mice as compared to their respective vehicle treated stressed group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) followed by the administration of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*), significantly

produced greater decrease in immobility time as compared to aminoguanidine, methylene blue and *Eriobotrya japonica per se.* (Figure 2).

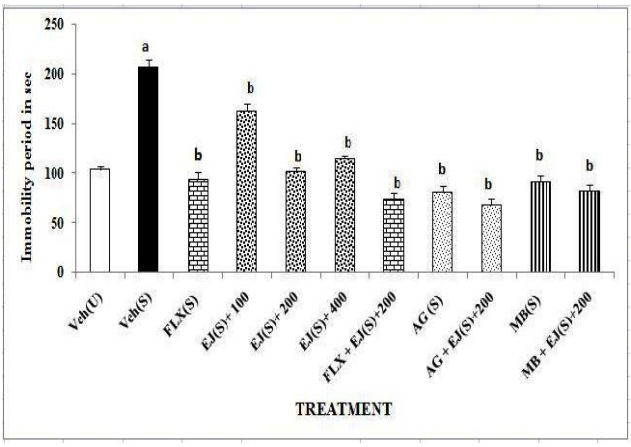

Figure 1: Effect of *Eriobotrya japonica* and its combinations with aminoguanidine and methylene blue on TST in stressed mice compared to respective stressed control group.

n = 6 mice in each group. Values expressed as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet *post-hoc* test, a= p<0.01 as compared to vehicle treated unstressed mice, b=p<0.01 as compared to vehicle treated stressed control group. Veh(U): Vehicle treated unstressed mice; Veh(S): Vehicle treated stressed mice; FLX(S): Fluoxetine (20 mg/kg, *ip.*) treated stressed mice; EJ(S): *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) treated stressed mice; FLX+EJ(S): Fluoxetine (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; AG (S): Aminoguanidine (50 mg/kg, *ip.*) treated stressed mice; AG +EJ(S): Aminoguanidine (50 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; MB (S): Methylene blue (20 mg/kg, *ip.*) treated stressed mice; MB+EJ(S): Methylene blue (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice.

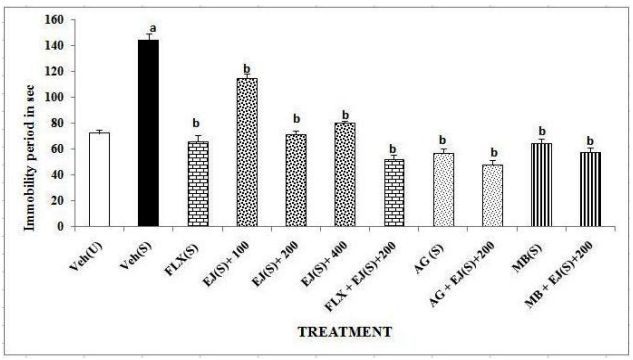

Figure 2: Effect of *Eriobotrya japonica* and its combinations with aminoguanidine and methylene blue on FST in stressed mice compared to respective stressed control group.

n = 6 mice in each group. Values expressed as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet *post-hoc* test, a= p<0.01 as compared to vehicle treated unstressed mice, b=p<0.01 as compared to vehicle treated stressed control group. Veh(U): Vehicle treated unstressed mice; Veh(S): Vehicle treated stressed mice; FLX(S): Fluoxetine (20 mg/kg, *ip.*) treated stressed mice; EJ(S): *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) treated stressed mice; FLX+EJ(S): Fluoxetine (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; AG (S): Aminoguanidine (50 mg/kg, *ip.*) treated stressed mice; AG +EJ(S): Aminoguanidine (50 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; MB (S): Methylene blue (20 mg/kg, *ip.*) treated stressed mice; MB+EJ(S): Methylene blue (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice.

Effect of Eriobotrya japonica and its combinations with aminoguanidine and methylene blue on brain nitrite levels in stressed mice compared to respective stressed control group.

Vehicle treated mice previously subjected to immobilization stress showed significant enhancement brain NO concentration as compared to unstressed control group, indicating depression-like behavior. *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) significantly decreased the brain NO concentration of stressed mice as compared to their respective stressed control group. From the three different doses of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) was found to be the most effective in decreasing the brain NO level. Fluoxetine (20 mg/kg, *ip.*) treatment in stressed mice showed significant decrease in brain NO concentration as compared to their respective stressed control group. Pre-treatment of stressed mice with aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) followed by the administration of EJ (200 mg/kg, *ip.*), significantly reduced the brain NO concentration as compared to their respective stressed control group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) significantly reduced the brain NO level in stressed mice as compared to their respective vehicle treated stressed group. Aminoguanidine (50 mg/kg, *ip.*) and methylene blue (20 mg/kg, *ip.*) followed by the administration of *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*), significantly produced greater decrease in the brain NO level as compared to aminoguanidine, methylene blue and *Eriobotrya japonica per se* (Figure 3)

Effect of Eriobotrya japonica and its combinations with aminoguanidine and methylene blue on locomotor activity in stressed mice compared to respective stressed control group.

Stressed control group (vehicle treated and subjected to immobilization stress) showed significant decrease in locomotor activity as compared to unstressed control group. *Eriobotrya japonica*, aminoguanidine, methylene blue and their combinations used in the present study did

not significantly affect the spontaneous locomotor activity of the stressed mice as compared to their respective stressed control groups. (Table 1)

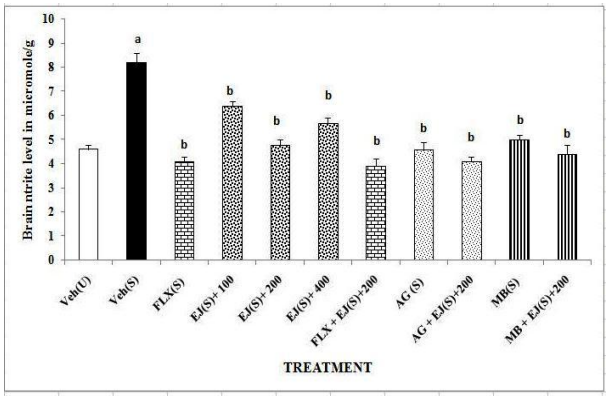

Figure 3: Effect of *Eriobotrya japonica* and its combinations with aminoguanidine and methylene blue on brain nitrite levels in stressed mice compared to respective stressed control group.

n = 6 mice in each group. Values expressed as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet *post-hoc* test, a= p<0.01 as compared to vehicle treated unstressed mice, b=p<0.01 as compared to vehicle treated stressed control group. Veh(U): Vehicle treated unstressed mice; Veh(S): Vehicle treated stressed mice; FLX(S): Fluoxetine (20 mg/kg, *ip.*) treated stressed mice; EJ(S): *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) treated stressed mice; FLX+EJ(S): Fluoxetine (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; AG (S): Aminoguanidine (50 mg/kg, *ip.*) treated stressed mice; AG +EJ(S): Aminoguanidine (50 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; MB (S): Methylene blue (20 mg/kg, *ip.*) treated stressed mice; MB+EJ(S): Methylene blue (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice.

n = 6 mice in each group. Values expressed as the mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnet *post-hoc* test, a= p<0.05 as compared to vehicle treated unstressed mice. Veh(U): Vehicle treated unstressed mice; Veh(S): Vehicle treated stressed mice; FLX(S): Fluoxetine (20 mg/kg, *ip.*) treated stressed mice; EJ(S): *Eriobotrya japonica* leaves extract (100 mg/kg, 200 mg/kg and 400 mg/kg, *ip.*) treated stressed mice; FLX+EJ(S): Fluoxetine (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; AG (S): Aminoguanidine (50 mg/kg, *ip.*) treated stressed mice; AG +EJ(S): Aminoguanidine (50 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice; MB (S): Methylene blue (20 mg/kg, *ip.*) treated stressed mice; MB+EJ(S): Methylene blue (20 mg/kg, *ip.*) and *Eriobotrya japonica* leaves extract (200 mg/kg, *ip.*) treated stressed mice.

Table 1: Effect of *Eriobotrya japonica* and its combinations with aminoguanidine and methylene blue on locomotor activity in stressed mice compared to respective stressed control group.

Treatment	Dose (kg–1)	Locomotor activity counts
Veh(U)	10 ml	360.5 ± 12.09
Veh(S)	10 ml	310.1 ± 4.09a
EJ(S)	100 mg	314.4 ± 9.81
EJ(S)	200 mg	305 ± 5.59
EJ(S)	400 mg	306.2±10.28
FLX(S)	20 mg	309 ± 12.23
FLX+EJ(S)	20 mg + 200 mg	305.9±5.51
AG (S)	50 mg	316± 3.45
AG +EJ(S)	50 mg + 200 mg	311±9.81
MB (S)	20 mg	323±10.33
MB+EJ(S)	20 mg + 200 mg	320.1 ± 12.28

DISCUSSION

In the present study, *Eriobotrya japonica* leaf extract (100, 200 and 400 mg/kg; *ip.*) was focused for its antidepressant-like activity in immobilization-induced stressed mice. This is the first study showing this activity of *Eriobotrya japonica.* Antidepressant-like activity was evacuated using TST and FST. These behavioral models are widely used in rodents to test antidepressant potential and here the decrease in immobility periods is recorded.[19] In FST, mice are subjected to an unavoidable and inescapable condition from which they cannot escape and ultimately are induced to a characteristic behavior of immobility. This characteristic behavior of immobility can be reversed by several antidepressants. Thus may be considered similar to human depression. Stress causes alterations in neurotransmitters[22] and neuroendocrine[33] systems and thus plays a key role in the pathogenesis of depression. Stress elicit the glutamate release and induces the expression of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in the brain.[7] Glutamate and IL-6 causes the activation of NMDA receptors[8] leading to the activation of nNOS, which is responsible for the production of NO[9], which further stimulate the production of enzyme sGC responsible for the production of cGMP.[34] The cGMP is known to produce the depressive effect by influencing 5-HT transporters. Enhanced expression of IL-1 and TNF-α enhances the expression of NF-κβ pathway, which further increases the expression of iNOS[11] leading to the continuous NO production. NO modifies the extracellular serotonin (5-HT) levels [13] by inhibiting the rate limiting enzyme involved in synthesis[14] leading to its reduced synthesis. NO also effects the release, reuptake, and function of 5-HT.[15] Reduced transmission of 5-HT contributes to depression as is responsible for the regulation of mood and behavior.[17] The exposure of rodents to unpredictable and uncontrollable immobilization stress induces depressive

behavior.[18] In the present study, it was seen that vehicle treated mice previously subjected to immobilization stress (2h daily for 7 days) showed significant enhancement in immobility time in FST and TST as compared to unstressed control group, indicating depression-like behavior. Also, it was found that the brain nitrite levels the vehicle treated stressed mice was significantly increased as compared to unstressed control group. *Eriobotrya japonica* leaves extract (100mg/kg, 200mg/kg and 400 mg/kg, *ip.*) significantly reduced the immobility period of stressed mice in both FST and TST as compared to their respective stressed control group. Out of the three doses of *Eriobotrya japonica* in use, 200 mg/kg, *ip.* was found to produce most significant antidepressant-like effect in stressed mice, hence this dose was further used for elucidating the probable mechanisms of antidepressant-like activity. *Eriobotrya japonica* did not show any significant change in locomotor functions of stressed mice as compared to their respective stressed control, so it did not produce any explicit motor effects. Thus we can purpose the hypothesis that the antidepressant-like effect of the *Eriobotrya japonica* is precise and not a false positive. The brain nitrite levels of the stressed mice were also reduced as compared to the vehicle treated stressed control group. The leaves of *Eriobotrya japonica* has been reported to inhibit the expression of iNOS[26] and also down regulate the NF-κβ pathways.[24] To check the possible role of the iNOS, in antidepressant activity, we administered the mice with the iNOS inhibitor aminoguanidine. It significantly reduced the duration of immobility period in both FST, TST, and the brain nitrite levels in stressed mice as compared to their respective vehicle treated stressed group. Administration of aminoguanidine (iNOS inhibitor) to stressed mice followed by *Eriobotrya japonica* (200 mg/kg, *ip.)*, significantly produced greater decrease in immobility time as compared to *Eriobotrya japonica* and aminoguanidine *per se*, indicating that *Eriobotrya japonica* might produce antidepressant-like activity in stressed mice by inhibition of inducible NOS. *Eriobotrya japonica* and aminoguanidine *per se* significantly decreased the brain nitrite levels in stressed mice. Enhancement of brain nitrite levels decreasing effect of *Eriobotrya japonica* by aminoguanidine in stressed mice further supports the involvement of inducible NOS inhibition for antidepressant-like activity of *Eriobotrya japonica* in stressed mice. The possible role of nNOS and sGC was determined by administrating the mice with methylene blue, a directly acting nNOS and sGC inhibitor. It significantly reduced the duration of immobility period in both FST, TST, and the brain nitrite levels in stressed mice as compared to their respective vehicle treated stressed group. Also in the previous studies it has been reported that methylene blue exerts antidepressant effect in rodents.[35]Administration of methylene blue (nNOS and sGC inhibitor) to stressed mice followed by *Eriobotrya japonica* (200 mg/kg, *ip.)*, significantly produced greater decrease in immobility time as compared to *Eriobotrya japonica* and methylene blue *per se*, indicating that *Eriobotrya japonica* might produce antidepressant-like activity in stressed mice by inhibition of nNOS and sGC. *Eriobotrya japonica* and methylene blue *per se* significantly decreased the brain nitrite levels in stressed mice. Enhancement of brain nitrite levels decreasing effect of *Eriobotrya japonica* by methylene blue in stressed mice further supports the involvement of nNOS inhibition for antidepressant-like activity of *Eriobotrya japonica* in stressed mice. Thus *Eriobotrya japonica* might produce antidepressant-like activity in stressed mice by inhibition of inducible NOS, neuronal NOS and sGC.

CONCLUSION

It is concluded that, immobilization stress (2h daily for 7 days) significantly increased the expression of iNOS and nNOS, which further stimulated the enzyme sGC responsible for the production of cGMP. The cGMP produced the depressive effect by influencing 5-HT transporters. *Eriobotrya japonica* showed antidepressant-like activity in stressed mice probably by its inhibitory action on iNOS, nNOS and sGS. Therefore, *Eriobotrya japonica* may be explored further for its potential in the management of clinical depression.

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