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Research Article

Syringic Acid Reversed Depression-resembling Behavior Induced by Chronic Unpredictable Mild Stress Paradigm in Mice

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ABSTRACT

The aim of the present investigation was to explore the anti-depressive potential of syringic acid in Swiss albino male mice. Mice were exposed to unpredictable stress for 21 sequential days. Imipramine (15 mg/kg, p.o.) and syringic acid (10 and 20 mg/kg, p.o.) were administered for three consecutive weeks to unstressed and stressed mice. Tail suspension test and sucrose preference test were used to evaluate antidepressant potential of the drugs. Syringic acid and imipramine significantly decreased immobility periods of stressed mice as compared to vehicle treated stressed mice in tail suspension test, indicating their antidepressant effects. Syringic acid (20 mg/kg) and imipramine also significantly restored the reduced sucrose preference (%) in stressed mice, which further substantiated their antidepressant effects. Syringic acid and imipramine did not produce significant antidepressant effects in unstressed mice. These drugs did not significantly affect locomotor activity scores of mice. Syringic acid (20 mg/kg) and imipramine significantly reversed chronic unpredictable mild stress (CUMS)-induced increase of plasma nitrite and corticosterone levels; brain malondialdehyde levels and monoamine oxidase (MAO(-A activity. Both the drugs also significantly reversed CUMS-induced decrease in brain reduced glutathione levels and catalase activity. Thus, **s**yringic acid showed significant antidepressant-like activity in mice subjected to CUMS through alleviation of oxidative and nitrosative stress; and inhibition of brain MAO-A activity. Further, antidepressant-like activity of syringic acid in mice subjected to CUMS might also be due to decrease in plasma corticosterone levels. Fig. 1 Illustrates further the scheme of protocol designed with various groups of animals.

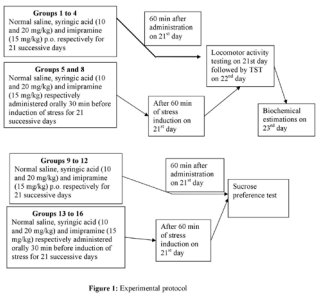

Figure 1: Experimental protocol

INTRODUCTION

Mood disorders have disturbances in mood as the predominant feature. Major depressive disorder is characterized by one or more major depressive episodes i.e., at least 2 weeks of depressed mood or loss of interest or pleasure in nearly all activities. A depressed individual has also variable symptoms such as changes in appetite or weight, sleep, and psychomotor activity; decreased energy; feelings of worthlessness or guilt; inability to think, concentrating, or making decisions; or recurrent thoughts of death or suicidal attempts.[1] It is assessed that approximately 264 million individuals worldwide are suffering from major depression.[2]

Depression is mainly due to neurotransmitter imbalances,[3] high oxidative stress in brain,[4] and hypothalamic-pituitary-adrenal axis (HPA) hyperactivity.[5] According to monoamine hypothesis, depletion and breakdown of monoamines like serotonin, norepinephrine and dopamine in the hippocampus, limbic system and frontal cortex are responsible for the depressive symptoms. MAO is the key enzyme

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which metabolizes monoaminergic neurotransmitters.[6] Chronic unpredictable stress enhances the reactive oxygen species formation in brain, which leads to oxidative damage and alterations in normal physiology of CNS.[7] There is impairment of L-arginine nitric oxide pathway in patients of depression.[8] Diminished antioxidant levels and increased oxidative stress were found in major depressive disorder patients.[9,10] Stressful conditions in mice significantly increased plasma nitrite levels, an index of nitric oxide production.[11]

Stress-induced hyperactivity of HPA axis leads to increased secretion of corticotrophin releasing factor which may be responsible for depressive symptoms.[5] High glucocorticoid levels may precipitate the symptoms of depression by impairing brain functions like neurogenesis, neuronal survival, neuronal excitability, and plasticity.[12] Hypofunction of GABAergic system and hyperfunction of the glutamatergic system can also lead to depression.[13]

Most frequently prescribed drugs for treatment of depression include selective serotonin-reuptake inhibitors (SSRI's) such as fluoxetine, sertraline, paroxetine, escitalopram; and serotonin-norepinephrine reuptake inhibitors like reboxetine. Other drugs used in depression treatment include tricyclic antidepressants like imipramine; and MAO inhibitors such as clorgyline and moclobemide.[14,15] Although these drugs are effective in treating most cases of depression, a considerable proportion of depressed patients do not show signs of improvement until 2–3 weeks after initiation of treatment. Furthermore, 10 to 30% of them do not improve or show a partial response coupled with functional impairment, poor quality of life, suicidal attempts, self-injurious behavior, and a high relapse rate.[16] In addition, these drugs can cause side-effects such as sedation, anticholinergic effects (urinary retention, constipation, blurred vision, dried mouth etc.), dizziness, postural hypotension, anxiety, impotence, seizures, cheese reaction (in case of MAO inhibitors) and dysrhythmias.[14] So there is alarming need to explore new strategies for treatment of depression.

Syringic acid is a natural phenolic compound, found abundantly in cereals such as rice, barley, millet, oat, rye, maize, sorghum, wheat and in plants such as *Raphanus sativus L.*[17] It is reported to possess various biological activities like antioxidant,[18] antihyperglycemic,[19] antiangiogenic [17] and neuroprotective.[20,21] It has been reported that syringic acid, a major phenolic constituent of *Morus nigra* aqueous extract, produced neuroprotective and antidepressant effects in mice,[22] but the effect of syringic acid on chronic unpredictable mild stress- induced depression in mice has not been explored till date. Further, the effects of syringic acid on brain MAO-A activity and stress-induced increase in plasma corticosterone levels have also not been reported. So, the present investigation was designed to study the effect of syringic acid on chronic unpredictable mild stress-induced depression in mice and to explore the possible underlying mechanisms for this effect of syringic acid.

Materials and Methods

Experimental Animals

Swiss male young albino mice (2–3 months old, weighing around 25–30 g) were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Estrogens (female sex hormones) have been found to have antidepressant effect, so we excluded female mice and used only male mice for the study.[23] Animals were housed separately in groups of 6 animals per cage (Polycarbonate cage size: 29×22×14 cm) an air-conditioned room with alternating light and dark cycle of 12 hours each. The animals were acclimatized for five days before behavioral experiments. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) of Guru Jambheshwar University of Science and Technology, Hisar in its meeting held on 8th Dec, 2015 (GJUST/IAEC/247-255, dated 08-12-2015). Animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Dept. of Animal Husbandry and Dairying, Government of India.

Drugs and Chemicals

Imipramine hydrochloride, syringic acid, p-nitroso-N, N-dimethylaniline (Sigma-Aldrich, USA), sulphanilamide, N-(1- Naphthyl) ethylene diaminedihydrochloride, meta-phosphoric acid, carboxy methyl cellulose (Hi-Media, Mumbai); normal saline (Claris Otsuka, Ahmedabad) were employed in this study.

Vehicle

Imipramine hydrochloride and syringic acid were dissolved/dispersed in normal saline.

Selection of Doses

Doses of syringic acid (10 and 20 mg/kg)[24,25] and imipramine hydrochloride (15 mg/kg)[11] were selected.

Chronic Unpredictable Mild Stress Paradigm

Mice were exposed to unpredictable continual mild stress (CUMS) as reported by Mao *et al*. (2009)[26] and Kumar *et al.* (2011)[27] with slight modifications. Animals were subjected to stress paradigm once a day for a period of 3 weeks as per the order mentioned in Table 1. Mice subjected to CUMS procedure were called as stressed mice. The unstressed mice were exposed to behavioral tests, and not subjected to CUMS procedure. Drugs were administered 30 minutes before CUMS procedure in case of stressed mice.

Laboratory models employed for behavioral tests

Tail Suspension Test (TST)

This behavioral test was conducted as per the method reported by Steru *et al.*, 1985[28] and as followed earlier in our laboratory.[11]

Table 1: Order of stressors for chronic unpredictable mild stress paradigm.

Weeks/Days	*Day-1*	*Day-2*	*Day-3*	*Day-4*	*Day-5*	*Day-6*	*Day-7*
Week-1	I	E	F	O	T2	X	T1
Week-2	I	O	X	T2	E	T1	F
Week-3	O	F	T1	X	T2	I	E

E – exposure to empty water bottles for 1-hour; F – exposure to foreign object for 24 hours (e.g. piece of plastic); I – immobilization for 2 hours; O – overnight illumination; T1 – tail pinch (30 s); T2 – tail pinch (60 s); X – tilted cage at 45° for 7 hours.

Sucrose Preference Test

Sucrose preference test was employed herein to determine anhedonia, one of the core symptoms of major depression in humans. The procedure was composed of training and testing courses. After 1 week of acclimatization, mice were trained to consume 1% (w/v) sucrose solution before the start of the CUMS protocol. In training course, mice were deprived of food and water for 48 hours and only exposed to 1% (w/v) sucrose solution. Three days later, after 23 hours food and water deprivation, 1-hour baseline test was performed, in which mice could select between two pre-weighed bottles, one with 1% (w/v) sucrose solution and the other with tap water. Then, the sucrose preference was calculated according to the following formula:

$$\text{SucrosePreference} = \frac{\text{Sucrosesolutionintake (g)}}{[\text{Sucrosesolutionintake (g)} + \text{waterintake (g)}]} \times 100$$

The test was again performed on the 21st day to evaluate the effect of stress as well as drug treatment.[29]

Measurement of Locomotor Activity:

To check and observe the effects of various drug treatments on locomotor activity, horizontal locomotor activities of control and test animals were recorded for a period of 5 min using photoactometer (INCO, Ambala, India).[11,27]

Experimental Protocol

The animals were divided in following 16 groups having 6 mice in each group:

Groups for Locomotor Activity and Tail Suspension Test (TST)

- *Groups 1 to 4 (n=6):* Vehicle (0.9% w/v normal saline), syringic acid (10 and 20 mg/kg) and imipramine (15 mg/kg) respectively were administered orally to mice for 21 successive days. After 60 min of vehicle/drug administration on 21st day, locomotor activity scores of mice were measured. The mice were subjected to TST on 22nd day.
- *Groups 5 to 8 (n=6):* Vehicle (0.9% w/v normal saline), syringic acid (10 and 20 mg/kg) and imipramine (15 mg/kg) respectively were administered orally 30 min before induction of stress to mice for 21 successive days. After 60 minutes of stress induction on 21st day, locomotor activity scores of mice were measured. On 22nd day, the mice were subjected to TST.

- Groups for Sucrose Preference Test
- *Groups 9 to 12 (n=6):* Vehicle (0.9% w/v normal saline), syringic acid (10 and 20 mg/kg) and imipramine (15 mg/kg) respectively were administered orally to mice for 21 successive days. After 60 minutes of vehicle/drug administration on 21st day, mice were subjected to sucrose preference test.
- *Groups 13 to 16 (n=6):* Vehicle (0.9% w/v normal saline), syringic acid (10 and 20 mg/kg) and imipramine (15 mg/kg) respectively were administered orally 30 minutes before induction of stress to mice for 21 successive days. After 60 min of stress induction on 21st day, mice were subjected to sucrose preference test.

Biochemical Assessments

In Plasma

The drug administration was continued up to 23rd day. One hour after the drug administration on 23rd day, blood (0.5–0.8 mL) was withdrawn from retro-orbital plexus of mice of groups 1–8. Blood samples were centrifuged at 2500 rpm for 10 minutes using refrigerated centrifuge (Remi, Mumbai, India) to separate the plasma, which was used for estimation of nitrite and corticosterone levels.

Estimation of Plasma Corticosterone Levels:

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979[30] and as followed in our laboratory.[11,31]

Estimation of Plasma Nitrite Levels

Plasma nitrite was measured by using the method of Green et al, 1982;[32] and as followed in our laboratory.[11,31]

Biochemical Estimations in Brain Homogenate

After withdrawing blood samples on 23rd day, mice were sacrificed by decapitation and their brains were isolated. The collected brain samples were washed with cold 0.25 M sucrose 0.1MTris-0.02M EDTA buffer (pH 7.4) and weighed. The buffer washed brain sample was homogenized in 9 volumes of cold 0.25M Sucrose-0.1M Tris-0.02M EDTA buffer (pH 7.4) buffer and centrifuged twice at 2500 rpm for 10 minutes at 4°C in a cooling centrifuge (Remi instruments, Mumbai, India). The pellet was discarded, and the supernatant was then centrifuged at 12000 rpm for 20 minutes at 4°C in a cooling centrifuge. This centrifuged supernatant was separated into two parts:

- *Part I:* The precipitates (mitochondrial fraction) were used for estimation of MAO-A activity.

- Part II: The remaining supernatant was used to assay lipid peroxidation, reduced glutathione and catalase levels.

Measurement of Brain MAO-A Activity

The MAO-A activity was assessed spectrophotometrically as per the method reported by Schurr and Livne, 1976,[33] Charles and McEwan, 1977,[34] and as followed earlier in our laboratory.[11,31]

Estimation of Protein Concentration

Total protein concentration in brain homogenate was estimated by Biuret method[35] and as followed earlier in our laboratory[11] by using a total protein kit (Siemens, Siemens Ltd., Vadodara, Gujrat), using semi-automatic autoanalyzer (Chem 5 plus-V2 semi-autoanalyzer; Erba Mannheim, Germany).

Estimation of Brain Lipid Peroxidation

The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances by the method of Wills, 1965,[36] and as followed earlier in our laboratory.[11,31]

Estimation of Brain Reduced Glutathione

Reduced glutathione in the brain tissue was estimated according to the method of Ellman (1959),[37] and as followed earlier in our laboratory.[38]

Estimation of Brain Catalase Activity

The catalase activity was estimated using method of Aebi (1984),[39] and as followed earlier in our laboratory.[38]

Statistical Analysis

All the results were expressed as mean ± SEM. Data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test using GraphPad Prism version 7.01 statistical software. $p < 0.05$ was considered as statistically significant.

Results

Effect of Syringic Acid and Imipramine on Immobility Periods of Mice in TST

The results of two-way ANOVA revealed that there was significant interaction between stress and drugs [F (3, 40) = 4.211; p < 0.05]. There was also significant difference among various treatments [F (3, 40) = 11.01; p < 0.0001] and effect of stress [F (1, 40) = 63.32; p < 0.0001]. Chronic unpredictable mild stress significantly (p < 0.0001) increased the immobility period as compared to vehicle treated unstressed mice. Imipramine and syringic acid administration for 3 successive weeks did not show any significant effect on immobility period of unstressed mice as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and syringic acid (10 and 20 mg/kg) administration for 3 successive weeks significantly (p < 0.0001, p < 0.05 and p < 0.01 respectively) decreased immobility period of stressed mice as compared to vehicle treated stressed mice (Fig. 1A).

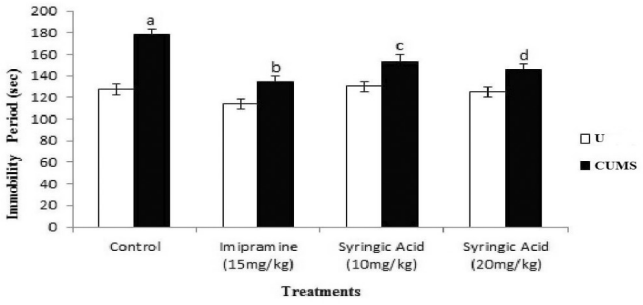
Effect of Syringic Acid and Imipramine on Sucrose Preference

For baseline test, the results of two-way ANOVA revealed that there was no significant interaction between stress and drugs [F (3, 40) = 0.08073; p>0.05] and no significant difference among various treatments [F (3, 40) = 0.5927; p>0.05] and effect of stress [F (1, 40) = 0.7068; p>0.05]. There was no significant difference in sucrose preference (%) among all the treatments in the baseline test.

After 21 days, there was significant interaction between stress and drugs [F (3, 40) = 7.174; p < 0.001], significant difference among various treatments [F (3, 40) = 10.98; p < 0.0001] and effect of stress [F (1, 40) = 480.6; p < 0.0001]. Exposure of mice to chronic unpredictable mild stress for 3 successive weeks significantly (p < 0.0001) decreased sucrose preference (%) as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and syringic acid (10 and 20 mg/kg) were administered for 21 successive days did not show any significant effect on sucrose preference (%) in unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid administered for 21 successive days significantly (p < 0.0001 and p < 0.01, respectively) restored the reduced sucrose preference in stressed mice but lower dose (10 mg/kg) of syringic acid did not show any significant effect on reduced sucrose preference (%) in stressed mice as compared to vehicle treated stressed mice [Figures 2 A and B].

Effect of Syringic Acid and Imipramine on Locomotor Activity

There was no significant interaction between stress and drugs [F (3, 40) = 0.03656; p > 0.05] and no significant



Fig, lA : Effect of syringic acid and imipramine on immobility period of mice in TST.

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 4.211; p < 0.05. Treatments/Row — F (3, 40) = 11.01; p < 0.0001. Stress/Column — F (1, 40) = 63.32; p < 0.0001. TST= Tail Suspension Test

a= p < 0.0001 as compared to vehicle treated unstressed mice. b, c, d= p < 0.0001, p < 0.05 and p < 0.01 respectively as compared to vehicle treated stressed mice.

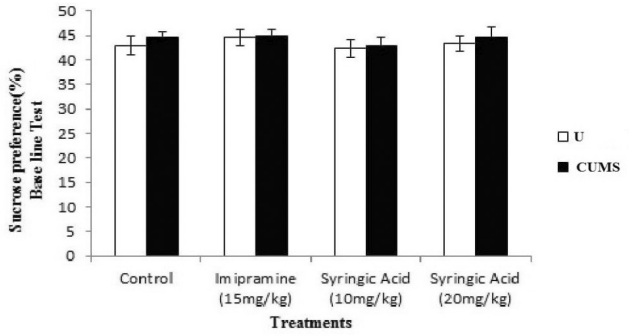


Fig. 2(A): Effect of syringic acid and imipramine on sucrose preference (%) baseline test.

n = 6 in each group. Values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 0.08073; p>0.05. Treatments/Row — F (3, 40) = 0.5927; p>0.05. Stress/Column — F (1, 40) = 0.7068; p>0.05.

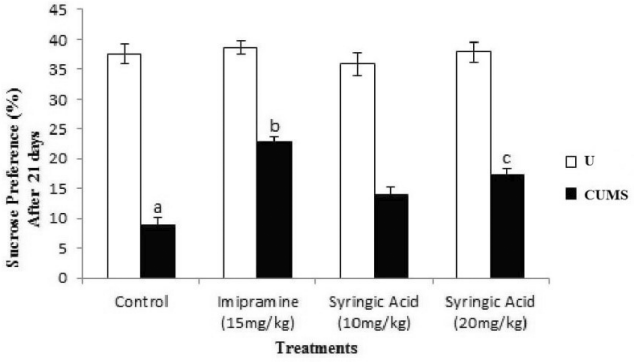


Fig. 2(B): Effect of syringic acid and imipramine on sucrose preference (%) after 21 days. U= Unstressed; CUMS= Chronic Unpredictable Mild Stress n = 6 in each group. Values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 7.174; p < 0.001. Treatments/Row — F (3, 40) = 10.98; p < 0.0001. Stress/Column — F (1, 40) = 480.6; p < 0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b, c= p < 0.0001, p < 0.01 respectively as compared to vehicle treated stressed mice.

difference among various treatments [F (3, 40) = 0.6327; p>0.05]. However, there was significant effect of stress [F (1, 40) = 13.37; p < 0.001]. Various treatment groups did not affect the locomotor activity scores of unstressed and stressed mice as compared to their respective vehicle treated controls (Fig. 3).

Effect of Syringic Acid and Imipramine on Plasma Corticosterone Levels

There was significant interaction between stress and drugs [F (3, 40) = 4.042; p < 0.05], also significant difference among various treatments [F (3, 40) = 5.744; p < 0.01] and effect of stress [F (1, 40) = 80.55; p < 0.0001]. Chronic unpredictable mild stress significantly (p < 0.0001) increased plasma corticosterone levels as compared to

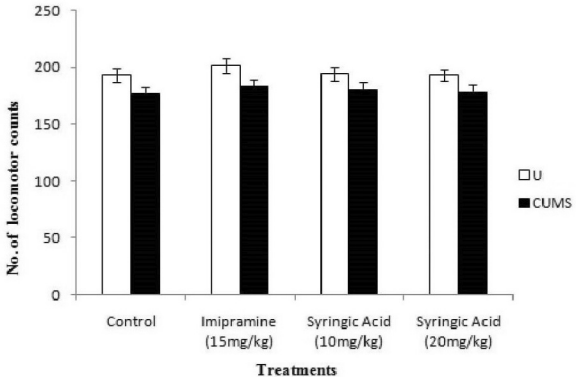


Fig. 3: Effect of syringic acid and imipramine on locomotor activity of mice

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 0.03656; p=0.9905 (Non-Significant). Treatments/Row — F (3, 40) = 0.6327; p=0.5983 (Non-Significant). Stress/Column — F (1, 40) = 13.37; p < 0.001

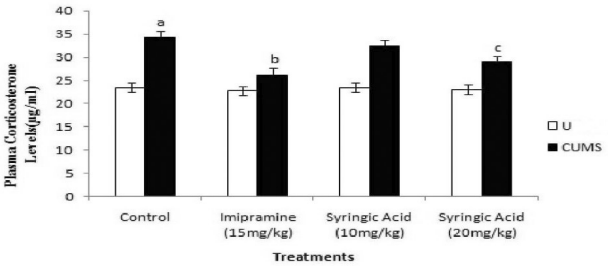


Fig. 4: Effect of syringic acid and imipramine on plasma corticosterone levels

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 4.042; p < 0.05. Treatments/Row — F (3, 40) = 5.744; p < 0.01. Stress/Column — F (1, 40) = 80.55; p < 0.0001

a= p < 0.0001 as compared to vehicle treated unstressed mice. 13, c= p < 0.001, p < 0.05 respectively as compared to vehicle treated stressed mice.

vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid administered for 3 successive weeks significantly (p < 0.001 and p < 0.05 respectively) decreased plasma corticosterone levels of stressed mice but lower dose (10 mg/kg) of syringic acid did not significantly decrease plasma corticosterone levels of stressed mice as compared to vehicle treated stressed mice. Imipramine and syringic acid administered for 3 successive weeks did not significantly decrease plasma corticosterone levels of unstressed mice as compared to vehicle treated unstressed mice (Fig. 4).

Effect of Syringic Acid and Imipramine on Plasma Nitrite Level

There was significant interaction between stress and drugs [F (3, 40) = 4.792; p < 0.01], significant difference among

various treatments [F (3, 40) = 7.383; p < 0.001] and effect of stress [F (1, 40) = 68.68; p < 0.0001]. Chronic unpredictable mild stress significantly (p < 0.0001) increased plasma nitrite levels as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid administered for 3 successive weeks significantly (p < 0.001 and p < 0.01, respectively) decreased the nitrite levels of stressed mice, but lower dose (10 mg/kg) of syringic acid did not significantly decrease plasma nitrite levels of stressed mice as compared to their vehicle treated control. Imipramine and syringic acid administered for 3 successive weeks did not show any significant effect on plasma nitrite levels of unstressed mice as compared to vehicle treated unstressed mice (Fig. 5).

Effect of Syringic Acid and Imipramine on Brain MAO-A Activity

There was no significant interaction between stress and drugs [F (3, 40) = 1.31; p>0.05], however there was significant difference among various treatments [F(3, 40) = 5.191; p < 0.01] and effect of stress [F (1, 40) = 102.3; p < 0.0001]. Chronic unpredictable mild stress significantly (p < 0.0001) increased brain MAO-A activity as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid administered for 3 successive weeks significantly (p < 0.05) decreased brain MAO-A activity in stressed mice, but lower dose (10 mg/kg) of syringic acid did not significantly decrease MAO-A activity in stressed mice as compared to vehicle treated stressed mice. Imipramine and syringic acid administered for 3 successive weeks did not show any significant effect on MAO-A activity of unstressed mice as compared to vehicle treated unstressed mice (Fig. 6).

Effect of syringic acid and imipramine on brain malondialdehyde (MDA) levels

There was significant interaction between stress and drugs [F (3, 40) = 6.005; p < 0.01], significant difference among various treatments [F (3, 40) = 8.722; p < 0.001] and effect of stress [F (1, 40) = 968.8; p < 0.0001]. Malondialdehyde levels were increased significantly (p < 0.0001) in mice subjected to stress as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid per se administered for three successive weeks significantly (p < 0.001 and p < 0.01, respectively) decreased malondialdehyde levels of stressed mice as compared to vehicle treated stressed mice. Lower dose (10 mg/kg) of syringic acid did not show any significant effect on malondialdehyde levels of stressed mice as compared to vehicle treated stressed mice. Imipramine and syringic acid administered for 3 successive weeks did not show any significant effect on malondialdehyde levels of unstressed mice as compared to vehicle treated unstressed mice (Fig. 7).

Effect of Syringic Acid and Imipramine on Brain Reduced Glutathione (Gsh) Levels

There was no significant interaction between stress and drugs [F (3, 40) = 1.492; p > 0.05], however there was significant difference among various treatments [F (3, 40) = 12.64; p < 0.0001] and effect of stress [F (1, 40) = 683.3; p < 0.0001]. Reduced glutathione levels were significantly (p < 0.0001) decreased in stressed mice as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid administered for 21 successive days significantly (p < 0.0001 and p < 0.05 respectively) increased reduced glutathione levels of stressed mice but lower dose syringic acid (10 mg/kg)

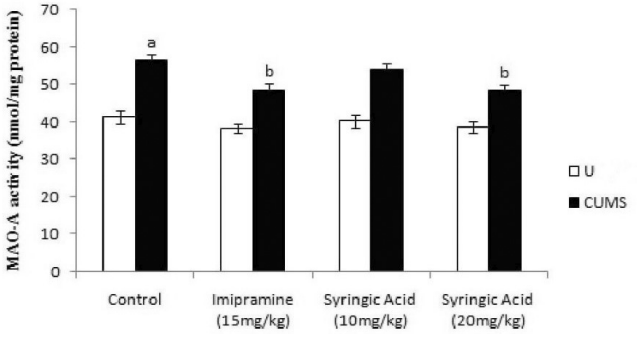


Fig. 5: Effect of syringic acid and imipramine on brain MAO-A activity

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 1.31; p=0.2844 (Non-Significant). Treatments/Row — F (3, 40) = 5.191; p < 0.01. Stress/Column — F (1, 40) = 102.3; p < 0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b= p < 0.05 respectively as compared to vehicle treated stressed mice.

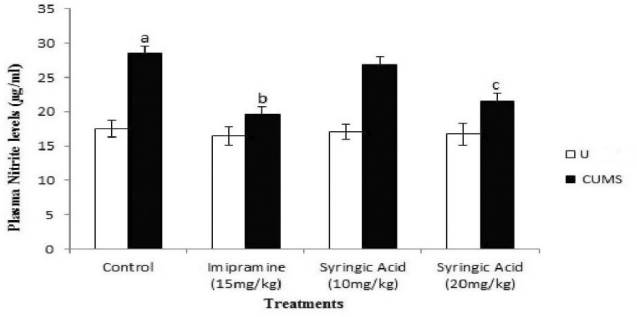


Fig. 6: Effect of syringic acid and imipramine on plasma nitrite levels

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 4.792; p < 0.01. Treatments/Row — F (3, 40) = 7.383; p < 0.001. Stress/Colunm — F (1, 40) = 68.68; p < 0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b, c= p < 0.001, p < 0.0I respectively as compared to vehicle treated stressed mice.

did not show any significant effect on reduced glutathione levels of stressed mice as compared to vehicle treated stressed mice. However, imipramine and syringic acid did not show any significant effect on the reduced glutathione levels of unstressed mice as compared to vehicle treated unstressed mice (Fig. 8).

Effect of Syringic Acid And Imipramine on Brain Catalase Activity

There was significant interaction between stress and drugs [F (3, 40) = 3.461; p < 0.05], significant difference among various treatments [F (3, 40) = 6.791; p < 0.001] and effect of stress [F (1, 40) = 140.5; p < 0.0001]. Brain catalase activity was significantly (p < 0.0001) reduced in stressed mice as compared to vehicle treated unstressed mice. Imipramine (15 mg/kg) and higher dose (20 mg/kg) of syringic acid per se administered for 21 successive days significantly (p < 0.01) increased brain catalase activity of stressed mice but lower dose (10 mg/kg) of syringic acid did not show any significant effect on the brain catalase activity of stressed mice as compared to vehicle treated stressed mice. Imipramine and syringic acid did not show any significant effect on brain catalase activity of unstressed mice as compared to vehicle treated unstressed mice (Fig. 9).

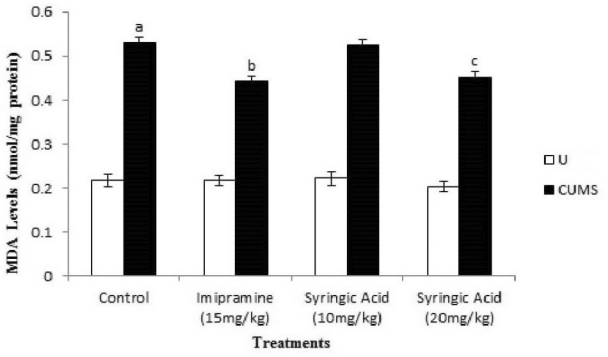


Fig. 7: Effect of syringic acid and imipramine on brain malondialdehyde (MDA) levels

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 6.005; p < 0.01. Treatments/Row — F (3, 40) = 8.722; p < 0.00I. Stress/Column — F (1, 40) = 968.8; p < 0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b, c= p < 0.001, p < 0.01 respectively as compared to vehicle treated stressed mice.

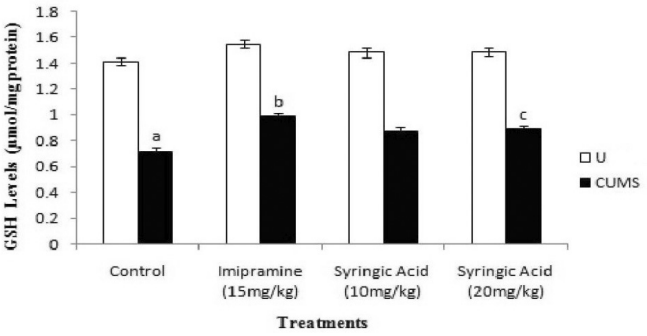


Fig. 8: Effect of syringic acid and imipramine on brain reduced glutathione (GSH) levels

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 1.492; p=0.2314 (Non-Significant). Treatments/Row — F (3, 40) = 12.64; p < 0.0001. Stress/Column — F (1, 40) = 683.3; p <0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b, c= p < 0.0001, p < 0.05 respectively as compared to vehicle treated stressed mice.

Discussion

In the present investigation, syringic acid administered for 21 successive days showed significant antidepressant-like activity in mice subjected to CUMS. Induction of depression using CUMS is widely accepted behavioral model of depression due to its excellent validity and reliable predictability. In CUMS model of depression, experimental animals are exposed sequentially, over a period of weeks, to a variety of mild stressors. CUMS-induced behavioral despair in rodents resembles depressive disorders in human beings. [40,41]

Tail suspension test (TST) and sucrose preference test were used as behavioral models to quantify depression in mice. In TST, mice that were exposed to CUMS exhibited greater immobility periods in TST as compared to control animals, thus stressed mice showed depression-like behaviour. Treatment with imipramine (15 mg/kg, p.o.) and syringic acid (10 and 20 mg/kg, p.o) per se for 21 successive days produced significant decrease in immobility periods of

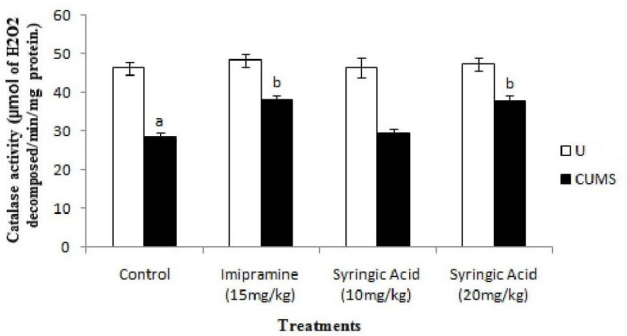


Fig. 9: Effect of syringic acid and imipramine on brain catalase activity

U= Unstressed mice; CUMS= Chronic unpredictable mild stress; n = 6 in each group, values are expressed as mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey's multiple comparison test. Interaction — F (3, 40) = 3.461; p < 0.05. Treatments/Row — F (3, 40) = 6.791; p < 0.001. Stress/Column — F (1, 40) = 140.5; p < 0.0001.

a= p < 0.0001 as compared to vehicle treated unstressed mice. b= p < 0.01 respectively as compared to vehicle treated stressed mice.

stressed mice in TST as compared to stressed control mice. The effect of syringic acid (20 mg/kg, p.o) on decrease of immobility period in TST was comparable to imipramine (15 mg/kg, p.o.). There was no significant effect of syringic acid on immobility period of unstressed mice in TST. These findings indicate significant antidepressant-like activity of syringic acid in mice subjected CUMS. Syringic acid did not affect the locomotor activity of the unstressed and stressed mice as compared to control, thus cutting out their CNS stimulant activity.

The second behavioral model used for evaluating depression-like behavior was sucrose preference test, which was used to detect anhedonia in animals. Anhedonia (loss of interest or pleasure) is a main symptom of human major depression. It was modeled by inducing a decrease in responsiveness to rewards reflected by a reduced consumption and/or preference of sweetened solutions.[29,41] In our study, vehicle treated stressed mice showed a decrease in sucrose preference as compared to unstressed control mice, thus showed depression-like behaviour. Reduced sucrose preference was significantly restored in stressed mice by chronic administration of imipramine (15 mg/kg, p.o.) and syringic acid (20 mg/kg, p.o.), suggesting their antidepressant-like actions. Thus, the results obtained from behavioral studies indicated that syringic acid produced significant antidepressant-like action in mice exposed to CUMS.

HPA axis is activated during stress, with resultant increase in circulating glucocorticoids such as corticosterone in rodents or cortisol in primates.[42] Hypothalamic hypersecretion of corticotropin-releasing hormone (CRH) contributes to the hyperactivity of the HPA system in patients with major depression.[5,43] Cortisol is known to regulate neuronal survival, neuronal excitability, and neurogenesis. High cortisol levels may thus contribute to the symptoms of depression by impairing these vital brain functions.[12] It has been reported that chronic antidepressant treatment in rodents reduced HPA hyperactivity.[44] Thus, restoring the hyperactive functional status of HPA axis to normal may be involved in the treatment of depression.[45] In the present study, CUMS increased plasma corticosterone levels, which is also supported by observations from other studies.[11,46] Plasma corticosterone levels were significantly reduced by imipramine (15 mg/kg, p.o.) and syringic acid (20 mg/kg, p.o.) in mice subjected to CUMS. There was no significant effect on plasma corticosterone levels in unstressed mice, strengthening the fact that hyperactivity of HPA axis is observed only in stressful conditions.

Activation of immune-inflammatory process, increased monoamine breakdown, and abnormalities in lipids may cause overproduction of reactive oxygen species, lipid peroxidation, and reduced antioxidant enzyme activities, and these processes may be related to altered physiology in depression.[47] CUMS impairs the antioxidant status of brain, through production of reactive oxygen species.[48] In present study, 21 days of exposure to different stressors resulted in increase of brain malondialdehyde levels and plasma nitrite levels; and decrease in brain reduced glutathione levels and catalase activity. This is supported by an earlier study where CUMS impaired the antioxidant status (increased lipid peroxidation and nitrite levels, decreased glutathione levels and catalase activity) in brain tissue, through production of excessive reactive oxygen species.[27] Chronic administration of imipramine (15 mg/kg, p.o.), and syringic acid (20 mg/kg, p.o.) significantly decreased brain malondialdehyde and plasma nitrite levels in stressed mice, but no notable change was observed in unstressed mice. Imipramine (15 mg/kg, p.o.) and syringic acid (20 mg/kg, p.o.) administered for 21 successive days significantly increased reduced glutathione levels and catalase activity in stressed mice. Thus, syringic acid and imipramine significantly alleviated oxidative stress in mice. Antioxidant activity of syringic acid and imipramine has been reported in literature.[18,49] Thus, antidepressant activity of syringic acid might be through protective effects against CUMS-induced oxidative stress. Further, CUMS led to increased activity of brain MAO-A, which is supported by the literature.[11] Chronic treatment with imipramine (15 mg/kg, p.o.) and syringic acid (20 mg/kg, p.o.) significantly inhibited brain MAO-A activity in stressed mice as compared to vehicle treated stressed mice. Thus, antidepressant-like activity of syringic acid in stressed mice might also be attributed to inhibition of brain MAO-A activity.

Administration of syringic acid for 3 successive weeks in unstressed mice did not show any significant change in various behavioral and biochemical parameters and this may be attributed partly due to absence of stress conditions.

Therefore, syringic acid produced significant antidepressant-like activity in mice subjected to chronic unpredictable mild stress possibly through inhibition of brain MAO-A activity, decrease in plasma nitrite and corticosterone levels; and also due to its antioxidant activity. Thus, syringic acid may be explored further for management of stress-induced depression.

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