Antidepressant-like Activity of Abietic Acid in Unstressed Mice and Chronic Unpredictable Mild Stressed Mice

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ABSTRACT

In the present study, abietic acid was evaluated for antidepressant-like activity in normal mice and chronic unpredictable mild stress (CUMS) –induced depressed mice. Swiss albino male mice were subjected to CUMS for 21 successive days. Abietic acid (7.5, 15, and 30 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) per se were given for 3 successive weeks to separate groups of normal mice and CUMS-induced depressed mice. Tail suspension test (TST) and sucrose preference test were employed to study the effect of the drugs on the depressive-like behavior of mice. CUMS produced depression-like behavior in mice. In TST, the immobility period was significantly decreased by abietic acid (30 mg/kg) and fluoxetine in normal mice as well as in CUMS-induced depressed mice as compared to their respective control groups. A decrease in sucrose preference due to CUMS was significantly restored by abietic acid (15 and 30 mg/kg) and fluoxetine. Locomotor activities of mice were not significantly changed by abietic acid and fluoxetine. Plasma nitrite, brain monoamine oxidase –A (MAO-A) activity, and brain malondialdehyde were significantly decreased; and brain catalase activity and reduced glutathione levels were significantly increased by abietic acid (15 and 30 mg/kg) and fluoxetine in both normal mice and CUMS-induced depressed mice. CUMS-induced increase in plasma corticosterone levels was significantly lowered by abietic acid (15 and 30 mg/kg) and fluoxetine. Abietic acid exerted significant antidepressant-like activity in both normal mice and CUMS-induced depressed mice possibly through mitigation of oxidative stress and decrease of brain MAO-A activity. Additionally, lowering of plasma corticosterone concentration by abietic acid in CUMS-induced depressed mice might also contribute to its antidepressant-like effect.

Key Words: *Abietic acid, Antidepressant, Chronic unpredictable mild stress, Depression, Sucrose preference test, Tail suspension test*

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INTRODUCTION

Depression is a common mental disorder worldwide, with more than 264 million people affected. In a depressed person, there is a decrease in mood, loss of interest and pleasure, low self-esteem, feeling of guilt, changes in sleep, and appetite [1-3]. It may be due to a decrease in norepinephrine, dopamine, and serotonin levels in the brain [4-6]. Stress has a major role in the development of human depression [7]. In a stressful situation, there is activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevation of the concentration of glucocorticoids like cortisol in primates or corticosterone in rodents, resulting in depression [8]. At present, the antidepressants available for clinical use include selective serotonin reuptake inhibitors, serotonin– noradrenergic reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors [9]. But these produce lots of side effects like cognitive impairment, sedation, fatigue, hypertensive crisis, sexual dysfunction [10, 11]. So plants and their bioactive compounds can be explored as an alternative and safe option for the treatment of depression. *Hypericum perforatum* has been proven to be an effective antidepressant for the treatment of depression in clinical studies [12]. Abietic acid is a tricyclic diterpene which is found in resins of different species of *Pinus* such as *P. mugo*, *P. palustris*, *P. strobus*, [13] *P. sylvestris*, *P. roxburghii*, [14] and also in *Abies alba* (Family – *Pinaceae*) [15]. Abietic acid has antioxidant and anti-cholinesterase, [16] antiepileptic, [17] anti-obesity [18] and anti-inflammatory [19] activities. But the effect of

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abietic acid on depression has not been mentioned in the literature. Therefore, abietic acid was studied for its effect on normal mice and CUMS-induced depressed mice.

MATERIALS AND METHODS

Experimental animals

Animals used were Swiss male albino mice (two to three months old and having body weight in the range 21-29 g). The mice were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Only male mice were used in the present study because the female sex hormone (estrogen) has been found to possess antidepressant activity [20]. The animals were housed in an air-conditioned room with a 12:12 h light-dark cycle. Drinking water and food were provided *ad libitum* to the animals, but the food was withdrawn 2 h before and 2 h after administration of the drugs, to rule out the effect of food on the absorption of the drugs [21]. The mice were acclimatized for 5 days before being used for the experiments. The experimental study was conducted between 9 AM and 5 PM. The experimental protocol and the number of animals to be used for the experiments were approved by Institutional Animals Ethics Committee (IAEC) in its meeting held on 6th October 2017 (Letter No. of Minutes- IAEC/2017/44-52, dated 02-11-2017).

Drugs and chemicals

Drugs used were abietic acid and fluoxetine (Sigma-Aldrich, St Louis, USA). Chemicals used were p-nitroso-N,N-dimethylaniline, N-(1- Naphthyl) ethylenediamine dihydrochloride, 5-hydroxytryptamine, thiobarbituric acid (HiMedia Laboratories Private Limited, Mumbai, India), sulfanilamide, metaphosphoric acid, potassium ferricyanide, hydrogen peroxide, trichloroacetic acid (CDH Private Limited, New Delhi, India), 5,5, Dithiobis-2-(nitro benzoic acid) (SRL Private Limited, Mumbai, India), sulfosalicylic acid (Spectrochem Private Limited, Mumbai, India). The kit was used for total protein estimation (Transasia Bio-medicals Ltd., Baddi (Solan), India).

Vehicle

The vehicle used for abietic acid was a 10% v/v solution of Tween 80 in distilled water. Normal saline was used as a vehicle for dissolving fluoxetine. An oral route of administration was employed for both the drugs.

Procedure for CUMS

CUMS was applied to the mice as per the method reported by Willner *et al*. (1987) with slight modifications [22]. The animals were exposed to various stressors only once a day for 3 successive weeks between 11 AM and 5 PM. The stressors were given to the mice in the following order:

Weeks	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
1st week	I	E	F	O	T2	X	T1
2nd week	I	O	X	T2	E	T1	F
3rd week	O	F	T1	X	T2	I	E

I— Immobilization for 2 h.
E— Exposure to an empty water bottle for 1 h.
F— Exposure to a foreign object (for example, a piece of plastic) for 24 h.
O— Overnight illumination.
T2— A tail pinch for 60 sec.
X— Tilted cage at 45 degrees for 7 h.
T1— A tail pinch for 30 sec.

Tail suspension test (TST)

The procedure for TST was the same as reported by Steru *et al.* [23]. The animals were suspended one by one fifty centimeters above the floor by adhesive tape which was placed approximately 1 cm from the tip of the tail. The immobility period of each mouse was recorded for 6 min. Immobility was characterized by lack of movements of limb or body except for respiratory movements which were produced during the passive and motionless hanging of animals.

Sucrose preference test

Sucrose preference test is mainly used to evaluate anhedonia, which is the core symptom of depression [24]. Initially, mice were exposed to a drinking water bottle containing 1% w/v sucrose solution until they are trained to take this solution. Then, the mice were not provided food and water for forty-eight hours but were allowed to take sucrose solution (1% w/v). After 3 days, mice were not given food and water for twenty-three hours; followed by a one-hour baseline test, where each mouse was exposed to two weighed bottles, one bottle filled with sucrose solution (1% w/v), and the other bottle filled with tap water. The sucrose preference was calculated as per the following formula:

$$\text{Sucrose Preference} = \frac{\text{Sucrose solution intake (g)}}{[\text{Sucrose solution intake (g)} + \text{water intake (g)}]} \times 100 \quad (1)$$

On the 21st day, the sucrose preference test was again performed to evaluate the effect of CUMS and drugs.

Measurement of locomotor activity

Horizontal locomotor activities of the mice were noted for 5 min [25] by using a photoactometer [INCO, Ambala (Haryana), India].

Details of experimental design

The animals were divided into the following 20 groups, each group having 8 mice.

For TST and locomotor activity

Groups 1–5: Tween 80 (10% v/v), abietic acid (7.5, 15 and 30 mg/kg) and fluoxetine (20 mg/kg) respectively were orally administered to mice for 3 successive weeks. The mice were subjected to TST one hour after vehicle/drug administration on the 22nd day, and this was followed by testing of their locomotor scores 30 min later.

Groups 6–10: Tween 80 (10% v/v), abietic acid (7.5, 15 and 30 mg/kg) and fluoxetine (20 mg/kg) respectively were orally administered 30 min before giving stressors to the mice for 3 successive weeks. The mice were tested by TST 60 min after administration of vehicle/ drug on the 22nd day, which was followed by testing of their locomotor scores 30 min later.

For sucrose preference test

Groups 11–20: Separate animals were used for this test, but the details of vehicle /drug treatments are the same as mentioned under groups 1 - 10. The test was carried out before the start of drug administration (baseline test) on the 1st day and 60 min after drug administration on the 21st day.

Estimation of various biochemical parameters

Collection of blood samples and separation of plasma

After performing TST and testing of locomotor activity of mice of groups 1–10 on the 22nd day, these mice were sacrificed on the 23rd day by cervical dislocation and a blood sample (1.0 - 1.2 ml) was withdrawn from the carotid artery. Separation of plasma from the blood samples was carried out using a cooling centrifuge (Remi, Mumbai, India) at the speed of 2500 rpm for 10 min. Plasma was used for the estimation of nitrite and corticosterone.

Analysis of plasma nitrite levels

Plasma nitrite was analyzed by following the method reported by Green *et al*., 1982 [26].

Analysis of plasma corticosterone levels

Plasma corticosterone levels were determined by the method of Bartos and Pesez, 1979 [27].

Estimation of biochemical parameters in brain homogenate

Following the withdrawal of blood samples on the 23rd day, the mouse brain was isolated. This was followed by washing of brain samples with cold buffer solution (pH 7.4) containing 0.25 M sucrose - 0.1 M Tris-0.02 M ethylenediamine tetra-acetic acid. Then, the brain samples were weighed. Each brain sample was homogenized in 9 volumes of the above-mentioned buffer, followed by its centrifugation two times at the speed of 2500 rpm for 10 min at 4°C using a refrigerated centrifuge (Remi Instruments, Mumbai, India). The supernatant collected was centrifuged at 12,000 rpm for 20 min at 4°C. The precipitates (mitochondrial fraction) collected were used for the determination of monoamine oxidase- A (MAO-A) activity. The supernatant obtained was used to analyze malondialdehyde and, glutathione (GSH) levels; and catalase activity.

Analysis of MAO-A activity

MAO-A activity was measured spectrophotometrically by the method as reported in the literature [28, 29].

Analysis of total protein

Total protein was determined in the brain homogenate by using a kit (Erba, Transasia, Baddi (Solan, H.P.), using semi-automatic auto-analyzer (Chem5 plus-V2 semi-AutoAnalyzer; Erba Mannheim, Germany) [30].

Estimation of malondialdehyde (lipid peroxidation)

Malondialdehyde levels were determined spectrophotometrically by quantifying thiobarbituric acid-reactive substances (TBARS) by the method reported by Wills, 1965 [31].

Analysis of reduced glutathione (GSH)

Reduced glutathione levels were analyzed by the method reported by Jollow *et al.*, 1974 [32].

Analysis of catalase activity

Catalase activity was determined by the method reported by Claiborne, 1985 [33].

Statistical analysis of data

The mean and standard error of the mean of the data were calculated using Graphpad Instant statistical software. Then, the results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test using the same statistical software.

RESULTS AND DISCUSSION

Effect on immobility periods of mice in TST

Mice exposed to CUMS markedly ($p < 0.01$; $q = 6.141$) enhanced immobility period of mice when tested in TST in comparison to control unstressed mice. Fluoxetine (20 mg/kg), the standard antidepressant drug employed, markedly ($p < 0.001$) reduced the immobility periods of both unstressed mice ($q = 16.764$) and stressed mice ($q = 13.694$) in comparison to their respective control groups. The lowest dose (7.5 mg/kg) of abietic acid did not markedly affect immobility periods of both unstressed mice as well as CUMS exposed mice. But abietic acid when used in higher doses (15 and 30 mg/kg) markedly ($p < 0.001$; $q = 7.922$ and 10.654 respectively) reduced immobility periods of CUMS exposed mice in comparison to their control group. On the other hand, abietic acid when used in the dose of 30 mg/kg markedly ($p<0.001$; $q=6.540$)) reduced immobility periods of unstressed mice in comparison to their control group **(Table 1)**.

Table 1. Effect of abietic acid and fluoxetine on immobility periods of mice in TST

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	Immobility Period (sec)
1.	Control	10 ml	151.87 ± 5.27
2.	Control + CUMS	10 ml	176.87 ± 3.67**
3.	Fluoxetine (U)	20 mg	83.62 ± 3.50***
4.	Abietic acid (U)	7.5 mg	144.38 ± 6.94
5.	Abietic acid (U)	15 mg	135.62 ± 3.03
6.	Abietic acid (U)	30 mg	125.25 ± 2.99***
7.	Fluoxetine + CUMS	20 mg	121.12 ± 3.50†††
8.	Abietic acid + CUMS	7.5 mg	171 ± 2.51
9.	Abietic acid + CUMS	15 mg	144.62 ± 3.77†††
10.	Abietic acid + CUMS	30 mg	133.5 ± 3.63†††

n=8 in each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9,70) = 42.24; p<0.05
**, *** = p< 0.01, p<0.001 respectively as compared to vehicle treated unstressed mice.
††† = p<0.001 as compared to vehicle treated stressed mice.

Effect on sucrose preference

Mice subjected to CUMS markedly (p <0.001; q= 9.714) reduced sucrose preference in comparison to control unstressed mice. In the baseline test, sucrose preference did not significantly differ among all the groups. In unstressed mice, all three doses of abietic acid, as well as fluoxetine (20 mg/kg), were devoid of any significant effect on sucrose preference. CUMS-induced decrease in sucrose preference was markedly reversed by abietic acid (15 and 30 mg/kg) and fluoxetine (20 mg/kg) [p <0.001; q= 10.088, 13.556 and 15.202 respectively] in comparison to CUMS exposed control mice **(Table 2)**.

Table 2. Effect of abietic acid and fluoxetine on sucrose preference

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	Sucrose preference (%)- Baseline test	Sucrose preference (%) - After 21 days
1.	Control	10 ml	61.13 ± 0.31	37.89 ± 3.19
2.	Control + CUMS	10 ml	60.89 ± 0.46	7.19 ± 0.65***
3.	Fluoxetine (U)	20 mg	61.76 ± 0.62	47.34 ± 5.30
4.	Abietic acid (U)	7.5 mg	61.35 ± 0.48	36.71 ± 0.28
5.	Abietic acid (U)	15 mg	61.28 ± 0.50	42.14 ±0.47
6.	Abietic acid (U)	30 mg	60.83 ± 0.41	47.33 ± 2.20
7.	Fluoxetine + CUMS	20 mg	61.45 ± 0.38	55.23 ± 4.29†††
8.	Abietic acid + CUMS	7.5 mg	61.42 ± 0.44	20.45 ± 3.34
9.	Abietic acid + CUMS	15 mg	60.64 ± 0.74	39.07 ± 2.55†††
10.	Abietic acid + CUMS	30 mg	61.09 ± 0.59	50.03 ± 4.42†††

n =8 each group, U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. The data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For Sucrose preference (%)- baseline test; F (9, 70) = 0.4246; p > 0.05.
For Sucrose preference (%)- after 21 days; F (9, 70) = 21.04; p < 0.05.
*** = p < 0.001, as compared to vehicle treated unstressed mice.
††† = p<0.001, as compared to vehicle treated stressed mice.

Effect on locomotor activity

Locomotor activities of unstressed mice and CUMS exposed mice were not significantly affected by abietic acid and fluoxetine in comparison to their respective controls **(Table 3)**.

Table 3. Effect of abietic acid and fluoxetine on locomotor activity

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	No. of locomotor counts
1.	Control	10 ml	246.87 ± 4.93
2.	Control + CUMS	10 ml	252.00 ± 3.12
3.	Fluoxetine (U)	20 mg	245.75 ± 4.61
4.	Abietic acid (U)	7.5 mg	254.75 ± 4.56
5.	Abietic acid (U)	15 mg	256.75 ± 7.00
6.	Abietic acid (U)	30 mg	238.37 ± 6.30
7.	Fluoxetine + CUMS	20 mg	256.62 ± 7.42
8.	Abietic acid + CUMS	7.5 mg	253.62 ± 6.83

9.	Abietic acid + CUMS	15 mg	269.12 ± 4.71
10.	Abietic acid + CUMS	30 mg	257.50 ± 6.71

n= 8 in each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
F (9, 70) = 2.049; p > 0.05

Effect on plasma nitrite levels

CUMS markedly (p <0.001; q= 10.993) elevated plasma nitrite levels in mice. There was a significant (p <0.001) decrease of plasma nitrite levels by fluoxetine (20 mg/kg) in both unstressed mice (q= 7.441) as well as CUMS exposed mice (q= 19.112) in comparison to their respective controls. Abietic acid used in the lowest dose (7.5 mg/kg) was devoid of any significant effect on plasma nitrite levels in unstressed mice as well as in CUMS exposed mice. Abietic acid used in the dose of 30 mg/kg markedly (p <0.001; q= 7.543) reduced plasma nitrite levels in unstressed mice in comparison to their control mice. Abietic acid used in higher doses (15 and 30 mg/kg) markedly (p<0.001; q= 10.298 and 16.769 significantly) reduced nitrite levels in mice exposed to CUMS in comparison to CUMS exposed control mice **(Table 4)**.

Table 4. Effect of abietic acid and fluoxetine on plasma nitrite and corticosterone levels

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	Plasma nitrite level (µg/ml)	Plasma Corticosterone Levels (µg/ml)
1.	Control	10 ml	22.83 ± 0.93	22.17 ± 0.57
2.	Control + CUMS	10 ml	31.68 ± 0.97***	28.18 ± 0.52***
3.	Fluoxetine (U)	20 mg	16.84 ± 0.91***	19.80 ± 0.62
4.	Abietic acid (U)	7.5mg	20.94 ± 0.88	21.87 ± 0.71
5.	Abietic acid (U)	15 mg	20.82 ± 0.68	20.68 ± 0.53
6.	Abietic acid (U)	30 mg	16.76 ± 0.37***	20.45 ± 0.58
7.	Fluoxetine + CUMS	20 mg	16.30 ± 0.61†††	22.13 ± 0.64†††
8.	Abietic acid + CUMS	7.5 mg	28.68 ± 0.88	25.16 ± 0.56†
9.	Abietic acid + CUMS	15 mg	23.40 ± 0.87†††	24.12 ± 0.47†††
10.	Abietic acid + CUMS	30 mg	18.19 ± 0.73†††	21.56 ± 0.48†††

n=8 in each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For plasma nitrite levels: F (9,70) = 41.726; p < 0.05
For plasma corticosterone levels: F (9, 70) = 18.289; p<0.05
***= p<0.001 as compared to vehicle-treated unstressed mice.
†, †††= p<0.05, p<0.001 respectively as compared to vehicle-treated stressed mice.

Effect on plasma corticosterone levels

CUMS exposed mice showed marked (p<0.001; q= 10.500) elevation in corticosterone levels in mice in comparison to control unstressed mice. Abietic acid at all the doses (7.5, 15 and 30 mg/kg) as well as fluoxetine (20 mg/kg) markedly (p<0.05, p<0.001, p<0.001 and p<0.001 respectively; q= 5.287, 7.046, 11.574 and 10.570 respectively) decreased corticosterone levels in CUMS exposed mice in comparison to CUMS exposed control mice. There was no significant effect of abietic acid and fluoxetine on corticosterone levels in unstressed mice in comparison to control unstressed mice **(Table 4)**.

Effect on brain MAO-A activity

CUMS markedly (p<0.001; q= 24.920) elevated MAO-A activity in comparison to control unstressed mice. There was a marked (p<0.001) decrease in MAO-A activity by fluoxetine (20 mg/kg) in unstressed mice (q= 8.293) as well as in mice subjected to CUMS (q= 43.979) in comparison to their respective controls. Abietic acid used in the lowest dose (7.5 mg/kg) was devoid of any marked effect on MAO-A activity in unstressed mice as well as in CUMS exposed mice. But abietic acid used in the dose of 30 mg/kg markedly (p<0.001; q= 8.644) reduced brain MAO-A activity in unstressed mice in comparison to their control. The higher doses (15 and 30 mg/kg) of abietic acid significantly (p<0.001; q= 9.533 and 41.154 respectively) reduced MAO-A activity in CUMS exposed mice in comparison to control mice exposed to CUMS **(Table 5)**.

Table 5. Effect of abietic acid and fluoxetine on brain MAO-A activity and malondialdehyde levels

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	MAO-A activity (nmol/mg protein)	Malondialdehyde levels (nmols/mg protein)
1.	Control	10 ml	67.97 ± 0.54	1.13 ± 0.003
2.	Control + CUMS	10 ml	89.87 ± 0.57***	2.20 ± 0.08***
3.	Fluoxetine (U)	20 mg	60.69 ± 1.23***	0.84 ± 0.04*
4.	Abietic acid (U)	7.5mg	66.92 ± 0.69	1.06 ± 0.02

5.	Abietic acid (U)	15 mg	64.48 ± 0.53	1.09 ± 0.06
6.	Abietic acid (U)	30 mg	60.38 ±0.57***	0.76 ± 0.03**
7.	Fluoxetine + CUMS	20 mg	51.22 ± 1.21†††	1.41 ± 0.05†††
8.	Abietic acid + CUMS	7.5 mg	87.80 ± 0.64	2.13 ± 0.12
9.	Abietic acid + CUMS	15 mg	81.50 ± 1.38†††	1.89 ± 0.05††
10.	Abietic acid + CUMS	30 mg	53.71 ± 0.84†††	1.58 ± 0.02†††

n=8 in each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For MAO- A activity: F (9,70) = 257.89; p<0.05
For Malondialdehyde levels: F (9,70) = 86.26; p<0.05
*, ** and ***= p<0.05, p<0.01, p<0.001 respectively as compared to vehicle-treated unstressed mice.
††, †††= p<0.01, p<0.001 respectively as compared to vehicle treated stressed mice.

Effect on brain malondialdehyde levels

There was a significant (p<0.001; q= 18.553) increase in malondialdehyde levels in CUMS exposed mice in comparison to unstressed control mice. Fluoxetine (20 mg/kg) and abietic acid (15 and 30 mg/kg) significantly (p<0.001, p<0.01 and p<0.001 respectively; q= 13.705, 5.366 and 10.731 respectively) decreased malondialdehyde levels in CUMS exposed mice in comparison to control mice subjected to CUMS. There was no significant effect of the lowest dose (7.5 mg/kg) of abietic acid on malondialdehyde levels in mice subjected to CUMS in comparison to control mice subjected to CUMS. Fluoxetine (20 mg/kg) and only the highest dose (30 mg/kg) of abietic acid markedly (p<0.05 and p<0.01 respectively; q= 4.978 and 6.228 respectively) reduced malondialdehyde levels in unstressed mice in comparison to their control **(Table 5)**.

Effect on brain reduced glutathione levels

There was a significant (p<0.001; q= 34.829) decrease in reduced glutathione levels in CUMS exposed mice in comparison to control unstressed mice. Fluoxetine (20 mg/kg) and only the highest dose (30 mg/kg) of abietic acid markedly (p<0.001; q= 61.698 and 8.274 respectively) increased brain reduced glutathione levels in unstressed mice in comparison to their control. Abietic acid (15 and 30 mg/kg) and fluoxetine (20 mg/kg) markedly (p<0.001; q= 8.558, 31.162 and 33.123 respectively) increased brain reduced glutathione levels in CUMS exposed mice in comparison to control mice subjected to CUMS **(Table 6)**.

Table 6. Effect of abietic acid and fluoxetine on brain reduced glutathione levels and catalase activity

S. No.	Treatment for 3 successive weeks	Dose (kg^{-1})	GSH Levels (µmol/mg protein)	Catalase activity (µg/mg protein)
1.	Control	10 ml	0.247 ± 0.007	45.47 ± 0.38
2.	Control + CUMS	10 ml	0.094 ± 0.001***	23.90 ± 1.00***
3.	Fluoxetine (U)	20 mg	0.517 ± 0.004***	89.60 ± 0.37***
4.	Abietic acid (U)	7.5 mg	0.247 ± 0.005	46.34 ± 0.44
5.	Abietic acid (U)	15 mg	0.264 ± 0.007	47.91 ± 1.55
6.	Abietic acid (U)	30 mg	0.283 ± 0.003***	72.39 ± 1.00***
7.	Fluoxetine + CUMS	20 mg	0.239 ± 0.003†††	68.58 ± 2.18†††
8.	Abietic acid + CUMS	7.5 mg	0.104 ± 0.003	28.58 ± 1.17
9.	Abietic acid + CUMS	15 mg	0.132 ± 0.002†††	31.43 ± 1.30†††
10.	Abietic acid + CUMS	30 mg	0.231 ± 0.003†††	40.82 ± 0.56†††

n=8 in each group. U = unstressed mice; CUMS = chronic unpredictable mild stress.
Values are expressed as Mean ± S.E.M. Data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison test.
For reduced glutathione: F (9,70) = 791.03; p <0.05
For catalase activity: F (9,70) = 344.54; p <0.05
*** = p<0.001 as compared to vehicle-treated unstressed mice.
†††= p<0.001 as compared to vehicle-treated stressed mice.

Effect on brain catalase activity

There was a significant (p<0.001; q=18.915) decrease in brain catalase activity in mice subjected to CUMS in comparison to control unstressed mice. Fluoxetine (20 mg/kg) and only the highest dose (30 mg/kg) of abietic acid markedly (p<0.001; q= 38.709 and 23.610 respectively) increased brain catalase activity in unstressed mice in comparison to their control. Abietic acid (15 and 30 mg/kg) and fluoxetine (20 mg/kg) markedly (p<0.001; q= 6.601, 14.837 and 39.162 respectively) increased brain catalase activity in CUMS exposed mice in comparison to control mice subjected to CUMS **(Table 6)**.

The present study demonstrated the effects of abietic acid (7.5, 15, and 30 mg/kg, *p.o*) on depression-like behavior in unstressed mice and CUMS-induced depressed mice using the behavioral models (TST and sucrose preference test). CUMS-induced model of depression is broadly employed for screening of drugs for antidepressant activity in mice and rats. This model results in depression-like behavior similar to that observed in humans [24, 34]. In TST, CUMS markedly increased immobility periods of mice, which

indicated the development of depression-like behavior in mice [35-39]. Immobility time in TST indicates despair behavior, which is a notable symptom of depression. There was a marked decrease in immobility periods of CUMS exposed mice by abietic acid (15 and 30 mg/kg) and fluoxetine (20 mg/kg), indicating antidepressant-like effects of these drugs. But in unstressed mice, fluoxetine (20 mg/kg) and only the highest dose (30 mg/kg) of abietic acid produced significant antidepressant-like activity. Fluoxetine was used as a standard drug to validate the employed animal models of depression. Abietic acid and fluoxetine did not produce any marked effect on locomotor activities of unstressed mice as well as in CUMS exposed in comparison to their respective controls, indicating that antidepressant-like effects of these drugs were specific and not due to their stimulant or depressant effects on the central nervous system. The sucrose preference test is considered the most reliable behavioral test to study the effects of drugs on CUMS-induced depression in mice and rats. A decrease in sucrose preference indicates anhedonia, that is, loss of interest or pleasure [24], which is commonly seen in depressive patients. There is damage to nerve cells by CUMS in the neural reward system linked to serotonergic and dopaminergic systems; and this leads to the abolition of experiencing happiness or pleasure [40]. In the present study, sucrose preference markedly decreased in CUMS exposed mice in comparison to control unstressed mice. Fluoxetine (20 mg/kg) and abietic acid (15 and 30 mg/kg) significantly reversed CUMS-induced decrease in sucrose preference. This indicated the antidepressant-like effects of abietic acid and fluoxetine in mice subjected to CUMS. Restoration of reduced sucrose preference by fluoxetine is also supported by the literature [41]. HPA axis plays an important role in bringing out various physiological changes in response to stressful stimuli [42]. Stress-induced activation of the HPA axis leads to a rise in plasma corticosterone levels in rodents. Cortisol levels elevate in depressive patients. Antidepressants such as fluoxetine decrease cortisol levels [43]. In the current study, elevated plasma corticosterone levels in CUMS exposed mice were markedly decreased by abietic acid and fluoxetine. In unstressed mice, no significant effect on plasma corticosterone level was shown which indicates that hyperactivation of the HPA axis occurs only in stressful conditions. Oxidative stress leads to the production of reactive oxygen species and decreases the levels of endogenous antioxidants, resulting in depression [44]. There is damage to lipids and proteins; a decrease in antioxidant enzymes and reduced glutathione levels in rodents' brains by repeated and unpredictable stress [45]. Catalase and glutathione are antioxidant defense systems against reactive oxygen species [46]. CUMS leads to a significant increase in reactive oxygen species accumulation in the brain which leads to abnormality in the normal physiology of the central nervous system [47]. In the present study, CUMS markedly increased brain lipid peroxidation and plasma nitrite levels; and diminished brain reduced glutathione levels and catalase activity. These findings are supported by earlier studies [48]. Abietic acid (15 and 30 mg/kg, p.o) markedly reversed CUMS-induced changes in oxidative stress parameters. But in unstressed mice, abietic acid in the highest dose (30 mg/kg) employed showed antioxidant activity *per se*. Thus, the antidepressant-like effect of abietic acid might be due to the amelioration of oxidative stress. CUMS markedly enhanced brain MAO-A activity. This finding is also supported by the literature [49]. Abietic acid (15 and 30 mg/kg) and fluoxetine (20 mg/kg) markedly decreased brain MAO-A activity in CUMS-induced depressed mice. But in unstressed mice, abietic acid in the highest dose (30 mg/kg) employed significantly reduced brain MAO-A activity. Therefore, the antidepressant-like activity of abietic acid in CUMS-induced depressed mice and also in unstressed mice might also be due to brain MAO-A inhibition. Literature also supports MAO-A inhibition by fluoxetine [50]. Thus, it may be concluded that the administration of abietic acid for 3 consecutive weeks exerted marked antidepressant-like activity in CUMS-induced depressed mice as well as in unstressed mice. This antidepressant-like effect of abietic acid might be to decrease oxidative stress, restorative antioxidant enzymes, and brain MAO-A inhibition. Additionally, in CUMS-induced depressed mice, abietic acid showed antidepressant-like activity through a decrease of plasma corticosterone levels.

CONCLUSION

In the present research study, abietic acid exerted significant antidepressant-like activity in both normal mice and CUMS-induced depressed mice possibly through alleviation of oxidative stress and decrease of brain MAO-A activity. Additionally, lowering of plasma corticosterone concentration by abietic acid in CUMS-induced depressed mice might also contribute to its antidepressant-like effect.

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