

## ANTIDEPRESSANT-LIKE ACTIVITY OF TRANS-ANETHOLE IN UNSTRESSED MICE AND STRESSED MICE

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### ABSTRACT

**Objectives:** The present study was undertaken to investigate the antidepressant potential of trans-anethole in unstressed and stressed male mice.

**Methods:** Swiss albino male mice were exposed to chronic unpredictable mild stress for 21 successive days. Simultaneously, trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) *per se* were administered for 21 successive days to separate groups of unstressed and stressed mice. The effect of drugs on depressive-like behavior of mice was tested by tail suspension test (TST) and sucrose preference test.

**Results:** Trans-anethole (25 mg/kg) and fluoxetine significantly decreased the immobility period of unstressed and stressed mice in TST as compared to their respective control. These drugs significantly restored the reduced sucrose preference (%) in stressed mice. Trans-anethole did not show any significant effect on locomotor activity of mice. Antidepressant-like activity of trans-anethole (25 mg/kg) was found to be comparable to fluoxetine. Trans-anethole and fluoxetine significantly inhibited brain monoamine oxidase-A (MAO-A) activity, decreased plasma nitrite, brain malondialdehyde, and increased brain reduced glutathione levels and catalase activity in unstressed and stressed mice. The drugs significantly reversed stress-induced increase in plasma corticosterone levels.

**Conclusion:** Trans-anethole produced significant antidepressant-like activity in unstressed and stressed mice, possibly through inhibition of brain MAO-A activity and alleviation of oxidative stress. Reversal of stress-induced increase in plasma corticosterone levels might also be responsible for antidepressant-like activity of trans-anethole in stressed mice.

**Keywords:** Antidepressant, Chronic unpredictable mild stress, Depression, Trans-anethole, Tail suspension test.

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### INTRODUCTION

Depression is a chronic mental disorder that causes changes in mood, thoughts, behavior, and physical health [1]. According to the World Health Organization (WHO, 2017), about 4.4% of global population suffered from depression in 2015 [2]. As per the WHO, 2018, more than 300 million people of all ages in the world suffer from depression [3]. Depression may be caused by decrease in neurotransmitter (norepinephrine, dopamine, and serotonin) levels in brain [4]. Monoamine oxidase (MAO) is a key enzyme that metabolizes these monoamines. This enzyme catalyzes the oxidative deamination of these monoamine neurotransmitters [5], which results in cell oxidative injury through production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), oxygen radicals, and aldehydes. Therefore, inhibition of MAO may result in protection against oxidative stress [6]. Decreased antioxidant status, elevated oxidative, and nitrosative stress are found in patients with major depression [7]. There are evidences showing prominent role of nitric oxide in the pathogenesis of major depression [8]. Stressed mice showed significantly high levels of plasma nitrite, indicating production of nitric oxide [9]. Depression may be caused by hyperactivation of hypothalamic-pituitary-adrenal (HPA) axis, which may be due to oversecretion of corticotropin-releasing hormone by hypothalamus, adrenocorticotrophic hormone by pituitary, and glucocorticoid by adrenal cortex [10,11]. Hyperactivation of HPA axis leads to decrease in synthesis of central monoamine neurotransmitters such as 5-hydroxytryptamine and norepinephrine; and also leads to hippocampal neuronal damage, which results in depression [10,12].

There are different classes of antidepressant drugs employed for the treatment of depression such as tricyclic antidepressant, MAO inhibitors, and selective serotonin reuptake inhibitor but these drugs

have also intolerable side effects such as slow onset, low response rate, toxic effects to organs, and drug resistance [13,14]. Approximately 50% of depressive patients do not respond to antidepressant treatment because of intolerable side effects [15,16]. Thus, newer and safer antidepressants need to be explored from plants. *Hypericum perforatum* has been proven to be effective antidepressant for the treatment of mild to moderate depression in clinical studies [17]. Hence, plants and their bioactive compounds can be explored as safer alternatives for the treatment of depression.

Anethole is a diterpene aromatic compound characterized by its distinct flavor and it is chiefly found in fennel, i.e., *Foeniculum vulgare* (Family: Umbelliferae), anise, i.e., *Pimpinella anisum* (Family: Apiaceae), and star anise, i.e., *Illicium verum* (Family: Schisandraceae) [18]. Anethole has been reported to possess antioxidant [19], anxiolytic [20], analgesic [21], anti-inflammatory [22], antidiabetic [23], gastroprotective [24], anticancer [25], estrogenic [26], muscle relaxant [27], antifungal [28], antiviral [29], antibacterial activities [30], and nootropic activities [31]. Anise oil has been reported to alleviate the symptoms of mild to moderate depression in patients suffering from irritable bowel syndrome [32]. Trans-anethole is a major component of anise oil [33]. However, the effect of trans-anethole on chronic unpredictable mild stress (CUMS)-induced depression has not been reported. Therefore, the present study was designed to explore the antidepressant potential of trans-anethole in unstressed and CUMS mice.

### METHODS

#### Experimental animals

Swiss male albino mice (2–3 months old), weighing around 21–27 g were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana,

India. Female sex hormone (estrogen) has been found to possess antidepressant activity [34], so the female mice were excluded from the present study, and only male mice were used. On arrival, mice were group-housed under standard housing conditions (room temperature 24–26°C and 12/12 h light/dark cycle) separately in groups of 8 per cage (polypropylene cage size: 29 cm×22 cm×14 cm). The animals had free access to standard diet and water, but food was withdrawn 2 h before and 2 h after drug administration, to maximize the absorption of the drugs. They were acclimatized for 7 days to the laboratory conditions before the study. All experiments were conducted between 9 a.m. and 5 p.m. All animal procedures were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals, Department of Animal Husbandry and Dairying, Ministry of Agriculture and Farmers Welfare, Government of India, New Delhi, for the care and use of laboratory animals and they were approved by the Institutional Animals Ethics Committee in its meeting held on October 6, 2017.

### Drugs and chemicals

Trans-anethole (high-performance liquid chromatography India, Mumbai), fluoxetine (Sigma-Aldrich, St Louis, USA), N-(1-Naphthyl) ethylenediamine dihydrochloride, p-nitroso-N,N-dimethylaniline, 5-hydroxytryptamine, thiobarbituric acid (HiMedia Laboratories Private Limited, Mumbai), sulfanilamide, metaphosphoric acid, potassium ferricyanide, hydrogen peroxide, trichloroacetic acid (CDH Private Limited, New Delhi), 5,5-Dithiobis-2-(nitrobenzoic acid) (SRL Private Limited, Mumbai, India), and total protein estimation (Transasia Bio-medicals Ltd., Baddi [Solan], India) were employed in the present study.

### Vehicle

Trans-anethole was suspended in 10% v/v solution of Tween 80 in distilled water and administered orally. Fluoxetine was dissolved in normal saline.

### CUMS procedure

CUMS procedure was carried out according to the method, as described earlier [35]. Mice were exposed to following stressors once a day for 3 successive weeks between 10:30 and 16:00 h. The order of stressors was as follows:

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

I—Immobilization for 2 h, E—Exposure to empty water bottles for 1 h, F—Exposure to foreign objects for 24 h (e.g., piece of plastic), O—Overnight illumination, T2—Tail pinch (60s), X—Tilted cage at 45 degrees for 7 h, T1—Tail pinch (30 s)

Mice subjected to CUMS procedure were called as stressed mice. Unstressed mice were exposed to behavioral tests, and not subjected to CUMS procedure. Drugs were administered 60 min before CUMS procedure in case of stressed mice.

### Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method of Steru *et al.* [36]. Individual mice were acoustically and visually isolated from other animals during the test. Mice were suspended one by one 50cm above the floor by adhesive tape placed approximately 1cm from the tip of the tail. The time during which the mice remained immobile were recorded in a test period of 6min. Mice were considered immobile only when they hung passively and completely motionless.

### Sucrose preference test

Sucrose preference test [37] is an important method that is mainly used to evaluate anhedonia, which is the core symptom of depression. The mice were trained to take a 1% w/v sucrose solution. Then, the mice were deprived of food and water for 48 h and allowed only to

take 1% w/v sucrose solution. After 3 days, mice were deprived of food and water for 23 h, which was followed by 1-h baseline test, in which individual mice were exposed to two pre-weighed bottles, one filled with 1% w/v sucrose solution and the other with tap water. Sucrose preference was measured according to the following formula:

$$\text{Sucrose preference} = \frac{\text{Sucrose solution intake (g)}}{\text{Sucrose solution intake (g)} + \text{water intake (g)}} \times 100$$

The test was again performed on the 21<sup>st</sup> day to evaluate the effect of CUMS and drug treatment.

### Measurement of locomotor activity

Horizontal locomotor activities of control and test mice were recorded for 5 min [38] using photoactometer (INCO, Ambala, India).

### Experimental protocol

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

### Groups for TST and locomotor activity

- Groups 1–5: Tween 80 (10% v/v), trans-anethole (12.5, 25, and 50 mg/kg), and fluoxetine (20 mg/kg), respectively, were administered p.o. to mice for 21 consecutive days. The mice were subjected to TST 60 min after administration of vehicle or drug on 22<sup>nd</sup> day, and then after 30 min, the mice were tested for locomotor activity.
- Groups 6–10: Tween 80 (10% v/v), trans-anethole (12.5, 25, and 50 mg/kg), and fluoxetine (20 mg/kg), respectively, were administered p.o. 30 min before exposure of stressor to mice for 21 consecutive days. The mice were subjected to TST 60 min after administration of vehicle or drug on the 22<sup>nd</sup> day, which was followed 30 min later by testing mice for locomotor activity.

### Groups for sucrose preference test

- Groups 11–20: Separate mice were used for this test, but the details of vehicle/drug treatments are the same as mentioned under Groups 1–10.

### Biochemical estimations

#### Collection of blood samples and separation of plasma

After TST and locomotor testing of mice of Groups 1–10 on 22<sup>nd</sup> day, these mice were sacrificed on 23<sup>rd</sup> day by cervical dislocation, and blood sample (1.0–1.3 ml) was withdrawn from carotid artery. Plasma was separated using a refrigerated centrifuge (Remi, Mumbai, India) at 2500 rpm for 10 min and this was used for the estimation of nitrite and corticosterone.

#### Estimation of plasma nitrite levels

Plasma nitrite was measured using the method of Green *et al.*, 1982 [39]. A mixture of 1% w/v sulfanilamide in 5% aqueous solution of m-phosphoric acid (1 part) and 0.1% w/v N-(1-Naphthyl) ethylenediamine dihydrochloride (1 part) were prepared and kept at 0°C for 60 min. 0.5 ml plasma was mixed with 0.5 ml of the above mixture and kept in the dark for 10 min at room temperature. The absorbance was read at 546 nm using ultraviolet (UV)-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST).

#### 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 [40]. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10% solution of p-nitroso-N, N-dimethylaniline in ethanol was added, and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 N sodium hydroxide was added. The tubes were plugged with cotton-wool and were let to stand at 0°C for 5 h, protected against light. To the above solution, 2.0 ml of

buffer for pH 9.8, 5.0 ml of 0.10 % solution of phenol in ethanol, and 0.50 ml of 1.0 % aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at  $20 \pm 2^\circ\text{C}$  for 10 min. The solution was read at 650 nm using UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST).

#### Biochemical estimations in brain homogenate

After withdrawing blood samples on 23<sup>rd</sup> day, the brain of mice was isolated. The collected brain samples were washed with cold 0.25 M sucrose – 0.1 M Tris-0.02 M ethylenediamine tetra-acetic acid buffer (pH 7.4) and weighed. The buffer washed brain sample was homogenized in 9 volumes of cold 0.25 M sucrose – 0.1 M Tris-0.02 M ethylenediamine tetra-acetic acid buffer pH 7.4 buffer and centrifuged twice at 2500 rpm for 10 min at  $4^\circ\text{C}$  in a cooling centrifuge (Remi Instruments, Mumbai, India). The pellet was discarded. The supernatant was then centrifuged at 12,000 rpm for 20 min at  $4^\circ\text{C}$  in a cooling centrifuge. This centrifuged supernatant was separated into two parts:

- Part I: The precipitates (mitochondrial fraction) were used for the estimation of MAO-A activity
- Part II: The remaining supernatant was used to assay lipid peroxidation, glutathione (GSH), and catalase levels.

#### Measurement of MAO-A activity

MAO-A activity was assessed spectrophotometrically (Schurr and Livne, 1976; Charles and McEwan, 1977) [41,42]. The mitochondrial fraction of the brain was washed twice with about 100 ml of sucrose-tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mixed well at  $4^\circ\text{C}$  for 20 min. Then, mixture was centrifuged at 15000 rpm for 30 min at  $0^\circ\text{C}$ , and the pellets were re-suspended in cold sodium phosphate buffer. Then, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100  $\mu\text{l}$  of 4 mM 5-hydroxytryptamine were mixed in a quartz cuvette which was placed in UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST). This is followed by the addition of 150  $\mu\text{l}$  solution of mitochondrial fraction to initiate the enzymatic reaction, and the change in absorbance was recorded at wavelength of 280 nm for 5 min against the blank containing sodium phosphate buffer and 5-HT.

#### Estimation of protein concentration

Total protein concentration was estimated in the brain homogenate using a total protein kit (Erba, Transasia, Baddi (Solan, H.P.), using semi-automatic auto-analyzer (Chem5 plus-V2 Semi-Automatic Auto Analyzer; Erba Mannheim, Germany). Total protein concentration was estimated by Biuret method at 546 nm wavelength. The procedure followed was as same as mentioned in the pamphlet supplied along with the kit [43].

#### Estimation of lipid peroxidation

Lipid peroxidation was estimated spectrophotometrically in brain tissue by quantifying thiobarbituric acid reactive substance (TBARS) by the method of Wills, 1965 [44]. Briefly, for the estimation of TBARS, 0.5 ml of the supernatant of tissue homogenate and 0.5 ml of Tris-HCl were incubated at  $37^\circ\text{C}$  for 2 h. After incubation, 1 ml of 10% trichloroacetic acid was added. Then, the solution was centrifuged for 10 min at 1000 rpm. 1 ml of supernatant was taken out and mixed with freshly prepared 1 ml of 0.67% thiobarbituric acid solution. The tubes were kept in boiling water for 10 min. After cooling, 1 ml of double-distilled water was added. Absorbance was measured at 532 nm using UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST). Thiobarbituric acid-reactive substances were quantified using an extinction coefficient of  $1.56 \times 10^5 \text{ M/cm}$  and expressed as nanomole of malondialdehyde (MDA) per milligram protein. Brain MDA content was expressed as nanomole of MDA per milligram of protein.

#### Estimation of reduced GSH

Reduced GSH was assayed by the method of Jollow *et al.*, 1974 [45]. Briefly, 1.0 ml of the supernatant of tissue homogenate was precipitated

with 1.0 ml of sulfosalicylic acid (4%). Then, the mixture was kept at  $4^\circ\text{C}$  for minimum 1 h. Then, centrifuged the solution at 1200 rpm for 15 min at  $4^\circ\text{C}$ . After that, 0.1 ml supernatant was taken out and mixed with 2.7 ml phosphate buffer (0.1M, pH 7.4), and 0.2 ml 5,5, dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm using UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST). GSH levels were calculated using the molar extinction coefficient of  $1.36 \times 10^4 \text{ M/cm}$  and expressed as micromole per milligram protein.

#### Estimation of catalase activity

Catalase activity was assayed by the method of Claiborne, 1985 [46]. Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml postmitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm using a UV-visible spectrophotometer (Varian Cary 5000 UV-VIS-NIR Spectrophotometer, the Netherland CHRIST). Catalase activity was calculated using the millimolar extinction coefficient of  $\text{H}_2\text{O}_2$  (0.07 mM) and expressed as micromoles of  $\text{H}_2\text{O}_2$  decomposed per minute per milligram protein.

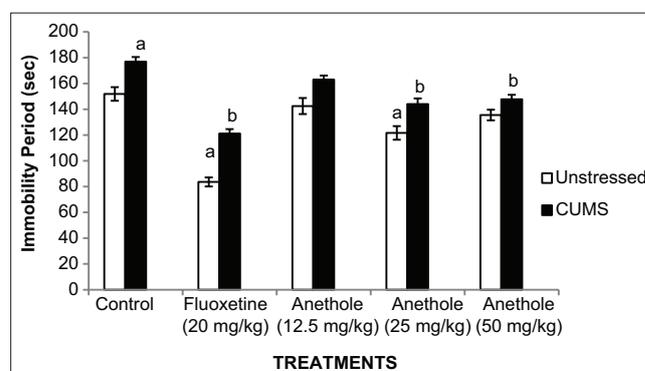
#### Statistical analysis

All the results were expressed as mean  $\pm$  standard error of mean (S.E.M). Data were analyzed by one-way analysis of variance followed by Tukey-Kramer multiple comparison test using GraphPad Instant statistical software.

## RESULTS

#### Effect of trans-anethole and fluoxetine on immobility periods of mice in TST

CUMS significantly increased the immobility period of mice as compared to vehicle-treated unstressed mice. Fluoxetine (20 mg/kg p.o.) administered for 3 successive weeks significantly ( $p < 0.001$ ) decreased the immobility period of unstressed mice and stressed mice as compared to their respective controls. Lowest dose (12.5 mg/kg p.o.) of trans-anethole administered for 3 successive weeks to unstressed mice and stressed mice did not show any significant effect on immobility period. However, the higher doses (25 mg/kg and 50 mg/kg p.o.) of trans-anethole administered for 3 successive weeks significantly decreased ( $p < 0.001$ ) the immobility period of stressed mice as compared to its vehicle-treated control. Only the middle dose (25 mg/kg p.o.) of trans-anethole significantly ( $p < 0.001$ ) decreased the immobility period of unstressed mice as compared to its vehicle-treated control (Fig. 1).



**Fig. 1: Effect of trans-anethole and fluoxetine on immobility periods of mice in tail suspension test. n=8 in each group. Values are expressed as mean  $\pm$  standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's post hoc test.  $F(9,70)=34.83$ ;  $p < 0.05$ ,  $a=p < 0.001$  as compared to vehicle-treated unstressed mice.  $b=p < 0.001$  as compared to vehicle-treated stressed mice**

**Table 1: Effect of trans-anethole and fluoxetine on sucrose preference (%)**

S. No.	Treatment for 3 successive weeks	Dose (kg <sup>-1</sup> )	Sucrose preference (%) -baseline test	Sucrose preference (%) -after 21 days
1.	Control	10 ml	61.13±0.3070	37.89±3.193
2.	Control+CUMS	10 ml	60.89±0.4616	7.19±0.6487 <sup>a</sup>
3.	Fluoxetine (U)	20 mg	61.76±0.6168	47.34±5.334
4.	Trans-anethole (U)	12.5mg	61.53±0.5137	36.81±1.135
5.	Trans-anethole (U)	25 mg	61.08±0.4666	37.99±0.8117
6.	Trans-anethole (U)	50 mg	61.42±0.5067	35.51±0.8330
7.	Fluoxetine+CUMS	20 mg	61.45±0.3833	55.23±4.293 <sup>d</sup>
8.	Trans-anethole+CUMS	12.5 mg	61.41±0.4777	22.58±1.962 <sup>b</sup>
9.	Trans-anethole+CUMS	25 mg	60.47±0.4779	52.52±4.255 <sup>d</sup>
10.	Trans-anethole+CUMS	50 mg	61.62±0.4372	24.61±1.296 <sup>c</sup>

n=8 each group, U: Unstressed mice; CUMS: Chronic unpredictable mild stress. Values are expressed as mean±standard error of mean. The data were analyzed by one-way analysis of variance followed by Tukey-Kramer multiple comparison test. For sucrose preference (%) -baseline test; F (9, 70) = 25.35; p>0.05. For sucrose preference (%) -after 21 days; F (9, 70) = 21.04; p<0.05. a=p<0.001, as compared to vehicle-treated unstressed mice. b, c, d=p<0.05, p<0.01, p<0.001, respectively, as compared to vehicle-treated stressed mice

### Effect of trans-anethole and fluoxetine on sucrose preference

Exposure of mice to unpredictable mild stress for 21 successive days significantly (p<0.001) decreased sucrose preference (%) as compared to vehicle-treated unstressed mice. There was no significant difference in sucrose preference (%) among all the groups in the baseline test. Trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) *per se* administered for 21 successive days did not show any significant effect on sucrose preference in unstressed mice. However, trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) *per se* significantly restored the reduced sucrose preference (%) in stressed mice as compared to their vehicle-treated control (Table 1).

### Effect of trans-anethole and fluoxetine on locomotor activity

Various treatments did not affect spontaneous locomotor activity scores of unstressed and stressed mice as compared to their respective vehicle-treated controls (Fig. 2).

### Effect of trans-anethole and fluoxetine on plasma nitrite levels

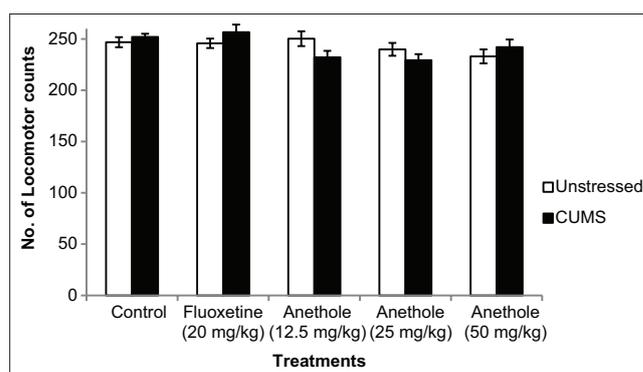
Plasma nitrite levels were significantly (p<0.001) increased in mice subjected to CUMS. Fluoxetine (20 mg/kg p.o.) administered for 21 successive days significantly (p<0.001) decreased plasma nitrite levels in both unstressed mice and stressed mice as compared to their respective controls. The lowest dose (12.5 mg/kg) of trans-anethole administered for 3 successive weeks did not show any significant effect on plasma nitrite levels of unstressed and stressed mice. However, higher doses (25 mg/kg and 50 mg/kg) of trans-anethole administered for 21 successive days significantly (p<0.001 and p<0.01) decreased plasma nitrite levels in stressed mice as compared to vehicle-treated stressed mice. On the other hand, only the middle dose (25 mg/kg) of trans-anethole significantly (p<0.01) decreased plasma nitrite levels in unstressed mice as compared to its vehicle-treated control (Fig. 3).

### Effect of trans-anethole and fluoxetine on plasma corticosterone levels

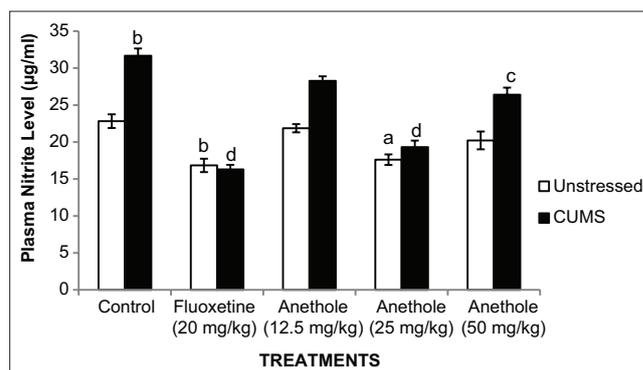
CUMS significantly (p<0.001) increased plasma corticosterone levels of mice as compared to vehicle-treated unstressed mice. Trans-anethole (25 mg/kg and 50 mg/kg) and fluoxetine (p<0.001) administered for 21 successive days significantly (p<0.001, p<0.01, and p<0.001, respectively) decreased plasma corticosterone levels in stressed mice as compared to their vehicle-treated control, but lowest dose (12.5 mg/kg) of trans-anethole did not significantly decrease plasma corticosterone levels in stressed mice. Trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) administered for 21 successive days did not significantly decrease plasma corticosterone levels of unstressed mice as compared to their vehicle-treated control (Fig. 4).

### Effect of trans-anethole and fluoxetine on brain MAO-A activity

CUMS significantly (p<0.001) increased brain MAO-A activity as compared to vehicle-treated unstressed mice. Trans-anethole (25 mg/kg)



**Fig. 2: Effect of trans-anethole and fluoxetine on locomotor activity. n=8 in each group. Values are expressed as mean±standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test. F (9, 70)=2.161; p>0.05**



**Fig. 3: Effect of trans-anethole and fluoxetine on plasma nitrite levels. n=8 in each group. Values are expressed as mean±standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test. F (9,70)=36.45; p<0.05. a, b=p<0.01, p<0.001, respectively, as compared to vehicle-treated unstressed mice. c, d=p<0.01, p<0.001, respectively, as compared to vehicle-treated stressed mice**

and fluoxetine (20 mg/kg) administered *per se* for 21 successive days significantly (p<0.001) reduced brain MAO-A activity of unstressed mice as compared to their respective control. However, the other doses (12.5 mg/kg and 50 mg/kg) of trans-anethole did not significantly decrease MAO-A activity in unstressed mice. Trans-anethole (12.5, 25, and 50 mg/kg) and fluoxetine (20 mg/kg) administered *per se* for 21 successive days significantly (p<0.05; p<0.001; p<0.001; and p<0.001,

respectively) reduced brain MAO-A activity in stressed mice as compared to their respective vehicle-treated control (Fig. 5).

**Effect of trans-anethole and fluoxetine on brain malondialdehyde levels**

Malondialdehyde levels were increased significantly ( $p < 0.001$ ) in mice subjected to CUMS as compared to vehicle-treated unstressed mice. Trans-anethole (12.5 mg/kg and 50 mg/kg) administered for 3 successive weeks did not show any significant effect on malondialdehyde levels of unstressed mice. However, the middle dose (25 mg/kg) of trans-anethole and fluoxetine (20 mg/kg) administered *per se* for 3 successive weeks significantly ( $p < 0.01$  and  $p < 0.05$ , respectively) decreased malondialdehyde levels in unstressed mice as compared to vehicle-treated unstressed mice. Trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) administered *per se* for 3 successive weeks significantly ( $p < 0.01$ ,  $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively) decreased malondialdehyde levels in stressed mice as compared to their respective vehicle-treated control (Fig. 6).

**Effect of trans-anethole and fluoxetine on brain reduced GSH levels**

Reduced GSH levels were significantly ( $p < 0.001$ ) decreased in stressed mice as compared to vehicle-treated unstressed mice. Trans-anethole (25 mg/kg and 50 mg/kg) and fluoxetine (15 mg/kg) *per se* administered for 21 successive days significantly ( $p < 0.001$ ) increased brain reduced GSH levels in stressed mice as compared to their vehicle-treated control. However, fluoxetine (20 mg/kg) and only the middle

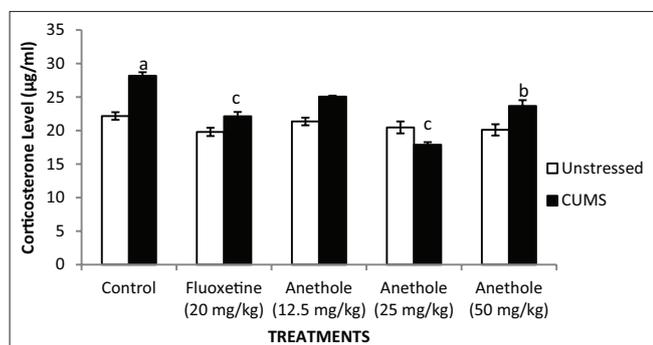
dose (25 mg/kg) of trans-anethole significantly ( $p < 0.001$ ) increased brain reduced GSH levels in unstressed mice as compared to their vehicle-treated control (Fig. 7).

**Effect of trans-anethole and fluoxetine on brain catalase activity**

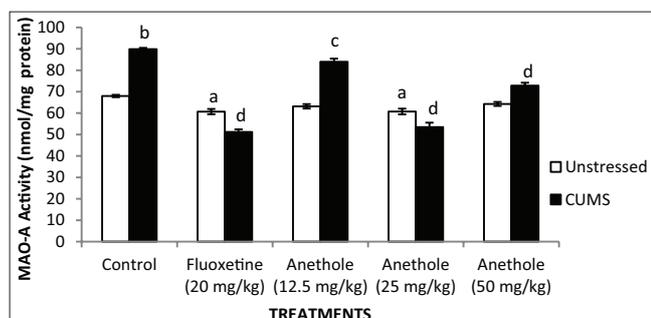
Brain catalase activity was significantly ( $p < 0.001$ ) decreased in stressed mice as compared to vehicle-treated unstressed mice. Trans-anethole (25 mg/kg and 50 mg/kg) and fluoxetine (20 mg/kg) administered *per se* for 3 successive weeks significantly ( $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively) increased catalase activity of unstressed mice as compared to vehicle-treated unstressed mice. Trans-anethole (12.5 mg/kg, 25 mg/kg, and 50 mg/kg) and fluoxetine (20 mg/kg) administered *per se* for 3 successive weeks significantly ( $p < 0.01$ ,  $p < 0.001$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively) increased catalase activity in stressed mice as compared to their respective vehicle-treated control (Fig. 8).

**DISCUSSION**

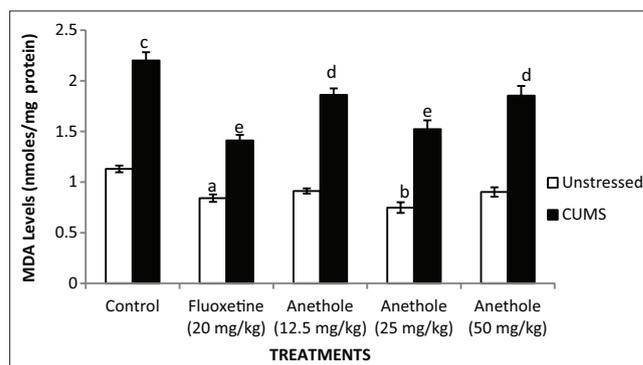
The present study investigated the effect of trans-anethole (12.5, 25, and 50 mg/kg, *p.o*) on depression-like behavior in unstressed mice and stressed mice. Unpredictable mild stress for 21 successive days triggers depression-like behavior in mice, and it is recognized as the most appropriate animal model of depressive behavior observed in



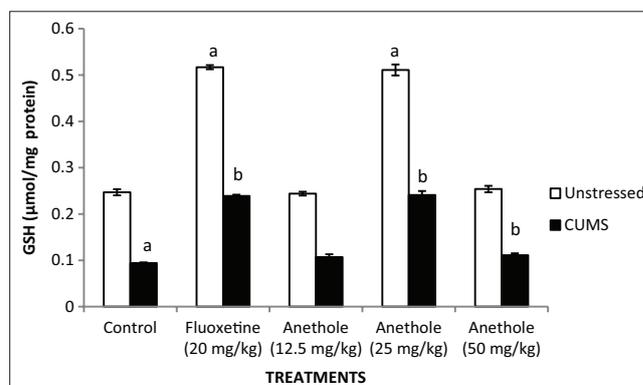
**Fig. 4:** Effect of trans-anethole and fluoxetine on plasma corticosterone levels.  $n=8$  in each group. Values are expressed as mean  $\pm$  standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test.  $F(9, 70)=22.87$ ;  $p < 0.05$ .  $a=p < 0.001$  as compared to vehicle-treated unstressed mice.  $b, c=p < 0.01, p < 0.001$ , respectively, as compared to vehicle-treated stressed mice



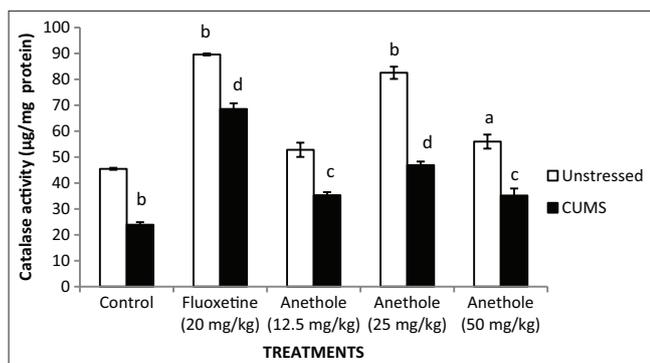
**Fig. 5:** Effect of trans-anethole and fluoxetine on brain MAO-A activity.  $n=8$  in each group. Values are expressed as mean  $\pm$  standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test.  $F(9, 70)=97.046$ ;  $p < 0.05$ .  $a, b=p < 0.01, p < 0.001$ , respectively, as compared to vehicle-treated unstressed mice.  $c, d=p < 0.05, p < 0.001$ , respectively, as compared to vehicle-treated stressed mice



**Fig. 6:** Effect of trans-anethole and fluoxetine on brain malondialdehyde levels.  $n=8$  in each group. Values are expressed as mean  $\pm$  standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test.  $F(9, 70)=68.08$ ;  $p < 0.05$ .  $a, b$  and  $c=p < 0.05, p < 0.01, p < 0.001$ , respectively, as compared to vehicle-treated unstressed mice.  $d, e=p < 0.01, p < 0.001$ , respectively, as compared to vehicle-treated stressed mice



**Fig. 7:** Effect of trans-anethole and fluoxetine on brain reduced glutathione levels.  $n=8$  in each group. Values are expressed as mean  $\pm$  standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test.  $F(9, 70)=55.6$ ;  $p < 0.05$ .  $a=p < 0.001$  as compared to vehicle-treated unstressed mice.  $b=p < 0.001$  as compared to vehicle-treated stressed mice



**Fig. 8: Effect of trans-anethole and fluoxetine on brain catalase activity.** n=8 in each group. Values are expressed as mean±standard error of mean. Data were analyzed by one-way analysis of variance followed by Tukey's *post hoc* test.  $F(9,70)=120.27$ ;  $p<0.05$ . a, b= $p<0.01$ ,  $p<0.001$ , respectively, as compared to vehicle-treated unstressed mice. c, d= $p<0.01$ ,  $p<0.001$ , respectively, as compared to vehicle-treated stressed mice

humans after long-term exposure to various stressors [47,48]. TST [36] and sucrose preference test [37] were used in the present study for evaluating the effect of drugs on depression-like behavior in mice. In the current study, CUMS significantly increased immobility periods of mice in TST, indicating induction of depression-like behavior. Immobility time in TST indicates hopeless behavior, which is notable symptom of depression [36]. On administration of trans-anethole (25 mg/kg and 50 mg/kg) and fluoxetine (20 mg/kg) *per se* for 21 successive days, there was significant decrease in immobility periods of stressed mice, indicating their antidepressant effect. However, in unstressed mice, fluoxetine, and only the middle dose (25 mg/kg) of trans-anethole produced significant antidepressant-like activity. Fluoxetine was used as a standard drug to validate the employed animal models of depression. There was no significant effect of trans-anethole and fluoxetine on locomotor activity of unstressed and stressed mice as compared to their respective controls, indicating that the drugs did not show any central nervous system stimulant activity.

The sucrose preference test is another model that measures anhedonia-like behavior in mice. Anhedonia is loss of interest or pleasure and is the main symptom of human major depression. Mice subjected to CUMS showed a decrease in sucrose preference as compared to unstressed mice. Fluoxetine (20 mg/kg) and trans-anethole administered for 3 successive weeks significantly restored the reduced sucrose preference in stressed mice, which further supported their antidepressant-like effect in mice subjected to CUMS. Restoration of reduced sucrose preference by fluoxetine is also supported by the literature [49].

Stress-induced depression is closely linked with disorders of the neuroendocrine system, mainly involved in the dysfunction of HPA-axis [50]. Stress-induced activation of HPA-axis leads to increase in plasma corticosterone levels in rodents. Antidepressant treatment relieves HPA-axis hyperactivity and decreases plasma cortisol levels [51]. In the present study, CUMS significantly increased plasma corticosterone levels, which is also supported by earlier studies [49]. Trans-anethole (25 mg/kg and 50 mg/kg) reduced CUMS-induced hyperactivity of HPA-axis in mice, as indicated by significant reduction of plasma corticosterone levels in stressed mice. There was no significant effect on plasma corticosterone levels in unstressed mice, which indicates that only in stressful conditions, hyperactivation of HPA-axis is observed.

CUMS induces the excessive production of oxygen free radicals and an imbalance of antioxidant capacity, which leads to major depression [52]. When excessive free radicals accumulate internally beyond an organism's antioxidant capacity, oxidative stress can severely damage all cells [53]. Oxidative stress results in lipid peroxidation. Malondialdehyde is an end

product of lipid peroxidation and considered as an index of free radical generation [54]. Stressful situations in rats have also been reported to significantly increase plasma nitrite levels [55]. Biological antioxidants which include antioxidant enzymes such as catalase and nonenzymatic antioxidants such as GSH can eliminate the excessive production of free radicals and activated oxygen molecules [56]. In the present study, CUMS resulted in increase in brain lipid peroxidation and plasma nitrite levels; and decrease in reduced GSH and catalase activity. This is also supported by earlier studies [57]. Trans-anethole (25 mg/kg and 50 mg/kg, *p.o.*) administered for 3 successive weeks significantly reversed CUMS-induced oxidative stress parameters, while only one dose (25 mg/kg) of trans-anethole significantly reversed oxidative stress parameters in unstressed mice. This is also supported by an earlier study, where trans-anethole showed antioxidant activity [19].

CUMS significantly increased brain MAO-A activity. This finding is also supported by earlier studies [58]. Chronic treatment with trans-anethole (12.5, 25, and 50 mg/kg, *p.o.*) and fluoxetine *per se* significantly decreased brain MAO-A activity in stressed mice. Thus, antidepressant-like activity of trans-anethole in mice subjected to CUMS might also be due to inhibition of brain MAO-A activity and subsequent increase in brain monoamine levels. However, in unstressed mice, only one middle dose (25 mg/kg) of trans-anethole and fluoxetine significantly decreased brain MAO-A activity. Thus, antidepressant-like activity of trans-anethole in unstressed mice might also be due to inhibition of brain MAO-A activity. Inhibition of brain MAO-A activity by fluoxetine is also supported by literature [59].

## CONCLUSION

Trans-anethole produced significant antidepressant-like activity in unstressed and stressed mice possibly through inhibition of brain MAO-A activity and alleviation of oxidative stress. In addition, antidepressant activity of trans-anethole in stressed mice might be through decrease in plasma corticosterone levels. Thus, trans-anethole may be explored further for the management of mental depression.

## CONFLICTS OF INTEREST

None.

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