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Antidepressant-like Activity of *Celastrus paniculatus* Seed Oil in Mice Subjected to Chronic Unpredictable Mild Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author RV collected the literature, performed experimental studies, statistically analysed the data, wrote the protocol, and wrote the first draft of the manuscript. Author DD designed and supervised the study; checked and finalized the manuscript.

Original Research Article

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ABSTRACT

Aims: The present study was done to evaluate the antidepressant-like effect of *Celastrus paniculatus* seed oil in Swiss young albino mice subjected to chronic unpredictable mild stress.
Study Design: Prospective.
Place and Duration of Study: Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India, between August 2012 to February 2013.
Methodology: The behavioral models such as forced swim test (FST) and sucrose preference test were used to evaluate the effect of seed oil on depression in mice. The oil (50, 100 and 200 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.) per se were administered for 14 successive days to unstressed and stressed mice. The effect of oil on locomotor activity of mice was also evaluated. In addition, the effects of oil on brain monoamine oxidase-A, malondialdehyde levels, reduced glutathione and catalase activities; and plasma corticosterone and nitrite levels were also assessed.
Results: The oil significantly decreased immobility period of both unstressed and stressed mice in FST as compared to the control. The oil also prevented the stress-induced decrease in sucrose preference, indicating significant antidepressant-like activity.

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There was no significant effect on locomotor activity of mice by the oil. Antidepressant-like activity of the oil was comparable to the standard drug, fluoxetine. The oil also significantly decreased monoamine oxidase-A (MAO-A) activity, malondialdehyde levels in both unstressed and stressed mice; and significantly prevented stress-induced decrease in reduced glutathione and catalase activities. It also significantly attenuated stress induced-increase in plasma nitrite and corticosterone levels.
Conclusion: *Celastrus paniculatus* seed oil showed significant antidepressant-like activity in both unstressed and chronic unpredictable mild stressed mice probably due to inhibition of MAO-A activity, decrease in plasma nitrite levels; and through scavenging of free radicals. In addition, the oil also showed antidepressant-like activity in stressed mice probably through decrease in plasma corticosterone levels.

Keywords: Celastrus paniculatus; corticosterone; depression; forced swim test; monoamine oxidase; sucrose preference test; chronic unpredictable mild stress.

ABBREVIATIONS

ANOVA: Analysis of variance; FST: Forced swim test; MAO: Monoamine oxidase; MDA: Malondialdehyde; CUMS: Chronic unpredictable mild stress.

1. INTRODUCTION

Depression is an important global public health problem due to both its relatively high lifetime prevalence and the significant disability that it causes. The demand for curbing depression and other mental health conditions is on the rise globally [1]. Current antidepressants, which target monoamines, only produce remission in 30% of patients [2] and most of the antidepressant drugs impose a variety of side effects. Thus, the search for more efficacious and well-tolerated drugs is in progress. *Hypericum perforatum*, a well known plant has been proven to be effective antidepressant in clinical studies [3]. Therefore, there is a constant need to identify newer natural antidepressants with efficacy more than synthetic antidepressants.

Celastrus paniculatus Willd. (Family: Celastraceae), commonly known as Malkangni (in Hindi) or Jyotishmati (in Sanskrit), was used to treat brain related disorders [4]. *C. paniculatus* seeds and seed oil have been used in Ayurvedic medicine for stimulating intellect and sharpening the memory [5]. Celastrus seeds have been reported to possess hypolipidemic and antiatherosclerotic [6], antispermatogenic [7], antioxidant [8], anxiolytic [9], antistress [10], and nootropic [11] activities.

Based upon use of *C. paniculatus* seeds to treat brain disorders in Ayurvedic medicine, the objective of the present study was to explore the antidepressant potential of *C. paniculatus* seed oil in mice subjected to chronic unpredictable mild stress and to investigate the probable underlying mechanisms of action.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The dried seeds of *C. paniculatus* were purchased from the commercial market, Hisar (Haryana, India) and were authenticated as *Celastrus paniculatus* Willd. from Raw Materials

Herbarium and Museum section of National Institute of Science Communication and Information Resources, New Delhi (Ref. No. NISCAIR/RHMD/Consult/2011-12/1779/79).

2.2 Preparation of *C. paniculatus* Seed Oil

The dried seeds were grounded to coarse powder. About 200 gm of powdered seeds were extracted with petroleum ether (60-80ºC) using soxhlet apparatus. The filtrate was concentrated using water bath. The oil was dark brown in color and the yield was 33.47% w/w. The Celastrus oil was stored in air tight container and then kept in a refrigerator [9].

2.3 Laboratory Animals

Swiss mice of either sex, weighing around 20-25 g were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Animals were housed separately in groups of 10 per cage (polycarbonate cage size: 29×22×14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each and have free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The animals were acclimatized for at least five days before behavioral experiments which were carried out between 09:00 and 17:00 h. The laboratory animals for using in this experimental study were approved by Institutional Animals Ethics Committee in its 22nd meeting held on 14th May, 2012. Care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

2.4 Drugs and Chemicals

Fluoxetine hydrochloride, prazosin hydrochloride, (±) sulpiride, DL para-chlorophenylalanine (*p*-CPA), p-nitroso-N,N-dimethylaniline and baclofen (Sigma-Aldrich, St. Louis, USA), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, tris, EDTA disodium salt AR, sucrose, 5-hydroxy tryptamine creatinine sulphate monohydrate (Hi Media laboratories Pvt. Ltd., Mumbai, India), dimethyl sulfoxide (Qualigens Fine Chemicals, Mumbai, India), acetic acid, boric acid, hydrochloric acid, gum acacia, potassium hydroxide, sodium hydroxide, tween-20 (CDH Ltd., New Delhi), total protein measurement kit (Coral Industries Ltd., India) were used in this study.

2.5 Vehicles

Fluoxetine was dissolved in normal saline (0.9% NaCl). The *C. paniculatus* seed oil was emulsified with 1% Tween-20 followed by dilution with dimethylsulfoxide each time before administration. Doses of the seed oil (50, 100 and 200 mg/kg) were selected based upon literature [10].

2.6 Chronic Unpredictable Mild Stress

10 groups (n = 10 each) of mice were subjected to chronic unpredictable mild stress as reported earlier [12-14] with some modifications. Animals were subjected to stress paradigm once a day over a period of two weeks between 0900 and 1700 h. The order of stressors used was as follows:

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	E	I	O	T	F	C	T1
Week 2	I	E	O	T1	C	T	F

C— Cage tilting at 45ºC for 7 hrs; E— Exposure to empty water bottles; I— Immobilization for 2 hrs; T— Tail pinch (60 s); F— Exposure to foreign object (24 h); O — Overnight illumination; 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.

2.7 Behavioral Tests

2.7.1 Forced swim test

FST (Forced swim test) is a most frequently used behavioral model for screening antidepressant-like activity in rodents [15]. The procedure was same as previously followed [16].

2.7.2 Sucrose preference test

Sucrose preference test [17] was employed herein to determine anhedonia, one of the core symptoms of major depression in human. 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 h and only exposed to 1% (w/v) sucrose solution. Three days later, after 23-h food and water deprivation, 1-h baseline test was performed, in which mice were housed in individual cages and were free to access two pre-weighted bottles, one with 1% (w/v) sucrose solution and the other with tap water. Then, the sucrose preference was calculated according to the 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 14th day to evaluate the effect of stress as well as drug treatment.

2.7.3 Measurement of locomotor activity

To rule out the effects of seed oil on immobility period, horizontal locomotor activities of control and test animals were recorded for a period of 10 min using photoactometer (INCO, Ambala, India).

2.8 Biochemical Estimations

Animals after subjecting to sucrose preference test on 14th day, were tested for locomotor activity on 15th day. One hour after testing for locomotor activity on 15th day, animals were sacrificed by cervical dislocation, and immediately brain samples were collected and analyzed for MAO-A, MDA, protein and reduced glutathione levels; and catalase activity. At the same time, blood samples were collected by carotid bleeding and centrifuged (Remi Centrifuge, Mumbai, India) at 2500 rpm for 10 min to separate plasma followed by estimation of corticosterone and nitrite levels.

2.8.1 Biochemical estimations in plasma

2.8.1.1 Estimation of corticosterone levels

The quantitative estimation of corticosterone level in the blood plasma was performed using UV-Visible-NIR Spectrophotometer (Varian Cary-5000, Christ, Netherland) using the procedure of Bartos and Pesez [18].

2.8.1.2 Estimation of nitrite levels

Plasma nitrite levels were measured by using the method of Green et al. [19].

2.8.2 Biochemical estimations in brain

Mouse brain mitochondrial fraction was prepared following the procedure described previously [20]. Briefly, the brain samples were collected immediately on an ice plate. Mouse brain mitochondrial fraction were prepared by cutting the brain sample into small pieces and rinsed in cold 0.25 M sucrose 0.1 M tris 0.02 M EDTA (pH 7.4) to remove blood. The pieces were homogenized for 45 seconds in a homogenizer with 400 mL of the same medium. The homogenate was centrifuged (Remi Centrifuge, Mumbai, India) at 800 rpm for 10 min at 4ºC and the pellets were discarded. The supernatant was then centrifuged at 12,000 rpm for 20 min in the same medium. This centrifuged supernatant was separated into two parts:

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

2.8.2.1 Measurement of MAO-A

MAO activity was assessed spectrophotometrically as described previously [21]. The precipitate (Part 1) was washed twice more with 100 ml of sucrose-tris-EDTA buffer and resuspended in 50 ml of the medium [22]. The assay mixture contained 100 µl of 4 mM 5-hydroxytryatpamine as the specific substrate for MAO-A, 250 µl solution of mitochondrial fraction and 100mM sodium phosphate buffer (pH 7.4) upto a final volume of 1ml. The reaction was allowed to proceed at 37 ^{0}C for 20 min, and stopped by adding 200 µl of 1M HCl, the reaction product was extracted with 5 ml of butyl acetate for MAO-A assay. The absorbance of the organic phase was measured at a wavelength of 280 nm using UV-Visible-NIR Spectrophotometer (Varian Cary-5000, Christ, Netherland). Blank samples were prepared by adding 100 µl of 4 mM 5-hydroxytrytpamine and 100 mM sodium phosphate buffer (pH 7.4) upto a final volume of 1ml and worked up subsequently in the same manner.

2.8.2.2 Estimation of protein content

Total protein was estimated in brain homogenate [23] by using Erba total protein kit from Coral Industries Ltd., India using colorimeter (Digital Photocolorimeter, Biomed).

2.8.2.3 Estimation of MDA content

The malondialdehyde content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid- reactive substances [24]. The brain malondialdehyde content was expressed as nanomole of malondialdehyde per milligram of protein.

2.8.2.4 Estimation of reduced glutathione content

Reduced glutathione was assayed by the method of Jollow et al [25]. The levels of reduced glutathione were calculated using molar extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} and expressed as micromole per milligram protein.

2.8.2.5 Estimation of catalase activity

Catalase activity was assayed by the method of Claiborne [26]. Catalase activity was quantified using the millimolar extinction coefficient of H_2O_2 (0.07 mM) and expressed as micromoles of H_2O_2 decomposed per minute per milligram protein.

2.9 Experimental Design

Animals were divided into 20 groups (n= 10 each group). The experiment was carried out in phases. Five groups of animals were treated as mentioned below and subjected to behavioural tests followed by biochemical estimations.

2.9.1 Groups for forced swim test (10 groups)

Group 1: Control Group (Unstressed mice) - Dimethyl sulfoxide was administered orally for 14 consecutive days and on 14th day, 60 min after administration; immobility period was recorded using FST.

Group 2: Control group (Stressed Mice) - Dimethyl sulfoxide was administered orally for 14 consecutive days 30 min before the induction of stress. On 14th day, 60 min after the administration; immobility period was recorded using FST.

Groups 3 and 4: Fluoxetine (Unstressed and stressed mice respectively) - Groups 3 and 4 were same as groups 1 and 2 except fluoxetine (20 mg/kg) was administered orally for 14 successive days followed by FST 60 min after administration of fluoxetine on 14th day.

Groups 5, 6 and 7: Celastrus seed oil (Unstressed Mice) - Seed oil (50, 100 and 200 mg/kg) was administered orally for 14 consecutive days followed by FST 60 min after administration of the oil on 14th day.

Groups 8, 9 and 10: Celastrus seed oil (Stressed Mice)- Seed oil (50, 100 and 200 mg/kg) was administered orally for 14 consecutive days 30 min before the induction of stress followed by FST 60 min after administration of the oil on 14th day.

2.9.2 Groups for sucrose preference test (10 groups)

Groups 11 to 20 were similar as mentioned under FST (Group 1 to 10) except the antidepressant activity was assessed using sucrose preference test.

2.9.3 Measurement of locomotor activity

Animals in groups 11 to 20 after subjecting to sucrose preference test on 14th day were assessed for locomotor activity on 15th day to rule out any effect on locomotion by the drugs.

2.10 Statistical Analysis

All the results were expressed as mean ± standard error mean (SEM). The data of all the groups were analyzed using one-way ANOVA (analysis of variance) followed by Tukey's post-hoc test using the software Graphpad Instat. In all the tests, the criterion for statistical significance was P <0.05.

3. RESULTS

3.1 Effect of Celastrus seed oil and fluoxetine on immobility periods in FST

Chronic unpredictable mild stress moderately increased the immobility period in FST as compared to control unstressed mice. Fluoxetine (20 mg/kg, p.o.) and Celastrus seed oil (50, 100 and 200 mg/kg, p.o.) per se administered for 14 successive days significantly decreased the immobility period in both unstressed and stressed mice as compared to the respective vehicle treated control. The effect of Celastrus oil (100 mg/kg) on reduction of immobility period was same (P < .01) in both unstressed and stressed mice (Fig. 1).

3.2 Effect of Celastrus Seed Oil and Fluoxetine on Sucrose Preference Test

There was no significant difference in sucrose preference (%) among all the groups in the baseline test (Fig. 2a). Celastrus seed oil (50, 100 and 200 mg/kg) and fluoxetine (20 mg/kg) per se administered for 14 successive days showed significant increase in sucrose preference (%) by unstressed mice. Exposure of the mice to stress for 14 days significantly decreased sucrose preference (%) in control stressed mice as compared to control unstressed mice. Reduced sucrose preference (%) in stressed mice was significantly restored by the administration of fluoxetine or seed oil. The effect of Celastrus oil (100 mg/kg) in restoring sucrose preference (%) was better in stressed (P < .01) than unstressed (P = .01) mice (Fig. 2b).

3.3 Effect of Celastrus Seed Oil and Fluoxetine on Locomotor Activity

Celastrus seed oil and fluoxetine per se administered for 14 successive days did not show any significant change in the locomotor function of mice as compared to the vehicle treated group in both stressed and unstressed mice (Fig. 3).

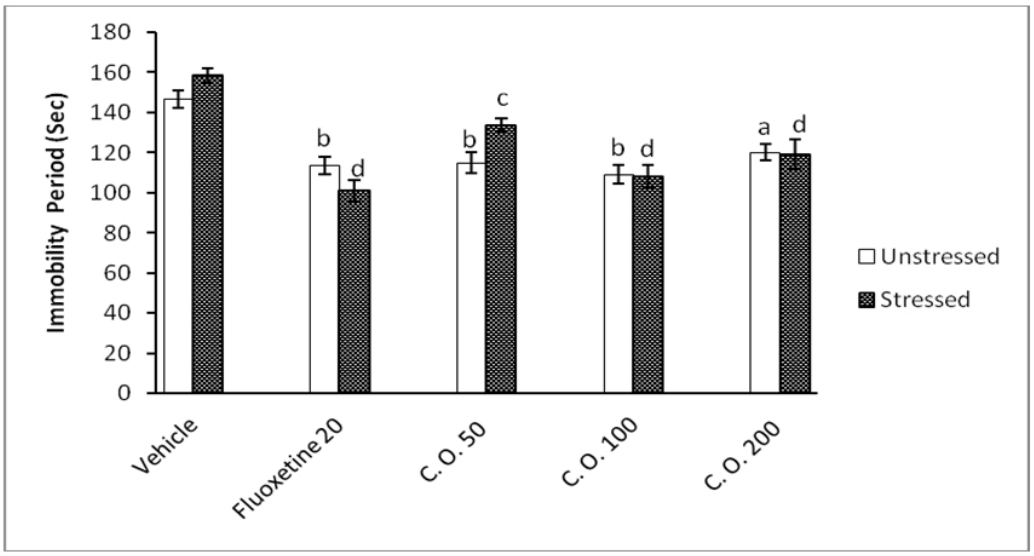

Fig. 1. Effect of *Celastrus paniculatus* seed oil on immobility period using FST

n=10 in each group; values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test.

F(9,90)= 12.067, *P*< .0001

[a]*P* = .05, [b]*P* < .01, when compared with vehicle treated unstressed mice;

[c]*P* = .05, [d]*P* < .01, when compared with vehicle treated stressed mice.

C.O. stands for Celastrus seed oil.

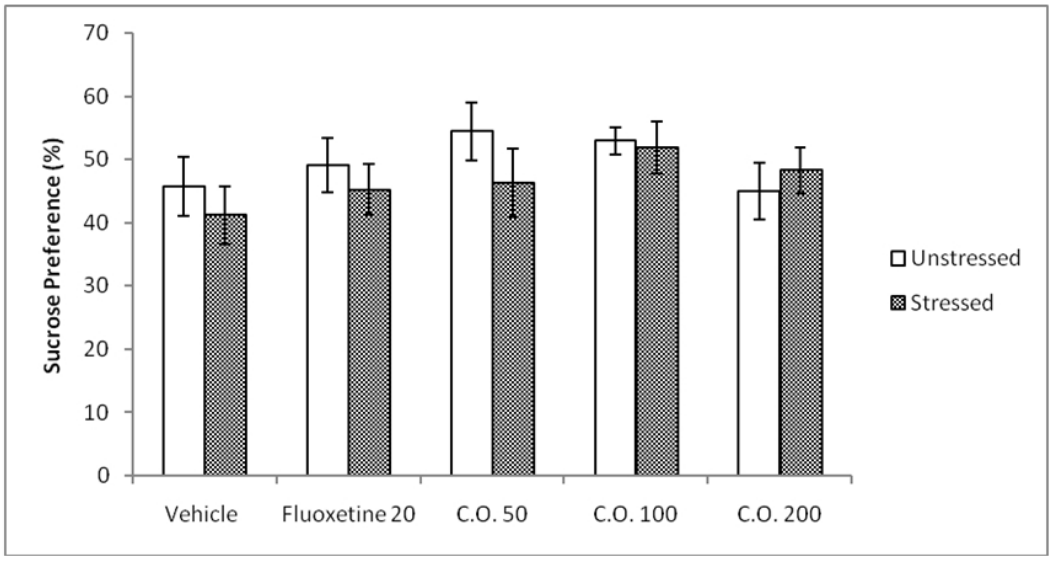

Fig. 2a. Effect of *Celastrus paniculatus* seed oil on (%) sucrose preference (Baseline test)

n=10 in each group; values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test;

F (9, 90) = 0.9379, P = .9379

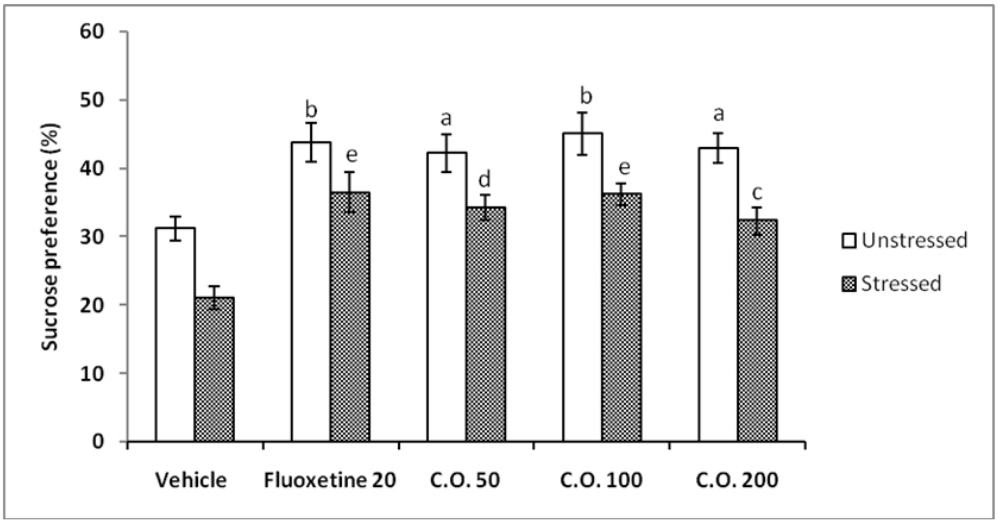

Fig. 2b. Effect of *Celastrus paniculatus* seed oil on (%) sucrose preference (after 14 days)

n=10 in each group; values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test;
F (9, 90) = 10.065, P < .0001;
[a]P = .05, [b]P = .01, when compared with vehicle treated unstressed mice;
[c]P = .05, [d]P = .01 , [e]P < .01 when compared with vehicle treated stressed mice.
C.O. stands for Celastrus seed oil.

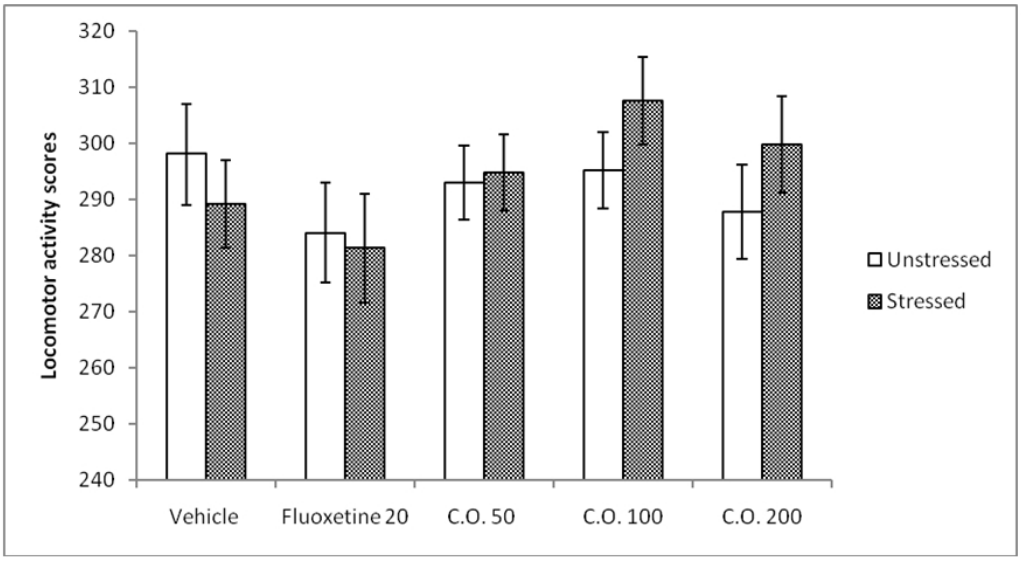

Fig. 3. Effect of *Celastrus paniculatus* seed oil on locomotor activity of mice

n=10 in each group; values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test.
F (9, 90) = 0.9353, P = .49
C.O. stands for Celastrus seed oil.

3.4 Effect of Celastrus Seed Oil and Fluoxetine on Plasma Corticosterone Levels

Fluoxetine (20 mg/kg) administered for 14 consecutive days significantly reduced plasma corticosterone content in both unstressed and stressed mice as compared to the respective controls. Celastrus seed oil (100 mg/kg) administered for 14 consecutive days to mice significantly reduced corticosterone levels in unstressed mice as compared to its control. In case of stressed mice, increase in corticosterone levels was significantly reduced by pretreatment with seed oil (50 and 100 mg/kg). The effect of Celastrus oil (100 mg/kg) on reduction of plasma corticosterone levels was better in stressed (*P = .01*) than unstressed (*P = .05)* mice (Fig. 4).

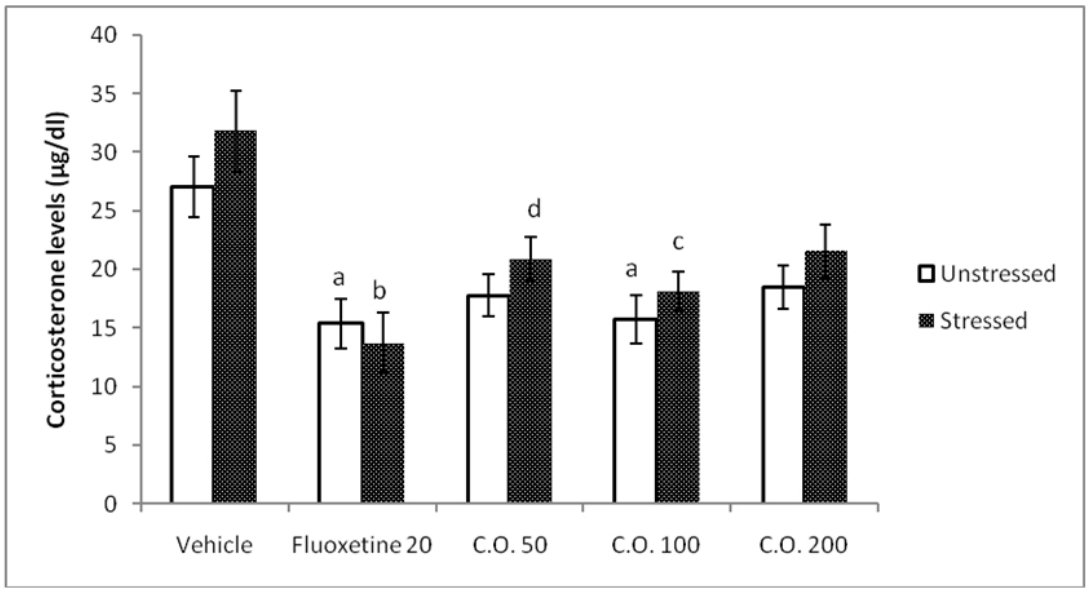

Fig. 4. Effect of *Celastrus paniculatus* seed oil on plasma corticosterone levels
n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.
F (9, 90) =6.022, P< .0001
[a]P = .05 when compared with vehicle treated unstressed mice;
[b]P < .01, [c]P = .01, [d]P = .05,when compared with vehicle treated stressed mice.

3.5 Effect of Celastrus Seed Oil and Fluoxetine on Plasma Nitrite Levels

Plasma nitrite levels were moderately elevated in mice subjected to CUMS. Celastrus seed oil and fluoxetine per se administered for 14 successive days significantly decreased plasma nitrite levels in unstressed and stressed mice as compared to respective controls. Among three doses of seed oil, dose of 100 mg/kg showed maximum decrease ($p<0.001$) in plasma nitrite levels of stressed mice, as compared to its control. The effect of Celastrus oil (100 mg/kg) on reduction of plasma nitrite levels was better in stressed (*P < .01)* than unstressed (*P = .05)* mice (Fig. 5).

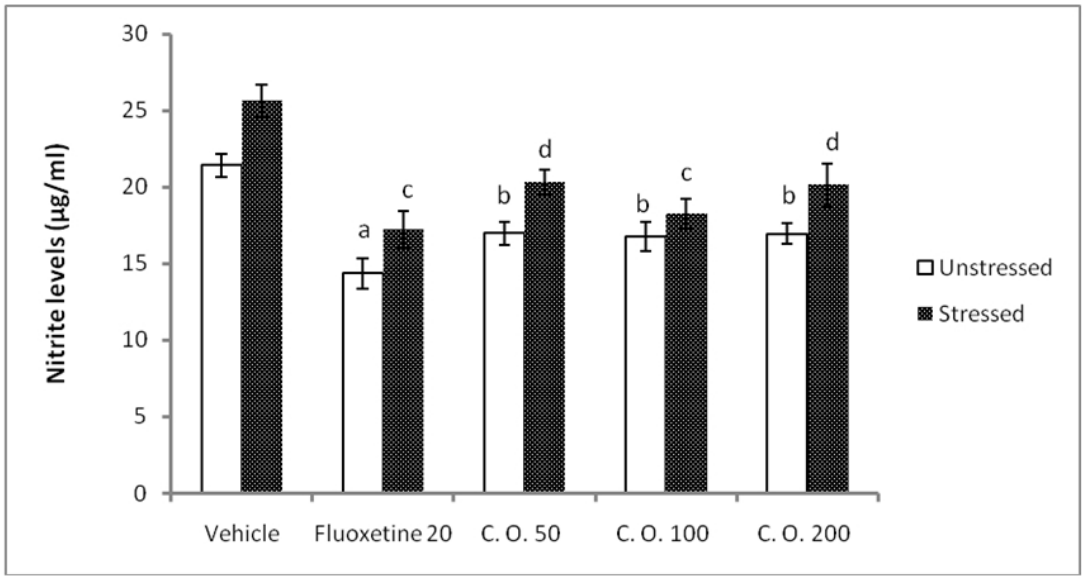

Fig. 5. Effect of *Celastrus paniculatus* seed oil on plasma nitrite levels

n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.

F (7, 72) =10.935, P < .0001

[a]P < .01, [b]P = .05, when compared to vehicle treated unstressed mice;

[c]P < .01, [d]P = .01 when compared to vehicle treated stressed mice.

3.6 Effect of Celastrus Seed Oil and Fluoxetine on Brain MAO-A Activity

Celastrus seed oil and fluoxetine per se administered for 14 consecutive days to mice, significantly reduced the brain MAO-A levels in both unstressed and stressed mice as compared to the respective controls. The efficacy of seed oil was found to be comparable to fluoxetine. The effect of Celastrus oil (50 and 100 mg/kg) on reduction of brain MAO-A levels was same ($P < .01$) in both unstressed and stressed mice (Fig. 6).

3.7 Effect of Celastrus Seed Oil and Fluoxetine on Brain Malondialdehyde Levels

Chronic treatment with seed oil (50, 100 and 200 mg/kg) produced a significant reduction in malondialdehyde levels in stressed mice as compared to its control. On the other hand, in unstressed mice, only a dose of 100 mg/kg of seed oil produced a significant reduction in malondialdehyde levels as compared to its control. The effect of Celastrus oil (100 mg/kg) on reduction of brain MDA levels was better in stressed ($P < .01$) than unstressed ($P = .05$) mice (Fig. 7).

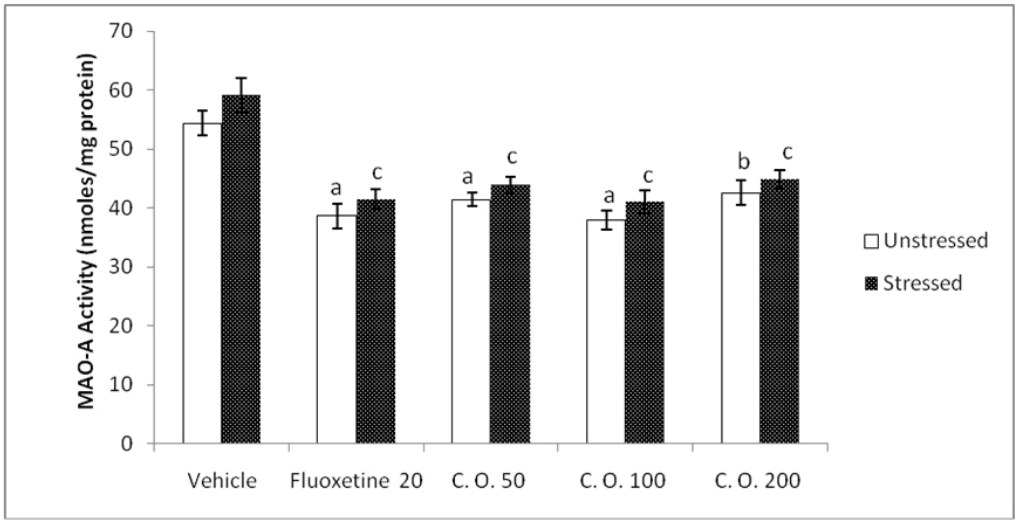

Fig. 6. Effect of *Celastrus paniculatus* seed oil on brain MAO-A activity

n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.
F (9, 90) =12.690, P< .0001
[a]P < .01, [b]P = .01 when compared to vehicle treated unstressed mice;
[c]P < .01 when compared to vehicle treated stressed mice.

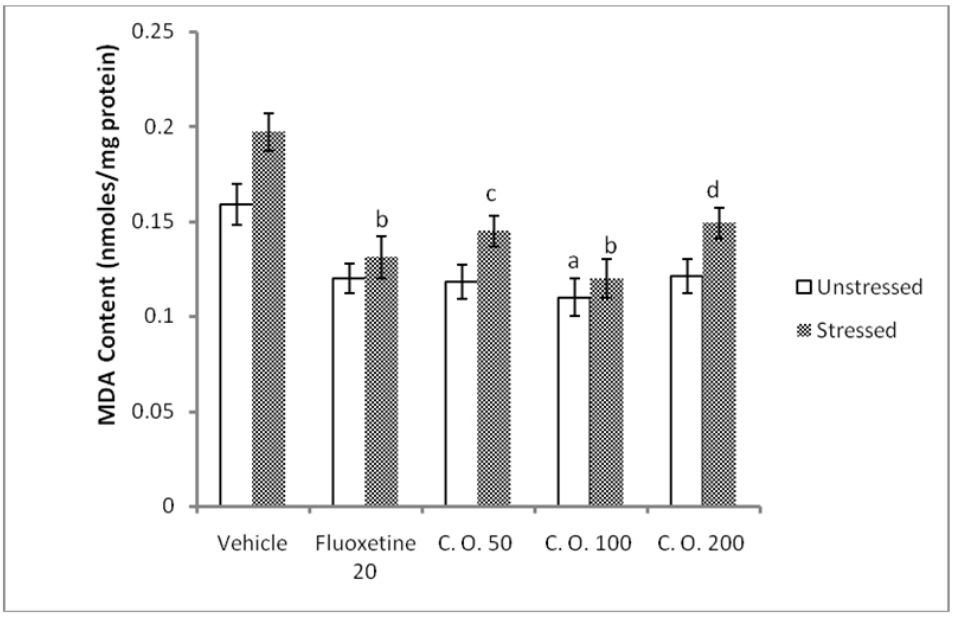

Fig. 7. Effect of *Celastrus paniculatus* seed oil on brain MDA content

n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.
F (9, 90) =7.753, P < .0001
[a]P = .05 when compared to vehicle treated unstressed mice;
[b]P < .01, [c]P = .01 , [d]P = .05 when compared to vehicle treated stressed mice.

3.8 Effect of Celastrus Seed Oil and Fluoxetine on Brain Reduced Glutathione and Catalase Activity

Reduced glutathione content and catalase activity were significantly decreased in brains of stressed mice as compared to respective vehicle treated unstressed controls. Fluoxetine produced a significant increase in the reduced glutathione in unstressed mice, and increase in catalase activity in both unstressed and stressed mice as compared to the respective controls. Celastrus seed oil (00 mg/kg) significantly increased the reduced glutathione content in unstressed mice as compared to its control. But in case of stressed mice, Celastrus seed oil produced a significant increase in the reduced glutathione at all the doses employed as compared to the control. Celastrus seed oil (100 mg/kg) significantly increased catalase activity in both unstressed and stressed mice as compared to the respective controls. The effect of Celastrus oil (100 mg/kg) on increase in brain reduced glutathione levels was better in stressed (*P = .01)* than unstressed (*P = .05)* mice. But the effect of Celastrus oil (100 mg/kg) on increase in brain catalase activity was same (*P = .05)* in both stressed and unstressed mice (Figs. 8 and 9).

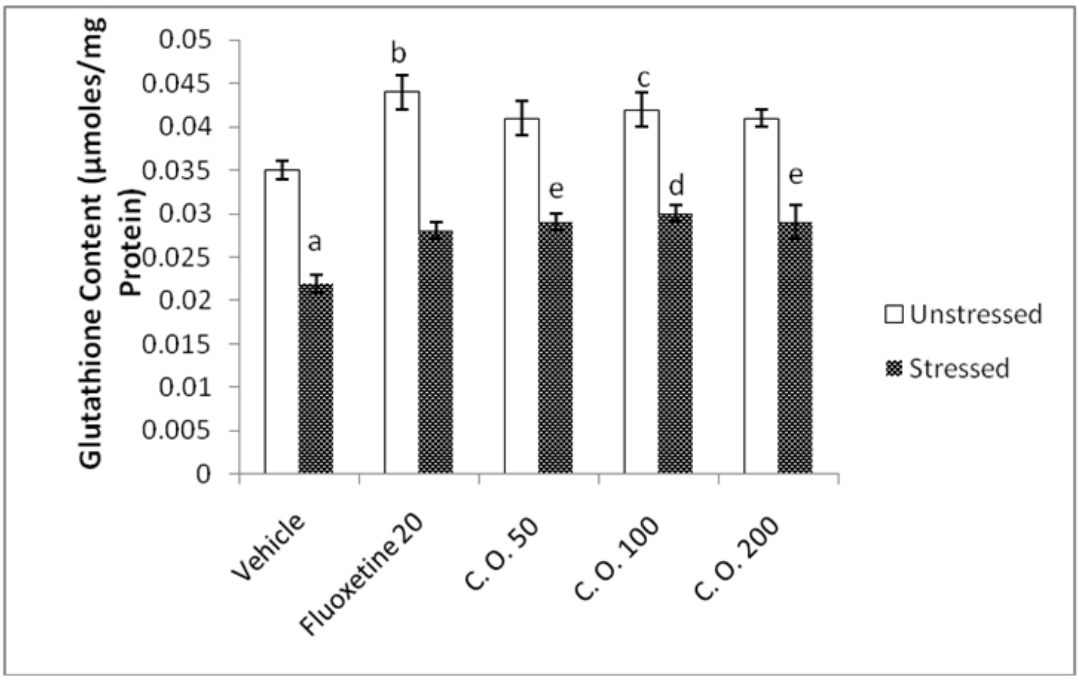

Fig. 8. Effect of *Celastrus paniculatus* seed oil on brain reduced glutathione content
n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.
F (9, 90) =25.702, P < .0001
[a]P < .01, [b]P =.01, [c]P = .05, when compared to vehicle treated unstressed mice;
[d]P = .01, [e]P = .05 when compared to vehicle treated stressed mice.

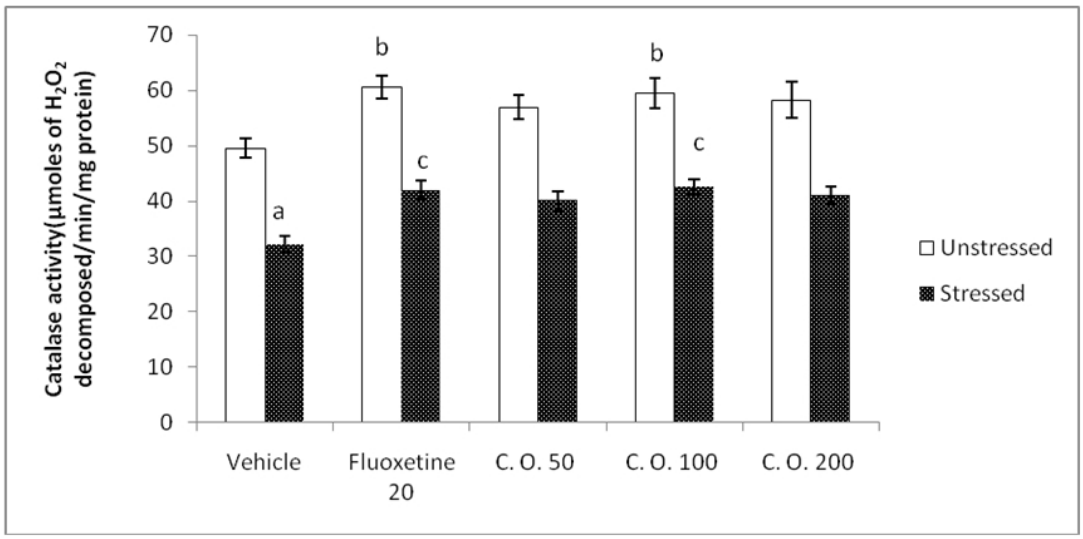

Fig. 9. Effect of *Celastrus paniculatus* seed oil on brain catalase activity

n=10 in each group; Values are in mean ± SEM. Data was analyzed by one way ANOVA followed by Tukey's test. C.O. stands for Celastrus seed oil.

F (9, 90) =23.642, P< .0001

[a]P < .01, [b]P = .05, when compared to vehicle treated unstressed mice;

[c]P = .05, when compared to vehicle treated stressed mice.

4. DISCUSSION

In the present study, mice that were exposed to CUMS exhibited greater immobility period in FST as compared to control animals and thus showed depression-like behavior. This is also supported by an earlier study [16]. *Celastrus paniculatus* seed oil (50, 100 and 200 mg/kg, p.o.) administered for 14 successive days to mice produced significant decrease in the immobility period of both stressed and unstressed mice in FST, thus showed significant antidepressant-like effect. The efficacy of the oil was found to be comparable to fluoxetine. FST is a commonly used behavioral despair model of depression. This model is widely employed in rodents to predict antidepressant potential by several different classes of antidepressant drugs [15]. Moreover, we employed another model, sucrose preference test to evaluate antidepressant-like activity of seed oil. This test is an indicator of anhedonia-like behavioral change, indicating loss of interest or pleasure. Anhedonia is a main symptom of major human depression. In the present study, when CUMS was applied, there is decrease in sucrose preference as compared to control unstressed mice. Celastrus seed oil and fluoxetine per se restored the decreased sucrose preference in both stressed as well as unstressed mice. CUMS has long been used as a model of depression. Most effects of CUMS can be reversed by antidepressant agents [17,27], illustrating a strong predictive validity. In rodents, CUMS also has a good face validity as it can elicit depression-like symptoms such as a lack of sucrose preference [17,28], interpreted as anhedonia, a core symptom of depression. Long term exposure to multiple stressors can cause depression in humans. Induction of depression using CUMS is considered as the most valid animal model of depressive conditions observed in humans after long-term exposure to multiple stressors [29]. Further, Celastrus seed oil did not show any significant change in locomotor functions of unstressed and stressed mice as compared to the respective control mice, so it did not

produce any overt motor effects. Thus, antidepressant-like activity of seed oil in mice is specific and not false positive.

Moreover, seed oil reduced the mouse whole brain MAO-A activity as compared to respective controls in both stressed and unstressed mice, so it indicated that seed oil inhibited the metabolism of monoamines, particularly serotonin and noradrenaline. Levels of monoamines like norepinephrine and serotonin are decreased in depression, so drugs like tricyclic antidepressants and MAO inhibitors, which enhance the levels of these monoamines, have been used as antidepressant drugs [2].

Long-time stress and elevated glucocorticoid levels leaded to the emergence of mood disorders [30]. Steroid hormones can modulate neuronal transmission by a variety of mechanisms. Hypersecretion of glucocorticoids and dysregulation of glucocorticoid receptor function are involved in the pathogenesis of depression [31,32]. It has been reported that high concentrations of blood glucocorticoid are maintained in patients with depression due to the dysfunction of feedback mechanism [33]. Several animal studies demonstrated that abnormally high corticosterone levels can induce depression-like behavior [34]. Our results demonstrated that pretreatment of mice with seed oil (50 and 100 mg/kg) and fluoxetine per se significantly reduced stress-induced elevated corticosterone levels. This indicated that antidepressant-like activity of celastrus seed oil might be due to reduction in hyperactivity of hypothalamic-pituitary-adrenal axis.

A number of studies have suggested that oxidative stress, characterized by the imbalance between production of free radicals and the antioxidant capacity of organism, may contribute to the neuropathology of neurological and psychiatric diseases, including major depression [35]. There are several reports showing that chronic stress can have a substantial impact on reactive oxygen species (ROS) generation and nitric oxide production in rat brain. It has been shown that repeated and unpredictable stress situations increase ROS production in the rat brain [36,37], which in turn results in oxidative damage in the central nervous system. Similarly, studies which investigated the oxidative stress profile in depressed patients found impairments in the blood levels of the antioxidants enzymes like superoxide dismutase, catalase and glutathione peroxidase and higher products of lipid peroxidation than healthy controls [38,39], strongly suggesting that major depressive disorder is accompanied by disturbances in the balance between pro- and anti-oxidative processes. Lipid peroxidation and antioxidant enzymes may be state markers of major depression because they returned to normal ranges after antidepressant treatment [14]. In the present study, 14 days of exposure to different stressors resulted in increase in malondialdehyde and nitrite levels and decreased endogenous antioxidant activity in mice. Chronic administration of Celastrus seed oil showed antioxidant activity in both unstressed and stressed mice, as indicated by decrease in brain malondialdehyde levels and increase in reduced glutathione and catalase activities. The antioxidant activity of seed oil has already been reported in the literature [8,40]. Reduction of nitric oxide levels within the hippocampus can induce antidepressant-like effects, thus implicating the role of nitrosative stress in the neurobiology of stress and depression [41]. Stressful situations in mice have also been reported to significantly increase plasma nitrite levels. Celastrus seed oil significantly reduced nitrosative stress as indicated by reduction in the plasma nitrite levels of both unstressed and stressed mice as compared to their respective vehicle-treated controls. Thus, Celastrus seed oil showed a strong protection against oxidative stress that plays key role in chronic mild stress-induced depression.

5. CONCLUSIONS

This is probably the first study which reports the antidepressant-like activity of *Celastrus paniculatus* seed oil in animal behavior despair models. The results of the present study indicate that *Celastrus paniculatus* seed oil showed antidepressant-like activity in unstressed and chronic unpredictable mild stressed mice probably through inhibition of brain MAO-A activity, decrease in plasma nitrite levels and due to its antioxidant activity. In addition, the seed oil also showed antidepressant-like activity in stressed mice possibly through decrease in plasma corticosterone levels. Thus *Celastrus paniculatus* seed oil may be explored further for its role in treatment of clinical depression.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that the experimental protocol was approved by IAEC in its 22nd meeting held on 14th May, 2012. and animal care was taken as per the guidelines of CPCSEA, Govt. of India (Registration No. 0436).

COMPETING INTERESTS

Authors have declare that we have no conflict of interest.

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