Behavioral and Biochemical Evidences for Antidepressant-Like Activity of *Celastrus Paniculatus* Seed Oil in Mice

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A B S T R A C T

Introduction: *Celastrus paniculatus* seed oil, commonly known as Malkangni or Jyotishmati, was in use from time immemorial to treat brain related disorders. *Celastrus paniculatus* seed oil has significant antidepressant-like activity in chronic unpredictable stressed mice. The present study was undertaken to evaluate the antidepressant-like effect of *Celastrus paniculatus* seed oil in unstressed mice and to explore its mechanism of action.

Methods: The seed oil (50, 100, and 200 mg/kg, PO) and fluoxetine per se were administered for 14 successive days to Swiss young albino mice. On the 14th day, 60 min after drug administration, animals were subjected to Tail Suspension Test (TST) and Forced Swim Test (FST). The mechanism of action was also studied.

Results: The oil significantly decreased immobility period of mice in both tail suspension test and forced swim test, indicating its significant antidepressant-like activity. The efficacy was found to be comparable to fluoxetine ($P<0.0001$). ED_{50} value of celastrus seed oil using FST and TST were 17.38 and 31.62 mg/kg, respectively. The oil did not show any significant effect on locomotor activity. It significantly inhibited brain MAO–A activity and decreased plasma corticosterone levels. Sulpiride (selective D_2-receptor antagonist), *p*-CPA (tryptophan hydroxylase inhibitor), and baclofen ($GABA_B$ agonist) significantly attenuated the oil-induced antidepressant-like effect, when assessed during TST.

Discussion: *Celastrus paniculatus* seed oil produced significant antidepressant-like effect in mice possibly through interaction with dopamine D_2, serotonergic, and $GABA_B$ receptors; as well as inhibition of MAO–A activity and decrease in plasma corticosterone levels.

1. Introduction

Depression is a neuropsychiatric disorder. Reduced monoamine signaling and monoamine metabolites level have been found in the cerebrospinal fluid of depressed individuals (Nutt, 2002). The levels of monoamine oxidase–A increase in depression, which in turn reduces levels of monoamines (Meyer et al., 2008). The use of alternative medicines is increasing worldwide and day by day due to their safety as compared to synthetic drugs. A number of plants have been explored for their antidepressant-like activity. *Hypericum perforatum*, a well known plant has been proven to be an effective antidepressant in clinical studies (Rahimi et al., 2009).

Celastrus paniculatus Willd. (Family: Celastraceae), commonly known as Malkangni (in Hindi) or Jyotishmati (in Sanskrit), was in use from time immemorial to treat brain-related disorders (Chopra et al., 1958). C. paniculatus seeds and seed oil have been used in Ayurvedic medicine for stimulating intellect and sharpening the memory (Gaitonde et al., 1957). Celastrus oil therapy

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in mentally-retarded children results in improvement in their I.Q. (Nalini et al., 1986). Celastrus seeds have been reported to possess hypolipidemic, antiatherosclerotic (Mathur et al., 1993), antispermatogenic (Bidwai et al., 1990), antioxidant (Kumar and Gupta, 2002), anxiolytic (Jadhav and Patwardhan, 2003), antistress (Lekha et al., 2010), and nootropic (Bhanumathy et al., 2010) activities. The seed oil contains a number of fatty acids such as oleic, linoleic, linolenic, palmitic, stearic, benzoic, and acetic acid as volatile acids and their glycerol esters mainly α, α' dipalmitoyl glycerol. The seeds also contain sesquiterpene alkaloids viz. celapanin, celapanigin, celapagin, and malkangunine (Sengupta and Bhargava, 1970; Zhang et al., 1998; Anonymous, 1999). We have already reported the antidepressant-like activity of the C. paniculatus seed oil in mice subjected to chronic unpredictable mild stress (Valecha and Dhingra, 2014), but its antidepressant activity in unstressed mice and underlying mechanisms of action have not been explored yet. So the aim of the present study was to evaluate the antidepressant-like activity in other behavioral models and to explore the probable underlying mechanisms of action of C. paniculatus seed oil.

2. Methods

2.1. Collection of plant material

The dried seeds of C. paniculatus were purchased from the local market of Hisar and were authenticated as *Celastrus paniculatus* Willd. by Raw Materials Herbarium and Museum section, 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 200g of powdered seeds were extracted with petroleum ether (60-80⁰C) using Soxhlet apparatus. The filtrate was concentrated using water bath. The oil was dark brown in color and the yield was 36.5% v/w. The celastrus oil was stored in air tight container in a refrigerator (Jadhav and Patwardhan, 2003).

2.3. Experimental animals

Swiss albino mice of either sex, weighing around 20-25g 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: 29×22×14cm) under laboratory conditions with alternating light and dark cycle of 12 h each having

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 5 days before behavioral experiments carrying out between 09:00 and 17:00. The experimental protocol was approved by Institutional Animals Ethics Committee and animal care was taken as per the guidelines of CPCSEA, Govt. of India (Registration No. 0436).

2.4. Drugs and chemicals

The following drugs and chemicals were used in this study: 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).

2.5. Vehicles

Fluoxetine, prazosin, sulpiride, and baclofen were separately dissolved in normal saline (0.9%). *p*-CPA was dissolved in minimum quantity of 0.1 N sodium hydroxide solution and pH was adjusted to 7.0 with 0.1 N hydrochloric acid. The C. paniculatus seed oil was emulsified with 1% Tween-20 (solubilising agent) followed by dilution with dimethylsulfoxide just before administration (Lekha et al., 2010). Doses of the seed oil (50, 100, and 200 mg/kg), prazosin, sulpiride, *p*-CPA, and baclofen were selected based upon the previous studies (Lekha et al., 2010; Dhingra and Valecha, 2007; Dhingra and Valecha, 2014).

2.6. Laboratory models employed for evaluation of antidepressant-like activity

2.6.1. Tail suspension test

Tail Suspension Test (TST) is a commonly employed behavioral model for screening antidepressant-like activity in mice (Steru et al., 1985). The test was conducted as previously followed (Dhingra and Valecha, 2014). Animals were moved from their housing colony to the laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 h. Each mouse was individually suspended to the edge of a table, 50 cm above

the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. The total period of immobility was recorded manually for 6 min. The animal was considered immobile when it did not show any body movement, was hung passively and became completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test.

2.6.2. Forced swim test

Forced Swim Test (FST) is another most commonly used behavioral model for screening antidepressant-like activity in rodents (Porsolt et al., 1977). The procedure was the same as previously followed (Dhingra and Valecha, 2014). Animals were moved from their housing colony to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 h. Mice were individually forced to swim in an open glass chamber ($25 \times 15 \times 25$ cm³), containing fresh water to a height of 15 cm and maintained at $26 \pm 1°C$. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered immobile when they ceased struggling and remained floating motionless in water, making only those necessary movements to keep their head above water. The test was conducted in a dim lighted room and each mouse was used only once in the test.

2.6.3. Measurement of MAO-A activity

Mouse brain mitochondrial fraction was prepared following the procedure described previously (Schurr and Livne, 1976). 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 and 0.02 M EDTA (pH 7.4) to remove the 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 4°C and the pellets were discarded. The supernatant was then centrifuged at 12000 rpm for 20 min in the same medium. The precipitate was washed twice more with 100 mL of sucrose-Tris-EDTA buffer and resuspended in 50 mL of the medium (Pan et al., 2005).

MAO activity was assessed spectrophotometrically as described previously (Yu et al., 2002). The assay mixture contained 100 μL of 4 mM 5-hydroxytryptamine as the specific substrate for MAO-A, 250 μL solution of mitochondrial fraction, and 100 mM sodium phosphate buffer (pH 7.4) upto a final volume of 1 mL. The reaction was allowed to proceed at 37°C for 20 min, and was stopped by adding 200 μL of 1M HCl. Then, the reaction product was boiled 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.

2.6.4. Estimation of protein content

Total protein was estimated in brain homogenate (Henry et al., 1974) using total protein kit (Coral Industries Ltd., Uttarakhand, India) and colorimeter (Digital Photocolorimeter, Biomed, India).

2.6.5. Estimation of corticosterone levels

Corticosterone levels were estimated in blood plasma by Bartos and Pesez method (Bartos and Pesez, 1979). Blood samples of animals were collected by carotid bleeding and centrifuged (Remi Centrifuge, Mumbai, India) at 2500 rpm for 10 min to separate plasma. To 1.0 mL of plasma sample, 1.0 mL of ethanol, 0.50 mL of 0.10 % solution of p-nitroso-N, N-dimethylaniline in ethanol were added and the tubes were immersed in ice water for 5 min, and then 0.50 mL of 0.1 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and let to stand at 0°C for 5 h, protected from light. To the above solution, 2.0 mL of Clark and Lubs buffer for pH 9.8 (prepared by mixing 50.0 mL of an aqueous solution of both boric acid and potassium chloride with 40.8 mL of 0.20M potassium hydroxide, and diluted to 200 mL with distilled water), 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 water bath at $20 \pm 2°C$ for 10 min. The absorbance of the solutions was read at 650 nm using UV-Visible-NIR Spectrophotometer (Varian Cary-5000, Christ, Netherland).

2.6.6. Measurement of locomotor activity

To rule out the effect of celastrus 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).

2.7. Experimental protocol

Animals were divided into 18 groups and each group comprised a minimum of 10 mice. The experimental protocol was consisted of the following groups:

2.7.1. Groups for tail suspension test

Group 1 (Control group): Vehicle (dimethyl sulfoxide) was administered orally for 14 consecutive days and after 60 min of administration, on the 14th day, the immobility periods of mice were recorded in TST.

Groups 2, 3, 4, and 5: Fluoxetine (20 mg/kg, PO) and celastrus oil (50, 100, and 200 mg/kg, PO) of C. paniculatus were administered for 14 successive days and after 60 min of administration on the 14th day, the immobility periods of mice were recorded in TST.

2.7.2. Groups for forced swim test

Groups 6 to 10 were similar as mentioned under TST groups except that the immobility periods of mice were recorded using FST.

2.7.3. Groups for investigating mechanisms of action by co-administration of various drugs modulating levels of monoamines and GABA employing TST

Groups 11 and 12: Vehicle (dimethyl sulfoxide) and celastrus oil (100 mg/kg, PO), respectively were administered for 14 consecutive days and after 45 min of administration on the 14th day, sulpiride (50 mg/kg, IP) was injected. After 45 min of injection, the animals were subjected to TST.

Groups 13 and 14: Vehicle and celastrus oil (100 mg/kg, PO), respectively were administered for 14 consecutive days and after 45 min of administration on the 14th day, prazosin (62.5μg/kg, IP) was injected and 45 min after the injection, the animals were subjected to TST.

Groups 15 and 16: Vehicle and celastrus oil (100 mg/kg, PO), respectively were administered for 14 consecutive days. Then, p-CPA (100 mg/kg, PO) was injected 45 min after administration of respective vehicle and oil from 11th day to 14th day. On the 14th day, 45 min after the injection of p-CPA, the animals were subjected to TST. Groups 17 and 18: Vehicle and celastrus oil (100 mg/kg, PO) were administered respectively for 14 consecutive days and after 45 min of administration on the 14th day, baclofen (10 mg/kg, IP) was injected and 45 min after the injection; the animals were subjected to TST.

2.7.4. Groups for estimation of MAO-A and corticosterone levels

Animals of groups 6 to 10 after being subjected to FST on the 14th day, were sacrificed by cervical dislocation on the 15th day, and immediately brain samples were collected and analyzed for MAO-A and protein levels. At the same time, blood samples were collected by carotid bleeding and corticosterone levels were measured.

2.7.5. Groups for measurement of locomotor activity

Animals in groups 1 to 5 after subjecting to TST on the 14th day were assessed for locomotor activity on the 15th day to rule out any effect on locomotion by the drugs.

2.8. Statistical analysis

All the results were expressed as mean±standard error mean (SEM). The data were analyzed by using 1-way ANOVA followed by Tukey's test for multiple comparisons using the software GraphPad Instat. In all tests, the criterion for statistical significance was P<0.05.

3. Results

3.1. Effect of celastrus seed oil and fluoxetine on immobility periods of mice in TST and FST

Celastrus seed oil (50, 100, and 200 mg/kg, PO) and fluoxetine (20 mg/kg, PO) per se administered for 14 consecutive days to mice significantly decreased the immobility periods in both TST and FST, indicating significant antidepressant-like activity. The efficacy of the oil was found to be comparable to fluoxetine (P<0.0001). ED_{50} value of celastrus seed oil using FST and TST were 17.38 and 31.62 mg/kg, respectively (Figure 1 and 2).

3.2. Effect of combination of celastrus seed oil with sulpiride, prazosin, p-CPA, and baclofen on immobility period in TST

Sulpiride (50mg/kg, IP), prazosin (62.5μg/kg, IP), p-CPA (100mg/kg, IP), and baclofen (10mg/kg, IP) alone significantly increased the immobility period as compared to control group. Pretreatment of animals with sulpiride or baclofen or p-CPA significantly reversed the decrease in immobility time elicited by celastrus oil (100 mg/kg). Pretreatment with prazosin did not show any significant effect on the immobility period elicited by seed oil (Table 1).

3.3. Effect of celastrus seed oil and fluoxetine on brain MAO-A levels

Celastrus seed oil (50, 100, and 200 mg/kg, PO) and fluoxetine per se administered for 14 consecutive days to mice, significantly reduced the brain MAO–A levels as compared to the vehicle treated group (Figure 3).
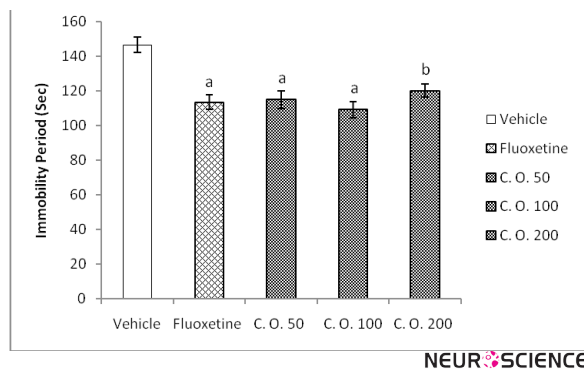
Figure 1. Effect of Celastrus paniculatus seed oil on immobility period using FST.

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

$F_{(4, 45)}=11.123$, $P<0.0001$.

[a]$P<0.001$, [b]$P<0.01$ as compared to vehicle treated group.

3.4. Effect of celastrus seed oil and fluoxetine on plasma corticosterone levels

Celastrus seed oil (50, 100, and 200 mg/kg, PO) and fluoxetine per se administered for 14 consecutive days to mice, significantly reduced the plasma corticosterone levels as compared to the vehicle treated group (Figure 4).

3.5. Effect of celastrus seed oil and fluoxetine on locomotor activity

Celastrus seed oil (50, 100, and 200 mg/kg, PO) and fluoxetine per se administered for 14 successive days did
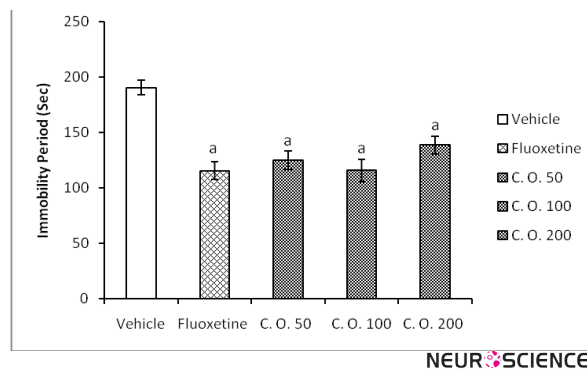

Figure 2. Effect of Celastrus paniculatus seed oil on immobility period using TST.

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 oil.

$F_{(4, 45)}=14.405$, $P<0.0001$.

ap<0.001 as compared to vehicle treated group.

not show any significant change in the locomotor activity of mice as compared to the vehicle treated group (Table 2).

4. Discussion

In the present study, *Celastrus paniculatus* seed oil (50, 100, and 200 mg/kg, PO) administered for 14 successive days to mice produced significant antidepressant-like effect in both TST and FST. This is the first study showing the antidepressant-like activity of celastrus seed oil. The efficacy of the oil was found to be comparable to fluoxetine. FST and TST are two commonly used behavioral

Table 1. Effect of Celastrus paniculatus seed oil (100 mg/kg) and its combination with sulpiride, prazosin, *p*-CPA and baclofen on immobility periods of mice using TST.

Sr. No.	Treatment for 14 days p.o.	Dose (Kg-1)	Immobility Period (Sec)	P-value
1	Vehicle	10 ml	190.66±6.62	
2	Celastrus oil	100 mg	115.83±9.92a	[a]P<0.001
3	Vehicle + Sulpiride	50 mg	194.33±9.63	
4	Oil+Sulpiride	100 mg+50 mg	220.33±6.89b	[b]P<0.001
5	Vehicle+Prazosin	62.5µg	196.5±7.49	
6	Oil+Prazosin	100 mg+62.5µg	145.66±10.36	
7	Vehicle + *p*-CPA	100 mg	191.50±5.81	
8	Oil+*p*-CPA	100 mg+100 mg	194.00±9.05b	[b]P<0.001
9	Vehicle+Baclofen	10 mg	207.33±10.28	
10	Oil+Baclofen	100 mg+10 mg	206.16±5.83b	[b]P<0.001

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n=10 in each group; values are in mean±SEM. Data was analyzed by one way ANOVA followed by Tukey's test.
$F_{(9, 90)}=14.081$; $P<0.0001$.
[a]P<0.001 as compared to vehicle treated group.
[b]P< 0.001 as compared to Celastrus oil (100 mg/kg) treated group.
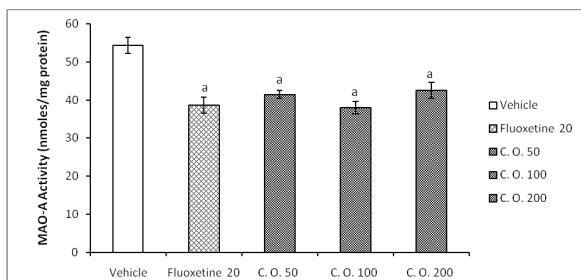
Figure 3. Effect of Celastrus paniculatus seed oil on MAO-A levels.

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 oil.

$F_{(4, 45)}=12.661$, $P<0.0001$.

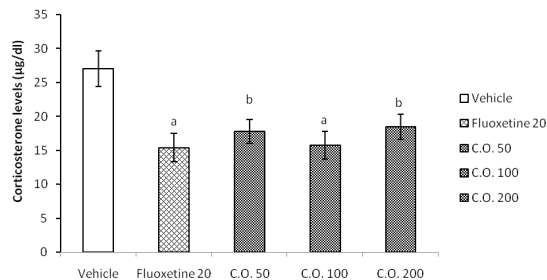[a]$P<0.001$ as compared to vehicle treated group.

Figure 4. Effect of Celastrus paniculatus seed oil on 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 oil.

$F_{(4, 45)}=5.080$, $P=0.0018$.

[a]$P<0.01$, [b]$P<0.05$ as compared to vehicle treated group.

despair models of depression. These models are widely employed in rodents to evaluate antidepressant potential through decreasing immobility period produced by different classes of antidepressant drugs (Steru et al., 1985; Porsolt et al., 1977). Celastrus seed oil did not show any significant change in locomotor function of mice as compared to control, so it did not produce any overt motor effects.

Thus, antidepressant-like effect of seed oil was specific and not the false positive. The antidepressant-like effect of seed oil was significantly reversed by pretreatment of animals with sulpiride (a selective dopamine D_2-receptor antagonist), *p*-CPA (a serotonin synthesis inhibitor) and baclofen (GABA$_B$ agonist), when tested in TST. This effect suggested that the celastrus seed oil might produce antidepressant-like effect by interaction with dopamine D_2-receptors, serotonergic and GABA$_B$ receptors, hence increasing the levels of brain dopamine and serotonin, as well as decreasing the levels of GABA. GABA$_B$ receptor antagonism may serve as a basis for the generation of novel antidepressants (Ghose et al., 2011). Prazosin (an α_1-adrenoceptor antagonist) did not significantly affect

the antidepressant-like effect of the seed oil. This issue indicated that antidepressant-like effect of seed oil might not be through interaction with α_1-adrenoceptors. Levels of monoamines like serotonin and dopamine decrease in depression, so antidepressant drugs enhance the levels of these monoamines (Zangen et al., 2001). The celastrus seed oil also significantly inhibited MAO-A activity compared to control, indicating that decreased metabolism of monoamines like serotonin, dopamine and noradrenaline might contribute to its antidepressant-like activity. MAO-A inhibitors have potent antidepressant activity (O'Donnell & Shelton, 2011).

Major depression has been linked with hyperactivity of the hypothalamic-pituitary-adrenal axis (Barden, 2004). High concentrations of blood glucocorticoid are maintained in patients with depression due to the dysfunction of this feedback mechanism (Johnson et al., 2006). High glucocorticoid levels cause pathological damage to the hippocampal neurons both in vitro and in vivo (Li et al., 2007; Murray et al., 2008) and can induce depression-like behavior in animals (Johnson et al., 2006; Murray et

Table 2. Effect of celastrus paniculatus seed oil on locomotor activity of mice.

Sr. No.	Treatment for 14 days p.o.	Dose (Kg-1)	Locomotion scores in 10 min.
1	Vehicle	10 ml	298.1±8.99
2	Fluoxetine	20 mg	284.1±8.87
3	Celastrus oil	50 mg	293.0±6.67
4	Celastrus oil	100 mg	295.2±6.87
5	Celastrus oil	200 mg	287.8±8.33

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

$F_{(4, 45)}=0.4985$; $P=0.7369$.

al., 2008). Our results demonstrated that celastrus seed oil significantly reduced the plasma corticosterone levels compared to the control group, so the antidepressant-like action shown by the oil may be due to the reduction of plasma corticosterone levels.

In conclusion, our present study shows that Celastrus paniculatus seed oil produced significant antidepressant-like effect in mice behavioral models like TST and FST probably by interaction with dopamine-D_2 receptors, serotonergic, and $GABA_B$ receptors, as well as MAO–A inhibition and reduction in plasma corticosterone levels.

Acknowledgements

Conflict of Interest

We declare that we have no conflict of interest.

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