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Possible Involvement of Monoaminergic Neurotransmission in Antidepressant-like Activity of *Emblica officinalis* Fruits in Mice

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SUMMARY

Aims: In this study, antidepressant-like activity of *Emblica offcinalis* Gaertn. fruits (Family: Euphorbiaceae) was evaluated in Swiss young male albino mice employing tail suspension test and forced swim test. **Methods**: Aqueous extract (200 and 400 mg/kg) of the fruits was administered orally for 14 successive days to mice. On day 14, 60 min after extract administration, animals were subjected to tail suspension test and forced swim test. **Results**: The extract significantly decreased immobility period in both tail suspension test and forced swim test, indicating significant antidepressant-like activity. The lower dose (200 mg/kg) of the extract showed better antidepressant-like action. The efficacy of the extract was found to be comparable to fluoxetine (20 mg/kg), imipramine (15 mg/kg), and phenelzine (20 mg/kg). The extract did not show any significant effect on locomotor activity of the mice. Prazosin (alpha$_1$-adrenoceptor antagonist), sulpiride (selective D_2-receptor antagonist), baclofen ($GABA_B$ agonist), and p-CPA (tryptophan hydroxylase inhibitor) significantly attenuated the extract-induced antidepressant-like effect. The extract also significantly decreased brain MAO-A levels. **Discussion**: The aqueous extract might produce antidepressant-like effect by interaction with α_1-adrenoceptors, dopamine D_2- receptors, serotonergic, and $GABA_B$ receptors. In this study, aqueous extract was found to contain 2.94% of ascorbic acid. So ascorbic acid and other constituents like flavanoids, tannoid principles, and polyphenolic substances present in the aqueous extract of *E. officinalis* might be responsible for its antidepressant-like activity. **Conclusions**: Thus, aqueous extract of *E. officinalis* showed antidepressant-like activity probably by inhibiting MAO-A and GABA; and also due to its antioxidant activity.

Introduction

Depression is an important global public-health issue, both because of the relatively high lifetime prevalence ranging from 2% to 15% and because it is associated with substantial disability [1]. To the present knowledge, antidepressant drugs used in the treatment of major depressive disorders are believed to act on the central monoaminergic systems mainly 5-HT and nor-adrenergic synaptic neurotransmissions. Selective serotonin reuptake inhibitors (e.g., paroxetine, fluoxetine, citalopram, escitalopram, fluvoxamine, sertraline) and nor-adrenaline reuptake inhibitors (e.g., reboxetine, desipramine) are the most common prescribed antidepressant drugs [2]. Although these are effective in treating most depressive episodes, a significant proportion of depressed patients do not display signs of mood improvement until 2–3 weeks after the start of the treatment. Furthermore, about one-third of these patients show only partial or no response to the treatment [3]. In addition, some side effects like sedation, anticholinergic effects (dried mouth, blurred vision, constipation, urinary retention, etc.), postural hypotension, seizures, impotence, agitation, insomnia, dizziness, anxiety, cardiac dysrhythmias, anorgasmia, weight gain, and cheese reaction (in case of MAO inhibitors) are very common with chronic treatment of antidepressants [4].

Monoamine oxidase (MAO) is an enzyme protein responsible for metabolic degradation of catecholamines, serotonin, and other endogenous amines in CNS. In case of depression, the level of MAO enzyme in brain is increased which in turn reduce the levels of monoamines [5]. It exists in two similar molecular forms—A and B. MAO-A has substrate preference for serotonin and is the main target for the antidepressant MAO inhibitors. MAO-B has substrate preference for phenylethyl amine. Both enzymes act on nor-adrenaline and dopamine. The type B is selectively inhibited by selegiline, which is used in treatment of Parkinsonism. Experimentally, selective MAO-A inhibitors (clorgyline, moclobemide) are found to more effective in treating major depression than MAO-B inhibitors like selegiline [6].

In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of MAO-A, Selective serotonin reuptake inhibitors, and nor-adrenaline reuptake inhibitors, depression continue to be a major medical problem [7].

Therefore, research for new antidepressants with greater effectiveness without any (or with least) adverse effects is still desirable. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Further, the use of alternative medicines is increasing worldwide day by day. Various herbal drugs (e.g., St. John's wort) have shown promising results in treating experimental as well as clinical depression [8] and many of these herbal drugs appear to be safe. Thus there is a constant need to identify newer natural antidepressants with greater efficacy, fewer side effects and to explore their potential over synthetic antidepressants. In light of the above background, our aim was to explore a plant for its antidepressant potential.

So we selected *E. officinalis* fruits for evaluating its antidepressant potential in mice. *E. officinalis* (Family: Euphorbiaceae), commonly known as Indian gooseberry, is common all over tropical and subtropical India and also found in Burma [9]. The fruits of *E. officinalis* have been reported to possess antioxidant [10], anticataleptic [11], antianxiety [12], memory enhancing [13], antistress [14], anticonvulsant [15], hepatoprotective [16], hypocholesterolemic [17], antiulcer [18], anticataract [19], antimicrobial [20], antiinflammatory [21], antitumor [22], analgesic, antipyretic [23], and antidiabetic [24] properties. The constituents of *E. officinalis* fruits include ascorbic acid [10]; tannins (emblicanin A, emblicanin B, punigluconin, and pedunculagin) [25,26]; terpenes (lupeol, gibberellin A-1, gibberellin A-3, gibberellin A-4, gibberellin A-7, and gibberellin A-9) [27]; flavonoids (kaempherol-3-O-ß-D-glucoside, quercetin-3-O-ß- D-glucoside) [28]; phenolics (gallic acid and ellagic acid) and sterols (ß-daucosterol) [29].

Therefore, this study has been undertaken to investigate the effect of *E. officinalis* fruits on depression in mice employing forced swim test and tail suspension test; and to explore the possible underlying mechanisms of antidepressant-like activity.

Materials and Methods

Collection of Plant Material

The dried fruits of *E. officinalis* were purchased from the local market of Hisar (Haryana, India). The crude drug was authenticated as *E officinalis* Gaertn. from Raw Materials Herbarium and Museum, National Institute of Science Communication and Informational Resources, New Delhi vide reference number NISCAIR/RHMD/Consult/-2010–11/1446/44.

Preparation of Aqueous Extract

E. officinalis (80 g) dried fruits were crushed into fine powder and extracted with 1 L boiling water for 30 min. The heated decoction obtained was allowed to cool at room temperature and filtered twice through fine filter paper. The filtrate was then evaporated to dryness on a water bath. The extract was brown in color and yield of the extract was 25.6% w/w. The extract was stored in a desiccator and used for the pharmacological studies by dissolving each time in distilled water. The two doses (200 and 400 mg/kg) of the extract were selected based upon the literature [15,24].

Animals

Swiss young male albino mice, weighing around 20–25 g were purchased from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana, India). Since estrogens (female sex hormones) have been found to have antidepressant effect, so we excluded female mice and used only male mice for the study [30]. 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. The animals had 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, which were carried out between 09:00 and 17:00 h. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. 0436).

Drugs and Chemicals

Prazosin HCl, (±) Sulpiride, DL-parachlorophenylalanine (p-CPA) and Baclofen (Sigma-Aldrich, St. Louis, USA); Imipramine hydrochloride, Fluoxetine hydrochloride, Phenelzine (Ranbaxy Laboratories, Gurgaon, India); sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, Tris, EDTA di sodium salt AR, Sucrose, 5-Hydroxy tryptamine creatinine sulphate monohydrate, m-phosphoric acid (Hi Media laboratories Pvt. Ltd., Mumbai, India), Butyl Acetate, Hydrochloric acid (Qualigens fine chemicals, Mumbai, India); Total Protein Kit (Crest Biosystems, Goa, India) were used in this study.

Vehicles

Imipramine, fluoxetine, prazosin, sulpiride, and baclofen were separately dissolved in normal saline (0.9% NaCl). 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 dried extract of *E. officinalis* was dissolved in distilled water each time before administration. Doses of prazosin, sulpiride, pCPA, and baclofen were selected on the basis of literature [31–33].

Estimation of Ascorbic Acid in Aqueous Extract

Total ascorbic acid was estimated by the method of Chinoy et al. [34]. The blue dye, dichlorophenolindophenol, is reduced to the colorless form on addition of ascorbic acid. The buffered HPO_3 extract was treated with dichlorophenol indophenol dye solution. The absorbance was read at 520 nm.

Laboratory Models Employed for the Evaluation Antidepressant-like Activity

Forced Swim Test (FST)

It is frequently used behavioral model for screening antidepressant-like activity in rodents [35]. The procedure followed was as previously described by our laboratory [36,37].

Briefly, mice were individually forced to swim in an open glass chamber ($25 \times 15 \times 25$ cm^3) containing fresh water to a height of 15 cm and maintained at $26 \pm 1^\circ$C. Water in the chamber was changed after subjecting each animal to FST because "used water" has been shown to alter the behaviour [38]. Mice placed in the chamber for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall, or diving to the bottom. After 2 min, activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating in water, making only those movements necessary to keep their head above water. Following the swimming session, mice were towel dried and returned to their housing conditions. The test was conducted in a dim lighted room and each mouse was used only once in the test.

Tail Suspension Test (TST)

It is commonly employed behavioral model for screening antidepressant-like activity in mice [39]. For the test, the mouse was individually suspended on the edge of a table, 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively, and completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test.

Estimation of MAO-A

After administration of drugs (aqueous extract of the *E. officinalis*, imipramine, fluoxetine, and Phenelzine) for 14 days, mice were sacrificed, and the brain samples were collected immediately on a 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 at 800 rpm for 10 min and the pellets were discarded. The supernatant was then centrifuged at 12,000 rpm for 20 min in the same medium. The precipitate was washed twice more with 100 mL of sucrose tris EDTA and resuspended in 50 mL of the medium [40,41].

MAO activity was assessed spectrophotometrically [42]. The assay mixture contains 4 mM of serotonin as the specific substrate for MAO-A, 250 μL solution of the mitochondrial fraction and 100 mM sodium phosphate buffer (pH 7.4) up to the final volume of 1 mL. The reaction was allowed to proceed at 37°C for 20 minutes and stopped by adding 1 M HCl (200 μL), the reaction product was extracted with 5 mL of butyl acetate, the organic phase was measured at wavelength of 280 nm in a spectrophotometer (Perkin–Elmer). Blank samples were prepared by adding 1 M HCl (200 μL) prior to the reaction and worked subsequently in the same manner.

Estimation of Protein

Total protein was estimated in brain homogenate [43] by using a total protein kit from Crest Biosystems, Goa, India using colorimeter (Photochem, AIMIL).

Measurement of Locomotor Activity

To rule out the effects of various drug treatments on immobility period, horizontal locomotor activities of control and test animals were recorded for a period of 10 min using Photoactometer (INCO, Ambala, India). The difference in the locomotor activity scores were noted before and after the drug treatment.

Experimental Protocols

The animals were divided into 27 groups and each group comprised a minimum of 6–10 mice.

Investigation of Antidepressant-Like Activity Using Behavioral Models

Groups for Forced Swim Test (FST)

Group 1 (n = 10)

Control group: Distilled water was administered orally for 14 consecutive days and 60 min after the administration on 14th day, immobility period was recorded.

Groups 2, 3, 4, 5, and 6 (n = 10 each)

Imipramine (15 mg/kg), fluoxetine (20 mg/kg), phenelzine (20 mg/kg), and aqueous extract (200 and 400 mg/kg) of *E. officinalis* respectively were orally administered for 14 successive days and 60 min after the administration on 14th day, immobility periods were recorded.

Groups for Tail Suspension Test (TST)

Groups 7, 8, 9, 10, 11, and 12 (n = 10 each)

These were same as groups 1–6, except the immobility periods were recorded using TST.

Investigation of Mechanisms of Action by Coadministration of Drugs Modulating Levels of Monoamines and GABA Employing TST

Group 13 (n = 10)

Distilled water was administered orally for 14 consecutive days and after 45 min of vehicle treatment on 14th day; sulpiride

(50 mg/kg, i.p.) was injected. After 45 min of injection, the animals were subjected to TST.

Group 14 (n = 10)

Aqueous extract (200 mg/kg, p.o.) of *E. officinalis* was administered for 14 consecutive days and after 45 min of extract treatment on 14th day, sulpiride (50 mg/kg, i.p.) was injected and 45 min after the injection, the animals were subjected to TST.

Group 15 (n = 10)

Distilled water was administered orally for 14 consecutive days and after 45 min of vehicle treatment on 14th day, baclofen (10 mg/kg, i.p.) was injected and 45 min after the injection, the animals were subjected to TST.

Group 16 (n = 10)

Aqueous extract (200 mg/kg, p.o.) of *E. officinalis* was administered for 14 consecutive days and after 45 min of extract treatment on 14th day, baclofen (10 mg/kg, i.p.) was injected and 45 min after the injection, the animals were subjected to TST.

Group 17 (n = 10)

Distilled water was administered orally for 14 consecutive days and after 45 min of vehicle treatment on 14th day, prazosin (62.5 μg/kg, i.p.) was injected and 45 min after the injection, the animals were subjected to TST.

Group 18 (n = 10)

Aqueous extract (200 mg/kg, p.o.) of *E. officinalis* was administered for 14 consecutive days and after 45 min of extract treatment on 14th day, prazosin (62.5 μg/kg, i.p.) was injected and 45 min after the injection, the animals were subjected to TST.

Group 19 (n = 10)

Distilled water was administered orally for 14 consecutive days. Then, pCPA (100 mg/kg, i.p.) was injected from 11th day to 14th day, 45 min after vehicle administration. On 14th day, 45 min after the injection of pCPA, the animals were subjected to TST.

Group 20 (n = 10)

Aqueous extract (200 mg/kg, p.o.) of *E. officinalis* was administered for 14 consecutive days. Then, pCPA (100 mg/kg, i.p.) was injected from 11th day to 14th day, 45 min after the extract administration. On 14th day, 45 min after the injection of pCPA, the animals were subjected to TST.

Estimation of MAO-A

Groups 21, 22, 23, 24, and 25 (n = 7 each)

Distilled water, Imipramine (15 mg/kg), Fluoxetine (20 mg/kg), Phenelzine (20 mg/kg), and aqueous extract (200 mg/kg) of *E. officinalis*, respectively were administered orally for 14 consecutive days and 60 min after the administration on 14th day, the animals were sacrificed under light ether anesthesia, and immediately brain samples were collected and analyzed for MAO-A.

Measurement of Locomotor Activity

Groups 26 and 27 (n = 6 each)

Distilled water and aqueous extract (200 mg/kg) of *E. officinalis*, respectively were administered orally for 14 successive days and 60 min after the administration on 14th day, locomotor activity was measured.

Statistical Analysis

All the results were expressed as mean ± standard error mean (SEM). The data of all the groups were analyzed by using one-way ANOVA followed by Dunnett's *t*-test using the software SigmaStat 3.5. The data for locomotor activity scores was subjected to Student's unpaired *t*-test. In all the tests, the criterion for statistical significance was $P < 0.05$.

Results

Total Ascorbic Acid in Aqueous Extract of *E. officinalis*

E. officinalis aqueous extract was found to contain 2.94% ascorbic acid.

Effect of Aqueous Extract of E. officinalis *on Immobility Periods in FST and TST*

Aqueous extract (200 mg/kg, p.o.) administered for 14 successive days to mice significantly decreased the immobility periods in both FST and TST, indicating significant antidepressant-like activity. But, the higher dose (400 mg/kg, p.o.) of the extract significantly decreased the immobility periods in TST only. The lower dose (200 mg/kg) of aqueous extract decreased the immobility period to a greater extent than the higher dose, thus showed better antidepressant-like action. Imipramine (15 mg/kg, p.o.), Fluoxetine (20 mg/kg, p.o.), and Phenelzine (20 mg/kg, p.o.) administered for 14 successive days to mice significantly decreased the immobility periods in both FST and TST as compared to control, thus showing significant antidepressant-like action (Tables 1 and 2).

Effect of Combination of Aqueous Extract with Sulpiride, Baclofen, Prazosin, and p-CPA on Immobility Period in TST

Sulpiride (50 mg/kg, i.p.), baclofen (10 mg/kg, i.p.), prazosin (62.5 μg/kg, i.p.), and p-CPA (100 mg/kg, i.p.) significantly increased the immobility period as compared to control group. Pretreatment of animals with sulpiride or baclofen or

Table 1 Effects of the aqueous extract of *E. officinalis*, fluoxetine, imipramine, phenelzine on immobility period of mice using Tail Suspension Test.

Group No.	Treatment for 14 days p.o.	Dose (kg^{-1})	Immobility Period (seconds) Mean ± SEM	*P* values
1.	Vehicle (Distilled Water)	10 mL	177.5 ± 5.28	
2.	Imipramine	15 mg	112.9 ± 6.86[a]	<0.0001
3.	Fluoxetine	20 mg	106.2 ± 6.48[a]	<0.0001
4.	Phenelzine	20 mg	117.2 ± 3.84[a]	<0.0001
5.	Aqueous extract	200 mg	121.3 ± 9.16[a]	<0.0001
6.	Aqueous extract	400 mg	143.9 ± 9.34[a]	0.0058

n = 10 in each group; Data was analyzed by one-way ANOVA followed by Dunnett's *t*-test.
F (5, 53) = 14.559; $P < 0.001$.
*P values as compared with vehicle treated group.

Table 2 Effects of the aqueous extract of *E. officinalis*, fluoxetine, imipramine, phenelzine on immobility period of mice using Forced Swim Test (FST)

Group No.	Treatment for 14 days p.o.	Dose (kg^{-1})	Immobility Period (seconds) Mean ± SEM	*P* values
7.	Vehicle (Distilled Water)	10 mL	161 ± 7.58	
8.	Imipramine	15 mg	105.3 ± 5.53[a]	<0.0001
9.	Fluoxetine	20 mg	89.6 ± 4.33[a]	<0.0001
10.	Phenelzine	20 mg	106 ± 4.7[a]	<0.0001
11.	Aqueous extract	200 mg	128.22 ± 10.2[a]	0.0189
12.	Aqueous extract	400 mg	139.4 ± 11.27	0.1292

n = 10 in each group; Data was analyzed by one-way ANOVA followed by Dunnett's *t*-test.
F (5, 52) = 11.780; $P < 0.001$.
[a]P values as compared with vehicle treated group.

prazosin or p-CPA significantly reversed the decrease in immobility time produced by aqueous extract (200 mg/kg) of *E. officinalis* (Table 3).

Effects of the Aqueous Extract of E. officinalis *on Brain MAO-A Activity*

Aqueous extract (200 mg/kg) administered for 14 consecutive days to mice, significantly reduced the brain MAO-A levels as compared to vehicle treated group. The efficacy of aqueous extract was found to be comparable to that of standard drugs (Table 4).

Effect on Locomotor Activity

Aqueous extract (200 mg/kg, p.o.) of *E. officinalis* administered for 14 successive days did not show any significant change ($P = 0.3258$) in the locomotor function of mice (644.5 ± 15.31) as compared to the vehicle treated group (666.17 ± 14.33).

Discussion

Antidepressant-like activity of *E. officinalis* was evaluated in mice employing FST and TST—two commonly used behavioral despair models. These models are widely employed in rodents to predict antidepressant potential by measuring the decreased immobility period produced by several different classes of antidepressant drugs [35,39]. In this study, aqueous extract (200 mg/kg, p.o.) administered for 14 successive days to mice produced significant antidepressant-like effect in TST as well as in FST. The lower dose (200 mg/kg) of aqueous extract decreased the immobility period to the greater extent than the higher dose (400 mg/kg), thus showed better antidepressant-like action. At higher dose of the extract, there might be saturation of receptors, so maximum effect was achieved at lower dose. There might also be sedative effect at higher dose of the extract, which might be responsible for less decrease in immobility periods as compared to control.

The efficacy of the extract (200 mg/kg) was found to be comparable to imipramine (15 mg/kg, p.o.), fluoxetine (20 mg/kg, p.o.), and phenelzine (20 mg/kg). Aqueous extract (200 mg/kg, p.o.) did not show any significant change in locomotor functions of mice as compared to control, so it did not produce any overt motor effects. This supports the hypothesis that the antidepressant-like effect of the extract is specific and not a false positive. The exact mechanisms by which aqueous extract of *E. officinalis* produces antidepressant-like effect are not completely understood. However, according to our results, the antidepressant-like effect of the extract (200 mg/kg) was significantly reversed by pretreatment of animals with prazosin (a α_1-adrenoceptor antagonist), sulpiride (a selective dopamine D_2-receptor antagonist), p-CPA (a serotonin synthesis inhibitor), and baclofen ($GABA_B$ agonist), when tested in TST. This suggested that the aqueous extract (200 mg/kg) might produce antidepressant-like effect by interaction with α_1-adrenoceptors, dopamine D_2- receptors, serotonergic, and $GABA_B$ receptors, hence increasing the levels of norepinephrine, dopamine and serotonin; and decreasing the levels of GABA in brains of mice. Rodrigues et al. [31] also employed prazosin, sulpiride, and pCPA to elucidate involvement of adrenergic, dopaminergic, and serotonergic systems in antidepressant-like activity of a plant extract. Moreover, aqueous extract reduced the mouse whole brain MAO-A activity as compared to control, so it indicated that this extract inhibited the metabolism of monoamines, particularly serotonin and noradrenaline. Thus, aqueous extract showed antidepressant-like activity probably by selectively inhibiting MAO-A activity.

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 [4]. The CSF concentration of homovanillic acid, the main metabolite of dopamine, is decreased in depressed patients. With regard to the specific action of antidepressants on dopaminergic systems, there is evidence

Table 3 Effect of combination of aqueous extract of *E. officinalis* with sulpiride, baclofen, prazosin, and p-CPA on immobility period of mice in Tail Suspension Test

Group No.	Treatment for 14 days p.o.	Dose (kg^{-1})	Immobility Period (seconds) Mean ± SEM	*P* values as compared to aqueous extract
1.	Vehicle (Distilled water)	10 mL	177.5 ± 5.28	
5.	Aqueous extract	200 mg	121.3 ± 9.16[a]	
13.	Vehicle + Sulpiride	10 mL + 50 mg	228 ± 2.40[a]	
14.	Aqueous extract + Sulpiride	200 mg + 50 mg	153.7 ± 1.73[b]	0.0027
15.	Vehicle + Baclofen	10 mL + 10 mg	200.8 ± 4.88[a]	
16.	Aqueous extract + Baclofen	200 mg + 10 mg	142 ± 1.41[b]	0.0384
17.	Vehicle + Prazosin	10 mL + 62.5 μg	191.9 ± 2.94[a]	
18.	Aqueous extract + Prazosin	200 mg + 62.5 μg	151.9 ± 1.75[b]	0.0041
19.	Vehicle + p-CPA	10 mL + 100 mg	205.5 ± 3.24[a]	
20.	Aqueous extract + p-CPA	200 mg + 100 mg	216.9 ± 3.68[b]	<0.0001

n = 10 in each group; Data was analyzed by one-way ANOVA followed by Dunnett's *t*-test.
F (4, 45) = 22.58; $P < 0.001$ (For vehicle treated groups 1, 13, 15, 17, and 19).
F (4, 45) = 60.72; $P < 0.001$ (For extract treated groups 5, 14, 16, 18, and 20).
[a]$P < 0.05$ when compared with vehicle treated group (1).
[b]$P < 0.05$ when compared with extract treated group (5).

Table 4 Effects of the aqueous extract of *E. officinalis*, fluoxetine, imipramine, phenelzine on MAO-A activity in mouse whole brain

Treatment for 14 days p.o.	Dose (kg^{-1})	MAO activity (nmol/mg protein) (Mean ± S.E.M.)	*P* values
Vehicle treated	10 mL	86.63 ± 8.08	
Imipramine	15 mg	52.60 ± 4.09[a]	0.0027
Fluoxetine	20 mg	51.28 ± 3.42[a]	0.0017
Phenelzine	20 mg	49.82 ± 5.86[a]	0.0031
Aqueous extract	200 mg	53.55 ± 4.17[a]	0.0034

n = 7 in each group; Data was analyzed by one-way ANOVA followed by Dunnett's *t*-test.
F (4, 30) = 8.242; $P < 0.001$.
*P values as compared with vehicle treated group.

that buproprion, amineptine, and nomifensin owe their antidepressant action by increasing central dopaminergic functions [44]. A decrease in $GABA_B$ neurotransmission may contribute to action of antidepressants [33]. Baclofen is $GABA_B$ agonist and it significantly reversed the antidepressant-like effect of the extract (200 mg/kg), so the antidepressant-like effect of the extract might be due to decrease in $GABA_B$ neurotransmission. It is clear from these *P* values mentioned in Table 3, serotoninergic pathway plays most prominent role in the antidepressant-like activity of aqueous extract of *E. officinalis*. Adrenergic and dopaminergic pathways play almost equivalent roles in the antidepressant-like activity of the extract. Role of GABAergic pathways is least prominent in the antidepressant-like activity of the extract. So there is major involvement of monoaminergic system and lesser involvement of GABAergic system in the antidepressant-like activity of the extract.

Reactive oxygen species like hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide, produced during normal cellular metabolic functions, produce oxidative damages in brain [45]. A series of studies performed in humans correlate depressive disorders with oxidative stress either in the brain or blood [46]. Constituents of aqueous extract of the fruits of *E. officinalis* like flavonoids, tannoid principles, and polyphenols have potent antioxidant activity by virtue of their superoxide scavenging, decreasing free radical production, and possessing neuroprotective effects [14,25]. In this study, aqueous extract of *E. officinalis* was found to contain 2.94% of ascorbic acid. It has also been reported that ascorbic acid showed antidepressant-like activity in mice through interaction with the monoaminergic system [32]. So ascorbic acid and other constituents like flavanoids, tannoid principles, and polyphenolic substances present in the aqueous extract of *E. officinalis* might be responsible for its antidepressant-like activity. However, further study is required to identify the particular component(s) present in the aqueous extract responsible for its antidepressant-like activity.

Thus, aqueous extract of *E. officinalis* showed antidepressant-like activity probably by inhibiting MAO-A and GABA; and also due to its antioxidant activity. Therefore, the aqueous extract of *E. officinalis* may have potential therapeutic value for the management of clinical depression.

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Conflict of Interest

The authors have no conflict of interest.

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