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ORIGINAL ARTICLE

Antidepressant Effect of *Hedyotis corymbosa* Extract in Olfactory Bulbectomy Rats

Anil T. Pawar, Gayatri D. Gaikwad, Bhanudas S. Kuchekar

Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Pune, Maharashtra, India

ABSTRACT

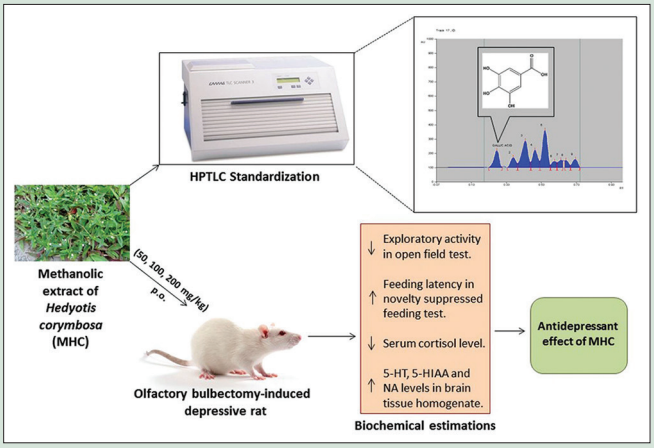
Background: *Hedyotis corymbosa* Linn. (family: *Rubiaceae*) has been used in the Indian indigenous system of medicine, Ayurveda, for treatment of various health aliments. **Objectives:** The present study was undertaken to evaluate the antidepressant activity of methanolic extract of whole plant of *H. corymbosa* (MHC) in olfactory bulbectomy rats. **Materials and Methods:** MHC was prepared and standardized to gallic acid by high-performance thin-layer chromatograph method. Effects of 14 days oral treatment of fluoxetine (30 mg/kg) and three doses of MHC (50, 100, and 200 mg/kg) were evaluated on olfactory bulbectomy (OBX)-induced alterations in behavioral and biochemical parameters in rats. **Results:** MHC treatment reversed the OBX-induced behavioral abnormalities such as increased exploratory activity in open-field paradigm and decreased feeding latency time in novelty-suppressed feeding test. Serum cortisol levels were restored near to normal by the MHC treatment. Further, treatment of MHC prevented the OBX-induced decline of brain levels of serotonin and nor-adrenaline in dose-dependent manner. **Conclusion:** MHC prevents behavioral and neurochemical changes in OBX-induced depression in rats. These results demonstrate the antidepressant effect of *H. corymbosa* and support its folklore claim.

Key words: Cortisol, depression, *Hedyotis corymbosa*, olfactory bulbectomy

SUMMARY

Methanolic extract of *Hedyotis corymbosa* (MHC) were administered orally at the dose of 20, 100 and 200 mg/kg for 14 days to olfactory bulbectomy-induced depressive rats. It was observed that MHC significantly improved behavioral parameters, serum cortisol level and brain monoamines contents. Therefore, it is concluded that the MHC possesses antidepressant activity against OBX-induced depression in rats.

Abbreviations Used: ANOVA: one-way analysis of variance; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; ELIZA: Enzyme-linked immunosorbent assay; HPTLC: High-performance thin-layer chromatography; MHC: Methanolic extract of Hedyotis corymbosa; NA: Noradrenaline; OBX: Olfactory bulbectomy; SEM: Standard error of mean; TLC: Thin layer chromatography; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid.



Correspondence:
Dr. Anil Tukaram Pawar,
Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune - 411 038, Maharashtra, India.
E-mail: anil_pawar31@yahoo.co.in

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INTRODUCTION

Depression is a chronic, recurring and potentially life-threatening illness that affects up to 17% of the world population.[1] Medications such as tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A, and specific serotonin-noradrenaline (NA) reuptake inhibitors are clinically employed for drug therapy. However, these drugs can impose a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder.[2] This indicates the need to develop alternative treatment option for the management of depression.

Medicinal plants are always remained important source of drugs. In the last decade, there is a growing interest in the therapeutic effects of plant-based products on mental disorders. Some medicinal plants and proprietary composite herbal formulations are reported to be effective in the experimentally-induced depression.[3] In Indian indigenous system of medicine, Ayurveda, several plants including *Hedyotis corymbosa* are claimed to be useful for the management of various health aliments including depression.[4] *H. corymbosa* Linn. (Synonym: *Oldenlandia corymbosa* Linn), a flowering plant in the family of *Rubiaceae*, is frequently found throughout India, Sri Lanka, Tropical East Asia to Java, and Philippines. The plant is reported to contain carbohydrates, proteins, phenols, tannins, flavonoids, saponins, steroids, terpenoids, and glycosides.[4] The plant been extensively studied for its various biological activities and therapeutic potentials such as hepatoprotective, antimalarial, antimicrobial, antioxidant, anticancer, antiulcer, and analgesic effects.[4] However, the rationale behind its usefulness in depression has not been established yet through the systematic pharmacological study. Therefore, the present study was planned to evaluate the effect of *H. corymbosa* extract in olfactory bulbectomy (OBX)-induced depression in rats.

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MATERIALS AND METHODS

Animals

Sprague–Dawley rats (male; body weight – 250–275 g) were used for this study. They were procured from National Institute of Biosciences, Pune, India. The animals were allowed for acclimatization for 10 days under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals. The animals were given standard diet supplied by Nutrivet Life Sciences, Pune. The animals were fed regularly and water *ad libitum*. The study protocols were approved by the Institutional Animal Ethics Committee (Ref. No.: MIP/IAEC/2012-13/M2/Appr/004).

Chemicals and apparatus

Fluoxetine hydrochloride and dopamine were purchased from Sigma-Aldrich, USA. Ketamine and anesthetic ether marketed by Research Lab, Mumbai, India, were used for the study. All other chemicals and reagents used are of analytical grade and procured from approved vendors. Apparatus, such as the metabolic cages (Biomedical Instruments, India), Cold centrifuge (BioEra Life Sciences Pvt., Ltd., Pune, RMC–260604), UV-spectrophotometer (CARY 100 Scan, EL08053091), and high-performance thin-layer chromatograph (HPTLC) (CAMAG TLC Scanner 3), was used in the study.

Collection of the plant material

The plant sample of *H. corymbosa* was collected from local region of Pune, Maharashtra, India in August 2012. It was authenticated by Dr. J. Jayanthi, Botanical Survey of India, Pune, Maharashtra, India (Voucher specimen: 2012-428).

Extraction of plant materials

The collected plant material was cleaned under tap water and dried under shade. The dried plant material was coarsely powdered and packed in to soxhlet column and extracted with 70% v/v methanol in water at 65°C–70°C for 22 h. The obtained methanolic extract of *H. corymbosa* (MHC) was evaporated at 45°C and then dried in vacuum oven. The dried extract was stored in airtight container.

Standardization of methanolic extract of *Hedyotis corymbosa* by high-performance thin-layer chromatograph

The MHC was standardized for the content of marker compound, gallic acid by HPTLC. Gallic acid and MHC were dissolved in methanol for the analysis. The sample solutions were applied on prewashed and activated precoated silica gel aluminium HPTLC plate $60F_{254}$ (20 cm × 10 cm with 250 µm thickness) in the form of band of 6 mm width with a Camag syringe (100 µl) using a Camag Linomat V sample applicator. HPTLC plates were then developed for 8 cm with 20 ml mobile phase consisting of toluene: ethyl acetate: Formic acid (4.7:3:0.3 v/v/v). Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber saturated with the solvent system. The chamber saturation time for solvent system was 15 min at room temperature 25 ± 2°C and relative humidity of 60% ± 5%. After chromatography, plates were dried in an air current. Densitometric scanning was performed using Camag TLC scanner III with winCATS software version 1.4.4 (Camag, Muttenz, Switzerland) at 278 nm.

Preliminary phytochemical analysis of methanolic extract of *Hedyotis corymbosa*

The MHC was subjected to qualitative analysis to detect the presence of different classes of phytoconstituents such as carbohydrates, proteins, amino acids, fats and oils, steroids, volatile oils, glycosides, saponins, flavonoids, alkaloids, tannins, and phenolic compounds by standard methods.[5] The qualitative results are expressed as (+) for the presence and (−) for the absence of phytoconstituents.

Antidepressant effect of methanolic extract of *Hedyotis corymbosa* in olfactory bulbectomy rat model

The three dose levels (50, 100, and 200 mg/kg) were used for the evaluation of antidepressant effect of MHC in this study.[6]

Bilateral olfactory bulbectomy surgery

The depression-like state was induced in rats by bilateral OBX surgery according to earlier reported method.[7] Briefly, male rat was anesthetized with ketamine (80 mg/kg, i.p.)[7] and placed in stereotaxic frame. Head of the rat was shaven and midline scalp sagittal incision (1 cm) was made and bilateral burr holes (2 mm diameter) were drilled 8 mm anterior from bregma and 2 mm lateral from the midline. Both main and accessory olfactory bulbs were aspirated through both burr holes using a blunt hypodermic needle attached to water pump without damaging frontal cortex. The burr holes were then plugged with a hemostatic sponge to control bleeding. All operated rats were administered with ibuprofen (30 mg/kg, p.o.)[8] on the day of surgery immediately after recovery from anesthesia, and this dose was repeated after 24 h. In addition, microbicidal powder (Povidone-Iodine powder 5% w/w) was applied topically on the wounds.[8] The rats were single-housed in their home cages and allowed to recover for 14 days.

Treatment schedule in olfactory bulbectomized rats

After recovery period of 14 days, animals were divided into six groups of six rats each and administered with drug treatment as follows: Group I was sham control (had surgery but no OBX) and was administered vehicle, i.e., 0.5% w/v gum acacia solution (5 ml/kg, p.o.). Group II was OBX-control and received 0.5% w/v gum acacia solution (5 ml/kg, p.o.). Group III was standard drug-treated control and was administered with fluoxetine (30 mg/kg, p.o.).[7] Group IV, V and VI were MHC-treated OBX rats and were administered with MHC at the dose of 50, 100, and 200 mg/kg, respectively. The MHC and standard drug were suspended in distilled water using 0.5% w/v gum acacia solution (5 ml/kg body weight) and given through oral route once a day at 8 a.m. for 14 days.

Behavioral assessment

One hour after last dose of treatment, animals were subjected to the behavioral tests. These tests were performed during the light cycle in the sequence of open-field test and novelty-suppressed feeding by keeping 30 min time interval between tests.

Open-field test

Open-field test was performed as per reported procedure.[9] The apparatus was consisted of a wooden box measuring 50 cm × 50 cm × 50 cm with white floor and luminescent walls. The animals were placed in the center of the apparatus and observed for the period of 5 min. Total distance traveled, time spent in the center, and numbers of rearings were evaluated by observer who was blind to the treatments.

Novelty-suppressed feeding

The novelty-suppressed feeding test was performed after 24 h of food deprivation (water available *ad libitum*) as per reported procedure.[9] The test was done in a dimly lit (30–50 lx) open-field apparatus (50 cm × 50 cm × 50 cm) containing clean wood chip bedding and with a home cage food pellet (2 g) placed on the center. During testing, rats were placed individually in one corner of the apparatus. The latency to begin a feeding episode was recorded by observer who was blind to the treatments.

Measurement of serum cortisol level

Immediately after behavioral tests, rats were anesthetized using diethyl ether and blood was collected through retro-orbital puncture. The serum was separated at 10,000 g for 10 min and used for estimation of cortisol using ELIZA kit (No: EIA-1887, DRG Diagnostics, Germany) as per manufacturer's instructions.

Brain monoamine estimation

The animals were sacrificed and brain tissue was isolated. The whole brain tissue was analyzed for levels of NA, 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) as per reported procedure.[10]

Statistical analysis

All the results were expressed as mean ± standard error of mean and analyzed with the help of software GraphPad Prism 7 (GraphPad Software, California, USA). The statistical significance between OBX-control group and sham control group was calculated by student's *t*-test, whereas one-way analysis of variance followed by Dunnett's comparison test was used to calculate statistical significance between drug-treated groups and OBX-control group. $P < 0.05$ was considered statistically significant.

RESULTS

Extraction and high-performance thin layer chromatograph standardization of methanolic extract of *Hedyotis corymbosa*

The yield of MHC was found to be 6.98% w/w. The HPTLC desitogram of MHC showed nine peaks and peak number 1 corresponding to Rf-0.28 was identified as gallic acid by comparing with HPTLC densitogram of reference standard of gallic showing peak at Rf-0.29 [Figure 1]. Based on peak area, percent content of gallic acid in MHC was found to be 0.02% w/w.

Preliminary phytochemical analysis

It revealed prominent presence of carbohydrates, proteins, steroids, saponins, flavonoids, tannins, and phenolic compounds [Table 1].

Effect on open-field activity in olfactory bulbectomy-rats

OBX induced a characteristic hyperactivity during open-field test in rats. The total distance traveled by sham control rats was 4.93 ± 0.67 m, which was significantly ($P < 0.001$) increased to 18.83 ± 1.40 m in the OBX-control rats [Table 2]. OBX-control rats also showed significant ($P < 0.001$) increase in the number of rearings from 7.50 ± 0.67 to 24.00 ± 1.65 [Table 2]. This increased in total distance traveled and number of rearings was significantly decreased in fluoxetine and MHC-treated rats dose dependently [Table 2]. Higher two doses of MHC (100 and 200 mg/kg) were found to be equipotent ($P < 0.001$) to the reference standard fluoxetine (30 mg/kg).

The total time spent by rats in the center of open-field apparatus was also significantly decreased ($P < 0.001$) from 7.83 ± 0.60 s to 3.00 ± 0.37 s in the OBX-control rats as compared to sham control rats [Table 2]. This decreased time spent by rats in the center was significantly increased in the fluoxetine and MHC-treated rats in dose-dependent manner [Table 2]. Higher two doses of MHC (100 and 200 mg/kg) were found to be equipotent ($P < 0.01$) to the reference standard fluoxetine (30 mg/kg).

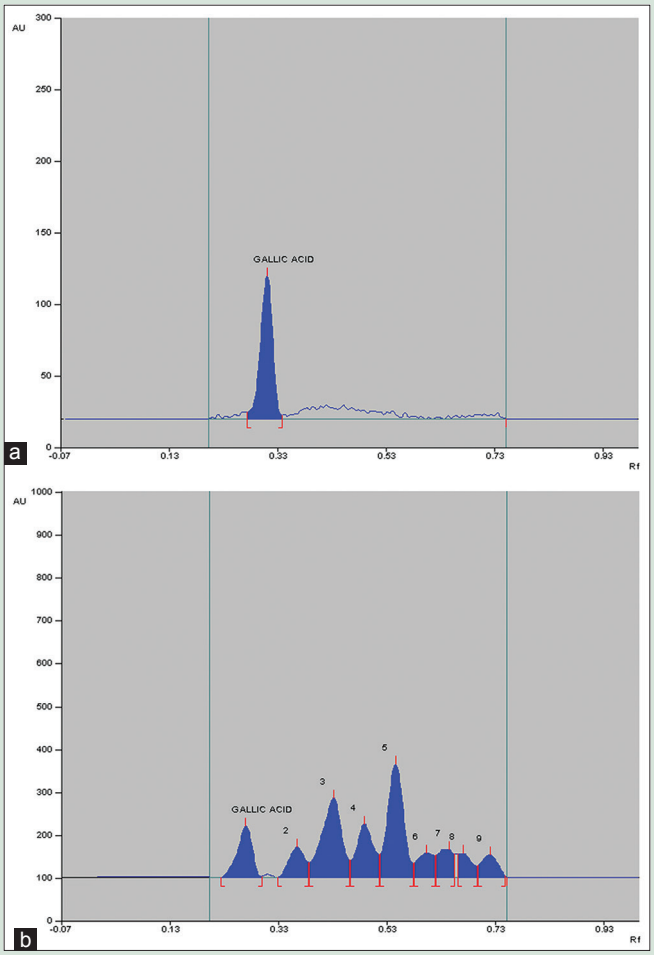

Figure 1: High-performance thin layer chromatograph densitogram of (a) gallic acid and (b) methanolic extract of *Hedyotis corymbosa* standardized to gallic acid (peak no. 1) scanned at 278 nm (AT COLUMN WIDTH)

Table 1: Phytochemical screening of methanolic extract of *Hedyotis corymbosa*

Phytochemicals	Test	MHC
Carbohydrates	Molisch's test	++
Protein	Biuret test	+
Amino acids	Ninhydrin reagent	-
Fats and oils	Spot test	-
Steroids	Salkowski reaction	++
Volatile oils	Stain test	-
Cardiac glycosides	Killer-Killiani test	-
Anthraquinone glycosides	Modified Borntrager's reagent	-
Cyanogenetic glycosides	Sodium picrate test	-
Coumarin glycosides	Fluorescence test	-
Saponins	Foam test	++
Flavonoids	Shinoda test	+++
Alkaloids	Mayer's test	-
Tannins and phenolic compounds	Test with ferric chloride solution	+

+: Slightly present; ++: Moderately present; +++: Highly present; –: Absent; MHC: Methanolic extract of *Hedytois corymbosa*

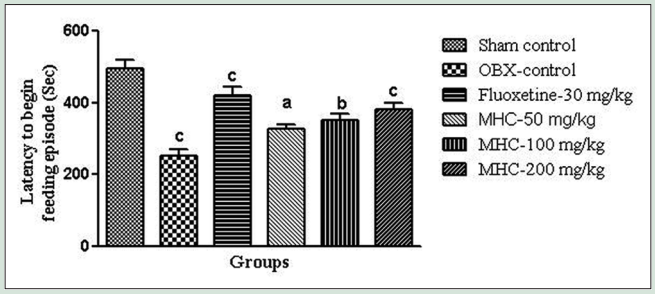

Figure 2: Effect of methanolic extract of *Hedyotis corymbosa* on novelty suppressed feeding in olfactory bulbectomy-rats (n = 6), P values: [a]<0.05; [b]<0.01; and [c]<0.001, values of olfactory bulbectomy-control were compared with sham control and those of drug-treated animals with olfactory bulbectomy-control (AT COLUMN WIDTH)

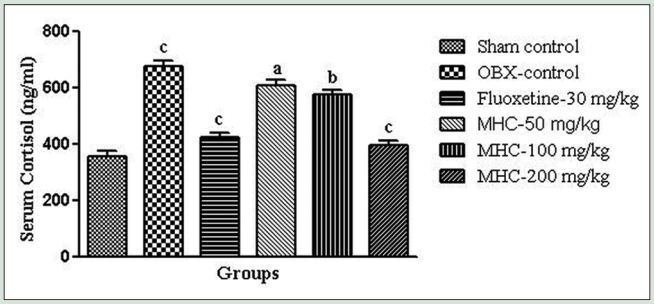

Figure 3: Effect of methanolic extract of *Hedyotis corymbosa* on serum cortisol level in olfactory bulbectomy-rats (n = 6), P values: [a]<0.05; [b]<0.01; [c]<0.001, values of olfactory bulbectomy-control were compared with sham control and those of drug-treated animals with olfactory bulbectomy-control (AT COLUMN WIDTH)

Effect on novelty suppressed feeding in olfactory bulbectomy-rats

OBX caused characteristic behavior, i.e., significant (P < 0.001) decrease in latency to begin feeding episode from 495.83 ± 23.75 s to 253.33 ± 17.50 s during novelty suppressed feeding test [Figure 2]. This decreased latency to feeding was significantly increased by the treatment with fluoxetine (30 mg/kg) and all doses (i.e., 50, 100, and 200 mg/kg) of MHC dose dependently [Figure 2]. The higher dose of MHC (200 mg/kg) was found to be equipotent (P < 0.001) to the reference standard fluoxetine (30 mg/kg).

Effect on serum cortisol level in olfactory bulbectomy-rats

Serum cortisol level of sham control rats was 354.80 ± 20.84 ng/ml. This serum cortisol level was significantly (P < 0.001) increased to 678.29 ± 18.32 ng/ml in the OBX control rats [Figure 3]. Treatment with fluoxetine (30 mg/kg) and all three doses (50, 100, and 200 mg/kg) of MHC significantly attenuated the increased serum cortisol levels as compared to OBX-control rats [Figure 3]. The higher dose of MHC (200 mg/kg) was found to be equipotent (P < 0.001) to the reference standard fluoxetine (30 mg/kg).

Effect on brain monoamine levels in olfactory bulbectomy-rats

OBX caused significant (P < 0.001) decrease in the 5-HT, 5-HIAA, and NA levels in brain tissue homogenate [Table 3]. This decreased brain levels of 5-HT, 5-HIAA, and NA were significantly increased by fluoxetine and MHC treatment. Higher two doses of MHC (100 and 200 mg/kg) were found to be equipotent to the reference standard fluoxetine (30 mg/kg) in increasing brain levels of 5-HT and 5-HIAA [Table 3]. All three doses of MHC (50, 100, and 200 mg/kg) were equipotent to the reference standard fluoxetine (30 mg/kg) in increasing brain NA levels.

Table 2: Effect of methanolic extract of *Hedyotis corymbosa* on behavioral parameters during open-field activity in olfactory bulbectomy-rats

Groups	Total distance traveled (m)	Rearings (*n*)	Time spent in center (s)
Sham control	4.93±0.67	7.50±0.67	7.83±0.60
OBX-control	18.83±1.40[c]	24.00±1.65[c]	3.00±0.37[c]
Fluoxetine-30 mg/kg	7.50±0.72[c]	9.83±0.91[c]	5.83±0.79[b]
MHC-50 mg/kg	13.17±1.62[b]	18.67±1.17[a]	5.17±0.31[a]
MHC-100 mg/kg	10.50±0.85[c]	16.00±1.18[c]	5.50±0.43[b]
MHC-200 mg/kg	8.33±0.56[c]	10.50±1.50[c]	5.67±0.49[b]

Values are expressed as mean±SEM, number of animals (n)=6, P values: [a]<0.05; [b]<0.01; [c]<0.001, values of OBX-control were compared with Sham control and those of drug-treated animals with OBX-control. OBX: Olfactory bulbectomy; MHC: Methanolic extract of *Hedyotis corymbosa*; SEM: Standard error mean

Table 3: Effect of methanolic extract of Hedyotis corymbosa on monoamine levels in the whole brain tissue homogenate of olfactory bulbectomy-rats

Groups	5-HT (ng/g)	5-HIAA (ng/g)	NA (ng/g)
Sham control	284.56±6.49	261.13±4.86	108.70±3.22
OBX-control	57.92±2.11[c]	86.66±2.08[c]	32.53±1.68[c]
Fluoxetine-30 mg/kg	232.83±4.98[c]	235.81±5.80[c]	95.33±2.17[c]
MHC-50 mg/kg	91.14±2.68[b]	108.95±2.31[b]	78.35±2.69[c]
MHC-100 mg/kg	194.47±5.61[c]	149.78±3.55[c]	92.32±2.23[c]
MHC-200 mg/kg	249.45±6.04[c]	219.73±4.42[c]	93.44±2.83[c]

Values are expressed as mean±SEM, number of animals (n)=6, P values: [a]<0.05; [b]<0.01; [c]<0.001, values of OBX-control were compared with sham control and those of drug-treated animals with OBX-control. OBX: Olfactory bulbectomy; MHC: Methanolic extract of *Hedyotis corymbosa*; NA: Noradrenaline; 5-HT: 5-hydroxytryptamine; 5-HIAA: 5-hydroxyindoleacetic acid; SEM: Standard error mean

DISCUSSION

A number of animal models using rodents have been used to induce depression.[11] The most reliable and hence commonly employed method is OBX in rats. Therefore, we evaluated the antidepressant effect of *H. corymbosa* OBX-induced depression in rats. In this animal model, bilateral removal of the olfactory bulbs results in several neurobiological and behavioral deficits that resemble key features of human depression.[12]

The behavioral symptoms of depression such as hyperactivity and exploratory behavior are reported in OBX rats.[9] Increased exploratory activity in open-field paradigm and decreased feeding latency time in novelty-suppressed feeding test in OBX-control rats as compared to sham-control rats found in the present study are indicative of increase in behavioral hyperactivity. These OBX-induced behavioral deficits were significantly reduced by treatment with MHC in a dose-dependent manner. This indicates that MHC causes reversal of the behavior deficits associated with the disease.

OBX in rats is known to associate with the hyperactivity of hypothalamic–pituitary–adrenal (HPA) axis.[13] This causes overexpression of corticotrophin-releasing factor and thereby elevation of blood cortisol level.[14] It is reported that the increased cortisol levels may lead to the behavior alterations including depression-like behavior.[15] In the present study, OBX caused significant increase in the serum

cortisol level. This suggests that the OBX-induced behavioral alteration observed in the present study may be due to the increased level of cortisol. Treatment of OBX rats with MHC decreased this elevation of serum cortisol level indicating prevention of hyperactive HPA axis by MHC in OBX-rats.

The signaling of several neurotransmitter systems such as 5-HT, 5-HIAA, and NA are deregulated in OBX rats.[16] In the present study, decreased levels of 5-HT, 5-HIAA, and NA in brain samples of OBX rats were observed as compared with sham-control rats. This OBX-induced decline of monoamine levels was significantly normalized by the MHC treatment. The effect of MHC on brain monoamine levels in individual brain part is required to be studied further for the detailed information.

Standardization of herbal extract is essential to ensure the herbal medicines of consistent quality and effects.[17] This is commonly performed by determining the content of one or more active constituents and marker compounds in the extract. HPTLC analysis of MHC revealed the presence of gallic acid in the MHC which is reported as an active antidepressant phytoconstitutent by earlier reports.[18,19] The gallic acid content of MHC might be considered as a useful parameter to ensure the quality and effectiveness of MHC.

Previously published studies revealed that the traditional claim, scientific observations, and presence of phytochemicals of herbal medicines have close relationship toward the actual therapeutic outcome.[20] Preliminary phytochemical analysis showed the presence of carbohydrates, proteins, glycosides, flavonoids, tannins, saponins, and phenolic compounds in the MHC. Out of these, flavonoids and saponins of MHC might be responsible for the observed antidepressant effect because earlier scientific studies have revealed that these phytochemicals are chiefly responsible for the antidepressant actions.[21,22]

Overall, this significant improvement in behavioral parameters, serum cortisol level, and brain monoamines contents by MHC in OBX-induced depression model itself suggest wide spectrum of activity of this plant extract covering all phases of pathogenesis. The magnitude of action exerted by plant material is largely governed by prominent presence of phytochemicals and their interaction with each other. The summary of the result is suggestive of antidepressant potential of MHC which might be due to the presence of gallic acid, flavonoids, and saponins.

CONCLUSION

The present investigation supports the use of *H. corymbosa* in folk medicine against depression. It is concluded that the administration of whole plant extract of H. *corymbosa* showed significant antidepressant activity against OBX-induced depression in rats by unknown mechanism(s). The further phytochemical exploration is required to establish the exact mechanism of action.

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Conflicts of interest

There are no conflicts of interest.

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