Antidepressant-like Potential of *Sida tiagii* Bhandari Fruits in Mice

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The present study was undertaken to investigate the effects of different fruit extracts of *Sida tiagii* Bhandari (Family: Malvaceae), popularly known as Kharinti, on depressive behaviors in mice using forced swim test (FST) and tail suspension test (TST). Extracts were prepared by partitioning of 90% alcoholic extract with *n*-hexane (HS) and ethyl acetate (EAS) successively and were administered orally for 20 successive days to separate groups of Swiss young male albino mice. HS showed a dose dependent effect on immobility period while residual ethanolic extract (RES) showed the most potent antidepressant effect at all three doses. Chronic administration of EAS showed a variable effect (ineffective in TST while reversal in FST) at 200 and 500 mg/kg doses. RES significantly reduced the immobility times of mice in both FST and TST, without any significant effect on locomotive activity at all doses (100, 200, 500 mg/kg). The efficacy of RES was found to be comparable to that of imipramine (15 mg/kg p. o.) and fluoxetine (20 mg/kg p. o.). Sulpiride (50 mg/kg i. p.; a selective D_2-receptor antagonist), baclofen [10 mg/kg, i. p., a gamma-aminobutyric acid ($GABA_B$) agonist] and prazosin (62.5 mg/kg i. p.; an α_1-adrenoceptor antagonist) significantly attenuated the extract (RES) induced antidepressant-like effect in TST. The monoamine oxidase inhibiting effect and lipid peroxidation inhibiting effect of *Sida tiagii* (*S. tiagii*) may contribute favorably to the antidepressant-like activity. Thus, it is concluded that *S. tiagii* extract may possess an antidepressant-like effect.

Key words —— Antidepressant, forced swim test, *Sida tiagii*, tail suspension test

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INTRODUCTION

Mental depression is a chronic illness that affects a person's mood, thoughts and physical health, and behavior and may range from a very mild condition, bordering on normality, to severe depression—sometimes called "psychotic depression"—accompanied by hallucinations and delusions. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically norepinephrine, serotonin and dopamine.[1]

In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has progressed constantly worldwide, demonstrating the psychotherapeutic effectiveness of different plant species in a variety of animal models.[2] These studies have provided useful information for the development of new pharmacotherapies from medicinal plants for use in clinical psychiatry. Further, the use of alternative medicines is also increasing day by day throughout the world. Various herbal drugs (*e.g.*, St. John's wort) have shown promising results in treating experimental as well as clinical depression and many of these herbal drugs appear to be safe.[3] 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.

Sida tiagii (*S. tiagii*) Bhandari (*Sida pakistanica*; Family: Malvaceae), a native species of the Indian and Pakistan desert area, popularly known as "Kharenti" in India, is used in folk medicine as a blood purifier, tonic and muscle strengthener.[4,5] Species from the *Sida* genus like *S. cordifolia*, *S. acuta*, *S. rhombhofolia* and *S. spinosa* are traditionally used as/in febrifuge, abortifacient, diuretic, in dysentery, vomiting, gastric disorders, asthma, fever, aches, pains, ulcers, skin disease, diarrhea during pregnancy, rheumatism, neurological modulators and an anti-worm medication.[6] These species have been known to possess properties of/as antidiuretic,[7] tonic, antidiabetic,[8] anti-inflammatory, analgesic,[9] antimalarial,[10] antiplasmodial,[11] antihypertensive[12] and antibacterial[13] and to be neuropsychiatric modulator.[14]

The literature reveals that different extracts of roots and leafs of these species of *Sida* have been reported pharmacologically, but no work has been reported on the fruit which is extensively used in traditional systems of medicine. The present study was undertaken to investigate whether the extracts from

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fruits of *S. tiagii* Bhandari produce behavioral modification in mice. Antidepressive-like effects were assessed in the forced swimming (FST) and tail suspension tests (TST). In addition, mice were treated with extract in combination with different antagonists involved in monoaminergic transmission. We attempted to analyze the mouse brain for monoamine oxidase (MAO)-A and -B activities *ex vivo* after extract treatment.

MATERIALS AND METHODS

Plant Material and Preparation of Extract —— *S. tiagii* Bhandari was collected from a local field in Rajasthan (India) and identified by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), India; a specimen was deposited in the Herbarium, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar (Voucher number PHARM/25/2007). *S. tiagii* Bhandari fruits were dried at 40 ± 1°C, ground into a granulated powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 4 kg of defatted seed powder with ethanol (95%) at 50°C for 72 hr in Soxhlet followed by filtration and concentration in a rotaevaporator at 50 ± 5°C to one third volume. The filtrate was partitioned with *n*-hexane (*n*-hexane extract; HS) and ethyl acetate (ethyl acetate extract; EAS); respective layers were separated and placed in a water bath at 30°C until dry (HS, 32.23 gm, Extract EAS, 26.68 gm). The residual ethanolic fraction (residual ethanolic extract; RES) was dried in a water bath separately (Extract RES; 104.10 gm). All the extracts were stored at temperature below 10°C and were freshly prepared with 2% Tween 80 in water for pharmacological experiments. Extract doses were chosen randomly as 100, 200 and 500 mg/kg body weight of mice.

Phytochemical Analysis —— Freshly prepared organic extracts were tested for the presence of alkaloids, steroids, triterpenoids, glycosides, tannins, flavonoids, carbohydrates, sterol and cardiac glycosides using standard procedures.[15)]

Animals —— Male Swiss mice (weighing 25–30 g, 90 days old) obtained from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University (CCHAU), Hisar, India, were maintained in a room with controlled temperature (21 ± 2°C) on a 12 hr light/dark cycle with free access to food and water. All experiments were conducted between 8 : 00 and 13 : 00 hr. Procedures were approved by the Institutional Animal Ethical Committee, Guru Jambheshwar University of Science and Technology (GJUS&T), Hisar, India. Four sets of mice were used in the study for locomotar activity for forced swim test for TST and for biochemical measurements. Each set of mice was further divided in to groups according to treatment consisting of eight mice in each group.

Drugs and Chemicals —— Fluoxetin hydrochloride (FLU; Cadila Pharmaceuticals, Ahmedabad, India), (±) sulpiride (SUL), prazosin hydrochloride, imipramine hydrochloride (IMI), baclofen (BAC; Sigma-Aldrich, St. Louis, MO, U.S.A.); serotonin EDTA, benzylamine, thiobarbituric acid (Hi Media Laboratories, Mumbai, India), disodium hydrogen phosphate, sodium hydroxide, sucrose (CDH Laboratory, New Delhi, India), sodium lauryl sulphate, acetic acid, chloroform, tris (S.D. Fine Chemicals, Mumbai, India); *n*-butanol, pyridine, hydrochloric acid (Qualigens Fine Chemicals, Mumbai, India) were used in present study. 2% Tween 80 solution in water was used as a vehicle.

Measurements of Locomotor Activity —— Animals were continuously treated with extracts (HS, EAS, RES; 200 mg/kg) orally for 20 days (once a day). Locomotor activity was measured on day 21 with a photocell activity meter for 15 min.

FST —— The FST, described by Porsolt *et al.*[16)] was used as modified by Benvenga and Leander.[17)] The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear plexiglass cylinder (20 cm high × 12 cm diameter) filled to 15 cm depth with water (24 ± 1°C). Two sessions were conducted: an initial 15-min training session (pretest session) followed 24 hr later by a 5-min test session. Following the training session mice were removed from the cylinder, towel dried, and placed under a lamp for 5 min then returned to the home cage for testing the next day. Fresh water was used for each animal to minimize any effect of soiled water from previous mice (possibly containing "alarm pheromones") inducing agitation and decreaseing motility. The mice were treated with different doses of extracts (100, 200 and 500 mg/kg, p. o.; $n = 8$), IMI and FLU (positive control, $n = 8$) and 2% Tween 80 (control group, $n = 8$) for 20 days once a day. In the pre-test session (day 20), every

animal was placed individually into the cylinder for 15 min, 24 hr prior to the 5 min swimming test. During the test session (day 21), behavioral response (immobility time: when the animal made no further attempts to escape, and made only movements to keep its head above the water) was recorded by a trained observer.

TST —— The TST was performed according to the method described by Steru *et al.*[18,19] with modifications. Briefly, mice were suspended by the tail at an elevated height (45 cm from the surface of the table) using adhesive tape in a sound-isolated room. The mice were at least 15 cm apart from each other and a Styrofoam divider was placed between them so that they could not see each other during testing. Mice were considered immobile only when they hung passively and were completely motionless. The duration of immobility was recorded for the last 4 min of the total 6-min observation period. Treatment was followed as describe above and immobility period was measured on day 21.

Biochemical Estimation —— After chronic treatment (20 days) with RES (100 mg/kg), mice were tested for brain MAO and lipid peroxidation level malondialdehyde (MDA) on day 21. They were exposed to the FST as mentioned previously (pretest session 24 hr followed by test session). Immediately after the FST mouse brain was excised followed by biochemical estimation for MAO and MDA.

MAO Assay —— Mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne.[20] MAO activity was assessed spectrophotometrically as described previously.[21] Briefly, the mitochondrial fraction suspended in 9 volume of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose), was mingled at 4°C for 20 min. The mixture was centrifuged at 4000 rpm for 10 min at 4°C, the supernatant was re-centrifuged to deposit the protein, which was resuspended in the same buffer. Protein concentration was estimated by the method of Lowry *et al.*[22] using bovine serum albumin as the standard and adjusted to 1 mg/ml. The assay mixtures contained 4 mM 5-HT or 2 mM β-PEA, specific substrates for MAO-A and MAO-B, respectively, 150 µl of the mitochondrial fraction, and 10 mM sodium phosphate buffer (pH 7.4) up to a final volume of 1 ml. The reaction was allowed to proceed at 37°C for 20 min, and stopped by adding 1 M hydrochloric acid (HCl, 200 µl). The reaction product was extracted with 4 ml of butylacetate (for MAO-A assay) or cyclohexane (for MAO-B assay), respectively. The organic phase was measured at wavelengths of 280 and 242 nm for MAO-A and MAO-B assay with a spectrophotometer. Blank samples were prepared by adding 1 M HCl (200 µl) prior to reaction, and were treated subsequently in the same manner. MAO-A and MAO-B values were expressed as U/g of protein.

MDA Assay —— MDA, an index of free radical generation/lipid peroxidation, was determined by the method described by Ohkawa *et al.*[23] Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5); 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of supernatant of brain homogenate as mentioned under estimation of the MAO-A and MAO-B activities. The mixture was made up to 4.0 ml with distilled water and heated at 95°C for 60 min. After cooling the contents under running tap water, 5.0 ml of *n*-butanol and pyridine (15 : 1 v/v) and 1.0 ml of distilled water were added. The contents were centrifuged at about 3000 rpm for 10 min. The organic layer was separated out and its absorbance was measured at 532 nm using double beam a UV-Visible spectrophotometer (Systronics 2203, Bangalore, India) against a blank having reaction mixture minus supernatant of the brain homogenate. The MDA content was expressed as nmol/mg protein. The protein concentration was estimated by the Lowry method using bovine serum albumin as the standard.[22]

Statistical Analysis —— All results are expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. In all the tests, the criterion for statistical significance was $p < 0.05$.

RESULTS

Preliminary Phyto-chemical Screening

Chemical groups identified in the extracts of *S. tiagii fruits* (Table 1).

Effect on Locomotor Activity

HS, EAS and RES (200 mg/kg, p.o.) administered for 20 successive days did not cause any significant change in the locomotor function of mice as compared to the vehicle treated group on day 21.

Table 1. Phytoconstituents of *S. tiagii* fruit extrats

Extract	Chemical class
Hexane	Alkaloids, Steroids, Triterpenoids
Ethyl acetate	Steroids, Triterpenoids and their Glycosides, Carbohydrates, Tannins, Flavonoids and their Glycosides
RES	Alkaloids, Triterpenoids and Their Glycosides, Carbohydrates, Tannins, Flavonoids and their Glycosides

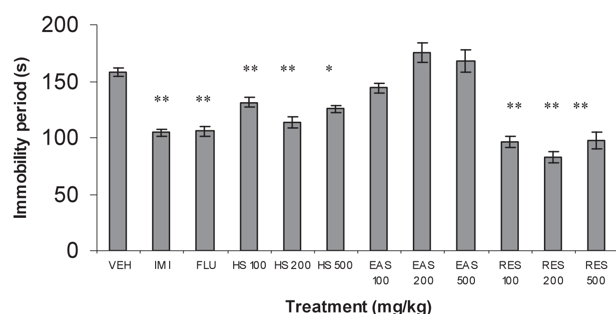


Fig. 1. Effect of *S. tiagii* on Immobility Period of Mice Using TST

Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared to control.

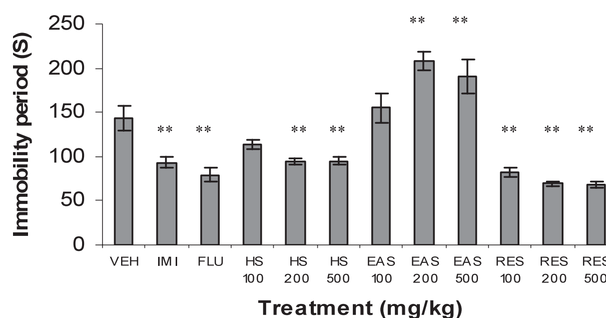


Fig. 2. Effect of *S. tiagii* on Immobility Period in Mice Using FST

Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{**}p < 0.01$ when compared with vehicle treated group.

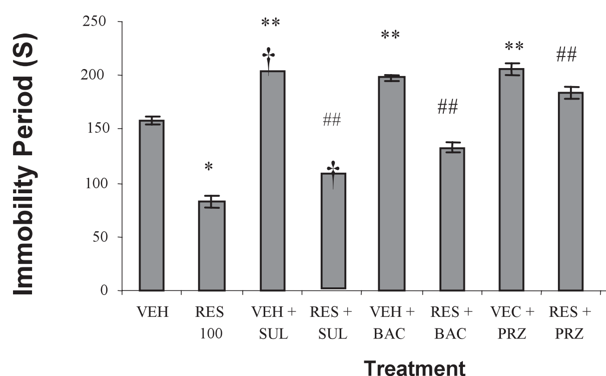


Fig. 3. Effect of Combination of RES with Sulpiride, Baclofen and Prazosin on Immobility Period of Mice in TST

Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{**}p < 0.01$ when compared with vehicle treated group; $^{\#\#}p < 0.01$ when compared with RES treated group.

Effect of Various Extracts of *S. tiagii* on Immobility Periods in TST and FST

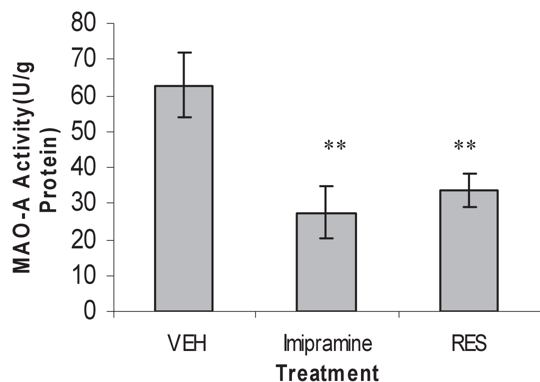
RES and HS (100, 200 and 500 mg/kg, p. o.) administered for 20 successive days to mice significantly decreased the immobility periods in both TST and FST on day 21, indicating significant antidepressant-like activity. HS showed a variable effect on immobility period while RES showed the most potent antidepressant effect all three doses. EAS showed no effect on the immobility period in TST at all three doses while it showed a depressant-like (increase in immobility period compared to control) effect in 200 and 500 mg/kg doses in FST. Imipramine (15 mg/kg, p. o.) and flueoxetine (20 mg/kg, p. o.) administered for 20 successive days to mice significantly decreased the immobility periods in both TST and FST as compared to control, thus showing significant antidepressant-like action (Figs. 1 and 2).

Effect of Combination of RES with Sulpiride, Baclofen and Prazosin on Immobility Period in TST

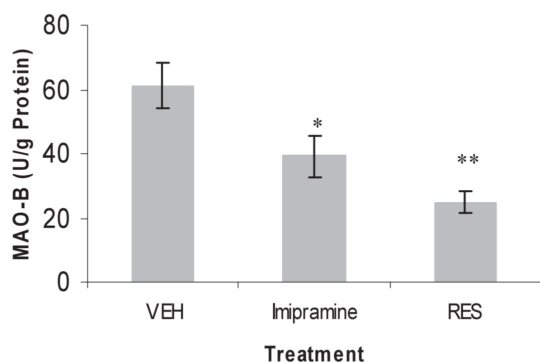
Among all extracts (100, 200 and 500 mg/kg of HS, EAS and RES) RES produced the optimum effect on immobility period in both TST and FST at all doses, hence 100 mg/kg was included for further study as well as for biochemical investigation. Sulpiride (50 mg/kg, i. p.), baclofen (10 mg/kg, i. p.) and prazosin (62.5 μg/kg, i. p.) alone significantly increased the immobility period as compared to the control group. Pretreatment of animals with sulpiride or baclofen or prazosin significantly reversed the effect on immobility time elicited by RES at the dose of 100 mg/kg (Fig. 3).

Effect of RES on Brain MAO and MDA Activities

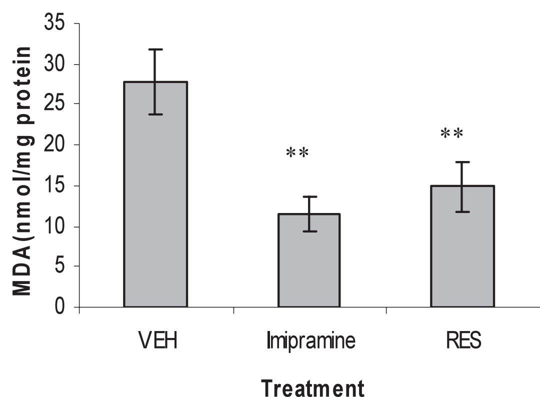
RES (100 mg/kg) administered for 20 consecutive days to mice significantly reduced the brain MAO-A (29.6 ± 3.7 U/g protein), MAO-B (32.9 ± 2.1 U/g protein) levels compared to the respective vehicle treated groups (62.03 ± 9.36 and 59.32 ± 5.22 respectively). The extract showed



(a) Effect of RES on MAO-A activity. Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{**}p < 0.01$ when compared with vehicle treated group.



(b) Effect of RES on MAO-B activity. Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with control group.



(c) Effect of RES on MDA level. Values are mean ± SEM ($n = 8$). Data was analyzed by one-way ANOVA followed by Dunnett's t-test. $^{**}p < 0.01$ when compared with control group.

Fig. 4. Effect of RES on MDA and MAO

more MAO-A inhibiting activity than to MAO-B inhibiting activity as indicated by higher decrease in the former. Lipid peroxidation level (MDA) in brain was found elevated in the control rats after exposure to stress (13.6 ± 1.97 nmol/mg protein to 27.29 ± 2.33 nmol/mg protein). The efficacy of RES towards these parameters was found to be comparable to imipramine (Fig. 4a, b, c).

DISCUSSION

Antidepressant-like activity of *S. tiagii* was evaluated in mice employing the FST and TST—two commonly used behavioral despair models of depression. These models are widely employed in rodents to predict antidepressant potential by decrease of immobility period produced by several different classes of antidepressant drugs.[16, 18, 19, 24–27] In the present study, HS, EAS and RES (100, 200 and 500 mg/kg, p. o.) were administered for 21 successive days to mice and among these RES (all doses) and HS (200 and 500 mg/kg dose) produced a significant antidepressant-like effect in TST as well in FST. RES showed the most potent result with highest decrease in immobility period in both tests. Thus, this extract was selected to investigate the possible underlying mechanisms of antidepressant-like action.

RES at the dose of 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 motor effects. This confirms the assumption that the antidepressant-like effect of the extract is specific and not a false positive. The precise mechanisms by which RES produced the antidepressant-like effect are not completely understood. However, according to our results, this effect of the extract (100 mg/kg) was significantly reversed by pretreatment of animals with prazosin (a α_1-adrenoceptor antagonist), sulpiride (a selective dopamine D_2-receptor antagonist) and baclofen [gamma-aminobutyric acid $(GABA)_B$ agonist], when tested in TST. This suggested that the RES might produce an antidepressant-like effect by interaction with α_1-adrenoceptors, dopamine D_2-receptors and $GABA_B$ receptors, hence increasing the levels of norepinephrine, dopamine and serotonin, and decreasing the levels of GABA in the mouse brain. Levels of monoamines like norepinephrine and serotonin are decreased in depression, so drugs like tricyclic antidepressants and monoamine oxidase inhibitors, which enhance the levels of these monoamines, have been used as antidepressant drugs.[28, 29] The Cerebrospinal Fluid (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 that buproprion, amineptine and nomifensin owed their antidepressant action to increasing central dopaminergic functions.[30] There are two GABA hypotheses of antidepressant action: an increase in $GABA_A$ neurotransmission or a decrease in $GABA_B$ neurotransmission may contribute to this action. Thus, $GABA_B$ receptor antagonism may serve as a basis for the generation of novel antidepressants.[31] Moreover, RES reduced the mouse whole brain MAO-A and MAO-B activities as compared to control, indicating that this extract inhibited the metabolism of monoamines, particularly serotonin and noradrenaline. Within CNS, MAO-A is expressed predominantly in noradrenergic neurons, while MAO-B is expressed in serotonergic and histaminergic neurons. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in CNS. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. MAO-A preferentially metabolizes adrenaline, nor-adrenaline and serotonin, while MAO-B metabolizes phenylethylamines. Dopamine is metabolized by both MAO-A and MAO-B. Experimentally, selective MAO-A inhibitors (clorgyline, moclobemide) have been found more effective in treating major depression than MAO-B inhibitors like selegiline.[32–34] Thus, RES extract showed antidepressant–like activity probably by inhibiting MAO activity. The reactive oxygen species like hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide, produced during normal cellular metabolic functions, produce oxidative damage in brain.[35,36] The pro-oxidant/antioxidant balance is crucial in neurodegenerative processes including cell death, motor neuron disease and neuronal injury.[37,38] The microsomal lipid peroxidation of polyunsaturated fatty acids (PUFA) produced MDA, lipid hydroperoxides and conjugated dienes.[39] The restraint stress reduced the levels of superoxide dismutase (SOD), catalase (CAT) and enhanced lipid peroxidation, thus increasing malondialdehyde.[40] The RES reduced the mouse brain malondialdehyde levels as compared to control, hence th antidepressant-like action of RES might be due to a decrease in lipid peroxidation in stress.

Thus, RES showed antidepressant-like activity probably by inhibiting MAO and lipid peroxidation, increasing the levels of monoamines like noradrenaline, serotonin and dopamine and decreasing the levels of GABA and oxygen free radicals. Therefore, the extract may have potential therapeutic value for the management of depressive disorders. According to results of phytochemical screening and the literature, the antidepressant-like action of HS and RES might be due to the presence of alkaloids, glycosides and flavonoids. HS showed low antidepressant–like potential which might be due to the absence of flavonoids. Further study is required to identify the particular component(s) present in this extract responsible for its antidepressant-like activity.

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