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Research Article

EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF *LAGERSTROEMIA SPECIOSA* LEAVES IN ALBINO WISTAR RATS

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ABSTRACT

Objective: The objective of the study was to evaluate the antidepressant activity of ethanolic extract of dried leaves of *Lagerstroemia speciosa* L. (EELS) on acute restraint stress (ARS)-induced depression-like behavior and biochemical alterations in albino Wistar rats.

Methods: Thirty rats were randomly divided into five experimental groups. Group-I (normal control) rats received normal saline (2.0 ml/kg, p.o.) daily for 14 days; Group-II (stress control) rats received normal saline (2.0 ml/kg, p.o.) daily for 14 days and subjected to restraint stress on the 13th day. Group-III (standard drug-treated) rats received imipramine (15 mg/kg, p.o.) daily for 14 days and subjected to restraint stress on the 13th day. Groups-IV and V rats were treated with EELS (100 mg/kg and 300 mg/kg, p.o.) daily for 14 days subjected to ARS on the 13th day. Stress-like behavior was assessed by subjecting the rats to behavioral paradigms such as tail-suspension test (TST) and open field test (OFT), 40 min post-restraint stress procedure. Pretest of 10 min for forced swim test (FST) was also given to each rat simultaneously. Then, 23.5 h later, the relevant samples were administered and the main test performed 30 min later. Oxidative stress parameters such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and extent of lipid peroxidation (LPO) were analyzed in restraint stress-induced animals and control group, following FST on the 15th day.

Statistical Analysis: Expression of data was done as a mean standard error of the mean. The normally distributed data were subjected to one-way analysis of variance followed by Dunnett's test. *$p<0.05$ was considered statistically significant.

Results: It was observed that *L. speciosa* L. showed a significant dose-dependent decrease in duration of immobility time in TST and FST when compared with the control group in a dose-dependent manner. The results of OFT also showed a dose-dependent increase in locomotor activity. In addition to behavioral tests, EELS also normalized oxidative stress markers such as CAT, SOD, MDA, and LPO in a dose-dependent manner.

Conclusion: The results suggest that the ethanolic extract of *L. speciosa* L. leaves possesses significant antidepressant property, may be recommended as a supplement for the antidepressant activity.

Keywords: *Lagerstroemia speciosa*, Antidepressant, Acute restraint stress, Tail-suspension test, Open filed test, Forced swimming test, Imipramine, Biochemical estimation.

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INTRODUCTION

Depression is a heterogeneous disorder that affects a person's mood, physical health, and behavior. It is caused not only by changing lifestyle as perceived by the general public but also by some of the allopathic drugs, for example, an anti-hypertensive drug, reserpine that depletes neuronal storage granules of norepinephrine (NE), serotonin, and dopamine (DA), cause clinically significant depression in more than 15% of patients. Patients with major depression have symptoms that reflect changes in brain monoamine neurotransmitters, specifically NE, serotonin, and DA. According to a World Health Report, about 450 million people suffer from a mental or behavioral disorder, yet only a small proportion of them receive even the basic treatment [1]. Depression accounts for about 12% of the global burden of disease which is expected to rise to 15% by 2020. The major problems of existing allopathic antidepressant drugs include delayed clinical benefit, serious side effects, and a response rate of <50%. Commonly used drugs for depression are monoamine oxidase (MAO) inhibitors and tricyclic antidepressants (TCAs). They increase the synaptic concentration of at least two of three neurotransmitters, namely, 5-hydroxytryptamine or serotonin (5-HT), NE, and DA. The combined effect of serotonin selective reuptake inhibitor (SSRI) and serotonin reuptake transporter inhibitor increases the synaptic concentration of 5-HT and its duration of action. Ethanolic extracts of most of the herbal drugs are rich in chemical constituents such as phenolic compounds, flavonoids, triterpenoids, and steroids. Ethanolic extracts have less harmful effects, availability, and lower cost of medicinal plants versus synthetic substances make them as outstanding and simple selection in the treatment of nervous diseases. Therefore, identification and validation of plant-derived substances for the treatment of various depressive disorders attract the attention of researchers [2].

Lagerstroemia speciosa L. is also known as the crepe myrtle or Pride of India or Banaba belongs to the family (Lythraceae). *Lagerstroemia* is named for the Swedish merchant, Magnus von Lagerstrom, was honored by Linnaeus, *speciosa* means showy, referring to the flowers varying in color from pink to purple. The tree is native to Southeast Asia and is called Banaba in the Philippines [3]. The flowers and leaves are used to make an herbal tea, as they contain corosolic acid, a chemical that has an insulin-like effect of lowering glucose levels in the body. It is also utilized in weight loss management [4].

METHODS

Collection and authentication of plant

The twigs (leaves, flowers, and fruits) of *L. speciosa* L. were collected from Mahapalika Garden, Bhayandar East, Thane. Sample specimen voucher was submitted to Dr. Rajendra D. Shinde, Director, Blatter Herbarium of St. Xavier's College, Mumbai. The leaves were compared and authenticated by comparison with the Blatter Herbarium Specimen No. JF-1532 of J. Fernandez.

The leaves were washed with tap water and shade dried at normal room temperature with the aid of circulating airflow using a fan, and dried leaves were ground to make a coarse powder.

Preparation of extract

The powdered plant leaves of *L. speciosa* L. were extracted with ethanol in the Soxhlet apparatus. The content of the round bottom flask was emptied in the Petri plate, and the solvent was allowed to evaporate. The extract was evaporated to obtain the dry powder of extract. This crude dry extract was stored in a suitable container and kept in the refrigerator (0–4)°C until use.

Physicochemical analysis of powdered leaves

Physicochemical parameters were carried out on powdered leaves of *L. speciosa* L. for loss on drying, total ash value, acid-insoluble ash value, water-soluble ash value, and determination of extractive values and were investigated as described by the well-established methods [5,6].

Preliminary phytochemical screening of the extract

Preliminary chemical tests were carried out on the ethanolic extract of *L. speciosa* L. for the determination of the presence of phytoconstituents such as alkaloids, flavonoids, cardiac glycosides, saponins, steroids, terpenoids, tannins, and phenolic compounds and were investigated as described by the well-established methods [5,6].

Experimental animals

Female albino Wistar rats (200–300 g) were used for the study. The animals were obtained from SA-FORD, Plot No.: V-10, MIDC, Taloja, Navi Mumbai, Dist. Raigad Maharashtra – 410 208. The use of these animals and the study protocols were approved by CPCSEA recognized the Institutional Animal Ethical Committee (IAEC) of Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400 705 under protocol no. OCP/IAEC/2018-19/08. Rats were kept at the animal house in polypropylene cages (three in each cage), at 22±2°C, with 12:12 h dark: light cycle. They were provided with commercial rat feed and water given *ad libitum*. The animals were allowed to acclimatize for 7 days before the study.

Selection of doses

In the literature survey, it was found that the ethanolic extract of *L. speciosa* L. was safe. LD_{50} of the ethanolic extract is reported to be 3000 mg/kg [7]. The plant is often eaten by animals, which is also an indicator to prove it is less toxic. Thus, for the purpose of the research study, the doses of ethanolic extract of *Lagerstroemia speciosa* (EELS) were finalized 100 mg/kg and 300 mg/kg.

Drugs and chemicals

Imipramine hydrochloride (Abbott, Mumbai, India) was used as reference standards for the antidepressant activity.

Experimental design

Thirty rats were randomly divided into five experimental groups. Group-I (normal control) rats received normal saline (2.0 ml/kg, p.o.) daily for 14 days; Group-II (stress control) rats received normal saline (2.0 ml/kg, p.o.) daily for 14 days and subjected to restraint stress on the 13th day. Group-III (standard drug-treated) rats received imipramine (15 mg/kg, p.o.) daily for 14 days and subjected to restraint stress on the 13th day. Groups-IV and V rats were treated with EELS (100 mg/kg and 300 mg/kg, p.o.) daily for 14 days subjected to acute restraint stress (ARS) on the 13th day.

Depression-like behavior was assessed by subjecting the rats to behavioral paradigms such as tail-suspension test (TST) and open field test (OFT), 40 min post-restraint stress procedure. Pretest of 10 min for forced swim test (FST) was also given to each rat simultaneously. Then, 23.5 h later, the relevant samples were administered and the main test performed 30 min later.

Oxidative stress parameters such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and extent of lipid peroxidation (LPO) were analyzed in restraint stress-induced animals and control group, following FST on the 15th day.

Procedure for ARS

ARS was accomplished by placing rats in an individual plastic rodent restraint device for 12 h. This restrained all physical movements without subjecting the animal to pain. Animals were deprived of food and water during the entire period of exposure to stress. After 12 h, the animals were released from their enclosure and 40 min post-release, the animals were subjected to behavioral tests and then to biochemical estimations. In the normal control group, the rats were kept in the animal cage in the experimental room [8,9].

Behavioral tests

TST

The animals were suspended individually by the end of the tail with micropore adhesive tape (approximately 1 cm) with the head 50 cm from the bottom in a suspension box 40 min post-restraint stress procedure. Rats were suspended for a total of 6 min. During the final 4 min interval of the test, the duration of immobility was recorded. Rats were considered immobile only when they will be hung passively and absolutely motionless. Antidepressant decreases the immobility of the rat in this test [10].

OFT

Open-field apparatus was made as reported, each rat was placed in the center of the open field, and its behavior observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures referred to as total (TL), peripheral (PL), and central locomotion, respectively. The numbers of leanings (one or two paws in contact with the wall), rearing (the mouse standing on its two hind paws without touching the walls), grooming (face cleaning, paw licking, fur licking, head scraping, and rubbing), and defecations were also recorded. At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel [11].

FST

On day 14, all the rats were allowed to swim individually for 10 min for adaptation. Then, 23.5 h later, the relevant samples were administered and the main test performed 30 min later, i.e., on day 15. Rats were forced to swim in a cylinder (diameter 40 cm, height 60 cm) containing

Table 1: Grouping of animals

S. No.	Groups	Test substances	Animal required per group (female albino Wistar rats)	Dose	Total number
1.	Normal control	Normal saline	6	2 ml/kg	6
2.	Stress control	Normal saline	6	2 ml/kg	6
3.	Standard control	Imipramine	6	15 mg/kg	6
4.	Test group 1	EELS	6	100 mg/kg	6
5.	Test group 2	EELS	6	300 mg/kg	6
Total animals required					30

*EELS: Ethanolic extract of *Lagerstroemia speciosa*

30 cm of freshwater maintained at 25°C±1°C. Water in the cylinder was changed after each animal to prevent behavioral alteration among animals due to used water. Each animal showed vigorous movement during the initial 2 min period of the test. Duration of immobility will be manually recorded during the next 4 min of a total 6 min testing period by the observer. Rats were considered to be immobile when they floated in an upright position, making only small movements to keep their heads above the water level. Following the swimming session, rats were dried using a cotton towel and returned to home cages after the experiment. A decrease in the duration of immobility is indicative of antidepressant-like effect, whereas an increase of immobility time, when compared with the control group, is associated with depressive-like effects [12,13].

Biochemical estimation

Preparation of brain tissue homogenate

All the animals were sacrificed by euthanasia using the CO_2 chamber as per rodent euthanasia guidelines, after behavioral observations. The brains were quickly removed, washed in ice-cold sterile isotonic saline, and weighed. A 10% (w/v) tissue homogenates were prepared with 0.1 Mphosphate buffer (pH 7.4). The supernatant was obtained by centrifugation of the homogenate at 1000 rpm for 20 min at 5°C and used for further biochemical estimation [14].

CAT activity

The supernatant (50 µl) was added to a cuvette containing 2.95 ml of 19 mM/L solution of H_2O_2 prepared in potassium phosphate buffer. The change in absorbance was monitored at 240 nm wavelength at the 1 min interval for 3 min. The presence of CAT decomposes H_2O_2 leading to a decrease in absorbance [15].

Sodium oxide dismutase activity

The SOD activity in the supernatant was measured by the method of Misra and Fridovich. The supernatant (500 µl) was added to 0.800 ml of carbonate buffer (100 mM, pH 10.2) and 100 µl of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 s. Parallel blank and standard were run for determination of SOD activity. The reaction mixtures are diluted 1/10 just before taking the readings in a spectrophotometer [16].

Determination of MDA formation

To 1 ml of suspension medium was taken from the 10% tissue homogenate. To 0.5 ml of 30% TCA will be added to it, followed by 0.5 ml of 0.8% thiobarbituric acid (TBA) reagent. The tubes were then be covered with aluminum foil and kept in shaking water bath for 30 min at 80°C. After 30 min, tubes were taken out and kept in ice-cold water for 30 min. These were then be centrifuged at 3000 rpm for 15 min.

The absorbance of the supernatant was read at 540 nm at room temperature against an appropriate blank. Blank consists of 1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA [17].

Determination of LPO assay

To 0.2 ml of the test sample, 0.2 ml of SDS, 1.5 ml of acetic acid, and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60 min. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and agitated smartly. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was scan at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA released/g wet tissue [17].

Statistical analysis

The data obtained from animal experiments were analyzed with InStat Software by GraphPad (version 3.10). It was expressed as mean±standard error of the mean. For statistical analysis, the data were subjected to analysis of variance followed by Dunnett's t-test. Results were considered to be statistically significant at $p \leq 0.05$. Significance levels were as follows:

*Indicates $p \leq 0.05$ as significant; **indicates $p \leq 0.01$ as highly significant; ***indicated $p \leq 0.001$ as very significant.

RESULTS

Physicochemical analysis of powdered leaves

Qualitative phytochemical screening

Antidepression evaluation

TST

Both the doses of the ethanolic extract of leaves of *L. speciosa* L. showed a dose-dependent decrease in immobility time when compared against

Table 2: Result of physicochemical analysis of powdered leaves of *Lagerstroemia speciosa*

Sr. No.	Test	Result (%)
1.	Loss on drying	60.67
2.	Total ash value	16.39
3.	Acid-insoluble ash value	6.24
4.	Water-soluble ash value	7.58
5.	Petroleum ether soluble extractive value	9.6
6.	Chloroform soluble extractive value	6.4
7.	Ethyl acetate soluble extractive value	2.4
8.	Ethanol soluble extractive value	8.8
9.	Water-soluble extractive value	15.2

Table 3: Result of quantitative phytochemical analysis of powdered leaves of *Lagerstroemia speciosa*

S. No.	Phytoconstituents	Test	Ethanolic extract of the leaves *Lagerstroemia speciosa* L.
1.	Carbohydrates	Molisch's test	+ve
		Fehling's test	+ve
2.	Proteins and free amino acids	Ninhydrin test	−ve
3.	Alkaloids	Mayer's test	−ve
4.	Cardiac glycosides	Keller–Kiliani test	+ve
5.	Steroids	Liebermann–Burchard test	+ve
6.	Terpenoids	Salkowski test	+ve
7.	Tannins	5% $FeCl_3$ solution	+ve
8.	Flavonoids	Shinoda test	+ve
9.	Phenols	Lead acetate test	+ve
10.	Saponins	Foam test	−ve

+ve: Present, −ve: Absent