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Evaluation of Antidepressant Activity of *Leucaena Leucocephala* Leaves in Swiss Albino Mice

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KEYWORDS

Depression,

Leucaena

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TST, FST

ABSTRACT:

Introduction: A common psychiatric condition that affects about 5% of the population is depression. Moreover, it is challenging to forecast which patient will react to a particular course of treatment. Many adverse effects, such as heart toxicity, hypopiesia, sexual dysfunction, weight gain, and sleep disorders, can be imposed by the drugs that people are now utilizing. For thousands of years, various plants and herbal remedies have been utilized in traditional medical systems to alleviate depression. In the current investigation, Swiss albino mice were used to test the ethanol extract of *Leucaena leucocephala* leaves for antidepressant-like properties.

Objectives: The purpose pf the study was to assess *Leucaena leucocephala* ethanolic extract's antidepressant potential.

Methods: *Leucaena leucocephala* ethanolic extract's antidepressant properties were examined utilizing the TST and FST. The standard drug was imipramine. Acute Restraint Stress was used to create stress in mice. Imipramine (10 mg/kg) and ethanol extract of the leaves (100, 200 mg/kg) were given for 14 days. In both models, the duration of immobility was documented.

Results: In our investigation, the period of immobility in both experimental models was dramatically shortened by imipramine and EELLL as compared to the animals in the control group. EELLL's antidepressant efficacy was compared to that of imipramine, a common medication. Thus, *Leucaena leucocephala* leaf ethanol extract significantly exhibited antidepressant-like effects in rats.

Conclusions: *Leucaena leucocephala* leaves were extracted using ethanol, phytochemical analysis determines the presence of coumarins, saponins, flavonoids, phenols, tannins, cardiac glycosides, terpenoids, steroids, and amino acids. Comparing the EELLL to ARS, the study indicates that the higher dose of 200 mg/kg of the extract had considerable antidepressant efficacy. It has been demonstrated to raise SOD and catalase levels as well as improve behavioural symptoms.

Introduction

One of the main causes of illness in the world today is depression [1]. Depression is a common but dangerous mood condition that manifests as low energy, difficulty concentrating, gloomy mood. [2]. It turns into a pessimistic prism through which the individual experiences and views the outside world. One experiences severe symptom that make it difficult to think, feel, or perform daily activities like sleeping, eating, or working. For a depression to be recognized, its symptoms must exist for at least two weeks. Over 350 million individuals worldwide, experience depression. Compared to men, women are almost twice as likely to experience serious depressive illness [2]. Thirty percent of those suffering from depression make an attempt at

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suicide. Depression is a result of neurotransmitter imbalance, specifically involving dopamine and serotonin. Depression is one of the mental health conditions that is most readily addressed. Eighty to ninety percent of depressed people who seek therapy eventually get well. The symptoms of depression worsen as people age. The most widely accepted explanation of depression, known as the monoamine theory, states that norepinephrine (NE) and serotonin (5-HT), two monoamine neurotransmitters in the brain, fluctuate in major depressive patients' symptoms [3]. Because of their antioxidant and neuroprotective qualities, phenolic compounds like flavonoids represent a promising class of naturally occurring substances that are significant in neuropharmacology [4]. Resveratrol is a potent antioxidant that has been demonstrated in a study to have antidepressant properties in animal screening models for depression by reversing the production of brain-derived neurotrophic factors (BDNF) in the hippocampus and frontal cortex [5]. The leguminous plant *Leucaena leucocephala* (Lam.) de Wit, sometimes referred to as leucaena, is native to Mexico and Central America. It is a member of the Fabaceae family (sub-family: Mimosoideae) [6].It is also referred to as the wild tamarind, white popinac, jumbay, and white lead tree. It is commonly referred to as kubabul or subabul in India [7]. The tree is useful for many things, including firewood, lumber, greens, feed, and green manure, shade, and soil erosion control [8]. Recently, there has been a particular focus on *Leucaena leucocephala*, a fast-growing shrub or tree that can adapt well to both dry and rainy climates. It also has highly favourable rates of reforestation and treatment of contaminated soils [9]. Leucaena provides so many different ecosystem services and has such strong ecological traits as fast growth, rapid regeneration, the ability to fix nitrogen in the soil, endurance to high temperatures, droughts, and salt [10].Following a phytochemical screening, various substances including flavonoids, saponins, tannins, steroids , amino acids, coumarins, cardiac glycosides, carbohydrates were found in fresh aqueous leaf extracts from *Leucaena leucocephala* [11]. A common allelopathic tree species in the tropics and subtropics is Leucaena leucocephala (Fabaceae). This legume species leaves have been shown to contain phytotoxic allelochemicals such as phenolic substances like gallic acid, p-hydroxycinnamic acid, quercetin, and protocatechuic acid [12]. 0.38% calcium, 2.9% phosphorus, and 23.3% DM crude protein are all present in *L. leucocephala* leaves[13].

Material & methods:

Experimental Animals:

For the investigation, 20–35-gram Swiss Albino mice in good health were employed. The animals obtained from the institute's primary animal facility. The College's Institutional Animal Ethics Committee gave its approval for the study of animal. Mice were placed in polypropylene cages at 22 ± 2°C and 12:12 hours of light and dark cycles at the animal house. They had unlimited access to water and rodent food.

Plant materials and extraction:

Leucaena leucocephala leaves were obtained from the surrounding area, and Mr. Mahesh Atale, MSc. Botany, verified their authenticity. The leaves of *Leucaena leucocephala* were collected in the vicinity. To get rid of all the dirt, the gathered leaves were washed under tap water. Then the leaves naturally dry at room temperature. A coarse powder was created by crushing the dried leaves. Using a Soxhlet extractor, 700 grams of coarse powder were extracted with ethanol over the course of five cycles. An electric water bath was used to concentrate the extract. The resulting thick greenish brown extract was kept dry and moisture-free in a desiccator [14].

Phytochemical Screening:

Fresh aqueous leaf extracts from *Leucaena leucocephala* were subjected to phytochemical screening, which discover the presence of phenols, flavonoids, coumarins, saponins, tannins, cardiac glycosides, steroids, and amino acids. The flavonoids found in leaves, particularly quercetin and naringenin, have antidepressant properties. The class of natural products called flavonoids is used to alleviate depression and anxiety. Reminiscent of an antidepressant, natural flavonoids.

Acute oral toxicity:

The safety of *Leucaena leucocephala's* ethanolic extract was determined by the literature review. It is stated that the ethanolic extract has an LD50 of 2000 mg/kg. Animal consumption of the plant is another indication that it is

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less poisonous. Therefore, the dosages of EELLL were determined to be 100 mg/kg and 200 mg/kg for the purposes of the research investigation [14].

Experimental design:

At random, five groups of thirty mice each were allocated to the experimental setup. The mice in Group II (stress control) were given 1.0 ml/kg of normal saline every day for 14 days, and on the 15th day, they were also subjected to restraint stress. For 14 days, mice in Group I (normal control) were given 1.0 ml/kg of normal saline daily. Imipramine (10 mg/kg) was given daily to Group III (Standard control) mice for duration of 14 days. After receiving EELLL (100 mg/kg or 200 mg/kg) for 14 days, Group IV, V, and the mice were exposed to ARS on the fifteenth day. By putting the mice through behavioural test including the tail suspension test (TST) and the 40-minute post-restraint stress treatment, stress-like behaviour was evaluated in the animals. Every mouse received a simultaneous pretest lasting ten minutes in preparation for the forced swim test (FST). The pertinent samples were then given out 23.5 hours later, and the primary test was conducted 30 minutes after that. After the forced swim test on the fifteenth day, oxidative stress measures like SOD, CAT, and MDA were examined in animals subjected to restraint stress as well as the control group.

Procedure for acute restraint stress:

Mice were placed in individual plastic rodent restraint devices for duration of 12 hours in order to achieve acute restraint stress. This prevented the animal from experiencing any pain while restricting its mobility. For the duration of their stress exposure, the animals were denied access to food and drink. The animals were taken out of their enclosure after 12 hours, and forty minutes later; they were tested for behaviour before being measured biochemically[15].

Behavioural study:

Tail-suspension test (TST):

The TST behavioural model is used to investigate the activity of mice that resembles antidepressants. The Tail suspension was carried out according to Steru et al.'s earlier description. with a few changes [16]. In short, the mice were placed in a dimly lit, acoustically and visually isolated room, with each mouse separately fastened to the edge of the table, 50 cm from the floor, using tape, about 1 cm from the tip of the tail. Each mouse's immobility was manually timed for six minutes, but the final four minutes of immobility were examined and shown. When an animal hung passively and without making any movement at all, it was said to be immobile. The observers documented the immobility of the animals [17].

Forced Swim Test (FST):

The FST, the mostly used behavioural paradigm for evaluating the effectiveness of antidepressants in rodents, was conducted using the methods described by Porsolt et al. with a few minor modifications [18]. In short, mice were raised to swim in a cylinder with a diameter of 15 cm, a height of 25 cm, and a temperature of 250C ± 10C that contained 15 cm of fresh water. To prevent changes in animal behaviour due to water usage, after each animal the water was refilled since mice could not survive at this level of water by touching the sidewalls or bottom of the cylinder with their paws and tails. Every animal moved vigorously during the test's first two minutes. The observers manually recorded duration of immobility for the remaining 4 minutes of the 6-minute testing time. When mice floated erect, moving only slightly to keep their heads above the water, they were thought to be motionless. After their swim, the mice were dried with a towel or a room heater before being put back in their cages. A decrease in immobility is suggestive of an antidepressant-like impact, whereas an increase in immobility time is associated to a depressive-like effect when compared to the control group.

Biochemical analysis:

Preparation of Brain homogenate:

After completing the behavioural study, animals were euthanized using CO2 chamber. The brains were extracted and immediately submerged in ice-cold salted water. The tissue was weight and homogenized using 0.1M Phosphate buffer (pH 7.5). Then centrifuge the sample the supernatant obtained was used for performing CAT, SOD & MDA activity.

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Catalase (CAT):

The Claiborne 1985 method was used to measure the amount of catalase. One ml of supernatant and nine ml of phosphate buffer (pH-7.5) were placed in a cuvette. Freshly made H_2O_2 was added to initiate the reaction. Changes in absorbance at 240 nm were used to determine the rate of H_2O_2 breakdown [19].

Superoxide Dismutase Level (SOD):

The Misra and Fridovich method were used to measure the SOD activity in the supernatant. A mixture of 0.8 ml carbonate buffer of pH 10.2, epinephrine 0.1 ml, and 0.5 ml of the supernatant was added. Each sample's absorbance change was then measured in a spectrophotometer at 480 nm [20].

Malondialdehyde formation (MDA):

1 ml of suspension medium was taken from the 10% tissue homogenate.0.5 ml of 30% TCA will be added to it, followed by 0.5 ml of 0.8% TBA reagent. The tubes were then covered with aluminium foil and kept in shaking water bath for 30 min at 80^0C. After 30 min tubes were taken out and kept in ice-cold water for 30 min. These were then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against appropriate blank. Blank consist of 1 ml distilled water, 0.5 ml of 30% TCA & 0.5 ml of 0.8 % TBA.[20].

Statistical analysis:

Software called GraphPad Prism (version 10.2.2) was used to evaluate the data gathered from the animal tests. To ascertain the statistical significance between the groups, the results presented as Mean ± SEM and subjected to one-way ANOVA and Dunnett's multiple comparison tests. Values in this study are expressed as Mean ± SEM of n=6 mice/treatment, with *p≤ 0.5 denoting significance. Imipramine group and Test group were compared with Acute Restraint Stress group (ARS), while Acute Restraint Stress (ARS)s group was compared with Normal control.

Results:

Phytochemical screening

Table 1. Phytochemical analysis of *Leucaena leucocephala* leaves extract

Sr. No.	**Phytoconstituents**	**Test**	**Inference**	***Leucaena leucocephala* Leaves extract**
1.	Carbohydrates	Molish test	No violet ring at the junction	-ve
		Benedicts test	No color change	-ve
2.	Flavonoids	Lead acetate test	Formation of yellow ppt	+ve
		Shinoda test	Pink or red solution	+ve
3.	Phenols and Tannins	Ferric chloride test	Deep black color	+ve
		Dragondroff reagent test	Rediish brown ppt	+ve
4.	Glycosides	Legal test	Formation of yellow precipitate	+ve
		Keller Kilani test	Formation of Brown ring	+ve
5.	Steroids	Sulphur powder test	Sulphur powder sink at bottom	-ve
6.	Alkaloids	Mayers test	Formation of white precipitate	+ve

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		Wagner's test	Appearance of reddish color	+ve
7.	Saponins	Foam test	Persistent foam	+ve

Antidepressant Evaluation:

Tail suspension test:

The doses of the Ethanolic extract of Leaves of *Leucaena leucocephala* showed a dose dependent decrease in immobility time when compared against stress control (ARS).

Table 2. Effect of EELLL on immobility time of TST in Swiss albino mice.

Sr. No.	Treatment	Duration of Immobility
1.	Normal	148.3± 2.364
2.	ARS	198.7± 3.556***
3.	Imipramine (10mg/kg) +ARS	97.67± 2.376**
4.	EELLL (100mg/kg)+ARS	128.7± 4.672*
5.	EELLL (200mg/kg)+ARS	113.6±3.684*

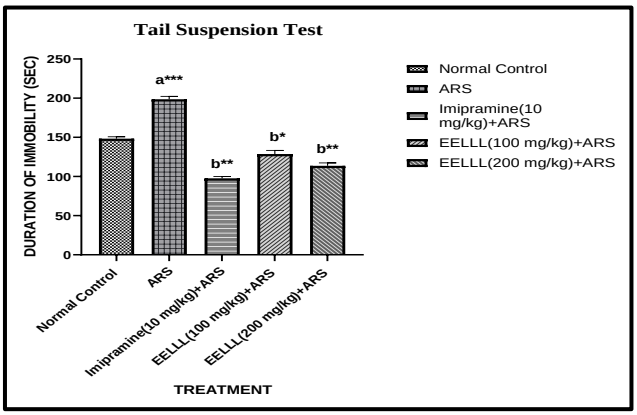

Figure 1. Effect of EELLL on immobility time of TST in Swiss albino mice. [F (4,25) = 128.6 p ≤ 0.5] ARS: Acute restraint stress; EELLL: Ethanolic extract of *Leucaena leucocephala* leaves. a versus normal control and b versus ARS.

Forced swim Test:

The dose of Ethanolic extract of *Leucaena leucocephala* leaves showed a dose dependent decrease in immobility time when compared against stress control (ARS).

Table 3. Effect of EELLL on immobility time of Forced swim test in Swiss albino mice.

Sr.No.	Treatment	Duration of Immobility
1.	Normal	128.4 ± 3.562
2.	ARS	179.4 ± 5.432 ****
3.	Imipramine (10 mg/kg) + ARS	86.30 ± 4.598 ***
4.	EELLL (100 mg/kg) + ARS	118.4 ± 5.982 *
5.	EELLL (200 mg/kg) + ARS	102.6 ± 4.632 **

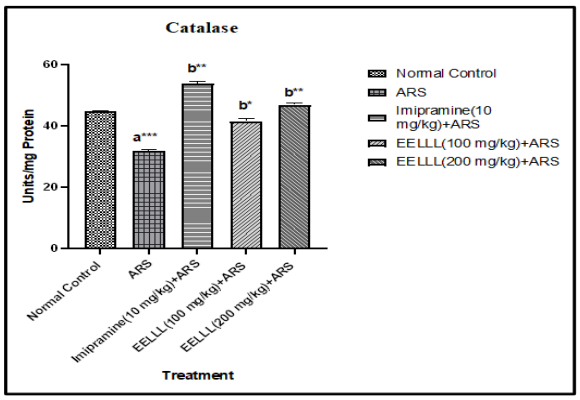

Figure 2. Effect of EELLL on immobility time of FST in Swiss albino mice. [F (4,25) = 51.78 p ≤ 0.5] ARS: Acute restraint stress; EELLL: Ethanolic extract of *Leucaena leucocephala* leaves. a versus normal control and b versus ARS.

Biochemical estimation:

Table 4. Effect of oxidative stress markers in brain.

Sr. No	Treatment	Catalase	SOD	MDA
1	Normal	44.67±0.146	2.9±0.120	0.049±0.004

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2	ARS	31.83± 0.549*	0.98± 0.129***	0.056± 0.005*
3	Imipramine(10mg/kg) +ARS	53.76± 0.654***	1.80± 0.134**	0.021± 0.002***
4	EELLL(100mg/kg) + ARS	41.54± 0.723**	1.2± 0.164*	0.036±0.004***
5	EELLL(200mg/kg) + ARS	46.89± 0.612**	1.5± 0.178*	0.027±0.003**

Catalase:

Evaluation of catalase activity revealed that acute restraint stress significantly decreased in the catalase levels which were significantly normalized by EELLL (100 mg/kg, 200 mg/kg) pre- treatment in a dose dependent manner.

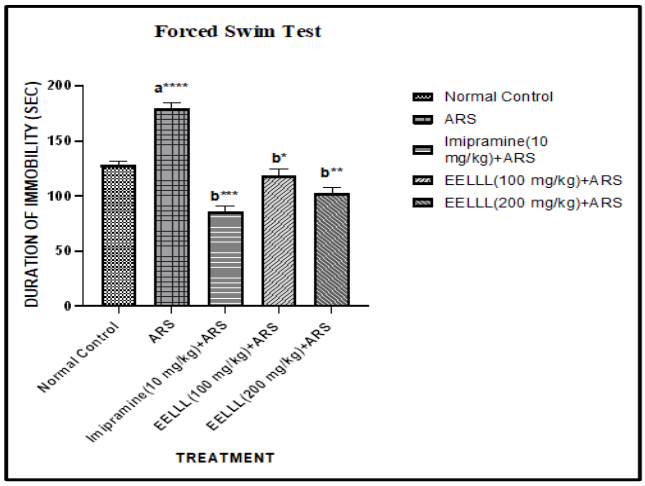

Figure 3. Effect of EELLL pre-treatment on ARS induced changes on catalase activity. [F (4,25) = 195.6 p ≤ 0.5] ARS: Acute restraint stress; EELLL: Ethanolic extract of *Leucaena leucocephala* leaves. a versus normal control and b versus ARS.

SOD:

In the mice pre-treated with EELLL (100 mg/kg, 200 mg/kg) the level of SOD were significantly increase as compared to acute restraint stress mice in dose dependent manner.

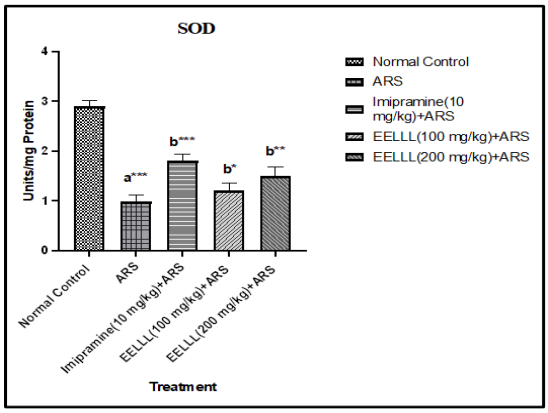

Figure 4. Effect of EELLL pre- treatment on ARS induced changes on Superoxide Dismutase (SOD) activity. [F (4,25) = 26.21 p ≤ 0.5] ARS: Acute restraint stress; EELLL: Ethanolic extract of *Leucaena leucocephala* leaves. a versus normal control and b versus ARS.

MDA:

Acute restraint stress significantly elevated the concentration of MDA levels in the brain which was significantly normalized by EELLL (100 mg/kg, 200 mg/kg) pre-treatment in dose dependent manner.

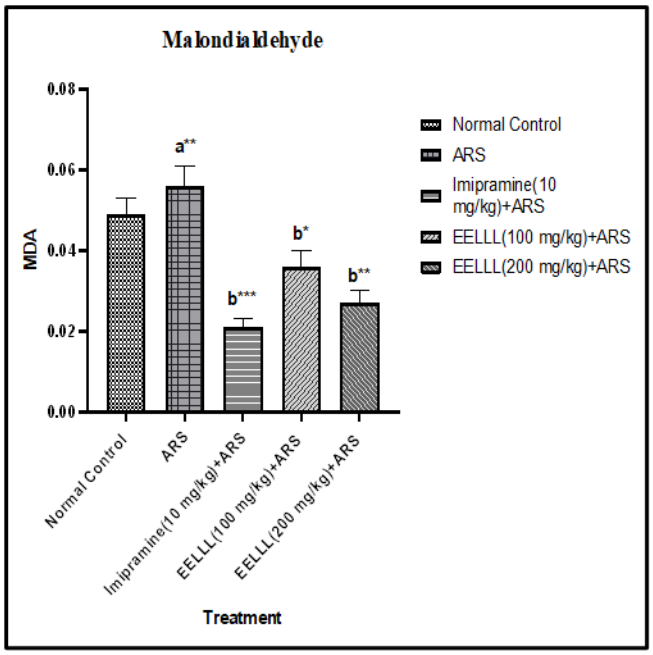

Figure 5. Effect of EELLL pre-treatment on ARS induced changes on Malondialdehyde (MDA) activity. [F (4,25) = 15.34 p ≤ 0.5] ARS: Acute restraint stress;

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EELLL: Ethanolic extract of *Leucaena leucocephala* leaves. a versus normal control and b versus ARS.

Discussion:

We assessed EELLL's antidepressant efficacy in TST and FST in the current study. When compared to ARS, the length of immobility was much shorter in TST and FST. It's analysed that decrease in the duration of immobility is good for antidepressant drugs. Additionally, oxidative stress indicators as SOD, Catalase, and MDA were normalized by EELLL. To comprehend the mode of action and pinpoint the chemical ingredient causing the antidepressant-like effects, more research is necessary.

Conclusion:

Leucaena leucocephala leaves were extracted using ethanol, phytochemical analysis determines the presence of coumarins, saponins, flavonoids, phenols, tannins, cardiac glycosides, terpenoids, steroids, and amino acids. Comparing the EELLL to ARS, the study indicates that the higher dose of 200 mg/kg of the extract had considerable antidepressant efficacy. It has been demonstrated to raise SOD and catalase levels as well as improve behavioural symptoms. The flavonoids in the leaf extract are what give it its antioxidant qualities. In order to comprehend the potential mode of action for antidepressant effect, more thorough research must be done.

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