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EVALUATION OF IN-VIVO ANTIDEPRESSANT ACTIVITY OF SOLID-LIPID NANOPARTICLES OF ALBIZZIA LEBBECK[L]

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ABSTRACT

The extraction of *Albizzia lebbeck* with ethanol were done and the percentage yield was calculated based on the dry weight of the extract.by using these methods to perform the phytochemical investigation of *Albizzia lebbeck* showed in the presence of alkaloids, carbohydrates, proteins, tannins, flavonoids and amino acids. Solid lipid nanoparticles of ethanolic extract *Albizia lebbeck* leaf extract were prepared by Co-Precipitation method. Various Behavioural studies through In-vivo antidepressant activity of the prepared nanoparticles 100mg/kg,200mg/kg,400mg/kg were done by tail suspension model, forced swimming model, open field model, locomotion model. Significant reduction in the depressive behaviour of Swiss albino mice were observed with decreased immobility in tail suspension model, increasing climbing & swimming in forced swimming model, increasing number of lines crossing in open field model and increasing count in locomotion model compared to control (Normal saline). may enhance mood depression-related behaviours, &, after behavioural parameter the Rats were sacrificed for Favourable histopathological findings—such as reduced neuronal damage, less inflammation, or normalization of brain structure in depression-related regions (e.g., hippocampus or prefrontal cortex)—support the antidepressant and neuroprotective effects of the compound and compared with standard drug Imipramine (15mg/ kg B.W). These findings indicate that the formulated solid lipid nanoparticles of *Albizia lebbeck* leaf extract have restored or protected brain tissue integrity. As depression is associated with various biochemical parameters, hence the study was continued for the estimation of oxidative stress, glutathione content, [18.48+_0.57µg/g wet tissue 17,35+*0.72µg/g wet tissue) superoxide dismutase [35.29+_0.63U/ml of protein,34.56+_0.86U/ml]*, catalase 1.39+_0.026*µg/g of protein*,1.26+_0.015*µg/g* protein] acetylcholinesterase. (56.43+_3.25 moles/min/mg tissue,49.57+_3.67 moles/min/mg tissue]

Keywords: *Antidepressant, Forced Swim Test, Tail Suspension test, Histopathology, Behavioural, psychoneurotic disorder.*

INTRODUCTION

Depression is a prevalent and debilitating mood disorder characterized by persistent feelings of sadness, fatigue, guilt, worthlessness, lack of concentration, and loss of interest in activities that were once enjoyable. Individuals with depression also often experience low self-esteem and recurrent thoughts of death. The World Health Organization (WHO) estimates that approximately 280 million people globally suffer from depression, which affects about 5% of the adult population and 5.7% of adults aged 60 and older [1]. A recent comprehensive review and meta-analysis showed a dramatic increase in depression prevalence during the COVID-19 pandemic, with rates rising to 33.7% in the general population, more than six times the pre-pandemic levels [2].

Beyond the emotional and cognitive distress, it causes, depression is also associated with severe disruptions in social interactions and a diminished quality of life. It significantly increases the risk of suicidal thoughts, cardiovascular diseases, and higher morbidity and mortality rates. The pathophysiology of depression remains complex and not fully understood, but several theories suggest that it may involve imbalances in cytokines and neurotrophins, which are critical to brain function and immune system regulation. Treatment for depression includes both psychological therapies, such as cognitive behavioural therapy (CBT), behavioural activation, interpersonal psychotherapy (IPT), and problem-solving therapy, and pharmacological interventions, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and other antidepressant medications [3]. However, despite the availability of these treatments, no single approach is without limitations. Current medications often have delayed therapeutic effects, and side effects such as sexual dysfunction, insomnia, tolerance, and physical dependence are common [4]. These challenges highlight the urgent need for new treatments that offer faster onset and improved efficacy with fewer side effects.

As a result, there is growing interest in exploring alternative treatment options, particularly herbal medicines and natural products, which have been used for centuries in traditional medicine to manage depression. Secondary metabolites from plants, such as polyphenols, alkaloids, terpenes, and flavonoids, have demonstrated potential antidepressant properties. These compounds are thought to work through mechanisms like enhancing serotonin (5-HT) and norepinephrine (NE) levels, increasing brain-derived neurotrophic factor (BDNF), and inhibiting monoamine oxidase (MAO) enzymes, all of which are involved in regulating mood and brain health. One such plant, *Albizia lebbeck* (L.) Benth., has shown promising antidepressant and anxiolytic effects in animal models, yet its clinical use has been limited due to challenges in effective delivery. To overcome these issues, researchers are investigating the use of solid lipid nanoparticles (SLNs) to enhance the bioavailability and therapeutic potential of *Albizia lebbeck*'s active compounds. This study aims to evaluate the antidepressant activity of *Albizia lebbeck* leaf extract in SLN form using predictive animal models of depression, based on its previously reported neuropharmacological properties, including anxiolytic effects and other broad pharmacological activities The findings could help establish *Albizia lebbeck* as a viable alternative or adjunct to current antidepressant treatments.

MATERIALS AND METHODS

Materials

Ethanol, Methanol, Chloroform, Acetone, Polyvinyl alcohol (PVA), Acetic anhydride, Tris-HCl buffer (pH 7.4) solution, Trichloroacetic acid (10%), Sodium phosphate buffer (0.2 M, pH 8.0),

Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Trisodium citrate (1%), Dithiol bis-2-nitrobenzoic acid, Metalloenzyme superoxide dismutase, Nitro blue tetrazolium (NBT) , Tris-buffer buffer, Imipramine, Haematoxylin & Eosin blue. Imipramine hydrochloride was obtained from Sigma Aldrich Chemicals Co. (St. Louis, USA). India. all the solvents and reagents used were of analytical grade.

Methods

Collection and Authentication of plant

Fresh leaves of *Albizia lebbeck* were collected from Tumkur gardens, and were authenticated by Central ayurveda research institute, Bangalore-560109. Ref. RRCBI-1637.

Ethanolic extraction of *Albizzia Lebbeck leaves*

The leaves of *Albizia lebbeck* were washed with double distilled water, shade dried for 7 days and pulverised. The powder was macerated with ethanol (1: 10) for 72 h. The ethanolic extract obtained was filtered followed by evaporation to dryness in a water bath at 60˚C. The yield of the extract was stored in a refrigerator at -4˚C until it was used for further experiment.

Phytochemical investigation

The preliminary phytochemicals tests were carried out for all the extract as per standard methods prescribed by Brain and Turner 1975 and Evans 1996.

Table.1

SL NO	Name of test	Procedure
1.	**Detectionof aloids**	Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids. a) Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids. b) Wagner's test: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.
2.	**Detection of Flavonoids**	a) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids') H2SO4 test: Extracts were treated with few drops of H2SO4. Formation of orange colour indicates the presence of flavonoids.
3.	**Detection of Steroids**	2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H2 SO4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.
4.	**Detection of Terpenoids**	Salkowski's test 0.2g of the extract of the whole plant sample was mixed with 2 ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish-brown coloration of the inner face indicates the presence of terpenoids.
5.	**Detection of Phenols**	a) Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol. b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol
6.	**Detection of Tannins**	A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric

		chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins
7.	**Detection of Carbohydrates**	Extracts were dissolved individually in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates
8.	**Detection of Saponins**	About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins

Preparation of solid lipid Nano-particles

30 mg of stearic acid (SA) was dissolved in a mixture of acetone and ethanol (10 ml each) by applying heat to 60 ºC in a water bath. 10 mg of ethanolic extract was added, stirred for 2 hours and sonicated for 30 min. The mixture was then poured into a 100 ml, cold solution of 1 % polyvinyl alcohol (refrigerated) under mechanical stirring. The solidified product was centrifuged, Ultrasonication at 1000 rpm and washed 3 times with deionized water. The solid lipid nanoparticles of (ASLNs) prepared were collected and used for further characterizations.

Animals

Swiss Albino mice (male) weighing 150-200 g were selected and maintained in the animal house of Sri Adichunchanagiri College of Pharmacy, Madya. The animals were maintained in the house at a temperature of 23±2oC, relative humidity 55±2% and 77 light and dark cycles of 12L: 12D. They were provided with standardized pellet feed and drinking water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) from Sri Adichunchanagiri college of pharmacy All the experimental procedures were carried out in accordance with the guidelines of CPCSEA.

Table.2 Animal grouping

Group	Treatment	No. of Animals
I	Control	6
II	Imipramine (15 mg/kg, p.o.)	6
III	Extract of *Albizia lebbeck* (200 mg/kg, p.o.)	6
IV	ASLNs (100 mg/kg, p.o.)	6
V	ASLNs (200 mg/kg, p.o.)	6
VI	ASLNs (400 mg/kg, p.o.)	6

Models to evaluate the antidepressant activity

Behavioural Test

The 36 male Wistar albino rats (150-200 g) were selected for the study. They were grouped into six different groups (n = 6) and consecutive 21 days treatment period. These rats owned free access to food and water for their life under normative conditions. Rats in Group 1 received a vehicle only in a single dose once for 21 days as a control group, Group-II Imipramine (15 mg/kg, p.o.) were utilized as the standard group. Group-III received extract of *Albizia lebbeck* (200 mg/kg, p.o.) and Group-IV to VI received ASLNs extract with the dose levels of 100, 200 and 400 mg/kg body weight/day displayed in Table 1.

Tail suspension test

The tail suspension test (TST) was performed according to the method described by Stern et al. The principle of this test is that suspending rats suspended upside down leads to characteristic behaviour immobility which resembles human depression. After the administration of the respective drug, rats were suspended on the edge of the table 50 cm above the floor by thread and adhesive tape placed approximately 2 cm from the tip of the tail. Immobility duration was recorded for the last 4 min during 6 min period. Rats were considered immobile when they hanged passively and completely motionless. Assessment of TST was performed on 1, 7th, 14 and 21st day.6

Forced swim test

Antidepressant activity of extract and ASLSs was assessed using a modified Porsolt test. Rats were placed individually in a plastic container (height 40 cm, 30 cm in diameter), which was filled with water to a height of 25 cm. Two swim sessions were conducted. An initial 15-min pre-test followed 24 hours later by a 6 min test. In the pre-test session, the rats which have not yet been treated were forced to swim in a glass cylinder for 15 min. In the second session, each mouse received a respective dose of sample 1 hour prior to test, and placed in the cylinders again for 6 min. The following behaviours were recorded during the last 4 min[7]

a. **Immobility:** Floating in water without swimming.
b. **Climbing**: Active movements of forelimbs on the container wall.
c. **Swimming**: Active movements of extremities and circling in the container.

Open field test

The open field test is widely used to determine the exploratory behaviour and general activity of the rats. The apparatus was composed of a clear plexiglass box (length 40 cm × width 40 cm × height 40 cm) with the floor divided equally into 16 squares. Each rat was placed into the centre of the field and was allowed to freely explore the area. The number of crossing (central and peripheral squares) was evaluated by counting the number the rats moved their positions to stay within a single square with four paw and activities were recorded for 5 min.8

Locomotor activity

The locomotor activity was assessed on naïve pretreated mice using an astrophotometer. operated on photoelectric cells which were connected in circuit with a counter. When the beam of light falling on the photocell was cut off by the animal, a count was recorded. These cutoffs were counted for a period of 10 min and the figure was taken as a measure of the locomotor activity of the animal.9

Histopathological study. The procedures as described by Molina et al. (1990) [18] was effective in inducing stress behaviour, and therefore adopted, with minor modifications in this study. Animals were subjected to stress paradigm once a day for a period of 21 days (D1-D21) as follows: cold swim (12°C, 5 min on days D1, D8, D13, and D19); tail pinch (30s on day D2/ 60s on days D7, and D17/ 90s on day D12); food and water deprivation (24h on days D3, D14 and D21); overnight illumination (on days D4, D9, D15, and D20); no stress (on days D5, D10, and D16); swimming at room temperature (23±2°C, 15 mins on day D6, and D18/ 10 mins on day D11). Rats were decapitated under anaesthesia after the behavioural analysis and their brains were removed. Brain samples were preserved in 10 % buffered neutral formalin solution for histopathological assessment. Brain tissues were processed and embedded in paraffin after fixing. Haematoxylin and Eosin (HE) dyes were used to stain the tissues, and then examined on a Trinocular microscope (Olympus India Pvt. Ltd. Model-CH20iBIMF) fitted with a camera. The photos were analysed for abnormal lesions, including interstitial enema, necrosis, inflammatory cell aggregation, steatosis, fibrosis, haemorrhage, and myocyte degeneration, were graded as none, few, mild, moderate, and severe based on the pathologist's subjective judgment.

Biochemical Experiments

On 21st day, at the end of behavioural study, rats were sacrificed for estimations of various biochemical parameters as follows**: -**

Estimation of oxidative stress

1. **Estimation of glutathione content (GSH)[10]**

Procedure:

- An equal volume of 10% TCA and brain tissue homogenate were taken and centrifuged at 5000 rpm for 10 mins.
- 0.1 ml of brain supernatant was combined with 2.0 ml of 0.6 mM DTNB reagent and 1.9 ml of 0.2 M phosphate buffer.
- Then absorbance was measured at 412 nm against a blank containing TCA (without supernatant).
- The amount of reduced glutathione is expressed as µg/g wet tissue.

Formula for estimation of GS

$$\text{GSH (µg/g wet tissue)} = \frac{(\text{O.D.} - 0.001)}{0.0008}$$

Estimation of superoxide dismutase activity[11]

Procedure:

- 0.1ml of brain supernatant was mixed with 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3) and 0.1 ml of 186 µM phenazine methosulphate, 0.3 ml of 300 µM NBT, and 0.2 ml of 780 µM NADH were added.
- The reaction mixture was then maintained at 30°C for 90 s. Then, 1 ml glacial acetic acid was added and stirred vigorously, followed by shaking with 4 ml n-butanol and centrifuged for 10 mins at 4000 rpm.
- The absorbance of organic layer was measured at 560 nm against blank.
- The blank value was determined using 0.1 ml distilled water that was free of tissue supernatant. At 560 nm, the color intensity was measured.

Formula for the estimation of SOD:

$$\text{SOD (U/ml of protein)} = \frac{\text{Control O.D.} - \text{Experimental O.D.} \times \text{DF}}{\text{Control absorbance/2}} \times \frac{1}{\text{Protein in mg}}$$

O.D. = Optical density
DF = Dilution factor

Estimation of catalase activity[12]

Procedure:

- 1 ml 30mM hydrogen peroxide was added to 0.1 ml brain supernatant with 1.9 ml 50 mM phosphate buffer.
- UV spectrophotometer was used to gauge the changes in absorbance at 240 nm, which led to the determination of the rate of H_2O_2 breakdown.
- A blank value (without supernatant) was obtained by using 0.1 mL distilled water.
- Catalase activity was measured in units of enzyme activity per milligrams of protein.

Formula for the estimation of CAT:

$$\text{CAT (µg/moles of } H_2O_2 \text{ metabolized/mg of protein)} = \frac{\delta \text{ O.D.}}{\text{E} \times \text{volume of sample (ml)} \times \text{mg of protein}}$$

δ O.D. – Change in absorbance/min

E – Extinction coefficient of H_2O_2 (0.071 mmol cm^{-1})

Measurement of acetylcholinesterase activity[13]

Procedure:

- The brain tissue was weighed and homogenized in 0.1 M phosphate buffer (pH 8.0)
- 0.4 ml aliquot of homogenate is added to a cuvette containing 2.6 ml phosphate buffer (0.1 M, pH 8.0) & 100 µl of DTNB.
- The contents in the cuvette are mixed thoroughly and absorbance is measured at 412 nm in a spectrophotometer.
- When absorbance reaches a stable valve, it is recorded as a basal reading.
- 20 µl of the substrate (acetylthiocholine) was added and a change in absorbance was recorded for 10 min at time intervals.
- Change in absorbance per min was noted. The final reading of enzyme activity is expressed as µ moles/min/mg tissue.

The enzyme activity is calculated using the following formula:

$$R= \frac{\text{absorbance/min}}{36. \quad 10^{-4}}$$

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- Change in absorbance per min was noted. The final reading of enzyme activity is expressed as µ moles/min/mg tissue.

The enzyme activity is calculated using the following formula:

$$R= \frac{\text{absorbance/min}}{1.36 \times 10^{-4}}$$

Statistical analysis

The results obtained were subjected to statistical analysis using the SPSS program. The results were subjected to statistical analysis using one way analysis of variance (ANOVA) followed by Dunnet's Post Hoc Test. The difference between groups were considered significant at a level of $P<0.05$ which is considered as statistically significant. The data were expressed as mean ± SEM (n=6).

RESULTS

Extraction of *Albizzia lebbeck* and Phytochemical investigations:

14. 6% yield was obtained with Soxhlet extraction of *Albizzia lebbeck*. Alkaloids, carbohydrates, proteins, tannins, flavonoids and amino acids were detected during the phytochemical investigation. A simple method has been established for the synthesis of SLN employing leaf extract of *Albizzia lebbeck*. different batches of solid nanoparticles were prepared by the *Albizzia* extract, with the different concentration of polymer. They were coded as AL1, AL2, AL3, AL4, AL5, AL6, AL7.

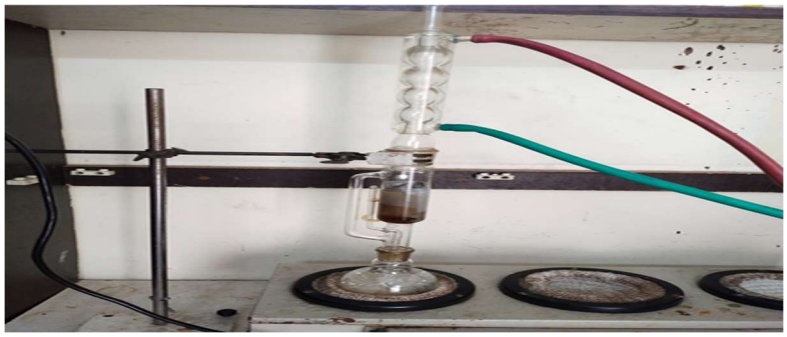


Fig.1 Preformulating studies

An FT-IR spectroscopy study has been carried out separately to check the compatibility between (*Albizia lebbeck*) and the lipids (Stearic acid) used for the preparation of Nanoparticles. The FT-IR was performed for drug, lipids, surfactants and physical mixture of drug and lipids. The spectra recorded by scanning in the wavelength of 500-4000 cm^{-1} Wavelength of 3338.89- N-H Stretch of Amine .1508.38-Aromatic (C=C) Stretch of alkenes.1220.9C-O Stretch vibration of aromatic ketones or carboxyl groups 2922.25-C-H Stretch of Aliphatic groups. The reaction progress for the formation of SLN using *Albizia lebbeck* leaf extract was monitored by visual colour change and UV–Vis spectral scanning.

Evaluation of solid lipid nano particles

The prepared nanoparticles were characterized by UV, FTIR, XRD, Particle size, zeta potential, Polydispersity index, SEM.: The X-ray diffractometer, Cu Kα radiation (λ = 1.5406 Å). Broad, yet sharp peaks in the range of 10°–35°confirmed the semi-crystalline nature of synthesized SLN.

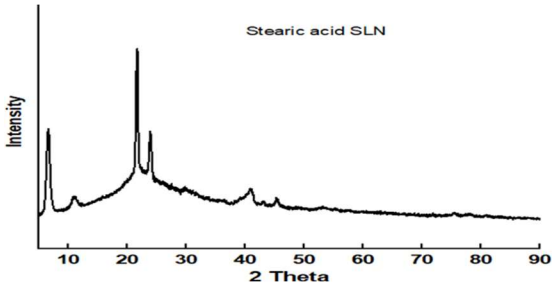


Fig.2 XRD pattern of SLN synthesized with *Albizzia lebbeck* leaf extract

A simple method has been established for the synthesis of SLN employing leaf extract of *Albizzia lebbeck*. The formation of solid-lipid nanoparticles was visually confirmed by SEM studies revealed the micrograms were obtained from JSM-7001F model. Obtained SLN were mounted on carbon tape and the FESEM pattern were analysed. The FESEM images confirm the shape of nano size of synthesized nanoparticles as their uniform distribution Among all the formulations, AL-2 formulation was found to be optimized and shown desired particle size, zeta potential and PDI. AL-2 formulation was further studied for various evaluation parameters. Surface plasmon resonance (SPR) of SLN was characterized by using Ocean Insight HR2000+ High Resolution UV Spectrophotometer. A characteristic peak was obtained at 285 nm. The X-ray diffractometer, Cu Kα radiation (λ = 1.5406 Å). Broad, yet sharp peaks in the range of 10°–35°confirmed the semi-crystalline nature of synthesized SLN The formation of solid-lipid nanoparticles was visually confirmed by SEM studies which revealed the micrograms obtained from JSM-7001F model Obtained SLN were mounted on carbon tape.

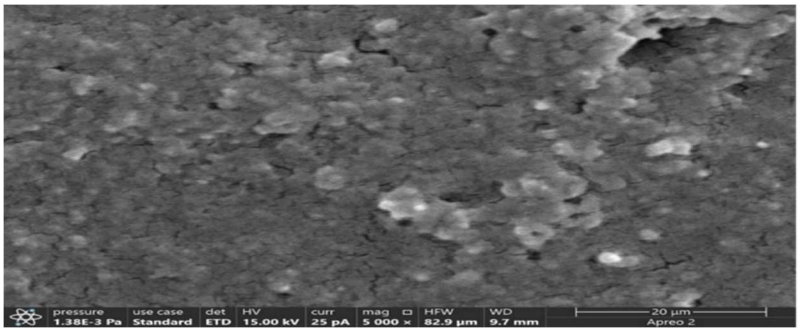


Fig.3 Detection of alkaloids

Behavioural activity

The antidepressant activity of behavioural response has relies upon Administration of normal saline, extract, ASLNs(High dose and low dose) and Imipramine[15 mg/kg p o] Albino Swiss mice groups i] forced swim test ii] Tail suspension test, iii] open field test were shown significant decrease in immobility time in tail suspension model, increasing climbing & swimming in forced swimming model, and increasing number of lines crossing in open field model and increasing count in locomotion model compared to control.

Fig.4 Tail suspension test

Fig.5 Forced swim test in rat model

Fig.6 Open field test in rat model

Fig.7 Locomotor activity in rat model

Table.3 Effect of drugs on tail suspension model

Group	Treatment	Immobility time at weekly interval (Sec)			
		Day 1	**Day 7**	**Day 14**	**Day 21**
I	Control receives vehicle	182.32±6.53	180.76±4.39	184.32±3.82	176.32±4.03
II	Imipramine (15 mg/kg, p.o.)	147.43±5.83**	132.43±5.04***	122.37±4.27***	105.39±3.08***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	173.24±5.54.	165.65±4.77**	153.65±3.85*	148.69±4.15**
IV	ASLNs (100 mg/kg, p.o.)	174.32±5.29.	168.25±4.04**	153.78±4.34**	145.37±3.58**
V	ASLNs (200 mg/kg, p.o.)	171.53±5.22.	163.15±3.97**	145.34±4.37**	132.12±3.14***
VI	ASLNs (400 mg/kg, p.o.)	166.38±5.29*.	152.32±4.78***	137.78±4.05***	118.26±3.76***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

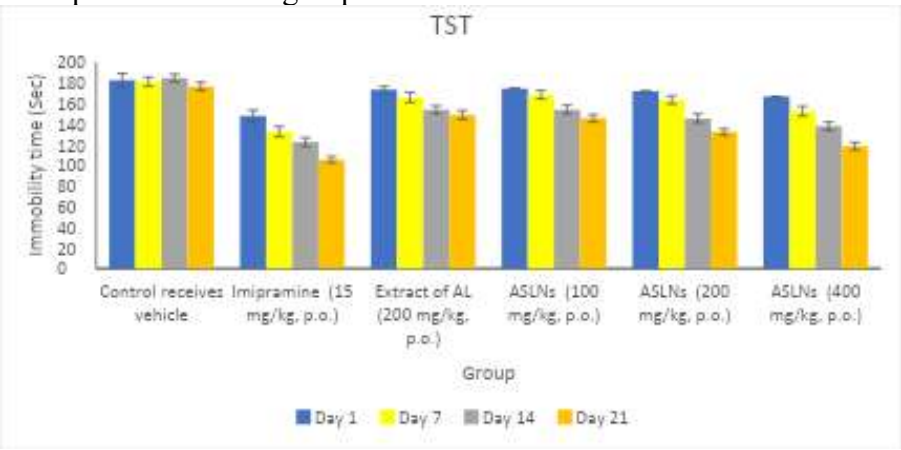


Fig.8 Effect of drugs on tail suspension in rats

Table.4 Effect of drugs on Forced swim test (Immobility)

Group	Treatment	Immobility at weekly interval (Sec)			
		Day 1	Day 7	Day 14	Day 21
I	Control receives vehicle	154.38±4.88	152.27±3.67	145.37±4.12	136.26±2.79
II	Imipramine (15 mg/kg, p.o.)	94.21±3.87***	87.21±3.03***	76.26±3.02***	63.07±2.34***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	120.12±3.02	101.38±3.22**	96.29±2.49**	80.23±2.08**
IV	ASLNs (100 mg/kg, p.o.)	123.53±3.44	105.53±3.58**	98.28±2.27**	82.17±2.25**
V	ASLNs (200 mg/kg, p.o.)	118.45±3.76**	102.67±3.24**	97.47±2.43***	79.42±1.65***
VI	ASLNs (400 mg/kg, p.o.)	113.53±3.44***	98.65±3.24***	92.25±2.65***	74.37±2.56***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

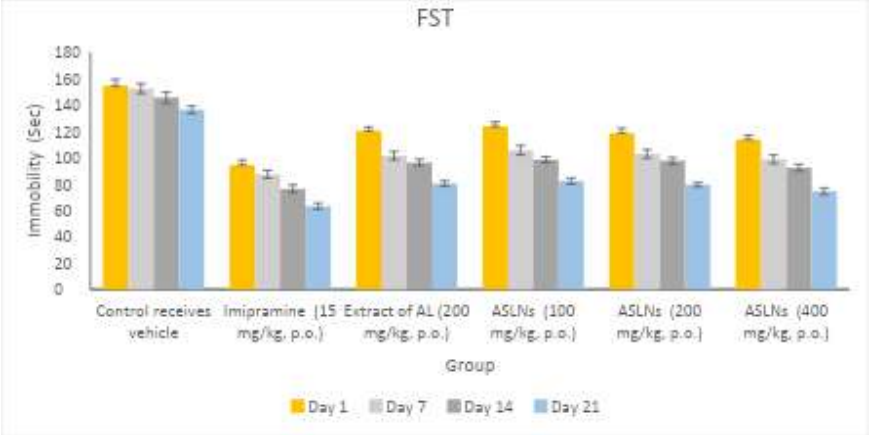


Fig.9 Effect of drugs on Forced swim test (Immobility)

Table.5 Effect of drugs on Forced swim test (Climbing)

Group	Treatment	Climbing at weekly interval (Sec)			
		Day 1	**Day 7**	**Day 14**	**Day 21**
I	Control receives vehicle	21.67±1.34	24.54±1.28	23.37±1.09	25.40±1.53
II	Imipramine (15 mg/kg, p.o.)	32.78±1.02	43.34±1.36**	48.52±1.56***	55.31±1.32***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	27.45±0.79	31.37±0.09	34.65±1.13*	37.05±1.27**
IV	ASLNs (100 mg/kg, p.o.)	28.43±0.88	33.08±0.75	37.32±1.02*	39.05±1.02**
V	ASLNs (200 mg/kg, p.o.)	27.29±1.04	33.19±1.12*	38.31±0.78**	44.05±1.23***
VI	ASLNs (400 mg/kg, p.o.)	28.54±0.97	36.27±1.05*	42.28±1.27***	49.27±1.46***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

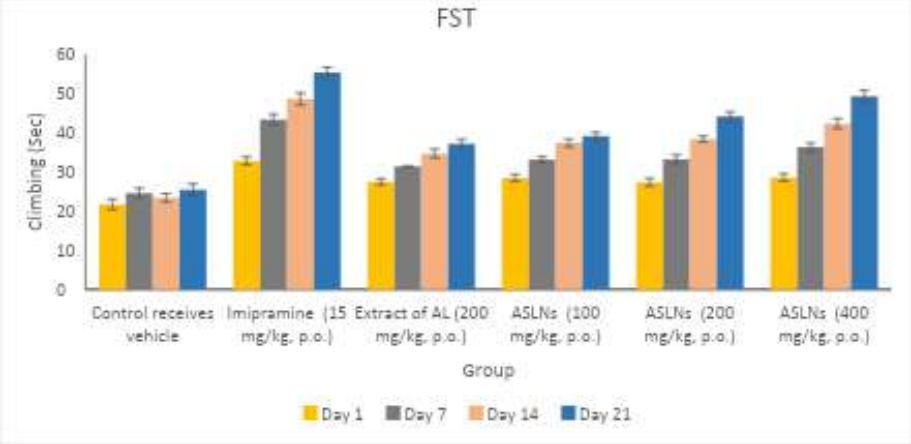


Fig.10 Effect of drugs on Forced swim test (Climbing)

Table.6 Effect of drugs on Forced swim test (Swimming)

Group	Treatment	Swimming at weekly interval (Sec)			
		Day 1	**Day 7**	**Day 14**	**Day 21**
I	Control receives vehicle	63.95±3.24	63.19±3.10	71.26±3.24	78.34±2.77
II	Imipramine	113.01±3.07***	109.45±3.78***	115.22±3.05***	121.62±3.12***

	(15 mg/kg, p.o.)				
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	92.43±2.77**	107.25±2.98**	109.06±2.78**	120.72±3.04***
IV	ASLNs (100 mg/kg, p.o.)	88.04±3.29*	101.39±3.14***	104.4±3.02***	118.78±2.69**
V	ASLNs (200 mg/kg, p.o.)	94.26±2.67**	104.14±2.69***	104.22±2.86***	116.53±2.65***
VI	ASLNs (400 mg/kg, p.o.)	97.93±2.78**	105.08±3.01***	105.47±2.87***	116.36±2.88***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

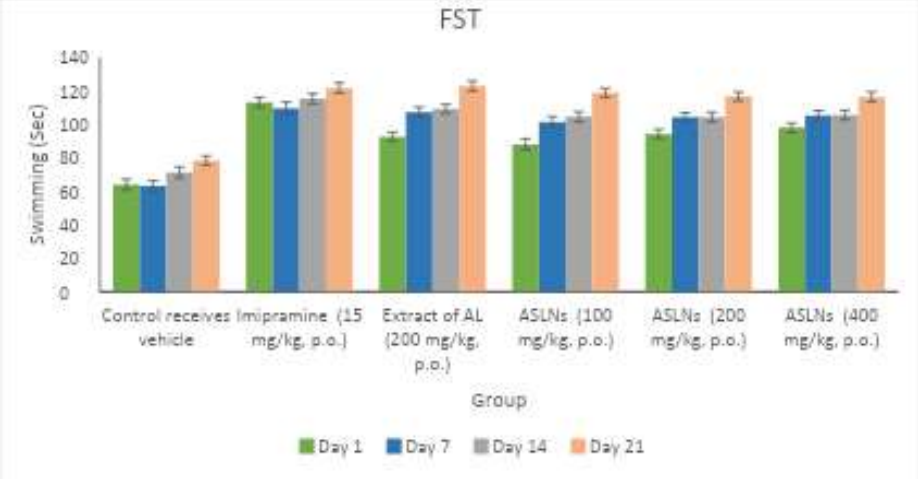


Fig.11 Effect of drugs on Forced swim test (Swimming)

Table.7 Effect of drugs on open field test

Group	Treatment	No. of line crossing at weekly interval (Sec)			
		Day 1	Day 7	Day 14	Day 21
I	Control receives vehicle	84.56±3.54	86.23±3.77	85.29±2.88	87.43±3.32
II	Imipramine (15 mg/kg, p.o.)	103.29±2.45*	108.51±3.65**	115.36±2.56**	118.02±3.67***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	94.06±4.06	96.76±3.35	99.62±3.56*	103.56±3.47*
IV	ASLNs (100 mg/kg, p.o.)	94.34±3.53	95.34±3.72	98.05±3.27	103.23±3.75*
V	ASLNs (200 mg/kg, p.o.)	93.25±4.87	96.46±3.87	103.45±3.02*	105.63±3.28**
VI	ASLNs (400 mg/kg, p.o.)	97.34±3.67	101.35±3.34*	107.76±3.44*	110.54±3.20***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

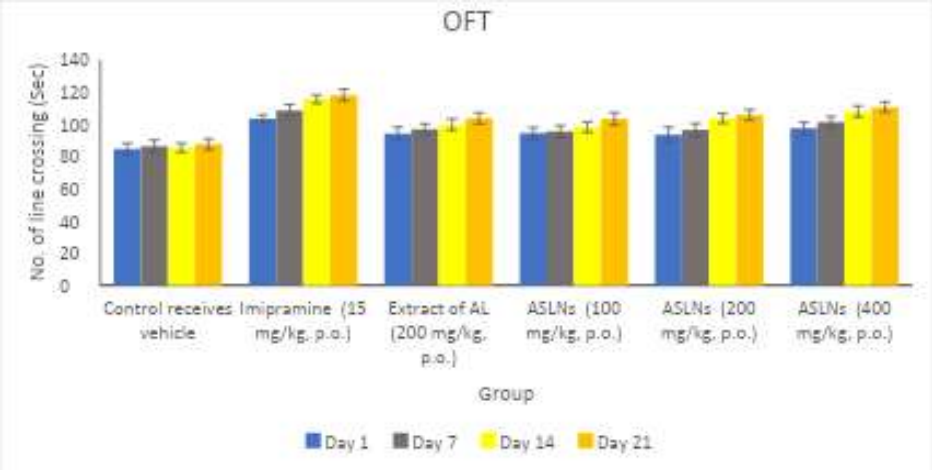


Fig.12 Effect of drugs on open field test

Table.8 Effect of drugs on locomotor activity

Group	Treatment	Count at weekly interval (Sec)			
		Day 1	**Day 7**	**Day 14**	**Day 21**
I	Control receives vehicle	175.35±5.28	169.43±4.27	175.37±3.13	179.26±3.37
II	Imipramine (15 mg/kg, p.o.)	242.03±4.87***	265.34±5.15***	287.35±4.06***	291.58±4.16***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	204.65±5.27**	233.43±4.27***	232.37±3.13***	242.26±3.37***
IV	ASLNs (100 mg/kg, p.o.)	203.35±5.28**	229.43±4.27***	235.37±3.13***	239.26±3.37***
V	ASLNs (200 mg/kg, p.o.)	222.23±4.36***	234.39±3.98***	243.76±3.37***	251.54±3.08***
VI	ASLNs (400 mg/kg, p.o.)	225.47±5.18***	248.15±4.75***	254.36±3.88***	268.56±3.23***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. *$P<0.5$, when compared to control group.

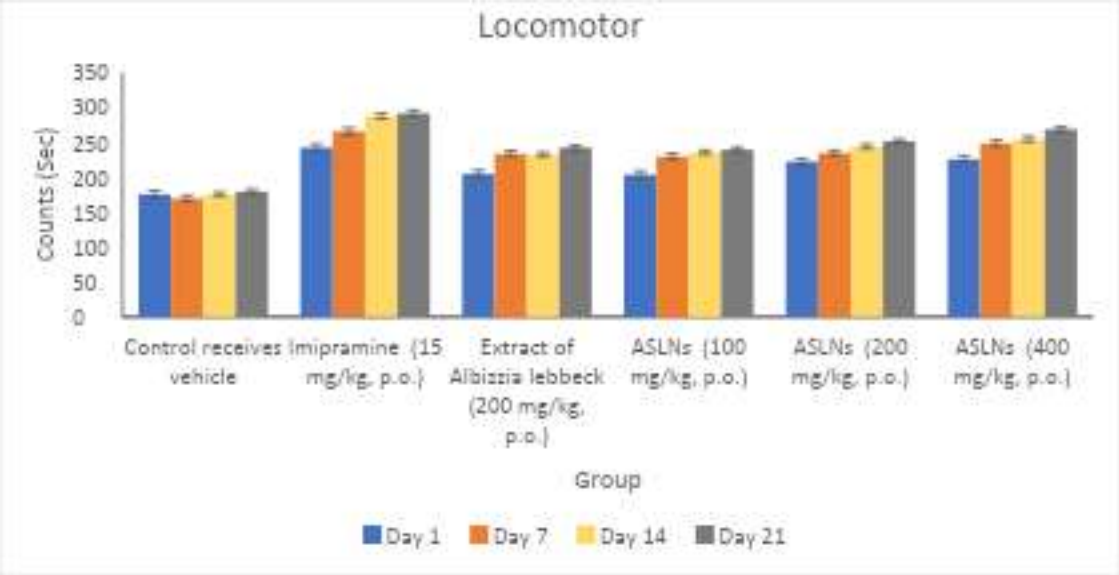
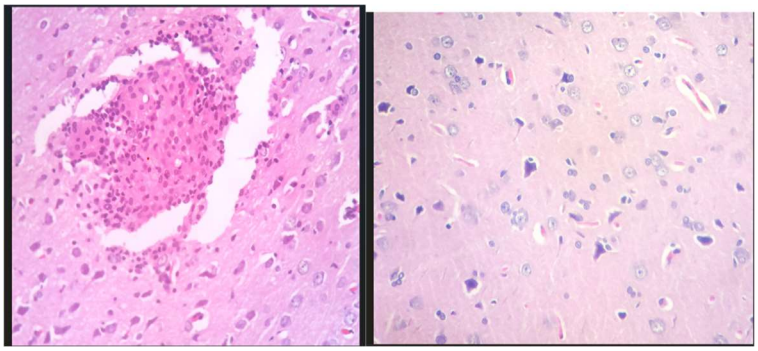


Fig.13 Effect of drugs on locomotor activity

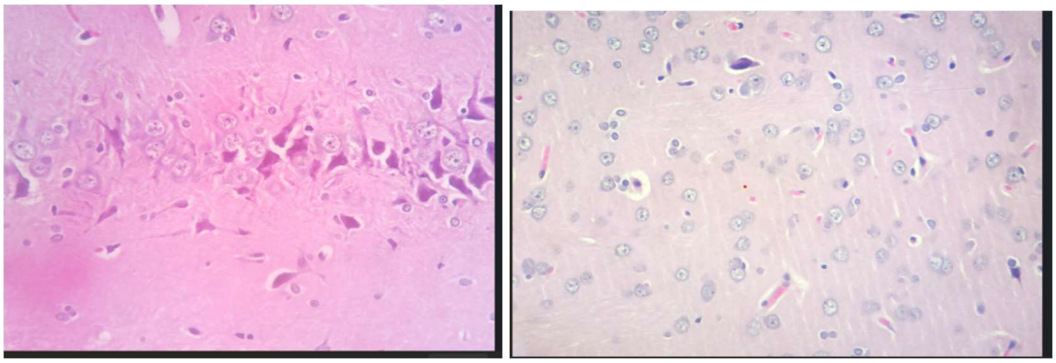
Histopathology: The brain tissue showed that several histopathological manifestations with marked neurodegeneration frontal cortex shows predominantly Group-1[Normal saline]. It is showing that the frontal cortex is predominantly degenerated cells and also moderately inflamed. Group-II[Imipramine] The frontal cortex shows intact pyramidal cells having round to oval vesicular nucleus with abundant cytoplasm, neuroglial cells, granular cells and intact blood vessels. Also seen are degenerated cells, few cells appear degenerated. Nucleus accumbent shows few degenerated cells. Section studied from the Amygdala appears within normal limits. Group-III [Extraction of *Albizzia Lebbeck*]- Section from the frontal cortex shows mild degenerated cells, congested vessels and mild inflammation. The Amygdala shows both viable and Moderate degenerated cells. Group-IV[ASLNs] The frontal cortex shows degenerated cells having shrunken and hyperchromatic nuclei, Mild inflammation and congested vessels are noted the Amygdala shows both viable and degenerated cells .Group-V[ASLNs]The frontal cortex shows degenerated cells [mild] having shrunken and hyperchromatic nuclei Focal inflammation and congested vessels are noted Group-VI[ASLNs]the frontal cortex shows mild degenerated cells having shrunken and hyperchromatic nuclei , Focal inflammation and congested vessels are noted.

Table.9

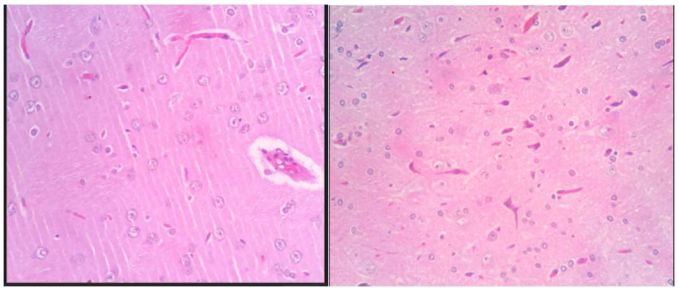
Sr. no	Groups	Observation
1	I	The frontal cortex shows predominantly degenerated cells and also moderately inflamed.
2	II	The frontal cortex shows intact pyramidal cells having round to oval vesicular nucleus with abundant cytoplasm, neuroglial cells, granular cells and intact blood vessels. Also seen are degenerated cells, few cells appear degenerated. Nucleus accumbent shows few degenerated cells. Section studied from the Amygdala appears within normal limits.
3	III	Section from the frontal cortex shows mild degenerated cells, congested vessels and mild inflammation. The Amygdala shows both viable and Moderate degenerated cells
4	IV	The frontal cortex shows degenerated cells having shrunken and hyperchromatic nuclei, Mild inflammation and congested vessels are noted the Amygdala shows both viable and degenerated cells
5	V	The frontal cortex shows degenerated cells [mild] having shrunken and hyperchromatic nuclei Focal inflammation and congested vessels are noted
6	VI	the frontal cortex shows mild degenerated cells having shrunken and hyperchromatic nuclei , Focal inflammation and congested vessels are noted



Group-I Normal saline **Group-II** standard



Group-III Extract of ASLNs **Group-IV** ASLNs 100 mg/kg,



Group-V ASLNs (200 mg/kg Group-VI ASLs 400mg/kg

Fig.14 Histopathology report

Biochemical parameters estimation:

Estimation of Oxidative Stress

Endogenous antioxidant capacity of rat brain tissue was assessed to measure the status of oxidative stress following induction of Antidepressant by Imipramine administration. The content of GSH and antioxidant enzyme activities in brain tissue is directly related to the development of oxidative stress in Antidepressant. These parameters were found to be saturated or severely declined in the Imipramine group, which reflects the oxidative stress The dose-dependent recovery of enzymatic activity and GSH content was observed in treatment controls groups There cover of SOD activity and GSH content was highly significant ($P < 0.001$) among all the treatment groups, including the STD group. These parameters were found to be severely compromised in the Imipramine treated group. However, the pattern of CAT activity recovery was not the same in all treatment groups. The highest recovery of CAT activity was observed in std 15mg ($P < 0.001$), while An Extract of *Albizzia lebbeck* (200 mg/kg and ASLNs 100,200, 400 were found to recover CAT activity comparable to STD ($P < 0.005$) Symptoms of depression can be induced through blockade/decrease of acetylcholinesterase (AChE). Thus, ACh signalling could contribute to the aetiology of mood regulation. Clinical studies suggested that increases in central acetylcholine could lead to depressed mood.

Table.10 Effect of drugs on brain tissue GSH

Group	**Treatment**	GSH (µg/g wet tissue)
I	Control receives vehicle	11.23 ± 0.68
II	Imipramine(15 mg/kg, p.o.)	18.48 ±0.57***
III	Extract of A*lbizzia lebbeck* (200 mg/kg, p.o.)	14.25±0.86
IV	ASLNs (100 mg/kg, p.o.)	13.76 ± 0.75
V	ASLNs (200 mg/kg, p.o.)	14.85 ± 0.84**
VI	ASLNs (400 mg/kg, p.o.)	17.35 ± 0.72***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. **P<0.5,* when compared to control group.

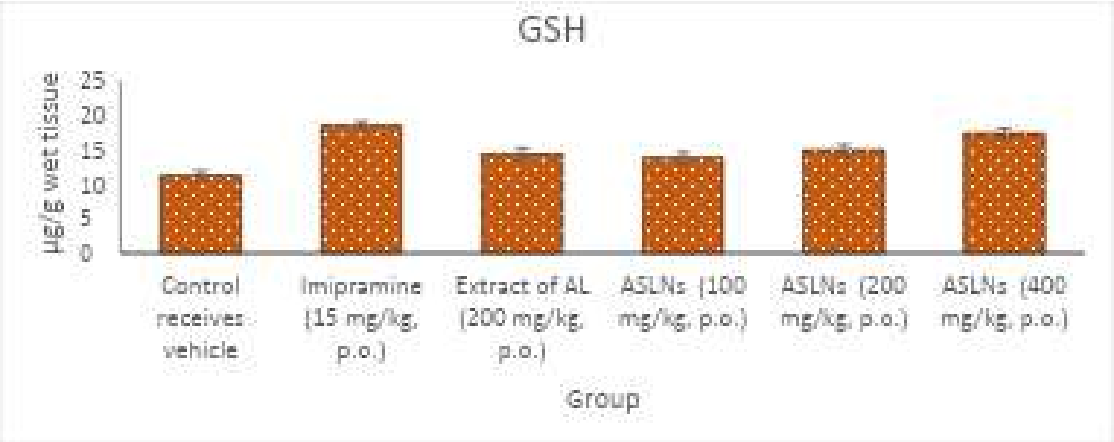


Fig .15 Effect of drugs on brain tissue GSH

Table.11 Effect of drugs on brain tissue SOD

Group	**Treatment**	SOD (U/ml of protein)
I	Control receives vehicle	23.36 ± 0.76
II	Imipramine (15 mg/kg, p.o.)	35.29 ± 0.63***
III	Extract of *Albizzia lebbeck* (200 mg/kg, p.o.)	29.62 ± 0.42
IV	ASLNs (100 mg/kg, p.o.)	28.37 ± 0.92
V	ASLNs (200 mg/kg, p.o.)	31.08 ± 0.79**
VI	ASLNs (400 mg/kg, p.o.)	34.56 ± 0.86***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. **P<0.5,* when compared to control group.

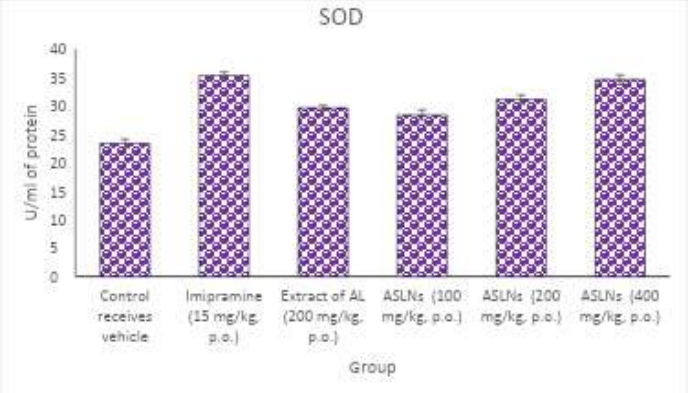


Fig.16 Effect of drugs on brain tissue SOD

Table.12 Effect of drugs on brain tissue catalase

Group	Treatment	CAT (µg/mg of protein)
I	Control receives vehicle	0.65 ± 0.026
II	Imipramine (15 mg/kg, p.o.)	1.39 ± 0.018***
III	Extract of A*lbizzia lebbeck* (200 mg/kg, p.o.)	1.09 ± 0.015*
IV	ASLNs (100 mg/kg, p.o.)	1.03 ± 0.015
V	ASLNs 200 mg/kg, p.o.)	1.15 ± 0.023**
VI	ASLNs (40 mg/kg, p.o.)	1.26 ± 0.015***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. **P<0.5,* when compared to control group.

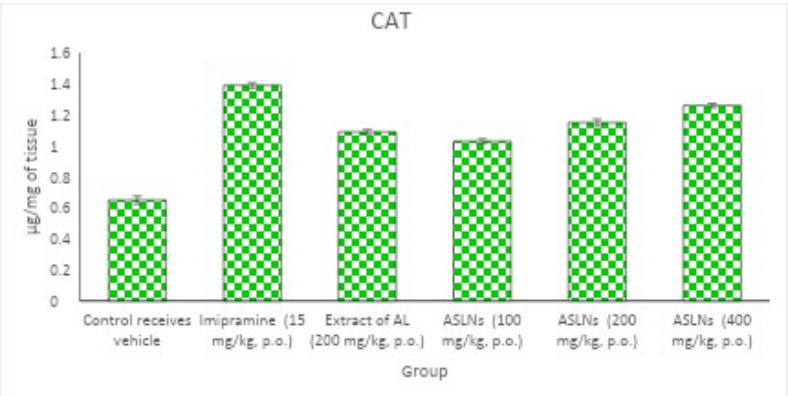


Fig.17 Effect of drugs on brain tissue catalase

Table.13 Effect of drugs on acetylcholinesterase enzyme in rat brain

Group	Treatment	AchE (µ moles/min/mg tissue)
I	Control receives vehicle	37.07±2.56
II	Imipramine (15 mg/kg, p.o.)	56.43±3.25**
III	Extract of A*lbizzia lebbeck* (200 mg/kg, p.o.)	45.87±2.30
IV	ASLNs (100 mg/kg, p.o.)	44.38±2.46
V	ASLNs (200 mg/kg, p.o.)	49.57±3.67*
VI	ASLNs (400 mg/kg, p.o.)	53.28±2.70**

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett test. **P<0.5,* when compared to control group.

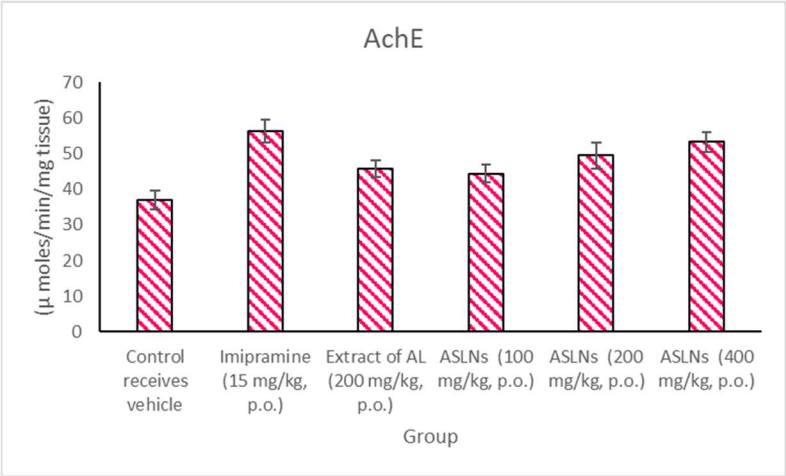


Fig.18 Effect of drugs on acetylcholinesterase enzyme in rat brain sample

SUMMARY & DISCUSSION

The SLNA, with stearic acid and the other with beeswax, were prepared. The selection of lipids was based on the solubility and miscibility of extract of with these lipids. It is generally accepted that the entrapment efficiency of the active constituent would be higher if it is miscible /soluble with the lipid phase used in SLN.In this study, evaluation of the effect of ethanol leaf extract of *Albizia lebbeck* on the central nervous system was carried out in mice using different models; Open field test, tail suspension test and force swimming test. The solid-lipid nanoparticles were found to cause significant dose-dependent increases in the frequency of line crossing, walling and rearing activities of the pretreated mice. It also significantly reduced the immobility time of the mice in

force swimming and tail suspension tests. Monitoring of locomotor activity of animals has been used to assess the effect of drug on the CNS. An increased movement is a measure of the level of excitability of the CNS while its decrease may be resulting from depression of the Central nervous system stimulants are known to increase locomotor activity, while agents with depressant activity cause reduction in movements. The leaf extract was found to increase significantly line crossing, walling and rearing activities during open field test suggesting stimulatory effect on the CNS
.
The CNS stimulatory effect of the leaf extract was further supported by its potential to reduce immobility time of mice during force swimming and tail suspension tests. Forced swimming and tail suspension tests are two of the most commonly used animal models of depression for antidepressant screening. In the forced swimming test, the development of immobility when mice are placed into an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior The tail suspension test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressants are able to reverse the immobility and promote the occurrence of escape related behaviour. Both models of depression are widely used to screen new antidepressants. These tests are quite sensitive to major antidepressant drugs including tricyclics, serotonin specific reuptake inhibitors, MAO inhibitors, and atypical antidepressants.

Forced swimming and tail suspension tests which represent the behavioural despair model, claimed to reproduce a condition similar to human depression The tests are based on the observation that animals, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli it is well known that clinically effective antidepressants (such as imipramine) typically increase the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test Similarly, the results of this study suggest that exhibited significant antidepressant activity with a strong psychomotor stimulation. Growing demand for herbal drugs for several disorders including that of CNS related neuro psychiatric conditions like depression. Paucity of studies about the antidepressant activity of the *Albizia lebbeck*, although being evaluated for anxiolytic activity and learning. Synthetic antidepressants have undesirable adverse effect in long term use the antidepressant activity of leaf powder of *Albizia lebbeck* was established in conventional most acceptable model of depression- behavioural despair model two doses (increasing) were selected for the study and was given orally to fasted animals. Duration of immobility, swimming and climbing was recorded in a 15 min session to record the antidepressant activity understand the likely mechanism of action. Ambulatory activity in the form of locomotor activity score was also recorded by placing the experimental animal in the central arena of the photo actometer to exclude false positive result, since psycho stimulants exerts indiscriminate motor stimulating activity in the dose employed for establishing antidepressant activity. The results of an antidepressant study recorded a statistically significant, dose dependent reduction in duration of immobility, on day 7th and 14th, and 21 days. compound to the day zero result and the effect of *Albizzia lebbeck* treatment on motor stimulating activity was ruled out, as indicated by insignificant change in locomotor activity score. when the experimental animals are exposed to herb for relatively longer periods of time, since is proposed that a treatment period of 21 days is a valid interval for demonstrating antidepressants were augmented following chronic the increase in

food intake and weight of experimental animals of all groups, some being significant suggest a positive influence on the depression. Tail suspension test, referred to as 'dry test 'was also employed the results was encouraging the duration of immobility displayed by test animals when subjected to unavoidable stress such as forced swimming and suspended from its tail reflects a state of despair or lowered mood, which one believed to the like the depressive disorder in human beings, suggesting the potential of herb to severe depressive states. Further, this investigation employed recording of duration of swimming and climbing when animals were subjected to forced swimming to give additional information on the possible mechanism mediating the antidepressant activity swimming is mediated by serotonergic neurotransmission, while climbing is mediating by norepinephrinergic neurotransmission. However, due to insignificant change, it was difficult to understand the possible mechanism of action. Using leaf powder is also proven to have its disadvantages, as the powder is a compound mixture of several phytochemicals and duration of study is insufficient to arrive at a conclusive result. Despite this limitation Albizia lebbeck holds potential to be a useful antidepressant.

CONCLUSION

The imipramine (15 mg/kg, p.o.) and ASLs (400 mg/kg, p.o.) significant antidepressant activity by decreasing immobility in tail suspension model, increasing climbing & swimming in forced swimming model, increasing number of lines crossing in open field model and increasing count in locomotion model compared to control. Symptoms of depression can be induced through blockade/decrease of acetylcholinesterase (AChE). Thus, ACh signalling could contribute to the aetiology of mood regulation. Clinical studies suggested that increases in central acetylcholine could lead to depressed mood.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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