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# Anxiolytic Activity of Methanolic Extract of *Albizia procera* Leaves in Albino Rats

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**ABSTRACT**

**Background and Objective:** Anxiety is an exaggerated feeling of uncertainty and fear. Anxiety refers to the experience of nervousness, panic restlessness and tension. Neurotransmitters like serotonin, dopamine, noradrenaline and GABA generates anxiety. Now a day's serotonin modulators and benzodiazepines are available to alleviate anxiety. Generally in adults, anxiety disorders are the most common class of mental disorders with a 12 months prevalence rate of 24.9%. The most common disorder was specific phobias and social anxiety disorder. As compared with adults aged between 18-64, the lifetime prevalence was generally less for panic disorder, GAD and SAD, but specific phobia and agoraphobia without a history of panic attacks were most common in adolescents between the age of $13^{-1}$ 7 years. The present paper discussed the anti-anxiety potential of *Albizia procera* leaves. *Albizia procera* leaves contain flavonoids, steroids, tannins, saponins and alkaloids. **Materials and Methods:** Flavonoids were the major constituent for the anxiolytic activity. The effect of methanolic extract of *Albizia procera* leaves (200 and 400 mg $kg^{-1}$, orally, daily, 21 days) on anxiolytic activity was assessed by using a Modified Elevated Plus-Maze Apparatus and Light-Dark Box Apparatus. Anxiety activity in rats was induced by restraint stress. **Results:** The results show that Open arm entries were increased and closed arm entries were decreased in Modified Elevated Plus-Maze Apparatus (MEPMA). Lightbox entries were increased and dark box entries were decreased in the Light and Dark Box model (LDB). **Conclusion:** Thus we can conclude that methanolic extract of *Albizia procera* leaves exhibits significant anxiolytic activity at a low dose (200 mg $kg^{-1}$) and high dose (400 mg $kg^{-1}$). Thus *Albizia procera* leaves is a promising herbal option in the pharmaceutical world.

**KEYWORDS**

*Albizia procera,* anxiety, anxiolytic activity, elevated plus maze apparatus, light and dark box apparatus



## INTRODUCTION

Anxiety is an inflated emotion of fear and uncertainty. Adnaik *et al.*[1] reported that anxiety is the condition of stress with an expectation of looming danger. Anxiety is the experience of panic, restlessness, nervousness, stress, fear and agitation. Its symptoms include headaches, faint trembling and sweating, probably elevated Blood Pressure (BP) and changes in other psychophysiological parameters like muscle tone, heart rate and skin conductance. Serotonin, noradrenaline, Gama Aminobutyric Acid (GABA), dopamine, Corticotropin-Releasing Factor (CRF), Melanocyte Stimulating Hormone (MSH), neuropeptides and neurosteroids are involved in the production of anxiety[2].

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The physical sign of anxiety includes dizziness, fatigue, insomnia, headache, palpitation and excessive perspiration. Anxiety is associated with almost all emotional disorders with a physical ailment. The amygdala is likely responsible for anxiety or dread and the termination of the dread prefrontal cortex has an important role in controlling the amygdale-mediated expression of dread but the molecular mechanism for a positive and negative control of the anxiety is not clear, several genes have been reported for anxiety or dread[3].

Generally in adults, anxiety disorders are the most common class of mental disorders with a 12 months prevalence rate of 24.9%. The most common disorders were specific phobias and social anxiety disorder. As compared with adults aged between 18-64, the lifetime prevalence was generally less for Generalized Anxiety Disorder (GAD) and Social Anxiety Disorder (SAD), panic disorder but agoraphobia and specific phobia without a history of panic attacks were the most common in adolescents between the age of 13-17 years[4].

The biggest epidemiological study conducted in the US, the Epidemiological Catchment Area (ECA) study, found that the commonest psychiatric disorder was specific phobia then after, Obsessive-Compulsive Disorder (OCD) ranked four. Another study by the National Comorbidity Survey, National Comorbidity Survey-Revised (NCS-R), reported identical prevalence rates. Other epidemiological studies conducted by different countries showed the same prevalence rates. A review of twenty-seven epidemiological studies in the European Union (EU) from 1990-2004, found that anxiety disorders were the most common psychiatric disorders in Europe, with a mean twelve-month prevalence of 2%[5].

In Central Nervous System (CNS), the main mediators of anxiety disorders symptoms are Gamma-Aminobutyric Acid (GABA), serotonin, norepinephrine, dopamine, other peptides and neurotransmitters such as Corticotropin-releasing Factor (CTRF). In the basolateral amygdala for the processing of anxiety neurons with dendritic arborization are responsible. Inhibitory control on action potentials and reduced arborization mediated by $SK_2$ potassium channels[6].

The plant is the main source of medicine and it plays a vital role in world health. Medicinal herbs or plants have been known to be a potential source of therapeutics. Medicinal plants are widely used and have got a major role in the health system throughout the whole world. The main reason for utilising the plant is due to their, better compatibility, adaptability and better cultural acceptability with the human body and yields lesser side effects. Some of the important drugs which are obtained from plants are atropine, quinidine, physostigmine, reserpine, tubocurarine, artemisinin, morphine, colchicine, quinine, digoxin, aspirin, pilocarpine, taxol, ephedrine, vinblastine and vincristine[7].

*Albizia procera* (Roxb.) Benth. is fast growing tropical and subtropical trees from the Mimosoideae subfamily of the Fabaceae family. *Albizia procera* is a traditional herb and it is widely utilised in Asian traditional medicine as an analgesic, antibacterial, antioxidant, antidiabetic and antidiarrheal drug[8].

The colour of the bark is brown, having a characteristic odour with a slightly bitter taste. The leaf is green in colour, having a slightly bitter taste with a characteristic odour[8].

Literature shows that *Albizia procera* leaves contain tannins, saponins, glycosides, steroids and flavonoids etc[9]. Flavonoids were the major constituents for anxiolytic activity[10].

Thus the present study aims to investigate the anxiolytic activity in the rat model by the administration of methanolic extract of *Albizia procera* leaves by administration of methanolic extract of *Albizia procera* leaves.

## MATERIALS AND METHODS

The study was carried out in the Research Laboratory of P. Wadhwani College of Pharmacy, Yavatmal from July, 2019 to April, 2020.

### Materials

**Animals:** About 8 weeks old, 30 healthy female Sprague-Dawley SD) rats with 150-250 g weight were used. Animals were housed in the animal house of P. Wadhwani College of Pharmacy, Yavatmal, India and were maintained under controlled conditions (12 hrs light, 12 hrs dark), 25±2°C temperature and 60±5% humidity, a standard pellet diet and water ad libitum, were used during the entire animal study as per the rules and regulations of CPCSEA and IAEC. The experiments were performed during 8.00-16.00 hrs (daytime). The protocol of the animal study was approved by the Institutional Animal Ethics Committee (IAEC) of P. Wadhwani College of Pharmacy, Yavatmal, India.

**Method:** The leaves of *Albizia procera* belonging to the family Fabaceae were collected in September from the local area of Yavatmal District, Maharashtra, India. The plant material was identified and authenticated by Prof. Mrs. A.M. Gaharwar and Asso. Dean of Vasantrao Naik College of Agriculture Biotechnology, Yavatmal (Ref No. VNCABT/Ytl/Hort/1030/2019).

Leaves were dried in a shade and then powdered to get a coarse powder. This powder was stored in an airtight container and used for extraction.

For the extraction of *Albizia procera* leaves methanol and water were used as a solvent. Methanol and water were used in the proportion of 7:3. Glass bottle was used for the process of extraction. Dried leaves of *Albizia procera* and water and methanol were poured into a glass bottle for extraction. In the maceration procedure, powdered leaves were macerated, it was occasionally stirred at regular intervals of time. It was then filtered and concentrated. Then it was dried by evaporation[10].

### Phytochemical screening

**Test for alkaloid:** About 1 mL of filtrate with 2 mL of Dragendroff's reagent gives turbid orange colour[11].

**Test for tannins:** About 1 mL of filtrate with 2 mL of ferric chloride gives a dark green colour[11].

**Test for saponin:** About 1 mL of filtrate with 2 mL distilled water is taken vigorously and allowed to stand for 10 min. The development of foam on the surface of the mixture, lasting for 10 min indicates the presence of saponin[11].

**Test for phenolic flavonide:** About 1 mL of filtrate with 2 mL of 10% lead acetate gives brown precipitate[11].

**Test for flavonoids:** About 1 mL of filtrate with 2 mL of dilute NaOH show the development of golden yellow colour[11].

**Experimental design:** For this study animals were divided into 5 groups:

- **Group I (Vehicle control group)** : Rats received only saline solution
- **Group II (Negative control group)** : Rats were subjected to restraint stress for 21 days using a saline bottle
- **Group III (Low dose group)** : Rats were subjected to restraint stress and treated with 200 mg $kg^{-1}$ methanolic extract of *Albizia procera* orally for 21 days

- **Group IV (High dose group)** : Rats were subjected to restraint stress and treated with 400 mg $kg^{-1}$ methanolic extract of *Albizia procera* orally for 21 days
- **Group V (Standard group)** : Rats were subjected to restraint stress and treated with 2 mg $kg^{-1}$ Diazepam for 21 days

**Induction of anxious state:** All groups were subjected for 21 days to restraint stress except the normal control group which was placed in normal conditions in an animal house. For the induction of anxiety, rats were packed in a saline bottle for 6 hrs daily for 21 days.

**Drugs and dosing:** Diazepam (2 mg $kg^{-1}$) was used as a standard drug. Diazepam was prepared by diluting with distilled water (1.5 mg/10 mL). Two different concentrations 200 mg $kg^{-1}$ (low dose) and 400 mg $kg^{-1}$ (high dose) of the leaves extract of *Albizia procera* were prepared by using distilled water. All solutions were prepared freshly on test days and administered orally according to the body weight of the rats. Low dose group extract was calculated at 200 mg $kg^{-1}$ and high dose group extract were calculated at 400 mg $kg^{-1}$ of rats. Then dosing were given to rats in the concentrations like 0.1, 0.2 and 0.4 mL etc.

Anxious behavioural states of animals after 21 days were checked by using an elevated plus-maze apparatus and a light and dark box model.

**Elevated plus-maze apparatus:** Elevated Plus-Maze Apparatus (EPMA) was widely used for the assessment of anxiolytic activity, rats were individually positioned in the centre of the EPMA facing one of the open arms before 30 min of the experiment. The number of entries and time spent in the open and closed arms were calculated for 5 min. When all four paws were in the arm, only such entries were considered and counted. The following formula was used for calculating the percentage of open arm entries[12] and the time spent in open arm:

$$\text{Time spent in open arm (\%)} = \frac{\text{Time in open arm}}{\text{Time in open arm} + \text{Time in the closed arm}} \times 100$$

$$\text{Open arms entries (\%)} = \frac{\text{Open arms entries}}{\text{Open arms entries} + \text{Closed arms entries}} \times 100$$

**Light and dark apparatus:** Light and dark apparatus was a commonly used model for the assessment of anxiolytic activity. This apparatus consisted of two compartments, dark (one-third of the total compartment) and light (two-thirds of the total compartment along with the light of 400 lux 35 cm above the box ). After 30 min of treatment with the extract, vehicle or diazepam, each rat was individually placed in the corner of the light compartment, facing away from the entry of the dark compartment. The rats were observed for 5 min and the following parameters were considered and calculated.

Latency of the first crossing from one compartment to the other, time spent in the light and dark compartment, the number of movements between the light and dark compartment[13].

## RESULTS

**Elevated plus-maze apparatus results:** Table 1 shows that there was a significant ($p<0.0001$) increase in the closed arm entries of negative control as compared to normal control and in the low dose, high dose treated group there was a significant ($p<0.0001$) decreased closed arm entries.

Table 1 showed that there was a significant ($p<0.0001$) decrease in the open arm entries of negative control as compared to normal control and in the low dose, high dose treated group there was a significant ($p<0.0001$) increased open arm entries.

Table 1: Effect of methanolic extract of *Albizia procera* leaves on elevated plus-maze test closed arm and open arm in anxious rats

| Groups | Number entries in closed arm (%) (0 day) | Number entries in closed arm (%) (21 days) | Number entries in open arm (%) (0 day) | Number entries in open arm (%) (21 days) |
|---|---|---|---|---|
| Normal control | 70.84±0.76 | 69.92±0.68 | 31.12±0.85 | 32.04±0.98 |
| Negative control | 64.27±2.22 | 73.90±9.80[@] | 37.53±2.08 | 28.14±3.16[@] |
| Low dose group | 66.66±3.59 | 60.52±6.63*** | 35.35±2.70 | 41.25±0.38*** |
| High dose group | 62.77±3.41 | 54.75±8.85*** | 39.70±1.90 | 47.20±1.02*** |
| Standard (Diazepam) | 68.05±0.70 | 52.51±15.54*** | 33.57±1.70 | 49.35±4.70*** |

Values are expressed in Mean±SEM (n = 6), [@]p<0.0001: Significant increase in the closed arm and decrease in open arm entries was observed compared to the normal control group, ***p<0.0001: Significant decrease in closed arm entries and an increase in open arm entries were observed compared to the negative control group and #p>0.05: Compared with negative control

Table 2: Effect of methanolic extract of *Albizia procera* leaves. on time spent in the closed arm and open arm in anxious rats

| Groups | Time spent in closed arm (sec) (0 day) | Time spent in closed arm (sec) (21 days) | Time spent in open arm (sec) (0 day) | Time spent in open arm (sec) (21 days) |
|---|---|---|---|---|
| Normal control | 43.25±0.98 | 40.25±3.20 | 53.10±9.87 | 54.40±7.52 |
| Negative control | 45.30±1.30 | 49.05±4.90[a] | 51.33±2.10 | 39.85±11.98[a] |
| Low dose group | 48.50±8.90 | 39.10±9.65*** | 44.22±2.70 | 44.98±0.88* |
| High dose group | 52.21±2.35 | 38.40±14.70*** | 51.18±1.93 | 49.89±1.27** |
| Standard (Diazepam) | 52.19±1.98 | 35.52±16.80*** | 43.07±3.53 | 50.58±7.56*** |

Values are expressed in Mean±SEM (n = 6), [@]p<0.0001: Significant increase in time spent in the closed arm and a decrease in time spent in the open arm was observed compared to the normal control group, *p<0.05: Significant increase in time spent in the open arm was observed compared to the negative control group, **p<0.001: Significant increase in time spent in the open arm was observed compared to the negative control group, ***p<0.0001: Significant decrease in time spent in the closed arm was observed compared to the negative control group and #p>0.05: Compared with negative control

There was a significant (p<0.0001) increase in the time spent in the closed arm of negative control as compared to normal control and in the low dose, high dose treated group there was a significant (p<0.0001) decreased time spent in closed arm entries (Table 2).

Table 2 shows that there was a significant (p<0.001) decrease in the time spent in the open arm of negative control as compared to normal control and in the low dose, high dose treated group there was a significant (p<0.05 and p<0.001) increased time spent in open arm entries.

The effect of *Albizia procera* Linn., on Transfer Latency (TL) in Elevated Plus-Maze (EPM) in anxious rats was shown in Table 3. There was a significant (p<0.01) increase in TL in the negative control group as compared to the control group. Whereas, *Albizia procera* Linn. In the low dose, high dose, standard treated groups there was a significant (p<0.01) decrease in TL as compared to the negative control group.

**Light and dark box apparatus results:** Table 4 shows that there was a significant (p<0.0001) increase in the dark box entries of negative control as compared to normal control, In the low dose and high dose treated group there was a significant (p<0.0001) decreased dark box entries.

Table 4 showed that there was a significant (p<0.001) decrease in the lightbox entries of negative control as compared to normal control the low dose and high dose treated group there was a significant (p<0.001) increase lightbox entries.

Table 5 showed that there was a significant (p<0.0001) increased in the time spent in the dark box of negative control as compared to normal control, the low dose and high dose treated group there was significant (p<0.05 and p<0.0001) decreased time spent in the dark box.

Table 5 showed that there was a significant (p<0.0001) decrease in the time spent in the lightbox of negative control as compared to normal control, In the low dose, and high dose treated group there was a significant (p<0.0001) increased time spent in lightbox entries.

Table 3: Effect of methanolic extract of *Albizia procera* on transfer latency of anxious rats on EPM

| Groups | Transfer latency in sec |
|---|---|
| Positive control | 28.0±0.93 |
| Negative control | 49.5±0.85[@] |
| Low dose (200 kg $mg^{-1}$) | 21.7±1.12** |
| High dose (400 kg $mg^{-1}$) | 19.5±1.45** |
| Standard (Diazepam) | 37.0±0.99** |

Values are Mean±SD, [@]p<0.01: Compared with the control group and **p<0.01: Compared with the negative control group

Table 4: Effect of methanolic extract of *Albizia procera* leaves on light and dark box model in a dark box and lightbox in anxious rats

| Groups | Number entries in darkbox (%) (0 day) | Number entries in darkbox (%) (21 days) | Number entries in lightbox (%) (0 day) | Number entries in lightbox (%) (21 days) |
|---|---|---|---|---|
| Normal control | 61.25±0.86 | 62.05±1.47 | 44.22±2.90 | 43.11±0.90 |
| Negative control | 64.15±1.05 | 69.35±4.95[a] | 41.18±3.80 | 36.25±4.70[a] |
| Low dose group | 63.12±1.02 | 64.18±0.92** | 42.62±2.10 | 41.55±1.48** |
| High dose group | 64.22±0.88 | 62.12±2.19*** | 41.21±1.95 | 43.15±0.08*** |
| Standard (Diazepam) | 63.53±0.95 | 59.82±4.15*** | 42.37±3.95 | 46.35±4.12*** |

Values are expressed in Mean±SEM (n = 6), [a]p<0.0001: Significant increase in a dark box and a decrease in lightbox entries was observed compared to a normal control group, **p<0.0001: Significant decrease in a dark box and an increase in lightbox entries were observed compared to the negative control group and ***p<0.001: Significant increase in lightbox entries was observed compared to the negative control group

Table 5: Effect of methanolic extract of *Albizia procera* leaves on time spent in a dark box and lightbox in anxious rats.

| Groups | Time spent in darkbox (sec) (0 day) | Time spent in darkbox (sec) (21 days) | Time spent in lightbox (sec) (0 day) | Time spent in lightbox (sec) (21 days) |
|---|---|---|---|---|
| Normal control | 52.75±3.10 | 60.83±9.44 | 248.36±5.90 | 242.00±6.17 |
| Negative control | 58.39±11.90 | 125.00±65.10[a] | 258.66±2.87 | 159.60±97.93[a] |
| Low dose group | 51.62±3.88 | 91.76±40.06* | 228.19±3.11 | 191.23±36.95*** |
| High dose group | 73.00±2.35 | 79.13±5.20*** | 239.50±4.70 | 209.12±31.02*** |
| Standard (Diazepam) | 41.27±3.22 | 61.52±19.95*** | 257.43±2.72 | 235.19±21.75*** |

Values are expressed in Mean±SEM (n = 6), [a]p<0.0001: Significant increase in time spent in a dark box and a decrease in time spent in a lightbox was observed compared to the normal control group, *p<0.05: Significant decrease in time spent in the dark box was observed compared to the negative control group and ***p<0.0001: Significant decreases in time spent in the dark box and increased time spent in the lightbox was observed compared to the negative control group

## DISCUSSION

Our study shows that methanolic extract of *Albizia procera* leaves exhibits significant anxiolytic activity in the stressed rat.

In our study rats showed anxiety after restraining in a plastic bottle and blocking its movement daily for 6 hrs. Anxiety can be produced by different methods such as using chemicals, social model and stress evoked sensory models, transitory model, restraint stress etc., a modified form of immobilization stress is restraint stress, which has been widely used as a model for chronic psycho-emotional stress to induce depression and anxiety-like behaviours, learning and memory deficits and hippocampal neuronal damage in rats. The restraint stress method is very economic, easy availability, easy induction of depression and anxiety and it also shows good results. Hence we choose restraint stress for the generation of anxiety in this work[14].

The physical and mental stress was induced by placing the rat in a plastic bottle to block the movements. This is one of the approved methods to produce psychological and physical stress simultaneously[15,16].

In modified elevated plus-maze apparatus, closed arm entries of control vehicle, low dose, high dose and standard group after 21 days were decreased as compared to 0 day entries. The only negative control group had more number entries in the closed arm as compared to 0 day entries. In the closed arm, time spent by rats in the control vehicle, low dose, high dose and standard group after 21 days were decreased as compared to 0 day readings time spent by rats. The only negative control group had more time spent in closed arms as compared to 0 day readings time spent by rats.

In the open arm, entries of control vehicle, low dose, high dose and standard group after 21 days were increased as compared to 0 day entries. The only negative control group had less number of entries in the open arm as compared to 0 day entries. In the open arm, time spent by rats in the control vehicle, low dose, high dose and standard group after 21 days were increased as compared to 0 days readings time spent by rats. Only the negative control group had less time spent in the open arm as compared to 0 day readings time spent by rats.

In the light and dark model, the number of entries and time spent by rats in the dark box was decreased by treated groups of rats compared with a negative control group. The number of entries and time spent by the rats in the lightbox was increased by treated groups of rats compared with negative control.

There are many models for the screening of anxiety-like modified elevated plus-maze apparatus, light and dark apparatus, elevated T maze, elevated zero mazes, open field test and a white black box. In this study for the assessment of anxiolytic activity, we have used a Modified Elevated Plus-Maze Apparatus (MEPA) and Light and Dark model (LDB) due to their economic, easily availability, popularity, accuracy, specificity and shows good results.

Our study confirms the presence of glycosides, saponins, tannins, steroids and flavonoids etc., in the methanolic extract of *Albizia procera* leaves.

Flavonoids present in the extracts may be responsible for their CNS depressant activity[10]. Literature shows that *Albizia procera* leaves contain saponins, steroids, tannins, glycosides and flavonoids etc.[9].

It is reported that many flavonoids and neuroactive steroids can be ligands for the GABA receptors and that can act like benzodiazepine-like molecules[10]. Activation of the GABAA receptor would have an anxiolytic effect[1]. Flavonoids were the major constituents of the anxiolytic activity[10]. This study gave birth to a new herbal drug for the treatment of anxiety with minimum side effects. But the active constituents responsible for activity and mechanism for the anxiolytic activity need to explore.

## CONCLUSION

The present findings indicated that the methanolic extract of *Albizia procera* leaves exhibits anxiolytic activity due to the presence of flavonoids as a major constituent moreover significant anxiolytic activity exist on a low dose (200 mg $kg^{-1}$) and high dose (400 mg $kg^{-1}$) of Diazepam drug. Thus *Albizia procera* leaves is a promising herbal option in the pharmaceutical world.

## SIGNIFICANCE STATEMENT

Now a day's life is very stressful, this stress may lead to several behavioural related problems and anxiety is one of the most important problems. Its treatment is very essential to avoid other associated problems. Treatment with a synthetic agent may produce adverse reactions hence treatment with herbs may be useful to overcome such issues. In our research methanolic extract of *Albizia procera* showed significant anxiolytic activity with minimum adverse effects compared to the synthetic agent.

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