Evaluation of anti-anxiety activity of *Alstonia scholaris Linn.* bark extract in mice

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ABSTRACT:

Context: Anxiety is a highly prevalent psychological and physiological state characterized by cognitive, somatic, emotional, and behavioural components and about 1/8 th of the world population is affected by anxiety disorders.

Aim: The present study was undertaken to evaluate the anti-anxiety activity of the ethanolic bark extract of *Alstonia scholaris L* in mice.

Materials and Methods: Anti-anxiety activity was evaluated using the Elevated plus maze, Light/dark transition test and Open field test in albino mice. Diazepam (2 mg/kg) was used as standard drug and *Alstonia scholaris L*(100,200,400mg/kg) was used as test drug.

Results: *Alstonia scholaris L* has shown significant anti-anxiety activity in Elevated plus maze, Light/dark transition test and Open field test.

Conclusion: *Alstonia scholaris L* has shown anti-anxiety activity in Elevated plus maze, Light/dark transition test and Open field test.

KEYWORDS: *Alstonia scholaris L*, Elevated plus maze, Light/dark transition test and Open field test.

INTRODUCTION:

Anxiety is actually an adaptive response which prepares a person to face the challenges of life and is characterized by emotionally unpleasant state, along with discomfort and concern of fear regarding some defined or undefined future threat[1]. Caused mainly by the interaction of biological, psychological and social factors, involving genetic factors, which react with situations, stress, or trauma resulting in clinically notable symptoms[2]. Panic disorder, generalised anxiety disorder (GAD), social anxiety disorder (SAD), post-traumatic stress disorder (PTSD), specific phobia, and obsessive-compulsive disorder (OCD) are all examples of anxiety disorders. Benzodiazepines are most commonly prescribed anxiolytic drugs, work as anxiolytics by acting as positive allosteric modulators on the gamma amino butyric acid ($GABA_A$) receptor. GABA inhibit or reduce activity of neurons thus helping to manage fear and anxiety when neurons become overexcited. Benzodiazepines potentiates effect of GABA on receptor $GABA_A$. Other medications like antidepressants mainly SSRIs and SNRIs are also prescribed[3].

Present study helps in evaluating anti-anxiety

action of *Alstonia scholaris L* preparation. Main classes of compounds used in anxiety are benzodiazepines. But, therapeutic uses are restricted by their negative effects such as drowsiness, confusion, fatigue and loss of coordination[4]. Creation of novel drugs with anxiolytic properties but no side effects would be extremely beneficial. Medicinal plants are a wonderful source to look for new treatments for various ailments. In view of this, current experiment was carried to investigate anti-anxiety activity of barks of *Alstonia scholaris L*. A review on earlier studies conducted, has shown to alter the levels of serotonin. Low serotonin levels are connected to depression and anxiety. Apparent mechanism of action is selective serotonin reuptake inhibition[5].

MATERIAL AND METHODS:

Plant material and preparations:

The barks of *Alstonia scholaris L.* were collected from Mangalore, Karnataka, India during the month of August,2019. These barks are dried and coarse powdered for extraction. This extract is further dried and then stored in a desiccator for future use.

Experimental animals:

Albino mice of either sex, 4-6 weeks old, weighing 20-30g were obtained from NUCARE, Paneer, Mangalore's central animal house. Animals were appropriately grouped and then sheltered in distinct cages. The cages were kept under standard lab conditions of temperature 25 ± 2° with appropriate dark and light cycle of 12 hours. Animals had full access to a standard dry pellet diet and water. Experiment was done according to the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi, India. Research work was permitted and approved by Institutional Animal Ethics Committee (**NGSMIPS/IAEC/MAY-2017/63**).

Drug treatment and acute toxicity:

The ethanolic extract of bark of Alstonia scholaris L was tested in an acute oral toxicity trial to determine the lethal dose, or LD50. This study was on Swiss Albino mice of 20 to 30g body weight by using "Up and Down Method" as per OECD 425 guidelines. The drug suspension (2000 mg/kg) was administered orally to overnight fasted mice. These animals were observed once in 30 minutes interval for about 4 hours in order to check any behavioural and neurological changes and finally till the death after 24 hours of administration[6].

Experimental design:

Albino mice of either sex weighing 20-30g were divided into five groups at rand Each group having six animals (i.e. n=6) were utilized to evaluate the anti-anxiety activity. The groups of animals are summarized below:

Group I (normal control): The animals were administered with single oral dose of the vehicle (gum acacia, 2% w/v, 10ml/kg p.o) on 7^{th} day.

Group ll (standard group): The animals were administered with single dose of Diazepam(2mg/kg i.p) on 7^{th} day.

v **Group lll:** The animals were daily administered with single oral dose of the *Alstonia scholaris L.*100 mg/kg, p.o once a

day for 7 days.

Group IV: The animals were daily administered with single oral dose of the *Alstonia scholaris L* 200mg/kg, p.o once a day for 7 days.

Group V: The animals were administered with single oral dose of the *Alstonia scholaris L* 400mg/kg, p.o once a day for 7 days.

About 1 hour after administration of drugs, trials were carried out and anti-anxiety activity was performed by using 3 models; Elevated plus maze, Light/dark transition test and Open field test.

Elevated Plus Maze Model:

Two open arms (16×5cm) and two closed arms (16×5×12cm) stretch from a shared central platform in the plus-maze apparatus. The maze is raised to a height of 25cms above the ground level. Mice were placed in a sound attenuated room, individually face one of the open arms in the maze's centre. The following characteristics were recorded during a five-minute test session: number of entries into open arms, and time spent in the open arms.. The parameters were expressed as a percentage (for example, % open arm entries =100×no.of open arm entries/ Total no. of entries) [7][13].

Light-dark transition test:

Light-dark apparatus is made up of a two-compartment chamber (40×60×20cm) with a brightly illuminated section and a dark area separated by a wall with opening at the bottom. During a 5-minute test session, mice were kept individually in illuminated part of the cage and metrics such as the number of transitions between the light and dark areas and time spent in the illuminated part of the cage were recorded. [8].

Open field test:

A wooden box is included (40x40x50 cm). The box's floor was divided into sixteen squares, with the centre of the field marked to distinguish it from the others. The equipment was lighted by a 60-W lamp suspended 100 cm above. During a 5-minute test session, the mice were kept in the centre of the arena, and metrics including total number of squares crossed, total number of rearings, and time spent in central square were observed and recorded. [9].

Statistical analysis:

All of the data is presented as Mean ± SEM. The ANOVA test will be used for statistical analysis, followed by a post hoc multiple comparison test using SPSS software. Statistical significance is defined as a P value of less than 0.05.

RESULTS AND DISCUSSION:

Ethanolic extract of *Alstonia scholaris L.* bark was shown to be nontoxic when given orally up to 2000 mg/kg body weight. The animals were confirmed to be stable after 24 hours. Because there were no deaths or evidence of toxicity, the extract was determined to be safe. Hence, for the present study, three dose levels i.e., 100 mg/kg, 200 mg/kg and 400 mg/kg body weight were chosen.

Anxiety is one of the highly prevalent nervous disorders that has affected about 1/8th of the world population. Anxiety is a psychological state usually caused by the perception of real or perceived danger that threatens the safety of an individual. Panic, fear and uneasiness, heart palpitations, shortness of breath, limb and muscle pain, skin tingling and numbness/ feeling weak, hot and cold flashes, sweating, shivering, dizziness, headaches, sleep issues, stomach sensations, blurred vision are some of the psychological and physical symptoms associated with anxiety[10]. In anxiety disorders, there will be changes in the levels of neurotransmitters and they play different roles. Norepinephrine, epinephrine, phenylalanine and dopamine levels are high

in anxiety patients, whereas, GABA and serotonin levels are low)[12]. The main classes of compounds used in anxiety are benzodiazepines. But, therapeutic uses are restricted by their negative effects. Creation of novel drugs with anxiolytic properties but no side effects would be extremely beneficial [4][11].

Diazepam is most commonly used as a standard drug. On the gamma amino butyric acid (GABAA) receptor, they mostly function as positive allosteric modulators, resulting in anxiolytic effects[13]. When neurons get overexcited, GABA helps to reduce fear and anxiety.. Benzodiazepines bind to the benzodiazepine binding site and potentiates the effect of the neurotransmitter GABA on the $GABA_A$ receptor which hyperpolarizes the cell and thereby produces inhibitory effect throughout the central nervous system)[3].

In the present study *Alstonia scholaris L* was used to evaluate antianxiety activity on mice, showed significant results when compared with control group and non-significant when compared with standard group. *Alstonia scholaris L* showed significant results by increasing % number of entries and % time spent in open arms in elevated plus maze; number of entries into the light chamber and time spent in the light chamber in Light/dark transition test; number of squares crossed, number of rearings and time spent in the central square in open field test. Average of each min is calculated in seconds. *Alstonia scholaris L* was proved to be as capable as the standard drug Diazepam in treating anxiety.

Table 1: Effect of *Alstonia scholaris L.* bark extract on Elevated Plus maze:

Groups	% number of open arm entries	% time spent in open arm
Control	14.2 ± 1.378	14.5 ± 1.558
Standard(Diazepam 2mg/kg)	43.27 ± 1.293 ***	43.67 ± 1.501***
Ethanolic extract(100mg/kg)	13.87 ± 1.678	16.17 ± 1.179
Ethanolic extract(200mg/kg)	27.73 ± 1.667 ***	31.07 ± 1.882***
Ethanolic extract(400mg/kg)	33 ± 1.726 ***	35.495 ± 1.376***

Table 2: Effect of *Alstonia scholaris L.* bark extract on Light/dark transition test:

Groups	Number of entries into light chamber	Time spent in light chamber
Control	13.1 ± 0.8238	105.7 ± 4.499
Standard(Diazepam 2mg/kg)	24.13 ± 1.552 ***	197 ± 8.609 ***
Ethanolic extract(100mg/kg)	14.03 ± 1.254	114.8 ± 7.7
Ethanolic extract(200mg/kg)	16.37 ± 0.458 *	144.2 ± 6.747 **
Ethanolic extract(400mg/kg)	21.68 ± 1.453 ***	182.8 ± 7.22 ***

Table 3: Effect of *Alstonia scholaris L.* bark extract on Open field test:

Groups	**Number of squares crossed**	**Number of rearings**	**Time spent in the central square**
Control	76.27 ± 5.029	15.93 ± 1.615	36.17 ± 4.152
Standard(Diazepam 2mg/kg)	172.2 ± 5.859 ***	36.33 ± 1.545 ***	82.17 ± 3.277 ***
Ethanolic extract(100mg/kg)	83.5 ± 4.267	18.53 ± 2.529	45.5 ± 3.127
Ethanolic extract(200mg/kg)	102.5 ± 4.308 **	25.33 ± 2.172 **	62 ± 3.568 ***
Ethanolic extract(400mg/kg)	153 ± 7.738 ***	32.5 ± 1.375 ***	73.5 ± 2.83 ***

The values are expressed as Mean ± SEM for each group of mice, with n=6 animals in each group. In comparison to the control, significance was $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

CONCLUSION:

The anti-anxiety efficacy of an ethanolic extract of *Alstonia scholaris L.* bark was demonstrated in this investigation. *Alstonia scholaris L.* bark extract showed considerable activity in the Elevated plus maze, Light/dark transition test, and Open field test in a dose-dependent manner.. From results it was concluded that the treatment with *Alstonia scholaris L* can help in increasing following parameters: % number of open arm entries and % time spent in open arms in elevated plus maze; number of entries into light chamber and time spent in light chamber in Light/dark transition test; number of squares crossed, number of rearings and time spent in central square in open field test. In future, this work can be extended by using more anxiety models to further justify the therapeutic benefits in the treatment of anxiety.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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