# ANTI-ANXIETY ACTIVITY OF *Alstonia scholaris* LINN. R.Br.,

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**Summary**

*Alstonia scholaris* (Family: Apocynaceae) is a medicinal plant which has been indicated for the treatment of various diseases including arthritis in folklore medicine. *Alstonia scholaris* is used in Nigeria to treat mental illnesses by traditional psychiatrists. The purpose of this study is to investigate the antianxiety activity of the leaves of this plant in experimental animal models. The ethanolic extract of *Alstonia scholaris* leaves (EEAS) was tested against various anti-anxiety models viz. elevated plus maze, open field, hole board, light dark, mirror chamber and foot shock induced aggression models. The false positive results were overcome by actophotometer and rotarod tests. The ethanolic extract of *the leaves* was found to be significantly active in all the above mentioned anti-anxiety models which emphasized their anti-anxiety activity. There was no significant change in the locomotion test which indicates that EEAS did not have stimulant or sedative effects. There was no significant change in the time spent in the rotarod test, which indicates its lack of motor or muscle in-coordination. The present study is suggestive that EEAS has prominent antianxiety activity which may be attributed to its phytoconstituents – alkaloids, saponins, triterpenoids and flavonoids.

**Key words**: *Alstonia scholaris*; Antianxiety, Behavioural tests.

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## Introduction

*Alstonia scholaris* Linn.R.Br., belonging to family Apocynaceae is native to India growing wild throughout in deciduous, evergreen forests and even in plains. Bark of *Alstonia scholaris* possess spectrum of pharmacological activity, ranging from bitter, astringent, thermogenic, laxative, antipyretic, anthelmintic to galactogoguic and cardiotonic properties, therefore used in fever, malarial fever, abdominal disorder, dyspepsia, leprosy, skin diseases, asthma, bronchitis, cardiopathy etc., (1,2). An antimalarial Ayurvedic preparation containing *Alstonia scholaris* is marketed Ayush-64 (3). Folklore use include application of milky juice of leaves on wounds, ulcers, and for rheumatic pain, as well mixed with oil and applied for earache (1).

Extracts of *Alstonia scholaris* is reported to possess several pharmacological activities of interest that include pronounced antiplasmodial activity of methanolic extract of plant (4), antimutagenic effect (5), immunostimulatory effect of bark extract in low dose and inhibition of delayed hypersensitivity reaction in higher dose of aqueous extract (6), hepatoprotective against $CCl_4$, β-D galactosamine, acetaminophen and ethanol induced hepatotoxicity (7) and a promising anticancer activity against sarcoma – 180 by echitamine, an indole alkaloid extracted from bark (8,9). Several compounds like echitamine, alstonine, villalstonine, lupeol acetate and scholaricine have been isolated from various parts of *Alstonia scholaris* (10). *Alstonia scholaris* is used in Nigeria to treat mental illnesses by traditional psychiatrists. The traditional use of alstonine is reported to be remarkably compatible with its profile in experimental animals (11). Hence, an attempt was made to establish the anti-anxiety activity of ethanolic extract of *Alstonia scholaris* Linn.

## Methods

### Plant Material and Extraction

The leaves of *Alstonia scholaris* (Family: Apocynaceae) were collected in the month of September – October 2006 from hills of Savanthwadi, Maharashtra, India. The plant material was taxonomically identified by the Botany Survey of India (BSI), Pune and the voucher specimen AS-1 is retained in herbarium of BSI, Pune for future reference. The dried powdered leaves (500 g) were defatted using petroleum ether and subjected to extraction in a Soxhlet apparatus by using ethanol. The solvent was removed from the extract under reduced pressure to obtain a semisolid mass and vacuum dried to yield solid residue (5.24 % w/w ethanol extract). The extract (EEAS) showed positive tests for alkaloids, saponins, glycosides, triterpenoids and flavonoids.

### Animals

Male Albino Wistar rats weighing between 150 – 200 g and male Swiss albino mice weighing between 20 – 35 g were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum.* The study was approved by Institutional Animal Ethics Committee (Reg. No. 100/1999/CPCSEA). CPCSEA guidelines were adhered to during the maintenance and experiment.

**Acute toxicity studies**

Acute toxicity study was carried out for the ethanol extract (12). The extract suspended in water with 2% v/v Tween 80 in the dose of 2 g/kg body weight was orally administered to overnight-fasted, healthy rats (n=3). The animals were observed continuously for 24 h for mortality.

**Anti-Anxiety Activity**

The animals were divided into five groups of eight animals each as follows:

Group I – Control, received 2% w/v Tween 80, p.o.
Group II – (Standard) Diazepam 1 mg/kg, p.o.
Group III – EEAS 100 mg/kg, p.o.
Group IV – EEAS 200 mg/kg, p.o.
Group V – EEAS 400 mg/kg, p.o,
The antianxiety activity was carried out using different models.

**Elevated plus Maze (13)**

The elevated plus maze consists of two open arms and two closed arms (44 cm x 15 cm) with the open arm perpendicular to the closed one. The maze is made of wood and is located 64 cm above a black floor. Respective treatment was given to the animals and 30 min later, the animals were individually placed at the center of the plus maze and observed for 5 min. The number of entries and time in seconds, spent by the animals in the open arm and closed arm were noted and compared with the control group.

**Open Field (13)**

The open field test was carried out on the dark grey floor subdivided into 16 equal parts in a wooden box (100 cm x 100 cm x 30 cm). After 45 min of the treatment with EEAS, the animals were individually placed in the corner square of the open field. Spontaneous ambulation (number of segments crossed at periphery), activity in the centre (number of central squares crossed) and total locomotion (total number of squares crossed), were observed for 5 min.

**Holeboard Test (13)**

Male adult Albino Wistar weighing between 150–200 g was placed on 2 consecutvie days for 10 min in a black Perspex box (50 cm x 50 cm, walls 30 cm high) with 16 equally spaced holes (2.5 cm diameter, 10 cm apart) in the floor. The number of head-dips was recorded. A reduction of head-dips on the second day was interpreted as habituation to an unfamiliar environment. The ratio of head-dips second: first day were expressed as a percentage.

**Light/dark exploration test (14)**

The apparatus consisted of two boxes (25 x 25 x 25 cm) joined together. One box was made dark by covering its top with plywood whereas a 40-W lamp illuminated the other

box. The light source was placed 25 cm above the open box. The mice were treated with respective treatment or vehicle 30 min before being placed in the lit box.

**Spontaneous locomotion in rats (15)**

Male, adult Albino Wistar rats weighing between 200 –250 g were individually placed for 12 min in transparent polycarbonate cages (45 x 30 x 20 $cm^3$) equipped with two rows of photocells 4 cm above the floor and 24 cm apart. The locomotion counts after EEAS or standard treatment were recorded over the 12 min session (count corresponds to the consecutive interruption of two infrared beams) and compared with control.

**Rota-Rod Test (16)**

Male, untreated mice of 25 – 34 g were placed on a horizontal wooden rod (32 mm diameter), rotating at a speed of 5 rpm. The animals remaining on the rod for 3 min or more in successive trials were selected for the study. The animals were administered with EEAS or standard and were placed on the rod at intervals of 30, 60, 90 and 150 min after treatment. The time taken for the mice to fall from the rotating rod was noted.

**Mirror chamber Test (17)**

Male mice, 20-25 g were administered with EEAS or standard and placed individually in the chamber of mirror at a fixed corner and stopwatch was started and latency to enter the chamber ie. time in sec. for first entry into the mirror chamber, number of entries in 5 min and total time in seconds spent in chamber during the 5 min test period were noted. Criterion for entry into the chamber was all four paws being placed on the floor panel of mirror chamber. The average time spent with each entry was calculated by dividing the total time spent with the number of entries.

**Foot shock induced aggression (17)**

Male mice weighing 20-30 g were selected. Respective treatment was given for the animals. Two mice from the same treatment group were placed in a box with a grid floor consisting of steel rods with a distance of 6 mm. A constant current of 0.6 mA was supplied to grid floor by LVE constant current shocker with associated scramble. A 60-Hz current was delivered for 5 sec followed by 5 sec intermission for 3 min. Each pair of mice were dosed and tested without previous exposure. Total number of fights for each pair was recorded during the 3 min period. The fighting behavior consisted vocalization, leaping, rearing, running and facing each other with some attempt to attack by hitting, biting and boxing.

**Statistical Analysis**

The data were subjected to ANOVA followed by Newman-Keul's multiple comparison test (15). The values of $P < 0.05$ were considered statistically significant.

## Results

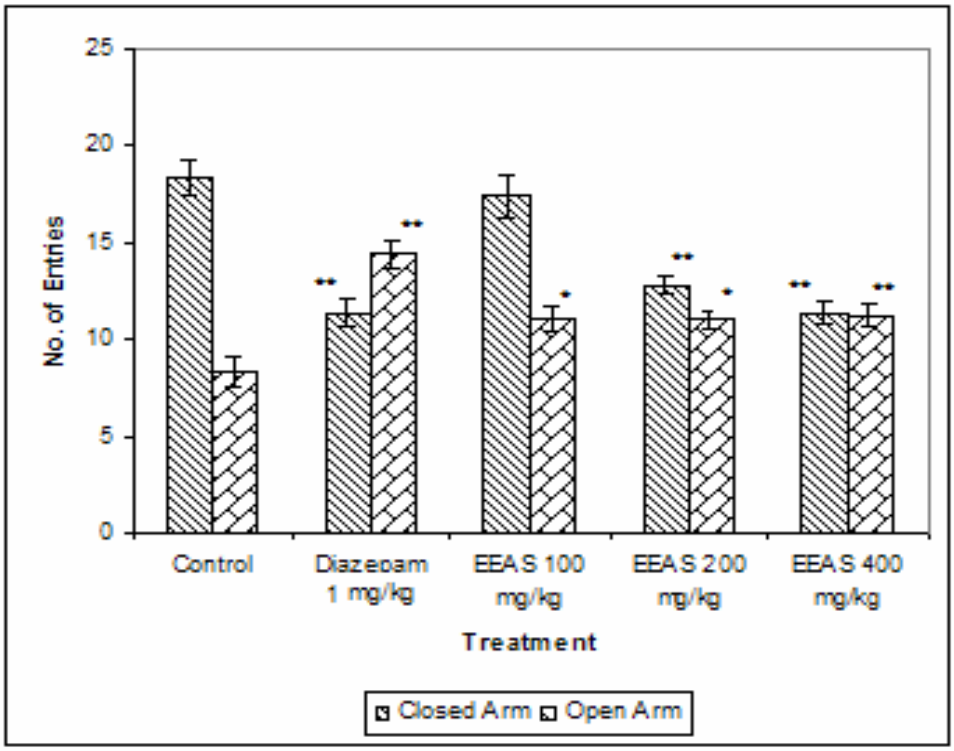
### Acute toxicity studies:

As suggested by OECD guidelines, the test animals were observed individually, after dosing at least once during the first 30 minutes, periodically during the first 24 h with special attention during first 4 h. The test animals did not exhibit any visible change and survived beyond recommended duration of observation. Hence EEAS was safe up to 2 g/kg. Doses of 100, 200 and 400 mg/kg were selected ($1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of 2 g/kg) for further experimentation (OECD guidelines, 2001).

### Elevated Plus Maze:

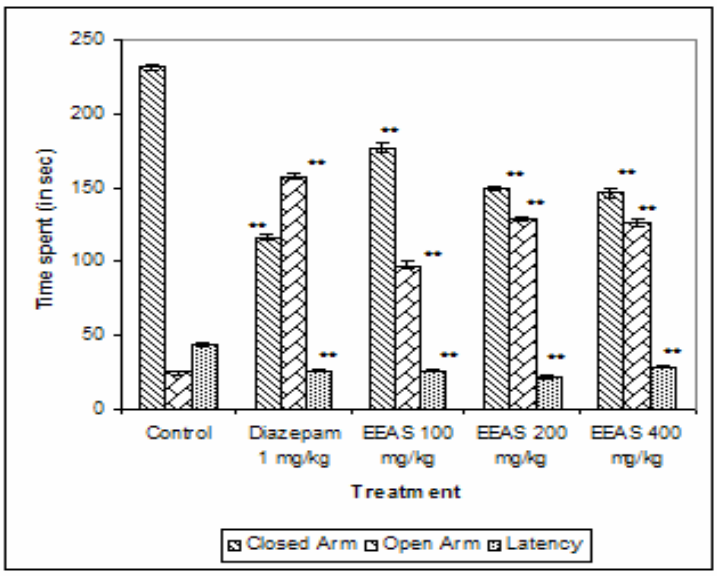
EEAS evoked a significant ($p<0.01$) dose dependent increase in the number of entries (Figure 1a) into open arms of the plus-maze without significant effect on total number of entries. EEAS also significantly ($p<0.01$) increased the time spent in the closed arm (Figure 1b).

Figure 1a. No. of entries in Elevated Plus maze




All values are represented as mean ± SEM (n = 8)
ANOVA followed by Student-Newman – Keul's multiple comparison test.
*$p<0.05$, **$p<0.01$ when compared to control.

Figure 1b. Time spent in Each arm in Elevated Plus maze

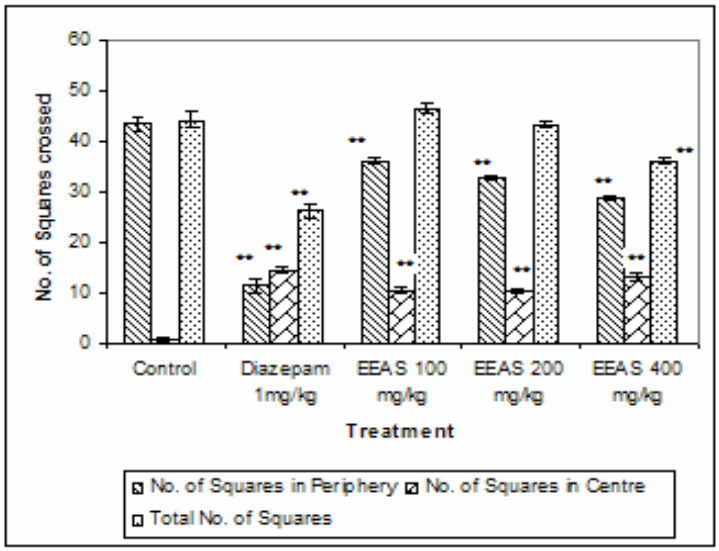



All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0.01 when compared to control.

**Open Field Test:**

EEAS significantly (p<0.01) increased the activity at center without significant effect on total locomotion. Diazepam (1 mg/kg) significantly (p<0.01) increased the ambulation activity at center (Figure 2).

Figure 2. Open Field Test – No. of squares crossed

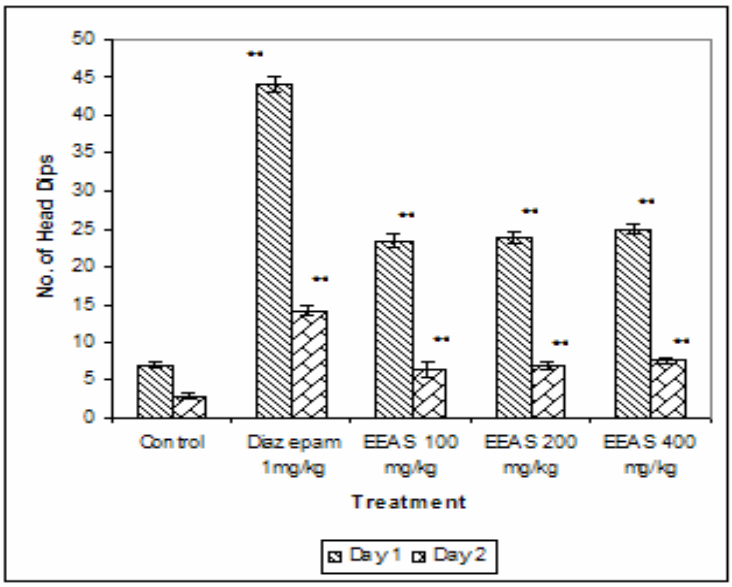



All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0.01 when compared to control.

**Hole board Test:**

There was a significant ($p<0.01$) increase in the number of holes explored in the diazepam and EEAS treated groups compared to control. There was a marked reduction of head-dips on the second day compared to the first day which indicate habituation of the animals to new and unfamiliar environment (Figure 3).

Figure 3. Hole Board Test – No. of Head Dips




All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **$p<0.01$ when compared to control.

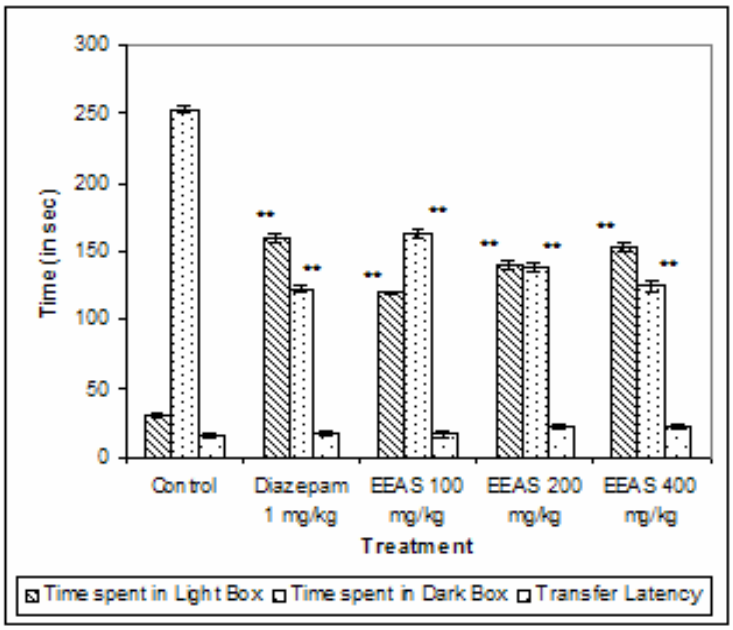
**Light Dark Exploration Test:**

There was a significant ($p<0.01$) increase in the time spent in the lighted box and a significant ($p<0.01$) decrease in the time spent in the dark box in the diazepam and EEAS treated groups compared to control (Figure 4a). There was no significant change in the transfer latency (Figure 4a) and the number of crossings (Figure 4b).

**Spontaneous locomotion in rats:**

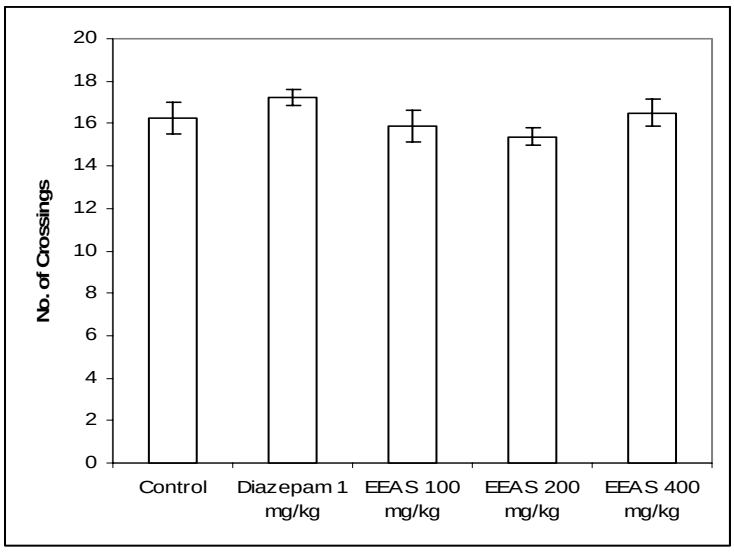
There was a significant ($p<0.01$) decrease in the total number of locomotion in diazepam (1 mg/kg) treated group. There was no significant change in locomotor activity in EEAS treated group compared to the control (Figure 5).

Figure 4a. Time spent in Light Dark Exploration Model



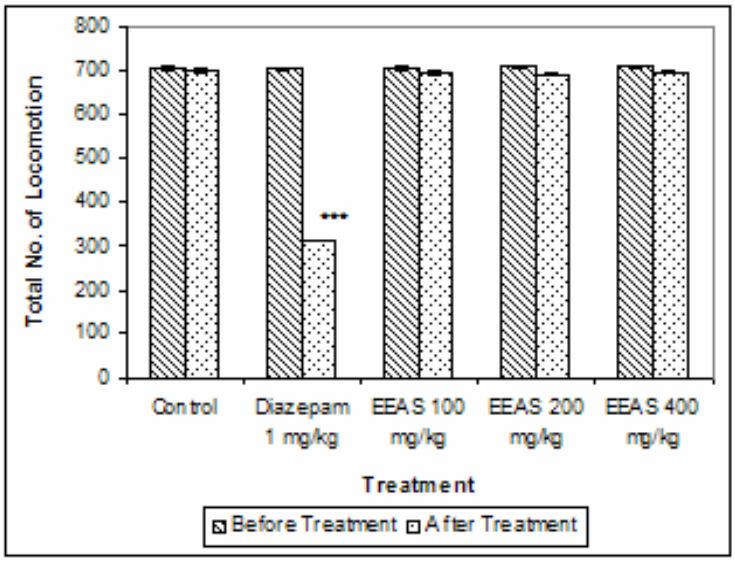

All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0.01 when compared to control.

Figure 4b. No. of Crossings in Light Dark Exploration Model




All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test.

Figure 5. Total no of locomotion in actophotometer

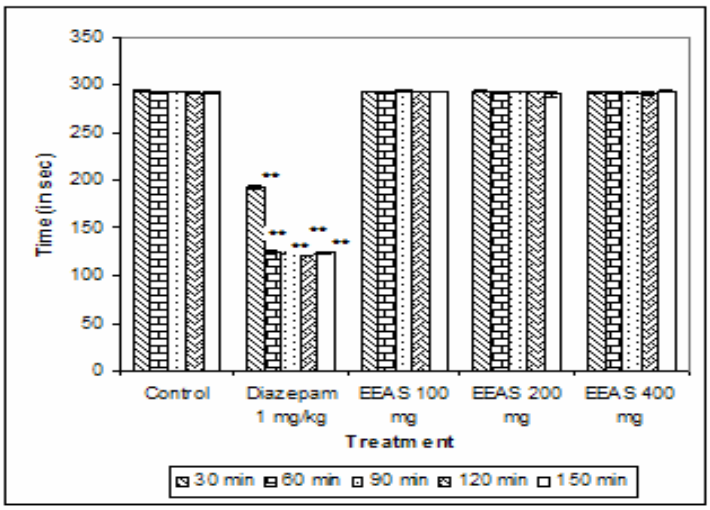



All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. ***p<0.001 when compared to control.

**Rotarod test:**

There was a significant (p<0.01) decrease in the time spent in the rotarod in diazepam (1 mg/kg) treated group. There was no significant decrease in the time spent in the rotarod in EEAS treated group compared to the control (Figure 6).

Figure 6. Time spent in Rotarod

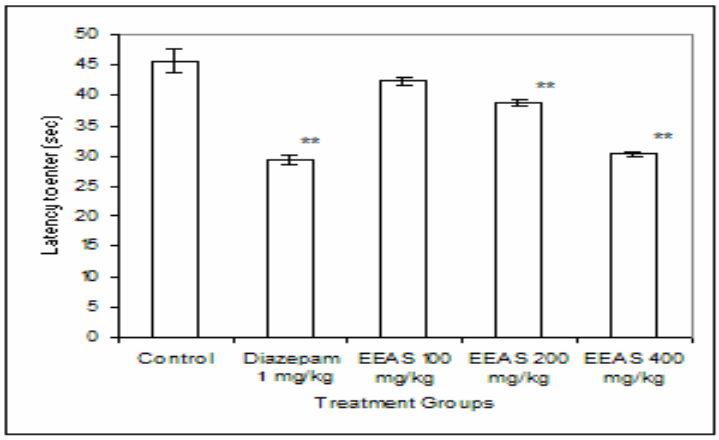



All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0. 01 when compared to control.

**Mirror chamber Test**

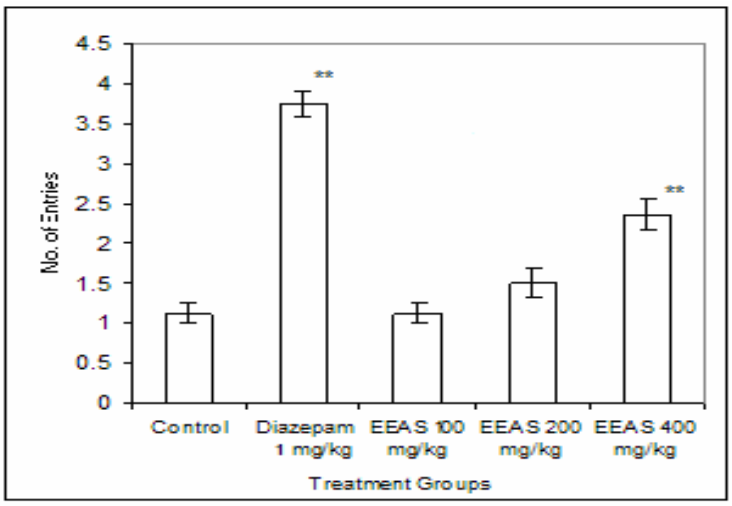
There was a significant ($p<0.01$) decrease in the latency to enter the mirror chamber in diazepam (1 mg/kg) and EEAS (200 mg/kg and 400 mg/kg) treated groups which was not observed in EEAS 100 mg/kg treated group (Figure 7a). There was also a significant ($p<0.01$) increase in the number of entries into the mirror chamber in diazepam (1 mg/kg) and EEAS (400 mg/kg) treated groups (Figure 7b). The time spent in the mirror chamber also increased significantly ($p<0.01$) in diazepam and EEAS (200 mg/kg and 400 mg/kg) treated groups (Figure 7c).

Figure 7a. Latency to Enter the Mirror Chamber



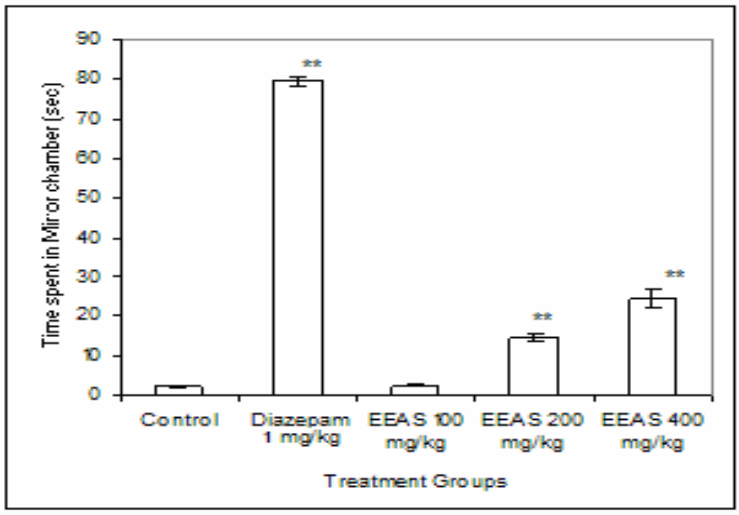

All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0. 01 when compared to control.

Figure 7b. No. of entries in the Mirror Chamber




All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0. 01 when compared to control.

Figure 7c. Time spent in each chamber

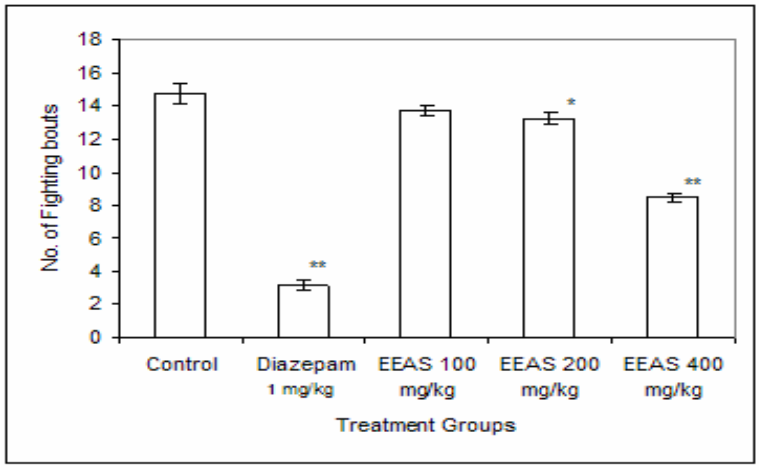



All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. **p<0. 01 when compared to control.

**Foot shock induced aggression**

Diazepam significantly (p<0.01) decreased the number of fighting bouts in foot shock induced aggression. There was a significant (p<0.05, p<0.01) decrease in the number of fighting bouts in foot shock induced aggression in EEAS 200 mg/kg and EEAS 400 mg/kg treated groups respectively. There was no significant change in EEAS 100 mg/kg treated group (Figure 8).

Figure 8. No. of Fighting bouts in Foot Shock Induced Aggression




All values are represented as mean ± SEM (n = 8); ANOVA followed by Student-Newman – Keul's multiple comparison test. *p<0.05, **p<0. 01 when compared to control.

## Discussion

Elevated plus maze and open field tests are based on spontaneous anxiety and are widely used test for screening of anxiolytic activity (18). It is based on the premise that exposure to an elevated plus maze evokes avoidance to conflict situation (such as fear of open space and height when approached to open arm) which is considerably stronger than that evoked when approached to an enclosed arm (19). Anxiolytics increase the time spent and number of entries into the open arm without changed locomotor activity (20). The closed arm entries are selectively correlated with locomotor activity and the drugs that cause stimulation and increase the locomotor activity were reported to increase the number of closed arm entries (21). In the present study there is a significant increase in the number of entries and time spent in the open arm on treatment with EEAS (Figure 1a, 1b). There is no significant increase in the closed arm entries, which proves the lack of stimulant effect.

In the open field test, forced confrontational situations induce anxiety behaviour in rodents. In such a situation, rodents spontaneously prefer the periphery of the apparatus and enter less in the central parts of the open filed. Indeed, mice and rats walk close to the walls, a behaviour called thigmotaxis (18). An increase in central locomotion or in time spent in the central part of the device without modification of total locomotion is interpreted as an anxiolytic effect (18). In the present study, EEAS (100 mg/kg and 200 mg/kg) increased the time spent in central part of the device without increasing the total number of squares crossed (Figure 2), whereas in diazepam (1 mg/kg) and EEAS (400 mg/kg), there is an increase in the time spent in central part of the device with a decrease in the total number of squares crossed which may be due to sedative effect (22).

In hole board test, there was a significant increase in the exploratory head dipping on the first day which shows anxiolytic like effect. There was a decrease in the number of head dips on the second day which indicates habituation of animals to new and unfamiliar environment (23).

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaivour of the rodents in response to mild stressors, that is, novel environment and light (24). Anxiolytics decrease the natural aversion to light and have been found to increase locomotion and time spent in the light zone, whereas anxiogenics decrease them (25). Results obtained in the present investigation showed increased time spent in the lighted box and decreased time spent in the dark box. This indicates the anxiolytic activity without increase in the locomotor activity. There is no significant decrease in the number of transitions and transfer latency which indicates the absence of sedation.

Locomotor activity is considered as an index of alertness and a decrease indicates a sedative effect where as increase in locomotor activity indicates a stimulant effect (26). In the present study, there is no change in locomotor activity with EEAS which strongly suggest that the anxiolytic activity is independent of stimulant or sedative effects.

There was no decrease in the time spent in the rotarod in EEAS treated groups which indicate the absence of skeletal muscle relaxant or motor inco-ordination of the extract (27).

EEAS shows antianxiety activity as evidenced by the decrease in latency to enter and increase in the number of entries in the mirror chamber (28).

Behavior characteristic of anger has been induced in various species of laboratory animals by a variety of experimental methods including foot shock. A mild foot shock has elicited fighting behaviour in rats. An anxiolytic agent reduces the number of fighting bouts (29). Decrease in the number of fighting bouts shown by EEAS indicated the anxiolytic activity of the extracts.

Previous studies on the chemical constituents of plants and their pharmacology suggest that plants that contain alkaloids, flavonoids, saponins and tannins possess activity against many CNS disorders (30). Phytochemical tests of EEAS revealed the presence of alkaloids, saponins, glycosides, triterpenoids and flavonoids. It is possible that the mechanism of anxiolytic activity of EEAS could be due to these phytochemicals.

The above results obtained in this study suggest that the ethanolic extract of the leaves of *Alstonia scholaris* possesses anxiolytic activity, which is devoid of sedative and muscle-relaxant activities. Thus, *Alstonia scholaris* has potential clinical application in the management of anxiety devoid of side effects. Further investigation of the mechanism(s) of action of the plant extract, and the active substance(s) responsible for its biological actions is underway.

## Acknowledgements

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