

Anti-anxiety and Anti-depressant Activity of Leaves of *Alstonia scholaris* Linn. R.Br.

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Abstract: Background: The antianxiety activity of the ethanolic extract of leaves of *Alstonia scholaris* (Family: Apocynaceae) has been well established. The objective of the present study is to evaluate the anti-anxiety activity of the ethyl acetate fraction from ethanolic extract of leaves of *Alstonia scholaris*. **Material and method:** EA fraction from *Alstonia scholaris* 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 change in brain monoamines was estimated. The possible serotonergic effect was tested by 5-Hydroxy Tryptophan (5-HTP) induced 'wet dog shake', tail suspension and modified forced swim tests. **Result:** EA was found to be significantly active in open field test, foot shock induced aggression and mirror chamber models of anxiety. However, lack of activity in elevated plus maze, light dark box, hole board test models, decrease in spontaneous motor activity, potentiation of 5-HTP, increase in 5-hydroxy tryptamine in brain indicate EA to have serotonergic effects. Decrease in immobility time in tail suspension test which was inhibited by reserpine and increase in swimming behaviour in forced swim test were conclusive evidence of selective serotonin reuptake inhibition. **Conclusion:** The present study suggests that EA possess antianxiety and antidepressant activity. The apparent mechanism of action is selective serotonin re-uptake inhibition.

Key words: Antianxiety, antidepressant, *Alstonia scholaris*, serotonin reuptake inhibition

INTRODUCTION

Alstonia scholaris Linn. R.Br., (Family: Apocynaceae) is native of 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 galactogogue and cardiogenic properties; therefore used in fever, malarial fever, abdominal disorder, dyspepsia, leprosy, skin diseases, asthma, bronchitis, cardiopathy etc. (Nadkarni, 1976; Kirtikar and Basu, 2002). An antimalarial Ayurvedic preparation, Ayush-64, containing *Alstonia scholaris* is marketed in India (Versha *et al.*, 2003). 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 (Nadkarni, 1976). Extracts of *Alstonia scholaris* is reported to possess several pharmacological activities of interest that include pronounced antiplasmodial activity (Keawpradub *et al.*, 1999) antimutagenic effect (Lim-Sylianco *et al.*, 1990), immunostimulatory effect,

inhibition of delayed hypersensitivity reaction (Iwo *et al.*, 2000), hepatoprotective against CCl₄, β-D galactosamine, acetaminophen and ethanol induced hepatotoxicity (Lin *et al.*, 1996), anticancer activity against sarcoma-180 (Saraswathi *et al.*, 1998, 1999) analgesic, anti-inflammatory (Arulmozhi *et al.*, 2007a), antidiabetic (Arulmozhi *et al.*, 2010) and antianxiety (Arulmozhi *et al.*, 2008) activities. Several phytoconstituents such as echitamine, alstonine, villalstonine, lupeol acetate and scholaricine have been isolated from *Alstonia scholaris* (Arulmozhi *et al.*, 2007b). *Alstonia scholaris* is used in Nigeria to treat mental illness by traditional psychiatrists. This traditional use of *Alstonia scholaris* is reported to be remarkably compatible with its profile in experimental animals (Costall and Naylor, 1995).

The methanolic extract of bark of *Alstonia scholaris* was reported to possess anti-anxiety activity in various stress models (Kulkarni and Juvekar, 2009). It was also found to be an antioxidant and improve cognition in passive avoidance models. Recently, we have reported the anti-anxiety activity of ethanol extract of leaves of

Alstonia scholaris Linn. (Arulmozhi *et al.*, 2008). In continuation of our work, an effort was made to identify the promising fraction of EEAS and to elucidate the possible mechanism behind the antianxiety activity.

MATERIALS AND METHODS

Collection and authentication of plant: The leaves of *Alstonia scholaris* (Family: Apocynaceae) were collected in the month of September-October, 2008 from hills of Sawantwadi, Maharashtra, India. The plant material was taxonomically identified by Dr. Rajesh Dabur, Regional Research Institute (RRI) (Ayurved), Pune and the voucher specimen 661 is retained in herbarium of RRI, Pune for future reference.

Preparation of ethyl acetate fraction from leaves of *Alstonia scholaris*: 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 EEAS). The EEAS was partitioned with petroleum ether (60-80°C), dichloromethane, ethylacetate and butanol in a separating funnel. The solvents were removed from the fractions under reduced pressure and vacuum dried to yield solid residues. The Ethyl Acetate fraction (EA) (Yield: 0.30 % w/w) showed the presence of tannins, flavonoids, triterpenoids and alkaloids in preliminary phytoanalysis. EA suspended in 2% w/v Tween 80 was used for the present study.

Chemicals and reagents: Reserpine, 5-hydroxy tryptophan (5-HTP), noradrenaline (NA), dopamine (DA) and 5-hydroxy tryptamine (5-HT) were procured from Sigma Aldrich, US. Fluoxetine was obtained from Palam Pharma Private Limited, Gujarat, India as gift sample. All other chemicals and reagents used for the study were of analytical grade procured from approved organizations.

Animals: Male Albino Wistar rats weighing between 150-180 g and male Swiss albino mice weighing between 20-25 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), Approval No. CPCSEA/45/2008. CPCSEA guidelines were adhered to during the maintenance and experiment.

Acute toxicity studies: Acute toxicity study was carried out for the EA following OECD guidelines (OECD The EA fraction suspended in water with 2%w/v Tween

80 in the dose of 5 mg kg⁻¹ body weight was orally administered to overnight-fasted, healthy rats (n = 3). The animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter for a total of 14 days. The acute toxicity study was repeated with doses of 50 and 300 mg kg⁻¹ body weight.

Anti-anxiety activity: The animals were divided into four groups of eight animals each as follows:

- Group I-Vehicle control, 2% w/v Tween 80, p.o.
- Group II-(Standard) Fluoxetine 30 mg kg⁻¹, p.o.
- Group III-EA 10 mg kg⁻¹, p.o.
- Group IV-EA 30 mg kg⁻¹, p.o.

The antianxiety activity was carried out using different models.

Elevated plus maze: Male Albino Wistar rats weighing between 150-180 g were used for the study. The elevated plus maze consisted of two open arms and two closed arms (44×15 cm) with the open arm perpendicular to the closed one. The maze was made of wood and was located 64 cm above a black floor. Respective treatment was given to the animals and 1h 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 (Rex *et al.*, 2004).

Open field: Male Albino Wistar rats weighing between 150-180 g were used for the study. The open field test was carried out in a wooden box (100×100×30 cm) which consisted of dark grey floor subdivided into 16 equal parts. After 1 h of treatment with EA, 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 (Rex *et al.*, 2004).

Holeboard test: Male adult Albino Wistar weighing between 150-180 g were administered with respective treatment. After 1 h, the animals were placed on a black Perspex box (50×50, walls 30 cm high) with 16 equally spaced holes (2.5 cm diameter, 10 cm apart) for 10 min for 2 consecutive days. The number of head-dips was recorded. A reduction of head-dips on the second day was interpreted as habituation to an unfamiliar environment (Rex *et al.*, 2004).

Light/dark exploration test: The apparatus consisted of two boxes (25×25×25 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. Male mice, 20-25 g were administered with respective treatment or vehicle 1 h before being placed in the lit box. The time spent in each box and number of crossings were recorded (Jain *et al.*, 2003).

Mirror chamber Test: Male mice, 20-25 g were administered with respective treatment and placed individually in the chamber of mirror at a fixed corner. The latency to enter the chamber (time in sec for first entry into the mirror chamber), number of entries in 5 min and total time 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 (Kulkarni, 1999).

Foot shock induced aggression: Male mice weighing 20-25 g were administered with respective treatment 1 h before the experiment. 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. 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 (Kulkarni, 1999).

Spontaneous locomotion in rats: Male, Albino Wistar rats weighing between 150-180 g were individually placed for 12 min in transparent polycarbonate cages (45×30×20 cm³) equipped with two rows of photocells 4 cm above the floor and 24 cm apart. The locomotion counts before and after 1 h of respective treatment were recorded over the 12 min session (count corresponds to the consecutive interruption of two infrared beams) and compared with vehicle control (Millan *et al.*, 2005).

Rota-Rod Test: Male, untreated mice of 20-25 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 respective treatment 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 (Dunham and Miya, 1957).

5-Hydroxy tryptophan (5-HTP) induced 'Wet Dog Shake' behaviour: Swiss albino mice of 20 to 25 g were divided into four groups and were administered with carbidopa (25 mg kg⁻¹) followed by which 5-HTP (100 mg kg⁻¹) was administered intraperitoneally. The number of head twitches for each animal was counted for a period of 5 min at 0 min, 15 min, 30 min, 1 h. The respective treatment was administered at 90 min (1 h and 30 min) of the administration of 5-HTP and the number of head twitches was counted till 6 h (Bedard and Pycocock, 1988).

Brain monoamine estimation: Brain mono amine levels were estimated using High performance liquid chromatography (Lakshmana and Raju, 1997). Once the animals completed the hole board task on day 2, they were sacrificed by decapitation under anesthesia, heads were dropped into ice-cold 0.1 M Perchloric acid (PCA) and the brains were removed on an ice-chilled petri dish. The brain tissues were weighed, homogenized in 2 mL of 30 ng mL⁻¹ isoproterenol in 0.1M PCA and centrifuged at 12,000 g for 15 min at 4°C. The supernatant was filtered through a 0.45 µm membrane (Sartorius) and 20 µL of the filtrate was injected onto a High Performance Liquid Chromatograph (HPLC) column. HPLC configured for dynamic mixing with a two-pump system PU 1580 (JASCO, Japan) was used. The rate of flow of mobile phase was kept at 0.9 mL⁻¹min. An auto sampler AS-1555 (JASCO, Japan) was used to introduce the sample to the column. Detection was done with a Fluorescent detector, JASCO, Japan. A guard column: RP-18, 33 mm Kromasil[®] and analytical column RP-18, 250 mm (5 µm) Vydac[®] were used at ambient temperature. Borwin chromatographic software was used for recording peaks and data integration. After separation, Noradrenaline (NA), dopamine, isoproterenol and 5-hydroxy tryptamine were detected at the excitation wavelength of 280 nm and an emission wavelength of 315 nm. The slit width was kept at 10/10 for excitation/emission respectively. The slit width is expressed as length and width of the sample-plane zone being quantified.

Tail suspension test: Male Swiss albino mice of 20 to 25 g were used for the study. The animals were administered with respective treatment before 1 h of the study. Each animal was suspended by the tail on the edge of a hook 50 cm above the floor using adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively

(Dhingra and Valecha, 2007). The study was also performed in a fresh set of animals which were reserpinized (4 mg⁻¹kg, i.p.) 4 h before administration of the respective treatment (Teste *et al.*, 1993).

Modified forced swim test: Male albino Wistar rats weighing between 150 -180 g were used for the study. The modified rat forced swim test was conducted (Cryan and Lucki, 2000). The rats were placed individually, for 15 min in Pyrex cylinders (21×46 cm) that were filled with water to a depth of 30 cm. They were removed after 15 min, dried and placed in their home cage. Animals were randomly assigned to groups receiving various drug treatments or to a vehicle control group. The respective treatment was administered three times (1, 5 and 23.5 h) before the test session. Twenty-four h after the first exposure, the animals were replaced in the swim apparatus for 5 min and the session was observed by a rater blind to the experimental conditions being scored. A time sampling technique was used whereby the predominant behavior in each 5 sec period of the 300 sec test was recorded. Climbing behaviour consisted of upward-directed movements of the forepaws along the side of the swim chamber. Swimming behaviour was defined as movement (usually horizontal) throughout the swim chamber, which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the rat's head above the water.

Statistical analysis: The data was subjected to one way ANOVA followed by Dunnet's 't' test. The values of p<0.05 were considered statistically significant.

RESULTS

Acute toxicity studies: The test animals did not exhibit any visible change and survived beyond recommended duration of observation. Hence EA was safe up to 300 mg kg⁻¹. Doses of 10 and 30 mg kg⁻¹ were selected for further experimentation (OECD Guidelines, 2001).

Effect of EA on elevated plus maze test: There was a significant (p<0.01) decrease in the number of open arm entries and time spent in the open arm on treatment with EA or fluoxetine when compared to vehicle control (Table 1). Treatment with EA or fluoxetine also significantly (p<0.01) reduced the number of closed arm entries (Table 1). The decrease in number of entries and duration spent in open arm thus indicate that there is an anxiogenic effect whereas decrease in number of closed arm entry indicates the decrease in motor activity.

Effect of EA on open field test: There was a significant increase (p<0.01) in the number of squares crossed in the centre with all the treatment groups compared to control. There was a significant decrease (p<0.01) in the number of squares crossed in the periphery with all the treatment groups compared to control. There was also a significant (p<0.01) decrease in the total number of squares crossed in all the tested groups when compared to control (Table 1), which indicates the decrease in motor activity.

Effect of EA on Light dark box: There was a significant (p<0.01) decrease in the time spent in the lighted box and a significant (p<0.01) increase in the time spent in the dark box in the fluoxetine or EA treated groups compared to control. There was also a significant decrease (p<0.01) in the number of crossings observed with all the tested groups when compared to control (Table 2).

Effect of EA on hole board test: There was a significant (p<0.01) decrease in the number of holes explored in all the treatment groups compared to control. There was a marked reduction of head-dips on the second day compared to the first day in all the groups including vehicle control, which indicate habituation of the animals to a new and unfamiliar environment (Table 2).

Effect of EA on foot shock induced aggression: There was a significant (p<0.01) decrease in the number of fighting bouts in foot shock induced aggression in the fluoxetine or EA treated groups compared to control (Table 2).

Table 1: Effect of EA on elevated plus maze test and open field test

Test	Parameter			
	Vehicle control	Fluoxetine 30 mg kg ⁻¹	EA 10 mg kg ⁻¹	30 mg kg ⁻¹
Elevated plus maze test				
No. of entries in closed arm	20.00±1.68	12.12±3.24**	7.24±2.88**	6.12±2.74**
No. of entries in open arm	7.74±0.98	3.24±0.36**	2.88±0.44**	2.74±0.44**
Time Spent in closed arm (sec)	232.74±3.96	263.24±1.14**	261.12±1.14**	263.62±1.22**
Time spent in open arm (sec)	24.24±1.92	13.50±0.62**	15.00±0.84**	14.12±0.82**
Transfer Latency (sec)	42.00±2.84	23.24±0.66	23.88±0.82	22.24±0.88
Open field test				
No. of squares crossed in Periphery	44.00±1.12	14.38±0.92**	16.50±1.28**	14.50±0.76**
No. of squares crossed in Centre	1.88±0.22	15.62±0.82**	12.74±0.56**	14.88±0.54**
Total No. of squares crossed	45.88±1.22	30.00±1.40**	29.24±1.04**	29.38±0.92**

Values are expressed as Mean±SEM, n = 8; One Way ANOVA followed by Dunnet's 't' test; **p<0.01 compared to Vehicle control; EA: Ethyl acetate fraction

Table 2: Effect of EA on Light dark box, hole board test and foot shock induced aggression

Test	Parameter			
	Vehicle control	Fluoxetine 30 mg kg ⁻¹	EA 10 mg kg ⁻¹	30 mg kg ⁻¹
Light dark box				
Time spent in Light Box (sec)	30.12±1.24	14.38±1.12**	19.00±0.94**	12.50±0.58**
Time spent in Dark Box (sec)	251.74±1.52	273.74±2.78**	265.38±2.18**	266.74±1.36**
Transfer Latency	18.12±1.46	11.88±2.36	15.62±1.84	20.74±1.86
No. of crossings	16.38±0.74	10.00±0.56**	9.88±0.58**	10.24±0.44**
Hole Board Test				
No. of head dips on Day 1	7.88±0.82	3.74±0.52**	4.74±0.24**	4.12±0.28**
No. of head dips on Day 2	3.00±0.18	2.24±0.36	2.50±0.26	2.12±0.22
Foot Shock induced aggression				
No. of Fighting bouts	16.00±0.56	2.74±0.44**	11.88±0.38**	5.24±0.36**

Values are expressed as mean ± SEM, n = 8; One Way ANOVA followed by Dunnett's 't' test; **p<0.01 compared to Vehicle control; EA:Ethyl acetate fraction

Table 3: Effect of EA on the Mirror chamber test and spontaneous locomotion in actophotometer

Test	Parameter			
	Vehicle control	Fluoxetine 30 mg kg ⁻¹	EA 10 mg kg ⁻¹	30 mg kg ⁻¹
Mirror chamber test				
Latency to enter Mirror chamber	45.38±2.66	30.74±1.14**	34.50±2.32**	31.00±1.72**
No. of entries in Mirror chamber	1.00±0.00	4.38±0.18**	3.38±0.26**	4.12±0.30**
Duration Spent in Mirror chamber	2.12±0.30	71.74±6.54**	18.00±1.92**	40.74±2.48**
Spontaneous locomotion in actophotometer				
No. of entries before treatment	543.38±10.54	527.62±8.02	534.00±6.18	531.00±7.24
No. of entries after treatment	530.88±9.82	329.00±6.10**	423.62±3.14**	424.88±4.18**
Tail suspension test				
Immobility Time (Non-reserpinized animals)	158.12 ±5.82	140.24±3.64*	145.74±4.94	138.62±24*
Immobility Time (Reserpinized animals)	205.24 ±4.06	204.50 ±4.26	205.62±3.44	206.00±2.92

Values are expressed as mean ± SEM, n = 8; One Way ANOVA followed by Dunnett's 't' test; *p<0.05, **p<0.01 compared to Vehicle control; EA:Ethyl acetate fraction

Effect of EA on mirror chamber: There was a significant (p<0.01) decrease in the latency to enter the mirror chamber in fluoxetine and EA treated groups when compared to control. There was also a significant (p<0.01) increase in the number of entries and duration spent in the mirror chamber in fluoxetine and EA treated groups when compared to control (Table 3).

Effect of EA on spontaneous locomotion in actophotometer: There was a significant (p<0.01) decrease in the total number of locomotion in fluoxetine (30 mg kg⁻¹) or EA (10 and 30 mg kg⁻¹) treated groups when compared to the control (Table 3).

Effect of EA on rotarod test: There was no significant decrease in the time spent in the rotarod in fluoxetine (30 mg kg⁻¹) or EA (10 and 30 mg kg⁻¹) treated groups when compared to the control (Fig. 1).

Effect of EA on 5-HTP induced 'Wet Dog Shake' behaviour: There was 'wet dog shake' type of twitching observed on injection of 5-HTP with all the treatment groups. The maximum number of wet dog shakes were observed at 15 min of injection of 5-HTP. The number of wet dog shakes with all the groups started to decrease after 1 h. On administration of EA or fluoxetine at 90 min, the number of wet dog shakes in EA or fluoxetine treated groups significantly (p<0.01) increased when compared to

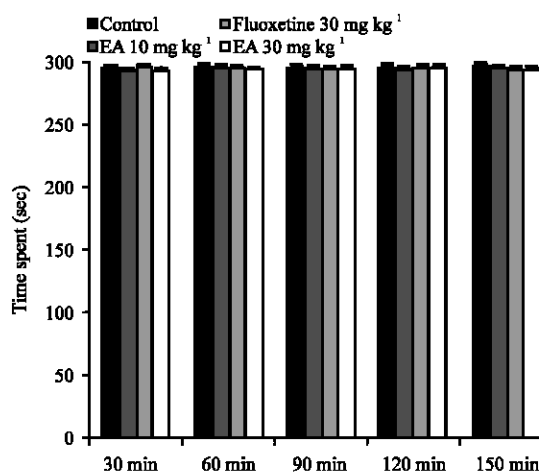


Fig. 1: Effect of EA on Rotarod test values are expressed as Mean±SEM, n = 8. One-way ANOVA followed by Dunnett's multiple comparison test EA-Ethyl acetate fraction

control (Fig. 2). This significant (p<0.01) increase in the number of wet dog shakes was observed with EA 30 mg kg⁻¹ and fluoxetine 30 mg kg⁻¹ till the end of the study, whereas the wet dog shakes was observed only till 3rd h in EA 10 mg kg⁻¹ treated group (Fig. 2). The results thus indicate that EA and fluoxetine have the ability to potentiate 5-HTP induced wet dog shake behaviour.

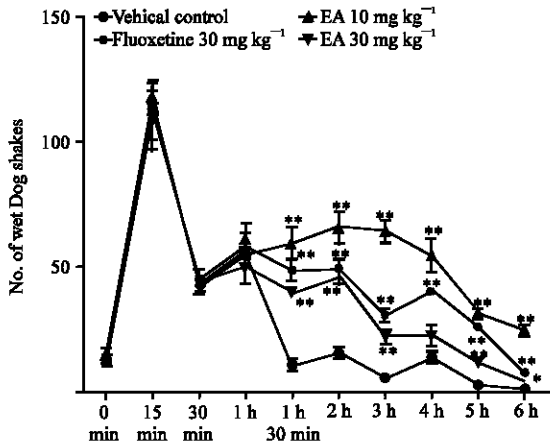


Fig. 2: Effect of EA on 5-HTP induced wet dog shake behaviour Values are expressed as Mean±SEM, n = 8 one-way ANOVA followed by Dunnet's multiple comparison test *p<0.05, **p<0.01 compared to vehicle control. EA-Ethyl acetate fraction 5-HTP-5 Hydroxy tryptophan

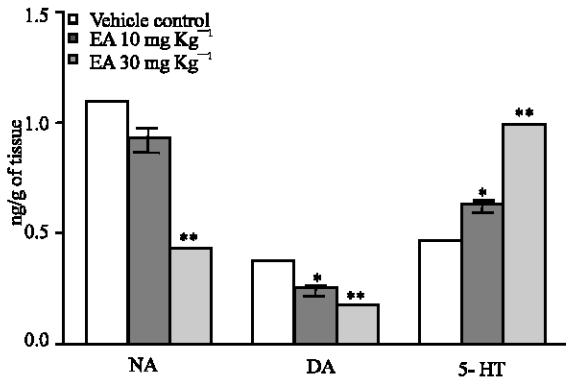


Fig. 3: Effect of EA on Brain Monoamines Values are expressed as Mean±SEM, n = 8 one-way ANOVA followed by Dunnet's multiple comparison test *p<0.05, **p<0.01 compared to vehicle control. NA- Noradrenaline, DA-Dopamine, 5-HT-5-hydroxy tryptamine, EA-Ethyl acetate fraction.

Effect of EA on brain monoamines: There was a significant decrease ($p<0.01$) in brain noradrenaline and dopamine levels on treatment with EA 30 mg kg⁻¹, whereas there was a non-significant decrease in the noradrenaline and significant ($p<0.05$) decrease in the dopamine levels on treatment with EA 10 mg kg⁻¹. The levels of 5-hydroxy tryptamine significantly ($p<0.05$ and $p<0.01$) elevated on treatment with EA 10 mg kg⁻¹ and 30 mg kg⁻¹, respectively (Fig. 3).

Effect of EA on tail suspension test: There was a significant ($p<0.05$) decrease in the immobility time on

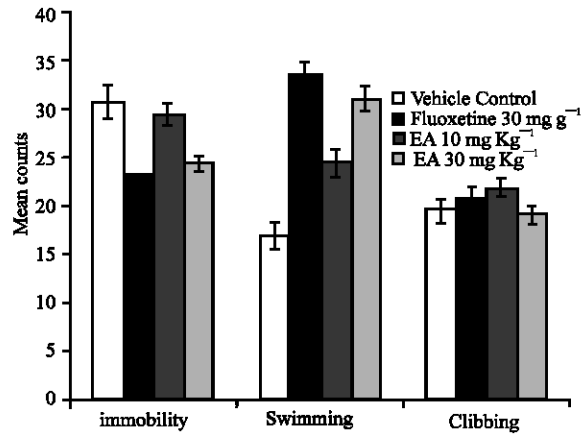


Fig. 4: Effect of EA on Modified Forced Swim Test. Values are expressed as Mean±SEM, n = 8 one-way ANOVA followed by Dunnet's multiple comparison test **p<0.01 compared to vehicle control. EA- Ethyl acetate fraction.

treatment with fluoxetine (30 mg kg⁻¹) or EA (30 mg kg⁻¹) in non-reserpinized animals when compared to the vehicle control (Table 3) while there was no change in the immobility time in reserpinized animals with any of the treatment groups when compared to reserpine control (Table 3).

Effect of EA on modified forced swim test: There was a significant ($p<0.01$) decrease in the mean immobility count on treatment with fluoxetine (30 mg kg⁻¹) or EA (30 mg kg⁻¹) when compared to the control (Fig. 3). There was also a significant ($p<0.01$) increase in the mean swimming count on treatment with fluoxetine or EA when compared to the control (Fig. 4). Treatment with EA 10 mg kg⁻¹ significantly ($p<0.01$) increased the mean swimming count but failed to modify the mean immobility count. None of the treatment groups exhibited any alteration in the climbing behaviour when compared to the control (Fig. 4).

DISCUSSION

Elevated plus maze test is based on spontaneous anxiety and is used for screening anxiolytic activity (Prut and Belzung, 2003). Anxiolytics increase the time spent and number of entries into the open arm without changed locomotor activity (Pellow *et al.*, 1985). In the present study, significant decrease in the number of entries and time spent in the open arm in EA treated group indicates anxiogenic activity. The decrease in the number of closed arm entry in the present study indicates decrease in the motor activity.

The number of head dips in hole board test gives an indication of exploratory tendency, an increase in which is an indication of anxiolytic activity (Sonavane *et al.*, 2002). In the present study, there was a decrease in the number of head dips on treatment with EA, which indicate anxiogenic-like activity.

The light dark test is based on the aversion of rodents to illuminated areas (Crawley and Goodwin, 1980). Anxiolytics decrease the natural aversion to light which is found by increase in time spent in the light zone, whereas anxiogenics decrease them. In the present study, there was a decrease in the time spent in the light zone, which indicate the anxiogenicity of EA.

In open field test, forced confrontational situations induce anxiety which makes rodents prefer the periphery, a behaviour called thigmotaxis (Prut and Belzung, 2003). An increase in central locomotion or in time spent in the central part of the device without increasing the total locomotion is interpreted as anxiolytic effect (Prut and Belzung, 2003). In the present study, there was an increase in the number of squares crossed in the centre on treatment with EA, which indicate anxiolytic activity. However, the decrease in the number of squares crossed in the periphery and decrease in the total number of squares crossed indicate the decrease in motor activity.

Aggression behaviour is induced in laboratory animals by a variety of experimental methods including foot shock, which is manifested as fighting behaviour. An anxiolytic agent reduces the number of fighting bouts (Tedeschi *et al.*, 1959). In the present study, the observed decrease in the foot-shock induced fighting behaviour with EA, indicate anxiolytic activity.

This is further substantiated by decrease in the latency to enter and increase in the number of entries into the mirror chamber, which offers an aversive stimulus.

The results thus obtained from elevated plus maze, hole board test and light dark test suggest anxiogenic effect whereas the open field test, foot shock induced aggression and mirror chamber test suggest anxiolytic effect of EA, which are contradictory to each other. There was also a significant decrease in locomotion observed in elevated plus maze, open field tests and actophotometer. However, there was no change in the time spent in the rotorod.

The main criterion for the use of elevated plus maze as an animal anxiety model is its sensitivity to the anxiolytic action of benzodiazepines, but it is known that several anxiety disorders, such as phobias and panic are resistant to benzodiazepines (Graeff *et al.*, 1993). Serotonergic drugs like fluoxetine are known to produce anxiogenic-like effect in elevated plus maze after acute administration (Kshama *et al.*, 1990; Handley *et al.*,

1993). Almost all researchers reported an anxiogenic effect with serotonergic drugs in elevated plus maze (Handley *et al.*, 1993). Acute fluoxetine has been shown to promote anxiogenic-like effects in rats tested in the elevated plus maze, in the hole-board and in a light aversion test (Silva *et al.*, 1999). The anxiogenic effect after acute Selective Serotonin Reuptake Inhibitor (SSRI) administration could be related to the increased extracellular 5-HT around sub-cortical structures (Silva *et al.*, 1999). These evidences suggest that the anti-anxiety activity of EA is not mediated through benzodiazepine receptor but through Selective Noradrenaline Reuptake Inhibition (SNRI) or selective serotonin reuptake inhibition (SSRI). However, the brain monoamine levels in the present study indicate an increase in the 5-HT and decrease in the noradrenaline and dopamine levels. Potentiation of 5-HTP induced wet dog shake by EA also substantiates the increase in the 5-HT levels, which is probably by 5-HT uptake inhibition activity (Takeuchi *et al.*, 1997). With these clues, the effect of EA on tail suspension test and modified forced swim test were performed to elucidate the possible mechanism of action of EA.

Tail suspension test is a model in which many antidepressants reduce the immobility time indicating that this is an Index of anti-depressant activity (Fujishiro *et al.*, 2001). In the present study, there was a significant decrease in immobility time in non-reserpinized mice whereas there was no change in immobility in reserpinized mice. Tail suspension test markedly reduces the turnover of NA in specific part of the brain in rats. Stress induced by tail suspension test increases the tissue content of noradrenaline, whereas serotonin is not affected (Teste *et al.*, 1993). Reserpine also depletes the noradrenaline, where SNRI are active in tail suspension test in reserpinized mice, whereas SSRIs show weak activity in tail suspension test in non-reserpinized mice and no activity in reserpinized mice (Teste *et al.*, 1993). Tail suspension test is likewise responsive to tricyclic antidepressants and SNRIs than to SSRIs (Millan *et al.*, 2001).

Most of the animal models of antidepressant activity are based on noradrenergic effects. Behavioural tests such as potentiation of effects of 5-HT precursors like 5-HTP induced twitches, detected alterations of serotonergic activity and were not tests for potential antidepressant activity (Lucki, 1997). Although, the forced swim test is sensitive to most antidepressants which increase noradrenergic transmission, the effects of SSRIs have not been readily detected using forced swim test. Hence, modified forced swim test is highly sensitive to acute antidepressant treatments, which differentiates

antidepressants from different classes, were used in the present study. In the present study, there was a significant increase in swimming behavior and significant decrease in immobility on treatment with EA, whereas the climbing behavior was not altered (Fig. 4). Antidepressants which selectively inhibit NE reuptake were shown to reduce immobility and selectively increase climbing without affecting swimming. In contrast, the SSRIs also reduced immobility but increased swimming without affecting climbing (Lucki, 1997; Cryan *et al.*, 2002, 2005). The results thus indicate that the mechanism of antidepressant action of EA is mediated through selective serotonin reuptake inhibition.

CONCLUSION

In summary, the proposed mechanism of antianxiety and antidepressant action of EA is mediated through selective serotonin reuptake inhibition. However, further studies are needed to identify, isolate and elucidate the bioactive principle(s) that are responsible for the activity that is underway.

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