Anxiolytic-Like Actions of Methanolic Extract of Solanum Torvum (Solanaceae) Seeds in Mice

Rehan Momin, Mahalaxmi Mohan*

Department of Pharmacology, MGV's Pharmacy College Panchavati, Nashik-422 002, Maharashtra, India.
*Priyadarshini College of Pharmaceutical Sciences, Chowdharyguda(V), Ghatkesar (M), Ranga Reddy (D), Hyderabad-501 088, India

*Corresponding author -Email: mm_nasik@yahoo.co.in
Mob no- (+091) 9581133007

Summary

Anxiety is one of the commonest psychiatric disorders and a large number of medicinal plants have been explored to treat this disorder.Anxiolytic-like action of methanolic extract of the seeds of *Solanum torvum* (ST) were studied in Swiss albino mice using elevated plus maze (EPM), light and dark transition apparatus (LDA), hole board apparatus (HBA) and marble burying test (MBT).In addition, the effect of Prazocin (62.5 µg/kg, i.p.), an alpha adrenoceptor antagonist; p-chlorophenylalanine (100 mg/kg, i.p.), an inhibitor of serotonin synthesis and haloperidol (50 µg/kg, i.p.), a D2 receptor antagonist on anxiolytic-like effect of ST in above four models were also studied. The effect of ST on PTZ (60 mg/kg, i.p) induced seizures was also studied. Methanolic extract of *Solanum torvum* (ST-10, 30 and 100 mg/kg) and its ethyl acetate fraction (EAF-10, 30 mg/kg) showed significant increase in open arm exploration, time spent in lit zone, number of head dips and significant decrease in no. of marbles buried in EPM, LDA, HBA and MBT respectively. Prazosin, p-chlorophenylalanine and haloperidol significantly reversed the ST induced anxiolytic effect in EPM, LDA, HBA and MBT. The extracts also protected animal against PTZ-induced seizures.These results suggest that anxiolytic like effect of ST facilitated the effects of adrenergic, serotonergic, dopaminergic and GABAminergic systems.

Keywords: Anxiolytic, Elevated plus maze, PTZ, *Solanum torvum*

Running title: Anxiolytic activity of *Solanum torvum*

Introduction

Anxiety disorders are the most common psychiatric conditions frequently seen [1]. A large number of population suffer from these conditions at some time during life. However, almost all pharmacological treatments used to diminish anxiety may produce side effects and efficacy of these drugs are very limited [2]. Therefore herbal therapies should be considered as an alternative/complementary medicine [3]. Consequently, because medicines are widely available

and used by the general public, more clinical research is needed to establish their safety and efficacy [4]. *Solanum torvum*, a plant in family solanaceae is very popular for health promotion in Thailand. It posseses antimicrobial,[5,6]antiviral,[7]immunosecretory,[8]antioxidant,[9] analgesic and anti-inflammatory activities.[10] Recently Arthan and co-workers reported that this plant contained high amount of flavonoids [7]. These substances have been reported to be a neuroprotector against various brain pathological conditions and serves as a valuable resource for treating neuropsychological diseases. Therefore this raises the possibility that *Solanum torvum* could be used in some neuropsychological diseases. The present study is carried out to determine the effect of the plant extract against anxiety disorder.

Materials

Plant material and preparation of extract: Dried fruits of *Solanum torvum* sw. (Solanaceae) were purchased locally and authenticated by Dr. Dasari, from Ayurvedic Seva Sangh, Panchavati, Nashik; India. Mature fruits were collected, sun dried and grounded. The powder obtained (950 gm) was defatted using pet ether (60-80^{0}C). The marc was macerated in ethanol for 3-4 days at room temperature. The filtrate was air dried and concentrated under reduced pressure to obtain 113 g, corresponding to a yield of 11.3 % w/w. The methanolic extract of *Solanum torvum* was subjected to column separation with neutral alumina as stationary phase using hexane, ethyl acetate and methanol successively to obtain hexane fraction (yield 0.29% w/w), ethyl acetate fraction (EAF) (yield 3.3% w/w) and methanol fraction (yield 2.1 % w/w) [7]. Appropriate concentrations of the extracts were made in distilled water.

Drugs and chemicals: Diazepam (Calmpose, Ranbaxy, India), Prazosin hydrochloride (Sigma chemicals, Mumbai), dl-p-chlorophenylalanine (Sigma chemicals, Mumbai), Haloperidol (Serenec, RGP Life Sciences LTD, Ankleshwar) and Pentylenetetrazol (Sigma chemicals, Mumbai) were used for the study.

Treatment: The methanolic extract of *Solanum torvum* (ST-10, 30, 100 mg/kg) and ethyl acetate fraction (10, 30 mg/kg) were administered intraperitoneally. Diazepam (1mg/kg) was administered intraperitoneally (i.p.) as standard. Pentylenetetrazole (60 mg/kg, i.p.) was used for producing convulsions in mice. Prazosin (62.5 µg/kg, i.p.) and Haloperidol (50 µg/kg i.p.) were given half an hour before administration of ST and p-CPA (100mg/kg, i.p.) was given for 4 consecutive days, the last injection being given 30 min before the administration of ST. The study was performed according to Irwin schedule (1969) and doses were selected from pilot studies performed in our lab. All the drugs were administered intraperitoneally in a fixed volume of 1 ml/100 g body weight. The drugs were administered 30 min before the animals were subjected to different behavioral tests.

Animals: Male albino mice (20-25g) were procured from Bharat serum and vaccines Ltd, Thane. Animals were housed into groups of five under standard laboratory conditions of temperature (25 ± 1ºC) with free access to food and water. The experiments were performed during the light portion (10-16h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

Experimental design:

Elevated plus-maze test (EPM)-This test has been widely validated to measure anxiety in mice [11]. Briefly, for mice, the apparatus consisted of a maze of two open arms (25 cm×5cm), crossed with walls (35 cm high) and central platform (5 cm×5 cm). The maze is suspended 50 cm above the room floor. The maze floor is constructed from black Plexiglas and the walls from clear Plexiglas. Thirty min after the i.p. treatment with Diazepam (1mg/kg) or ST (10, 30, 100mg/kg) or EAF (10,30mg/kg), each animal was placed at the centre of the maze, facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded. Entry into an arm was defined as the point when the animal places all four paws into the arm. After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution). In another experiment the effect of pretreatment of Prazosin (62.5 µg/kg i.p.), p-CPA (100mg/kg i.p.) and Haloperidol (50 µg/kg i.p.) in ST (30 and 100mg/kg) or EAF (10, 30mg/kg) treated animals were also studied and the same above parameters were determined.

Light/dark transition test: The light/dark box consisted of two compartments: one light area (27 L × 27 W × 27 H cm, 400 lx) illuminated by 100-W desk lamp was painted white, and the other dark area (18 L×27 W×27 H cm, 4 lx) was painted black. The two compartments were separated by a partition with a tunnel to allow passage from one compartment to the other. Mice (5 per group) were randomly assigned into experimental groups (vehicle control, Diazepam-1 mg/kg or ST-10, 30, 100 mg/kg or EAF-10, 30 mg/kg). Drug administration was given i.p. and 30 min prior to the test. Animals were placed in the centre of the lit area facing the wall opposite to the tunnel. The following parameters were recorded during 5 min: (1) the number of crossings between the light and the dark compartment, (2) the total time spent in the illuminated part of the box. The apparatus was cleaned thoroughly between trials [12]. In another experiment, the effect of pretreatment of Prazosin (62.5µg/kg, i.p.), p-CPA (100mg/kg, i.p.) and Haloperidol (50µg/kg, i.p.) in ST (30 and 100mg/kg) or EAF (10, 30 mg/kg) treated animals were also studied and the same above parameters were determined.

Hole board test: The hole-board apparatus consisted of a gray Perspex panels (40 cm×40 cm, 2.2 cm thick) with 9 equidistant holes 3 cm in diameter in the floor. The board was positioned 15 cm above a table. The number of head-dips were measured, increase in number of head-dips revealed as positive anxiolytic effect. Animals were placed singly in the centre of the board facing away from the observer and animal behavior and head-dip numbers were recorded over 5 min. Mice subjected to this test were administered ST (10, 30 or 100 mg/kg) or EAF (10, 30 mg/kg); i.p, 30 min before the test [13]. In another experiment the effect of pretreatment of Prazosin (62.5 µg/kg i.p.), p-CPA (100mg/kg i.p.) and Haloperidol (50 µg/kg i.p.) in ST (30 and 100mg/kg) or EAF (10, 30mg/kg) treated animals were also studied and the same above parameters were determined.

Marble-burying test: This test was carried out in Plexiglas cage (42 cm×26 cm×15 cm) with a glass lid. The floor was covered with a 2-cm layer of sawdust and 25 glass marbles were distributed throughout the cage. Thirty min after i.p. treatment with Diazepam (1mg/kg) or ST (10, 30, 100 mg/kg) or EAF (10,30 mg/kg), male mice were individually placed in the cage for 10 min, after which they were removed and the burying response quantified by counting the number of marbles that were more than two thirds covered with sawdust. A diminution of the

burying response revealed a positive anxiolytic-like effect [14]. In another experiment the effect of pre-treatment of Prazosin (62.5 µg/kg i.p.), p-CPA (100mg/kg i.p.) and Haloperidol (50 µg/kg i.p.) in ST (30 and 100mg/kg) or EAF (10, 30mg/kg) treated animals were also studied and the same above parameters were determined.

Pentylenetetrazole induced seizures: Mice of either sex were randomly allotted to different control and test groups. The control mice were administered with PTZ (60 mg/kg, i.p.) 30 min after normal saline 10 ml/kg, p.o. Test group of mice received Diazepam(10mg/kg, i.p.), ST (10, 30 and 100 mg/kg, i.p.) 30 min before PTZ (60mg/kg, s.c.). Onset to straub tail, myoclonic jerks, hind limb extension, clonic convulsion and stupor were recorded. The onset and number of death after showing tonic hind limb extension were also recorded. Mice that did not convulse 30min after PTZ administration were considered protected [15].

Statistical analyses: All analyses were performed using the software Primer for windows. All data are represented as mean ± SEM values. Data were analyzed by one-way ANOVA followed by Dunnett's t-tests. The level of statistical significance adopted was $P < 0.05$.

Results

Elevated plus maze test: In this model, analysis of behaviour of mice in the elevated plus-maze revealed that treatment of ST (10, 30 and 100mg/kg), EAF (10 and 30mg/kg), and Diazepam (1mg/kg) produced significant ($p<0.05$) increase in time spent and number of entries in open arm; decrease in time spent in closed arm as compared to vehicle treated group (Table 1). Prazosin (62.5 µg/kg, i.p.), p-CPA (100 mg/kg, i.p.) and Haloperidol (50 µg/kg, i.p.) alone significantly decreased the time spent in open arm as compared to vehicle treated group. Pretreatment of prazosin or haloperidol or p-CPA in ST (30 and 100 mg/kg) or EAF (10, 30 mg/kg) treated animals produced significant ($p<0.05$) increase in time spent and number of entries in open arm; decrease in time spent in closed arm as compared to prazosin or haloperidol or p-CPA treated groups respectively (Table 1).

Table 1: Effect of methanolic extract of *Solanum torvum* (ST) on open and enclosed arms of elevated plus maze in mice

Sr. no	**Treatment**	**Open arm**		**Closed arm**	
		Time spent (sec)	No. of entries	Time spent (sec)	No. of entries
1	Vehicle	52.8 ± 3.6	3.8 ± 0.37	194 ± 9.22	8.8 ± 9.22
2	Diazepam (1mg/kg)	132 ± 3.78*	7 ± 0.57*	156 ± 9.07*	3.6 ± 0.88*
3	ST (10mg/kg)	76.3 ± 6.69*	4.66 ± 0.66	146 ± 8.91*	7.33 ± 0.8
4	ST (30mg/kg)	94 ± 3.6*	5.33 ± 0.33*	150 ± 6.35*	8 ± 6.35
5	ST (100mg/kg)	97 ± 4.9*	6.66 ± 0.33*	146 ± 8.91*	7.66 ± 1.02
6	EAF (10mg/kg)	75.5 ± 2.10*	5.0 ± 0.4	164 ± 2.6*	11.5 ± 1.2
7	EAF (30mg/kg)	90 ± 3.9*	6.5 ± 0.95*	155.5 ± 3.6*	9 ± 0.9
8	Prazosin (62.5µg/kg)	22.1 ± 1.6*	2.33 ± 0.4	134.5 ± 4.5*	7.3 ± 0.4

9	Prazosin (62.5µg/kg) + ST (30mg/kg)	23.1 ± 1.5[a]	4.5 ± 0.5[a]	139.5 ± 1.5	8 ± 1.0
10	Prazosin (62.5µg/kg) + ST (100mg/kg)	30.5 ± 8.5	5.5 ± 0.5[a]	141 ± 4	7 ± 1.0
11	Prazosin (62.5µg/kg) + EAF(10mg/kg)	19.5 ± 0.5	3 ± 0	146 ± 2[a]	9 ± 1.0
12	Prazosin (62.5µg/kg) + EAF (30mg/kg)	25 ± 3[a]	4 ± 1[a]	142.5 ± 0.5[a]	8.5 ± 0.5
13	p-CPA (100mg/kg)	22.3 ± 2.3*	2 ± 2.3	215.7 ± 5.9*	10.8 ± 0.9
14	p-CPA (100mg/kg) + ST (30mg/kg)	37.5 ± 6.5[b]	2 ± 0.1	184.5 ± 3.5[b]	9 ± 1.0
15	p-CPA (100mg/kg) + ST (100mg/kg)	42.5 ± 4.58	3.5 ± 0.5	182.5 ± 3.5[b]	9.5 ± 0.5
16	p-CPA (100mg/kg) + EAF(10mg/kg)	27.5 ± 0.5[b]	2.5 ± 0.5	191.5 ± 2.5[b]	27.5 ± 0.5
17	p-CPA (100mg/kg) + EAF(30mg/kg)	46 ± 1.0[b]	4 ± 1.0	169.5±11.5[b]	9 ± 1.0
18	Haloperidol (50µg/kg)	33 ± 5.09*	3.7 ± 0.6	192.8±19.4*	11.5 ± 0.28
19	Haloperidol (50µg/kg) + ST (30mg/kg)	46 ± 3.0[c]	3.5 ± 0.5	115 ± 7.7[c]	12 ± 1.0
20	Haloperidol (50µg/kg) + ST (100mg/kg)	49 ± 7.0[c]	4 ± 0.5	185.5 ± 4.5[c]	12 ± 0.5
21	Haloperidol (50µg/kg) + EAF (10mg/kg)	35.5 ± 5.5[c]	4.5 ± 0.5	161 ± 3[c]	11.5 ± 0.5
22	Haloperidol (50µg/kg) + EAF (30mg/kg)	45 ± 1.0[c]	4.5 ± 0.5	149.5 ± 1.5[c]	11.5 ± 0.5

All values are in mean ± S.E.M. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's t-test.

* $p<0.05$ when compared with control [a] $p<0.05$ when compared with Prazosin

[b] $p<0.05$ when compared with p-CPA [c] $p<0.05$ when compared with Haloperidol

Light-dark test: Analysis of behaviour of mice in the light and dark transition model revealed that treatment of ST (10, 30 and 100 mg/kg), EAF (10 and 30 mg/kg), and Diazepam (1 mg/kg) produced significant ($p<0.05$) increase in time spent in lit area and decrease in time spent in dark area as compared to vehicle treated group (Table 2). Prazosin (62.5 µg/kg, i.p.), p-CPA (100 mg/kg, i.p.) and Haloperidol (50 µg/kg, i.p.) alone significantly decreased the time spent by mice in the lit area as compared to vehicle treated group. Pre-treatment of prazosin or haloperidol or p-CPA in ST (30 and 100 mg/kg) or EAF (10, 30 mg/kg) treated animals produced a significant ($p<0.05$) increase in time spent in lit area and decrease in time spent in dark area as compared to prazosin or haloperidol or p-CPA treated groups respectively (Table 2).

Table 2: Effect of methanolic extract of *Solanum torvum* on time spent, number of crossing, in light and dark model in mice

Sr. no.	Treatment	Time spent in lit box (sec)	Time spent in dark box (sec)	No. of crossings
1	Vehicle	74.4 ± 6.00	148.8 ± 3.39	16.2 ± 2.2
2	Diazepam (1mg/kg)	161 ± 3.21*	96.67 ± 2.96*	17.33 ± 2.02
3	ST (10mg/kg)	106.3 ± 6.93*	78.67 ± 3.18*	12 ± 1.15*
4	ST (30mg/kg)	120.7 ± 3.75*	87.67 ± 3.5*	12.67 ± 0.88
5	ST (100mg/kg)	133.3 ± 6.22*	106.1 ± 2.72*	9.66 ± 0.88
6	EAF (10mg/kg)	82.75 ± 1.93*	120.5 ± 2.46*	17.5 ± 0.64
7	EAF (30mg/kg)	120 ± 6.41*	81.75 ± 3.7*	7.25 ± 0.85
8	Prazosin (62.5µg/kg)	30.17 ± 0.94*	126.7 ± 2.4*	12.3 ± 0.4
9	Prazosin (62.5µg/kg)+ ST (30mg/kg)	45.5 ± 3.5[a]	116.5 ± 2.5[a]	12.5 ± 0.5
10	Prazosin (62.5µg/kg)+ ST (100mg/kg)	57 ± 2.0[a]	112.5 ± 3.5[a]	13.5 ± 1.5
11	Prazosin (62.5µg/kg)+ EAF (10mg/kg)	42 ± 2.0[a]	122 ± 1.0[a]	13.5 ± 1.5
12	Prazosin (62.5µg/kg)+ EAF (30mg/kg)	49.5 ± 1.5[a]	117 ± 4.0[a]	13 ± 1.0
13	p-CPA (100mg/kg)	37.8 ± 1.7*	198.2 ± 3.9*	17.6 ± 1.7
14	p-CPA (100mg/kg)+ ST (30mg/kg)	59 ± 5.0[b]	180 ± 1.0[b]	19.5 ± 0.5
15	p-CPA (100mg/kg)+ ST (100mg/kg)	73.5 ± 8.0[b]	169 ± 8.0[b]	14.5 ± 0.5
16	p-CPA (100mg/kg)+ EAF (10mg/kg)	53.5 ± 5.5[b]	156 ± 2.0[b]	21 ± 1.0
17	p-CPA (100mg/kg)+ EAF (30mg/kg)	72.5 ± 8.5[b]	150 ± 2.0[b]	20.5 ± 0.5
18	Haloperidol (50µg/kg)	42.25 ± 3.1*	184.5 ± 5.6*	21.2 ± 1.2
19	Haloperidol (50µg/kg)+ ST (30mg/kg)	50 ± 2.0[c]	144.5 ± 3.5[c]	19 ± 1.0
20	Haloperidol (50µg/kg)+	63.5±0.5[c]	130±1.0[c]	20.5±1.5

	ST (100mg/kg)			
21	Haloperidol (50µg/kg)+ EAF (10mg/kg)	53 ± 2.0[c]	160 ± 1.0[c]	20.5 ± 1.5
22	Haloperidol (50µg/kg)+ EAF (30mg/kg)	62.5 ± 1.5[c]	149.5 ± 0.5[c]	20.5 ± 0.5

All values are in mean ± S.E.M. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's t-test.

* $p<0.05$ when compared with control [a] $p<0.05$ when compared with Prazosin

[b] $p<0.05$ when compared with p-CPA [c] $p<0.05$ when compared with Haloperidol

Hole board test: Analysis of behavior of mice in the Hole board test revealed that treatment of ST (10, 30 and 100mg/kg), EAF (10 and 30mg/kg), and Diazepam (1mg/kg) produced significant ($p<0.05$) increase in number of head poking as compared to vehicle treated group (Table 3). Prazosin (62.5 µg/kg, i.p.), p-CPA (100 mg/kg, i.p.) and Haloperidol (50 µg/kg, i.p.) alone significantly decreased the number of head poking as compared to vehicle treated group. Pre-treatment of prazosin or haloperidol or p-CPA in ST (30 and 100mg/kg) or EAF (10, 30 mg/kg) treated animals produced significant ($p<0.05$) increase in number of head poking as compared to prazosin or haloperidol or p-CPA treated groups respectively (Table 3).

Marble burying test: Analysis of behaviour of mice in the Marble-burying test revealed that treatment of ST (10, 30 and 100mg/kg), EAF (10 and 30mg/kg) and Diazepam (1mg/kg) produced significant ($p<0.05$) decrease in number of burying response as compared to vehicle treated group (Table 3). Prazosin (62.5 µg/kg, i.p.), p-CPA (100mg/kg, i.p.) and Haloperidol (50µg/kg, i.p.) alone significantly increased the number of marbles buried as compared to vehicle treated group. Pre-treatment of prazosin or haloperidol or p-CPA in ST (30 and 100mg/kg) or EAF (10, 30mg/kg) treated animals produced a significant ($p<0.05$) decrease in number of burying response as compared to prazosin or haloperidol or p-CPA treated groups respectively (Table 3).

Table 3: Effect of methanolic extract of *Solanum torvum* on number of head poking in hole board apparatus and on burying response in marble burying test in mice

Sr. no	**Treatment**	**No. of head poking**	**No. of marbles buried**
1	Vehicle	27.4± 0.92	10.33 ± 0.88
2	Diazepam (1mg/kg)	41 ± 1.73*	1.75 ± 0.25*
3	ST (10mg/kg)	32 ± 2.30*	7.25 ± 0.85*
4	ST (30mg/kg)	36 ± 1.55*	4.75 ± 0.47*
5	ST (100mg/kg)	40 ± 6.55*	1.75 ± 0.47*
6	EAF (10mg/kg)	39 ± 0.91*	7 ± 0.57*
7	EAF (30mg/kg)	44.5±2.75*	4.75 ± 0.75*
8	Prazosin (62.5µg/kg)	15.6 ± 0.8*	13.83 ± 0.60*
9	Prazosin (62.5µg/kg) + ST (30mg/kg)	22.5 ± 1.5[a]	9.0 ± 1.0[a]
10	Prazosin (62.5µg/kg) +ST (100mg/kg)	26 ± 2.0[a]	6.5 ± 1.5[a]

11	Prazosin(62.5µg/kg) + EAF(10mg/kg)	19.5 ± 1.5	12 ± 2.0[a]
12	Prazosin(62.5µg/kg) + EAF(30mg/kg)	22.5 ± 2.5[a]	9.5 ± 0.5[a]
13	p-CPA (100mg/kg)	16 ± 0.89*	14 ± 0.68*
14	p-CPA(100mg/kg)+ ST (30mg/kg)	25.5 ± 0.5[b]	7.5 ± 0.5[b]
15	p-CPA(100mg/kg) + ST (100mg/kg)	25.5 ± 0.5[b]	5.0 ± 1.0[b]
16	p-CPA(100mg/kg) + EAF (10mg/kg)	31 ± 2.0[b]	8.5 ± 0.5[b]
17	p-CPA(100mg/kg) +EAF (30mg/kg)	37.5± 1.58[b]	6.0 ± 1.0 [b]
18	Haloperidol (50µg/kg)	19 ± 0.7*	14.5 ± 0.64*
19	Haloperidol (50µg/kg) +ST (30mg/kg)	22 ± 2.0	9.0 ± 1.0[c]
20	Haloperidol (50µg/kg)+ST(100mg/kg)	28.5 ± 4.5[c]	5.5 ± 1.5[c]
21	Haloperidol (50µg/kg) + EAF(10mg/kg)	22 ± 4.0	10.5 ± 0.5[c]
22	Haloperidol (50µg/kg) + EAF(30mg/kg)	27.5 ± 0.5[c]	9 ± 1.0[c]

All values are in mean ± S.E.M. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's t-test.
* $p<0.05$ when compared with control [a] $p<0.05$ when compared with Prazosin
[b] $p<0.05$ when compared with p-CPA [c] $p<0.05$ when compared with Haloperidol

Pentylenetetrazole (PTZ)-induced seizures: The vehicle treated group showed 100 % mortality. Diazepam (10mg/kg) showed total protection against the onset of straub tail, tonic extensor and clonic convulsion. ST (10, 30 and 100 mg/kg) and EAF (10, 30 mg/kg) showed significant ($p<0.05$) increase in latency for straub tail, tonic extensor and clonic convulsion as compared to vehicle treated group (Table 4).

Table 4: - Effect of methanolic extract of *Solanum torvum* (ST) and ethyl acetate fraction (EAF) on PTZ induced convulsions in mice

Sr. No	Treatment	Onset of Straub tail (sec)	Onset of Extensor (sec)	Onset of Myoclonic jerk (sec)	Onset of Clonic convulsion (sec)	Onset of Stupor (sec)
1	Control	35.5± 1.38	64± 5.2	88.5± 2.5	234± 3.06	299± 4.5
2	Diazepam (10 mg/kg)	–	–	–	–	–
3	ST (10 mg/kg)	98.3± 11.2*	154.3± 4.22*	294± 4.8*	421.3± 3.5*	439± 16..09*
4	ST (30 mg/kg)	144.8± 13.83*	168.5± 4.27*	289.8± 5.1*	463± 27.64	472.2± 27.04
5	ST	190.3± 3.7*	215.2± 10.77	291.2± 4.7*	511.5± 5.5*	521.8± 6.65*

	(100 mg/kg)					
6	EAF (10 mg/kg)	101.4±4.81*	159.8± 8.2*	281.5±3.5*	438.3±4.81*	451.3±12.85
7	EAF (30 mg/kg)	143± 4.53*	214± 4.22*	301± 5.8*	476.3± 4.5*	501.3± 11.1

All values are in mean ± S.E.M. Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's t-test. * $p<0.05$ when compared with control.

Discussion

In the present study, the methanolic extract of *Solanum torvum* produced significant anxiolytic effect in mice in EPM, LDA, HBA and in MBT. All these models of anxiety are widely used to screen the new anxiolytic drug. These models are quite sensitive and relatively specific to all major classes of anxiolytic drugs. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, i.e., the fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform [16]. Moreover, it is known that anxiolytic agents increase the frequency of entries and the time spent in open arms of the EPM [11]. In the present study, we found that administration of ST (10, 30 and 100 mg/kg) and EAF (10 and 30 mg/kg) increased both of these parameters as compared to vehicle treated group. Light/dark box is also widely used in rodents as a model for screening anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light [12]. In the present study, ST (10, 30 and 100mg/kg) and EAF (10 and 30mg/kg) significantly increased time spent in the light area and decreased time spent in dark area. The hole-board model has been frequently used to detect and evaluate anxiolytic/anxiogenic properties of drugs [17]. The increase in the number of head-dips is the most reliable parameter in the hole-board model [18]. In the present study, head-dip numbers were dose dependently increased by ST (10, 30 and 100mg/kg) and EAF (10 and 30mg/kg) treatment as compared to vehicle treated group. Animals, such as mice and rats, often bury objects in the bedding material of their cage. One example of this type of behavior occurs when mice are placed in an environment, similar to their standard housing, which contains glass marbles as unfamiliar objects. Typically, mice housed in group (5–30/cage), when placed in the test environment on an individual basis proceed to bury, within 30 min, approximately 70% of the marbles [13,19]. Animals that are pre-treated with an antianxiety agent, however, will bury significantly fewer marbles. The administration of diazepam or buspirone, for example, inhibits marble-burying behaviour (i.e. more marbles remain uncovered) at doses that do not disrupt motor activity [20]. In the present study, ST (10, 30and 100mg/kg) and EAF (10 and 30mg/kg) significantly blocked marble-burying behavior of mice as compared to vehicle treated group. These observations indicate that the anxiolytic effect of ST and EAF are selective, and not simply the result of either a general stimulation of locomotor activity or of exploratory behavior consequent to exposure to a novel environment. Pentylenetetrazole induced seizures in all the mice used. Pentylenetetrazole may elicit seizures by inhibiting gabaergic mechanisms. Standard antiepileptic drugs, diazepam and phenobarbitone, are believed to produce their effects by

enhancing GABA mediated inhibition in the brain.[21] It is, therefore, possible that the anticonvulsant effects shown in this study by the drugs against seizures produced by PTZ might be due to the activation of GABA neurotransmission. The results of the present study thus indicate that ST and EAF possess anticonvulsant activity in mice. The fact that the extract protected animal against PTZ-induced seizures may suggest that the plant extract contains compound(s) that facilitate GABAergic transmission.The anxiolytic-like effect of ST seems not to be associated with any motor effects, since it did not show significant change in locomotor function of mice as compared to control. This indicates that increased motor activity was not involved in the action seen in EPM, LDA, HBA and MBT, and confirms the assumption that the anxiolytic effect of the extract is specific. The precise mechanisms by which ST produced anxiolytic effect are not completely understood. However, according to our results, the anxiolytic effect of ST was significantly reversed by the treatment of animals with Prazosin (an alfa-1 adrenoceptor antagonist), p-CPA (a serotonin synthesis inhibitor) and Haloperidol (a dopamine D2-receptor antagonist) when tested in EPM, LDA, HBA and MBT. This suggests that ST might produce anxiolytic effect by interaction with alfa-adrenoceptors, serotonergic and dopamine receptors, thereby increasing the level of norepinephrine, serotonin and dopamine in brains of mice. This suggests that ST might produce anxiolytic-like effect by interaction with alpha1-adrenoceptors, serotonergic and dopamine D2 receptors, thereby increasing the levels of norepinephrine, serotonin and dopamine in brains of mice. The fact that the extract protected animal against PTZ-induced seizures may suggest that the plant extract contains compound(s) that facilitate GABAergic transmission. In summary, the present study demonstrates that ST has an anxiolytic-like effect, and further suggests that this effect involves the adrenergic, serotonergic, dopaminergic and GABAergic nervous system. However, its underlying mode of action remains to be elucidated.

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