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Neuropsychopharmacological Profiling of Flunarizine: A Calcium Channel Blocker

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Abstract: This study was designed to investigate the effect of flunarizine, a calcium channel blocker, a piperazine derivative in several neuropsychopharmacological experimental models. Effect of the drug flunarizine was evaluated in Pentobarbitone – induced sleeping time, Forced swim test, Actophotometer test, Elevated plus maze test for nootropic activity, Elevated plus maze for anxiety, Hole board test, Rotarod test, Apomorphine – induced stereotypy, Haloperidol – induced catalepsy and Pentylenetetrazole – induced convulsions using mouse as animal model. The result of this work provided evidences that flunarizine may be active as an anxiolytic agent and suggestive profile of atypical antipsychotic with a anticonvulsant activity. Flunarizine was found to produce alteration of general behavioural pattern, significant reduction in spontaneous locomotor activity, significant inhibition of stereotypic behavior induced by apomorphine (APM), significant increase in open arm entry – time spent in open arm without any alteration in the closed arm parameters, significant reduction in number of nose poking and line crossing in hole board apparatus, significant reduction in transfer latency (TL) in EPM, and a mild catalepsy at the highest dose among the selected doses for the study. Again a considerable decrease in immobility time in forced swim test was also observed which is not at par with the expected profile of flunarizine. Flunarizine also did not show any disturbances in motor co-ordination.
Keywords: Flunarizine, Calcium channel blocker, Apomorphine, Neuropsychopharmacology.

1. Introduction

The calcium channel blocker flunarizine is a lipophilic diphenyl piperazine derivative. It is non selective voltage-dependent Ca^{2+} channel blocker in smooth muscle and neuronal cells. [1] It is derived from cinnarizine and the only difference is that it contains a piperazine moiety which is also found in neuroleptics and antihistaminics.[2] Flunarizine has the ability to cross the blood brain barrier , antagonise calcium influx and to interfere with the neurotransmitter system.[3] Flunarizine may therefore suppress hyperexcitability, produce antiepileptogenic activity and alter neurotransmitter actions. Flunarizine has similar chemical features as trifluoperazine which is an antipsychotic drug.[4] Flunarizine is found to possess neuroprotective effect, vasodialating effect, antiserotoninergic effect and antivertiginous effect. [5,6,7,8] Flunarizine is also found to be used in vertigo, migraine and cyclic vomiting syndrome. [9] In India flunarizine is used as an antimigraine drug. [10] Despite all these facts, there is no any clear scientific work regarding the neuropsychopharmacological profiling of flunarizine and its possible use in different psychological disorders. Therefore in our effort to cultivate the concept of drug repositioning, we have studied the neuropsychopharmacological profiling of flunarizine in laboratory animals.

2. Materials and methods

2.1 Animals used

All the pharmacological experiments were conducted using adult Swiss albino mice (25 ± 2 gm; n = 6). The animals were housed separately in diffusely illuminated room with 60-70% relative humidity and

approximately 22° C temperature in polypropylene cages. The animals received a standard rodent diet and water *ad libitum*. They were acclimatised for at least 7 days before the start of experiments. All the experiments were carried out in a diffusely illuminated sound attenuated room. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).

2.2 Drugs and chemicals

Flunarizine was obtained from Torrent Pharmaceuticals, India as a gift sample for the study. Apomorphine hydrochloride, Pentylenetetrazole and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other drugs, chemicals and reagents were of analytical grade. (SRL, Mumbai, E.Merck, India, Himedia, India etc.)

2.3 Vehicle for Flunarizine (Flunarizine Hydrochloride)

Flunarizine was suspended in a 1% w/v solution of tween 80. Before injecting the drug, the drug solution was shaked well for uniform dosing.

2.4 Neuropharmacological experiments

2.4.1 Pentobarbitone induced sleeping time [11]

Flunarizine at three doses (5, 10, 20 mg/kg; i.p) was administered 30 min. prior to pentobarbitone injection to three different groups of mice. Each group consists of 6 mice for each dose. The time interval between the loss and regaining of righting reflex (fall asleep) was measured as sleeping time. Noted the time of recovery from sleep as the animal turns to recover its normal posture. Calculated the onset and duration of action of pentobarbitone and its combination with flunarizine.

2.4.2 Forced swim test [12]

The forced swim test or behavioral despair swim test has been used as a test for depression like behavior. This test is sensitive to all major classes of antidepressant drugs. The procedures for the forced swim test (FST) were similar to those first described by Porsolt et al. (1977). Mice were placed individually in glass cylinder containing water. Animals (mice) were forced to swim individually for 15 minutes, in glass cylinder containing 15 cm water at room temperature .Animals were individually trained in 15 minute sessions. This was "pre- test session". 24 hours later, the animals were treated with flunarizine at 5, 10, 20 mg/kg doses in 3 groups. And the control group was treated with vehicle (1% aqueous solution of Tween 80) and each animal was again forced to swim in similar environment for 5 minutes in a "test-session" and duration of immobility time for each mouse was recorded. Each experimental group consisted of 6 animals (mice) and was chosen by means of completely randomized schedule.

2.4.3 Spontaneous Locomotor Activity (SMA) using Actophotomer [13]

Spontaneous locomotor activity was evaluated by using a photocell activity cage. Degree of depression was determined by this test. The actions of flunarizine on spontaneous locomotor activity were measured automatically by using Actophotometer (Medicraft photo actometer, model No.600-40, S.No: P A-0149, India). The units of activity counts were arbitrary and based on the beam breaks by movement of the mice. The spontaneous locomotor activity of each mouse was recorded individually for 10 minutes using the Actophotometer. Flunarizine was administered at 3 doses (5, 10, 20 mg/kg; i.p) to 3 different groups of mice each with 6 mice 30 minutes before the test and chlorpromazine (3 mg/kg, i.p.) as a standard drug was also given to a separate group of mice 30 min before the test.

2.4.4 Elevated plus maze model for Nootropic activity [14]

The elevated plus maze apparatus consists of two open arms (30×5 cm) and two closed arms (30×5×15 cm) that extend from a common central platform (5×5 cm). The floor of each arm is wooden and the walls of the closed arms are also wooden. The open arm edges are 0.5 cm in height to keep the mouse from falling down and the edges of closed arms are 15 cm in height. The entire maze is elevated to a height of 38 cm above the floor level, as has been validated and described (Pellow S., 1986). Four groups of mice were taken for the study (each group contains 6 mice). The control group received vehicle only. Experiments were carried out in a sound attenuated room illuminated only by dim light. The animals were placed individually on the maze 30 minutes after drug (flunarizine) administration. The animal was placed at the end of the open arms facing away from centre of the maze and the time to move from the open arm to the closed arm was recorded as transfer latency (TL). The recording was done on the first day and after 24 hours for 90 seconds. TL on the first day served as a measure of acquisition learning and TL after 24 hrs for retrieval or explicit learning.

2.4.5 Elevated plus maze model of anxiety [13]

The elevated plus maze apparatus used was same as described earlier. Five groups of mice (each group contains 6 mice each) were taken. A standard 5 minutes test was employed for each mouse. Mice were placed on the maze 30 min after diazepam and flunarizine administration. The animal was placed at the centre of the maze, facing one of the closed arms. During 5 min test period the following measures were taken-

- The number of entries into the open arm.
- The number of entries into the closed arm.
- Time spent in the open arm.
- Time spent in the closed arm.

Arm entries were counted when the animal placed all of its four paws on it. The procedure was conducted in a sound attenuated room.

2.4.6 Hole board apparatus [15]

Standard hole board apparatus was taken for the test. The hole board apparatus consists of a wooden box (40×40×25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to the height of 25 cm. Five groups of mice (each group contains 6 mice) were taken for the study. 30 minutes after drug administration each animal was placed on the hole board apparatus and tested for five minutes. Number of line crossing and nose poking behavior was noted. Diazepam (1 mg/kg; i.p.) was used as the standard anxiolytic drug in separate group of animals and to a control group vehicle (1% w/v tween 80) was also given.

2.4.7 Rotarod test for neurotoxicity [13]

Rotarod test was used to determine the effect of drugs on motor co-ordination. A custom build Rotarod apparatus (Medicraft Rota Rod, Model No. 519/E-2C, S. No: MRA-036, Medicraft electro medicals (P) Ltd., Lucknow.) was used for the experiment. The instrument (a horizontal rotation device) was set at a rate of 20 rpm. Four groups of mice were taken for the study (each group contains 6 mice). Mice were placed on the rod and those animals that remained on the rod for 5 minutes were selected for the study. The animals were then evaluated for motor coordination at an interval of 30, 60, 180, and 360 min. after i.p administration of flunarizine at 3 doses (5, 10, 20 mg/kg, i.p) to 3 different groups of animals. If the animal fails more than once to remain on the rotating rod for 5 minutes then was considered to be positive. The time each animal falls off from the rod was noted.

2.4.8 Apomorphine (APM) - induced stereotypy in mice [16]

Three test groups of mice were pretreated with flunarizine at 3 doses (5, 10, 20 mg/kg; i.p) half an hour prior to apomorphine (1mg/kg; i.p.) administration and mice were observed for stereotypy behavior for next 50 min. Separate positive control group of mice was also maintained to which only apomorphine was administered. One vehicle control (1% aq. Solution of Tween 80) was also taken. The intensity of stereotyped behavior was assessed at 5 minutes intervals throughout the duration of experiment. Each group contains 6 mice each.

Scoring was done as follows-

- Score 0: no change than control.
- Score1: discontinuous sniffing, constant exploratory activity.
- Score 2: continuous sniffing, periodic exploratory activity.
- Score 3: continuous sniffing, discontinuous biting, gnawing or licking,
  Very brief periods of locomotor activity.
- Score 4: continuous biting, gnawing or licking, No exploratory activity.

2.4.9 Haloperidol (HP) induced-catalepsy in mice [17]

Catalepsy is defined as the acceptance and retention of abnormal imposed posture, and is measured by means of the bar test. In case of haloperidol–induced catalepsy; haloperidol (1 mg/kg) was used to induce catalepsy. Haloperidol 1mg/kg dose was selected so that it could elicit a moderate degree of catalepsy and thus enable the detection of either attenuation or potentiation of the phenomenon. Four groups of mice were taken for the study (each group contains 6 mice). Animals were tested for the presence of catalepsy by placing both front paws on a 4 cm high wooden block (1 cm diameter), a cataleptic animal maintaining this posture for a period of time dependent upon the degree of catalepsy. If the animal (mouse) maintained the imposed posture for at least 20 seconds it was said to be cataleptic and was given one point. For every further 20 seconds it continued to maintain the cataleptic posture , one extra point was given , thus the animal scored 2 points if it maintained the cataleptic posture for 40 second, 3 point for 60 second, and so on. Flunarizine was administered half an hour before haloperidol administration. The animals were tested for catalepsy 30 minutes after haloperidol administration. Catalepsy was assessed at 30 min interval until 120 min. and at the end of 240 min. by means of the standard bar test. The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal (mice) moved its head in an exploratory manner. A cut off time of 1100 seconds were applied. Between determinations the mice were returned to their individual cages. All observations were made between 10.00 and 16.00 hrs in a quiet room at 23-25 ^{0}C.

2.4.10 Pentylenetetrazol (PTZ) – induced convulsions in mice [18]

Five groups of mice were used for the study (each group cantains 6 mice). The unstrained mice were injected with pentylenetetrazol (50 mg/kg, i.p) 30 minutes after test drug and standard drug (Phenytoin) and the occurrence of the first generalized clonus (repeated clonic seizures of the fore- and hind limbs lasting over 5 sec. with an accompanying loss of righting reflex) or jerky movements was recorded during individual observation for 30 min. The onset and duration both were observed. Reduction in such movements was selected as the criteria to support antiepileptic activity of the drug.

2.5 Statistical Analysis

Values were expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by one way ANOVA followed by Dunnett's test as ***post hoc*** and $p<0.05$ was considered significant. In some model, data were analyzed by paired-*t* test where necessary. All the statistical analysis was done by using the Graphpad INSTAT statistical software package.

3. Results

3.1 Effect of flunarizine on Pentobarbitone (PB) - induced sleeping time in mice

Flunarizine at the employed doses did not show any significant potentiation of sleeping time induced by pentobarbitone. The sleeping time of mice treated with pentobarbitone intraperitoneally and in combination with flunarizine at 3 doses (5, 10, 20 mg/kg, i.p) are shown in **table 1.**

3.2 Effect of flunarizine on Forced swim test in mice

The acute treatment of flunarizine showed mild decrease in immobility time at 10 mg/kg dose of flunarizine but at 20 mg/kg dose of flunarizine there was a significant decrease in immobility time observed and at this dose only alteration of the swimming behavior was also observed. Flunarizine at the dose of 20 mg/kg showed an antidepressant like activity. Results are presented as mean ± SEM. Experimental data in **table 2** were analyzed by one way **ANOVA** followed by ***post hoc*** Dunnett's test.

3.3 Effect of flunarizine on Spontaneous Locomotor Activity (SLA) using Actophotometer in mice

The effect of flunarizine on spontaneous locomotor activity was measured in a 10 minutes test using Actophotometer. Flunarizine at dose of 20 mg/kg significantly decreased the spontaneous locomotor activity. Flunarizine at 5 and 10 mg/kg doses did not show any significant alteration in the spontaneous locomotor activity. In the standard drug group, treated with chlorpromazine (3 mg/kg; i.p), 23 % reduction of spontaneous locomotor activity was observed and on the other hand 17 % reduction of spontaneous locomotor activity was observed in the group which received 20 mg/kg dose of flunarizine. Data are presented in **table 3**.

3.4 Effect of flunarizine on Elevated plus maze as model for Nootropic activity in mice

The transfer latency (TL) was recorded for the control group and for other three test groups on day-1 and after 24 hrs on day-2. The group treated with flunarizine at dose of 20 mg/kg showed significant decrease in transfer latency on day-1 and on day-2 as compared to control group respectively. On the other hand TL on day-2 was further decreased as compared to day-1. Only 20 mg/kg dose of flunarizine seems to be effective in this animal model. Data are presented in **table 4.**

3.5 Effect of flunarizine on Elevated plus maze model of anxiety in mice

Behavior observed in elevated plus-maze showed the anxiolytic activity of diazepam reported previously. As positive control diazepam (1 mg/kg; i.p.) increased the open arm entries and time spent in open arms significantly but diazepam did not significantly alters the closed arm entry and time spent in closed arm. Flunarizine at doses of 10 mg/kg and 20 mg/kg significantly increased open arm entry and time spent in open arms as compared to control (1% aqueous solution of Tween 80) group of mice. But flunarizine at a dose of 5 mg/kg did not show any significant increase in open arm entries and time spent in open arms. On the other hand there was no significant alteration observed in closed arm entry and time spent in closed arms in all the test groups of mice at these particular doses (5, 10, 20 mg/kg; i.p.).The data are shown in **table 5.**

3.6 Effect of flunarizine on Hole board test apparatus in mice

The effect of the drug flunarizine upon the hole board test was performed by measuring the number of nose poking and number of line crossing in the apparatus. As a positive control diazepam (1 mg/kg; i.p.) decreased the number of nose poking and line crossing significantly as compared to control group. Flunarizine at all the studied doses (5, 10, 20 mg/kg) showed significant decrease in the number of line crossing and nose poking behaviour as compared to control group, which is comparable to the standard drug (diazepam) treated group. Data are shown in **table 6.**

3.7 Effect of flunarizine on motor impairment assessment in Rotarod test in mice

The fall off time of mice administered with flunarizine at doses (5, 10, 20 mg/kg; i.p.) did not differ significantly from control group which received 1% aqueous solution of tween 80 (vehicle). All the mice retained in the rod for more than 5 minutes, suggesting that flunarizine did not induce disturbances in motor co-ordination at these particular doses (5, 10, 20 mg/kg). The data are shown in **table 7**.

3.8 Effect of flunarizine on Apomorphine – induced stereotypy in mice

The maximum intensity of stereotypy induced by apomorphine (APM) was observed at 15 min. Between 5 min and 15 min there was no any significant reversal of the stereotypy induced by APM in any of the test group treated with flunarizine (at doses 5, 10, 20 mg/kg; i.p). But after 20 min and up to 50 min significant reversal of stereotypy induced by APM was observed at these particular doses of flunarizine in all the test groups. Data for 5, 20, 35 and 50 min are only

presented in **table 8** and all the data are presented in **figure 1**.

3.9 Effect of flunarizine on Haloperidol (HP) – induced catalepsy in mice

Flunarizine at doses (5, 10, 20 mg/kg) did not show any potentiation of the catalepsy time induced by haloperidol at 30 min. But after 90 min of haloperidol administration, when catalepsy scoring was done, flunarizine at a dose of 20 mg/kg showed a significant potentiation of catalepsy time as compared to control. The data are presented in the **table 9**.

3.10 Effect of flunarizine on Pentylenetetrazol (PTZ) - induced convulsions in mice

Pentylenetetrazol (50 mg/kg; i.p.) in control group of mice produced jerky movements and clonic convulsions in all the animals within 3 minutes which lasted for nearly 10 minutes on an average, PTZ produced convulsions in all the animals pre-treated with flunarizine (20 mg/kg; i.p.) but with a decrease in duration of convulsions and increase in onset time of convulsions. The onset time in standard antiepileptic drug (Phenytoin) treated group showed significant increase as expected and prescribed as compared to control group. Flunarizine at dose of 20 mg/kg showed significant increase in onset time of convulsions induced by PTZ. On the other hand flunarizine at doses of 10 and 20 mg/kg showed significant decrease in duration of convulsions in mice in a dose-dependent manner. But in control group only, 50 % animals died due the convulsions after 10 min on an average and in 5 mg/kg flunarizine test group 30 % animals died. Other groups are totally protected from death due to convulsions. The data are shown in **table 10**.

Table No. 1: Effect of flunarizine on Pentobarbitone - induced sleeping time in mice

Treatment (mg/kg,i.p)	Duration of Immobility (seconds)
Control (vehicle)	155.40 ± 21.115
Flunarizine (5)	170.40 ± 10.458
Flunarizine(10)	112.00 ± 7.436
Flunarizine(20)	99.800 ± 3.929*
ANOVA Values	F(3,16)=7.319, P=0.0026

Values are mean ± SEM of 6 animals a group. *ns* = non significant
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 2: Effect of flunarizine on Forced swim test in mice.

Treatment (mg/kg,i.p)	Sleeping Time (Min.)
Control PB (45 mg/kg,i.p)	79.667 ± 4.104
Flunarizine (5)	80.333 ± 2.951 *ns*
Flunarizine (10)	82.500 ± 2.717 *ns*
Flunarizine (20)	87.333 ± 1.961 *ns*

Values are mean ± SEM of 6 animals a group. *$p<0.05$
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 3: Effect of flunarizine on Spontaneous Locomotor Activity (SLA) using Actophotometer in mice.

Treatment (mg/kg,i.p)	Photocell activity count	
	Before administration	After administration
Chlorpromazine (3mg/kg)	541.33±23.647	421.83±18.112*** [a]
Flunarizine (5)	539.83±23.793	505.17± 41.898
Flunarizine (10)	502.00± 41.874	499.17±41.867
Flunarizine (20)	537.33±23.647	447.50±19.500** [b]
a = ANOVA Values	t (5)= 21.562, P< 0.0001	
b= ANOVA Values	t (5)= 7.006, P=0.0009	

Values are mean ± SEM of 6 animals a group. ** $p<0.001$, *** $p<0.0001$ as compared with basal value.

Table No. 4: Effect of flunarizine on Elevated plus maze as model for Nootropic activity in mice.

Treatment (mg/kg,i.p)	Transfer latency in seconds	
	Day-1	Day-2
Control	53.500±3.160	33.833±3.156
Flunarizine (5)	48.333±4.310	28.333±4.310
Flunarizine (10)	45.667±3.721	25.667±3.721
Flunarizine (20)	39.000±3.246*	20.667±2.155*
Day-1 ANOVA Values	F(3,20)= 2.751, P= 0.0696	
Day-2 ANOVA Values	F(3,20)= 2.560, P= 0.0838	

Values are mean ± SEM of 6 animals a group. * $p<0.05$
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 5: Effect of flunarizine on Elevated plus maze model of anxiety in mice.

Treatment (mg/kg,i.p)	Mean no of entries in		Mean time spent in	
	Open arm	Closed arm	Open arm(s)	Closed arm(s)
Control	0.8333±0.3073	5.833±0.6009	31.167±3.420	182.00±4.824
Diazepam (1mg/kg,i.p)	3.667±0.3333**	5.667±0.3333	49.667±1.909**	180.50±5.903
Flunarizine (5)	2.167±0.4773	6.000±0.7303	34.333±4.279	164.00±12.798
Flunarizine(10)	2.500±0.4282*	6.000±0.8165	43.167±3.371*	184.00±7.220
Flunarizine (20)	3.167±0.3073**	6.000±0.6831	47.500±2.693**	181.00±6.055
ANOVA Values	F(4,25)= 8.242 P= 0.0002	F(4,25)=0.05195 P= 0.9946	F(4,25)=6.292 P= 0.0012	F(4,25)=1.057 P= 0.3982

Values are mean ± SEM of 6 animals a group. * $p<0.05$, ** $p<0.01$
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 6: Effect of flunarizine on Hole board test in mice.

Treatment (mg/kg,i.p)	No. of (Reading)	
	Nose poking [a]	Line crossing [b]
Control	53.333±2.813	37.667±3.630
Diazepam (1mg/kg,i.p)	30.333±2.333**	3.833±0.9458**
Flunarizine (5)	35.833±3.628*	17.333±4.984**
Flunarizine (10)	30.667±6.692**	14.333±5.439**
Flunarizine (20)	28.833±2.994**	5.167±1.447**
a = ANOVA Values	F(4,25)= 6.420, P= 0.0011	
b = ANOVA Values	F(4,25)=13.083, P< 0.0001	

Values are mean ± SEM of 6 animals a group. *p<0.05, **p<0.01
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 7: Effect of flunarizine on motor co-ordination in Rotarod test in mice

Treatment (mg/kg,i.p)	Fall off time	
	Before	After
Control	347.50± 17.770	356.67± 20.621
Flunarizine (5)	373.50±16.852	362.62±19.349
Flunarizine (10)	409.17±27.052	369.17±21.280
Flunarizine (20)	370.17±19.752	365.83±18.320

Values are mean ± SEM of 6 animals a group. *ns* = non significant as compared to basal.

Table No. 8: Effect of flunarizine on Apomorphine – induced stereotypy in mice.

Treatment (mg/kg,i.p)	Scoring for stereotypic behaviour			
	05 min	20 min	35 min	50 min
Control	3.000±0.3651	3.167±0.3073	2.667±0.3333	2.833±0.1667
Flunarizine(5)	2.833±0.3073	1.833±0.3073**	1.667±0.3333	1.667±0.2108**
Flunarizine(10)	2.333±0.4944	1.667±0.2108**	1.333±0.2108**	1.333±0.2108**
Flunarizine (20)	2.167±0.1667	0.5000±0.2236**	0.6667±0.2108**	0.8333±0.1667**

Values are mean ± SEM of 6 animals a group. **p<0.01
(one way ANOVA followed by Dunnett's test as compared to control group)

Table No. 9: Effect of flunarizine on Haloperidol – induced catalepsy in mice.

Treatment (mg/kg,i.p)	Catalepsy score after 30 min.	Catalepsy score after 90 min.
Control(HP) (1mg/kg,i.p)	30.333±1.282	37.667±2.319
Flunarizine (5)+HP	27.167±1.721	38.667±1.585
Flunarizine (10) +HP	30.500± 1.455	39.833±3.081
Flunarizine (20) +HP	31.167±1.939	48.000±3.347*
ANOVA Values	F(3,20)= 1.218 P= 0.3290	F(3,20)= 3.121 P= 0.0489

Values are mean ± SEM of 6 animals a group. *p<0.05
(one way ANOVA followed by Dunnett's test as compared to control group)

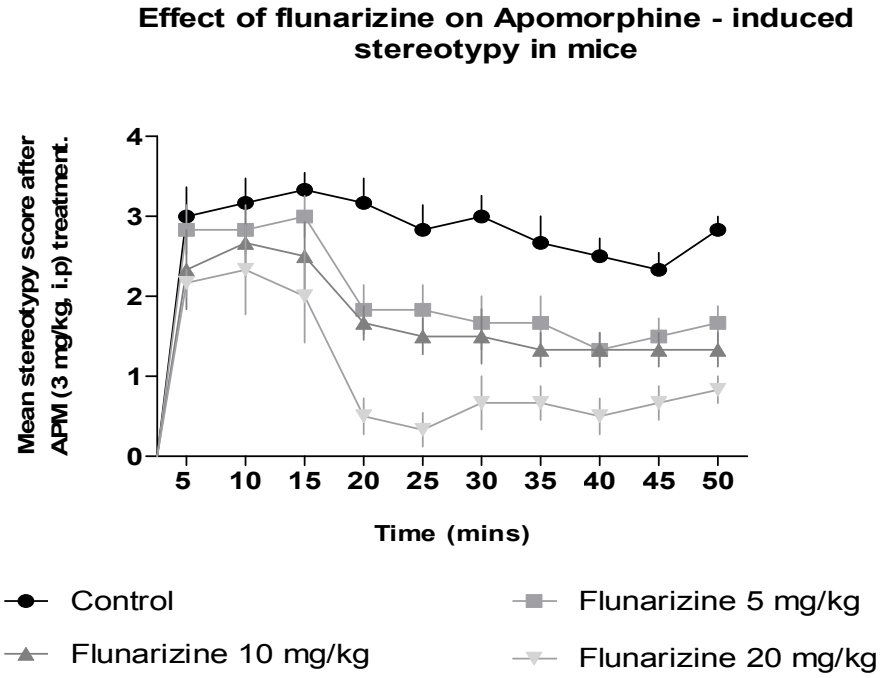

Figure No. 1

Table No. 10: Effect of flunarizine on Pentylenetetrazol (PTZ)- induced convulsions in mice.

Treatment (mg/kg,i.p)	Clonic Convulsions or Jerky movements		Status of the animal
	Onset time (sec.)	Duration (sec.)	
Control	136.17± 2.822	610.00± 47.539	3 died
Phenytoin	241.17± 15.856**	286.00± 27.140**	All alive
Flunarizine (5)	151.50± 3.364	500.50± 42.991	2 died
Flunarizine (10)	170.00± 5.556	448.33± 29.990*	All alive
Flunarizine (20)	236.83± 18.337**	312.83± 21.561**	All alive
ANOVA Values	F(4,24)= 18.718 P < 0.0001	F(4,24)= 14.527 P < 0.0001	

Values are mean ± SEM of 6 animals a group. **p*<0.05, ***p*<0.01
(one way ANOVA followed by Dunnett's test as compared to control group)

4. Discussion and conclusion

The present work investigated the effect of flunarizine, a calcium channel blocker, a piperazine derivative in several neuropsychopharmacological experimental models. The result of the present work provided evidences that flunarizine may be active as an anxiolytic agent and suggestive profile of atypical antipsychotic with an anticonvulsant activity. Flunarizine was found to produce alteration of general behavioural pattern, significant reduction in spontaneous locomotor activity, significant inhibition of stereotypic behavior induced by apomorphine (APM), significant increase in open arm entry – time spent in open arm without any alteration in the closed arm parameters, significant reduction in number of nose poking and line crossing in hole board apparatus, significant reduction in transfer latency (TL) in EPM, and a mild catalepsy at the highest dose among the selected doses for the study. Again a considerable decrease in immobility time in forced swim test was also observed which is not at par with the expected profile of flunarizine. Flunarizine also did not show any disturbances in motor co-ordination.

The efficiency of the most remedies is attributed to the fact that flunarizine readily crosses the blood brain barrier (BBB) and interferes with various neurotransmitter systems, e.g., it exerts antihistaminergic and antiserotoninergic activities; modulating effects on dopaminergic; adenosine and

opioid transmission.[19] Flunarizine is actually derived from cinnarizine, and differs from it by the fact that it has a piperazine radical in its molecule, which can also be found in Neuroleptics and Antihistamine drugs. [20] Again flunarizine is structurally similar to neuroleptic trifluoperazine. Flunarizine exhibits a complex interaction with the dopaminergic system. It inhibits ^{3}H-tyramine binding, a putative marker for a vesicular dopamine (DA) transporter, in striatal membranes (k_i 0.5 uM) and potentiates cocaine-induced dopamine release in the striatum. it also inhibits to binding of both ^{3}H-spiperone (a D_2 receptor ligand) and ^{3}H-SCH23390 (a D_1 receptor ligands) to the striatum with k_i values of 112 ± 9 and 532 ± 39 nM, respectively; these data suggest that flunarizine can act as a moderate dopamine receptor antagonist. [21]

On the other hand it was advocated that a group of compounds, blocks Na^+ and Ca^{2+} channels to prevent the overloading of the cell with Ca^{2+} ions under pathological and ischemic conditions, defined as Ca^{2+} overload blockers and discriminated from conventional Ca^{2+} entry blockers. Although only a few types of Na^+ and/or Ca^{2+} channel blockers, including flunarizine, lomerizine (KB-2796) and lifarizine (RS-87476) have been reported as Ca^{2+} overload blockers and shown to prevent neuronal cell death in animal models of ischemia, they have in common a diphenylmethylpiperazine moiety which not only causes the blockade of ion channels but also has significant affinity for dopamine D_2 receptors. [22] In our models the results followed the same pattern as above and indicate this dopamine antagonism.

A recent survey of psychiatric experts classified calcium channel blockers as adjuncts to other mood stabilizers for treatment–resistant mania which includes nimodipine and other calcium channel blockers. With respect to the safety profile and relative lack of side effects of calcium channel blockers and their potential efficacy in the treatment of mania, it is proposed that they may be an alternative choice to lithium. [23] The present study showed an antidepressant like activity at a dose of 20 mg/kg of flunarizine which support its possible use as a adjunct to benzodiazepines in post traumatic stress disorder. Flunarizine also found to produce extrapyramidal side effects identical to those induced by Neuroleptics, i.e.; parkinsonism like symptoms, tardive dyskinasia, akathysia etc. but those symptoms occur in elderly patients (above 65 years of age), especially if they are females [22].The dopaminergic tone is also decreased in old age when an extrapyramidal side effect occurs. The present work also showed a considerable increase in catalepsy time in haloperidol induced catalepsy model only at dose of 20 mg/kg only. This supports the statement regarding extrapyramidal side effect of flunarizine. But this can be overruled by the potential use of flunarizine as anxiolytic and as atypical antipsychotic in young. The present work showed that flunarizine potently inhibited hyper locomotion and stereotypic behavior induced by APM, (a model with predictive validity for antipsychotics) at doses (5, 10 mg/kg; i.p.) that produced no considerable hypo locomotion and cataleptic behaviour, a characteristic suggestive of atypical antipsychotics. [22] But at 20 mg/kg dose, flunarizine showed hypo locomotion and a mild catalepsy, whereas at doses (5, 10, 20 mg/kg; i.p.) flunarizine was equally effective in reversing the Apomorphine induced stereotypy. Therefore the results suggest a potential role of flunarizine as an atypical antipsychotic against schizophrenia and other psychotic disorders.

The present study showed no statistically significant sedative effect in pentobarbitone induced sleeping time model. But duration of sleeping time is increased dose dependently. It is generally believed that locomotor activation results from brain activation, which manifests as an excitation of central neurons and as an increase in cerebral metabolism, while different neurochemical metabolisms are involved in brain activation. Dopamine (DA) appears to play an essential role. Various environmental challenges that induce locomotor activities also increase DA transmission, inhibit spontaneous locomotion and greatly attenuate behavioural activation independent of triggering mechanisms. Hyper locomotion and stereotypy also occurs during pharmacological increase in DA transmission induced by both direct i.e.; (bromocriptine, APM) and indirect (i.e.; amphetamine, methamphetamine, cocaine) dopamine agonist.[24] Being dopamine antagonist, flunarizine was expected to inhibit hyper locomotion and stereotypy induced by APM. The present work also in accordance with this fact as stated.

The elevated plus maze is a well established animal model for testing anxiolytic drug.[12] Diazepam, standard anxiolytic used clinically, is also employed in behavioural pharmacology as a reference compound for inducing anxiolytic – like effects, even when the compound being screened does not act via benzodiazepine receptors.

In present study significant increase in number of open arm entry and time spent in open arm was observed and there were no any alteration in closed arm parameters, this indicate anxiolytic- like a activity of flunarizine. The observed activity might be due to the interaction of flunarizine with different neurotransmitter system in the brain, particularly the serotoninergic system. However further studies are required to confirm the exact mechanism of action of flunarizine as an anxiolytic agent.

The Rotarod method was used to test the neurotoxicity of flunarizine and this test was also used to determine

the effect of drugs on motor co-ordination. The flunarizine treated animals had not showed any alterations in Rotarod model suggesting that the drug flunarizine is devoid of neurotoxicity at these particular studied doses (5, 10, 20 mg.kg; i.p.)

Flunarizine at the employed doses exhibited a dose dependent inhibition of PTZ-induced convulsions. At 10 and 20 mg/kg doses, the drug significantly decreased the duration of convulsions and also at 20 mg/kg dose the onset time also increased. Further flunarizine protected all the animals from death at 10 and 20 mg/kg doses. These results support the work of Wolfgang Fischer et al., 2004; P. Sahadevan and M.N. Rema., 2002, dealing with the anticonvulsant activity with respect to the Na^+ and Ca^{++} blocking action.

Therefore the experimental data suggested that flunarizine exerts multiple effects on different neurotransmitter system in the brain particularly the dopaminergic system. Results also suggested that flunarizine has putative antipsychotic profile without major motor side effects, similar to atypical antipsychotics. Flunarizine showed comparatively weak antagonistic activity towards dopamine receptors particularly D_2 receptor as compared to D_1 receptors which supports the concept of atypical antipsychotic profile of flunarizine. However the long half life of flunarizine also may prevent abrupt exacerbation of symptoms in case of discontinuing the treatment. Another notable advantage of flunarizine is its cost, which is 10-30 times lower in comparison with atypical antipsychotics. Since the animal studies indicate a role of flunarizine in anxiety and schizophrenia without any significant motor side effect as part of simple interpretation of the results obtained from different animal models, detailed animal studies are required to establish the efficacy of flunarizine in molecular level, so that the data will be to be strong enough to consider this drug for some clinical trials and to facilitate the therapeutic repositioning of this drug in near future.

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