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# AN EXPERIMENTAL EVALUATION OF FLUNARIZINE IN ANIMAL MODELS FOR NEUROPSYCHOPHARMACOLOGICAL DISORDERS

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**ABSTRACT**

Objective of the present study to neuropsychopharmacological profile of flunarizine in laboratory animals. The study was conducted using adult Swiss albino mice (n = 6 in each group) and was approved by Institutional Animal Ethics Committee. Separate experiments were carried out to study effects of flunarizine at two doses (10, 20 mg/kg; i.p.) on thiopentone induced sleeping time, on spontaneous locomotor activity (SLA), on neurotoxicity (rota rod apparatus) and on haloperidol (1 mg/kg) induced catalepsy. The data was analyzed by paired t test and one way ANOVA followed by Dunnett's test and $p<0.05$ was considered significant. Flunarizine did not show any significant potentiation of sleeping time induced by thiopentone. 69 % reduction of SLA in the control group (chlorpromazine 3 mg/kg; i.p) and 43 % reduction in 20 mg/kg dose of flunarizine were observed. The number of falls of mice did not differ significantly from control and there was no potentiation of haloperidol induced catalepsy. Results indicate that flunarizine which is a potent candidate as future atypical antipsychotic and anti-anxiety agent does not cause sedation, neurotoxicity and motor side effect. However, detailed studies are required to establish its benefits at molecular level.

**Keywords:** Flunarizine, Atypical anti-psychotic, Anti-anxiety agent.

## INTRODUCTION

The calcium channel blocker flunarizine is a lipophilic diphenyl piperazine derivative. It is non selective voltage-dependent Ca2+ 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 is found to possess neuroprotective effect, vasodialating effect, antiserotoninergic effect and antivertiginous effect [4-7]. Flunarizine has similar chemical features as trifluoperazine which is an antipsychotic drug [8] and several animal studies indicate a role of flunarizine in anxiety and schizophrenia. Therefore in our effort to cultivate the concept of drug repositioning, we have studied the neuropsychopharmacological profiling of flunarizine in laboratory animals.

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## MATERIALS & METHODS

All the pharmacological experiments were conducted using adult Swiss albino mice (n = 6). The animals were housed separately in diffusely illuminated room with 60-70% relative humidity and approximately 22° C temperature in cages. The animals received a standard rodent diet and water *ad libitum*. They were acclimatized for at least 7 days before the start of experiments. The protocol was approved by Institutional Animal Ethics Committee of Smt. Kashibai Navale Medical College & Hospital, Pune. The care and use of laboratory animals was strictly in accordance with the guidelines prescribed by the

Institutional Animal Ethical Committee constituted under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India).
The study was carried out in 4 different parts. First part was to study of effects of flunarizine on thiopentone induced sleeping time. Flunarizine at two doses (10, 20 mg/kg; i.p) was administered 30 min prior to thiopentone sodium injection to two different groups of mice. Each group consisted of 6 mice for each dose. The time interval between the loss and regaining of righting reflex (fall asleep) was measured as sleeping time.

Second part was to study of effects of flunarizine on spontaneous locomotor activity (SLA). Spontaneous locomotor activity was evaluated by using a Digital Photoactophotomer. The action of flunarizine on spontaneous locomotor activity was measured automatically by photoactophotometer. The units of activity counts were based on the beam breaks by movement of the mice. The spontaneous locomotor activity of each mouse was recorded individually for 10 minutes. Flunarizine was administered 30 minutes before the test at 2 doses (10, 20 mg/kg; i.p) to 2 different groups of mice with 6 mice in each group and chlorpromazine (3 mg/kg, i.p.) as a standard drug was also administered to a separate group of mice 30 min before the test.

Third part of the study was to study the effects of flunarizine on neurotoxicity using rota rod apparatus. Rotarod test was used to determine the effect of drugs on motor co-ordination. The rod of the instrument (a horizontal rotation device) was set at a rate of 20 rpm. Three groups of mice were taken for the study (6 mice in each group). 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, 90 and 180min. after i.p administration of flunarizine at 2 doses (10 and 20 mg/kg, i.p) to 2 different groups of animals. The number of falls in 5 minutes was counted.

Fourth part of the study was to study the effects of flunarizine on haloperidol induced catalepsy. Catalepsy is defined as the acceptance and retention of abnormal imposed posture. 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. Three groups of mice were taken for the study (6 mice in each group). 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. 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 test. The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the mice moved the head in an exploratory manner.

**RESULTS**

The sleeping time of mice treated with thiopentone intraperitoneally and in combination with flunarizine at 2 doses (10 and 20 mg/kg, i.p) are shown in table 1. Flunarizine at the employed doses did not show any significant potentiation of sleeping time induced by thiopentone. Values are mean ± SEM of 6 animals a group. (One way ANOVA followed by Dunnett's test as compared to control group)

As shown in table no 2, the effect of flunarizine on spontaneous locomotor activity was measured in a 10 minutes test using Digital Photoactometer. Flunarizine at dose of 20 mg/kg significantly decreased the spontaneous locomotor activity. Flunarizine at 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), 69 % reduction of spontaneous locomotor activity ($p<0.001$) was observed and on the other hand 43 % reduction of spontaneous locomotor activity ( $p<0.05$) was observed in the group which received 20 mg/kg dose of flunarizine. Values are mean ± SEM of 6 animals a group.

As shown in figure 1,the number of falls of mice administered with flunarizine at doses (10 and 20 mg/kg; i.p.) did not differ significantly from control group, suggesting that flunarizine did not induce disturbances in motor co-ordination at these particular doses.

As shown in figure 2, flunarizine at doses (10 and 20 mg/kg) did not show any potentiation of the catalepsy time induced by haloperidol at different time intervals.

**Table 1. Effect of Flunarizine on Thipentone - induced sleeping time in mice**

| Treatment Group | Duration of Immobility (seconds) |
|---|---|
| Control | 161.5 ± 13.8 |
| Flunarizine 10 mg/kg i.p. | 151.7 ± 8.8 |
| Flunarizine 20 mg/kg i.p. | 141.7 ± 13.6 |

ANOVA values: $F= 0.65$, $p= 0.537$
Values are mean ± SEM of 6 animals a group. (one way ANOVA followed by Dunnett's test as compared to control group)

**Table 2. Effect of Flunarizine on Spontaneous Locomotor Activity (SLA) using Digital Photoactometer in mice**

| Treatment Group | Digital Photoactometer count | |
|---|---|---|
| | Baseline | After Drug |
| Chlorpromazine 3mg/kg i.p. | 481.16 ± 25.24 | 147.83 ± 31.48*** |
| Flunarizine 10 mg/kg i.p. | 427.33 ± 32.43 | 361.83 ± 15.61 |
| Flunarizine 20 mg/kg i.p. | 430.66 ± 58.40 | 241.5 ± 34.77* |

Values are mean ± SEM of 6 animals a group. *$p$<0.05, ***$p$<0.001 as compared with basal value.

**Fig 1. Effect of flunarizine on motor co-ordination in Rotarod test in mice**

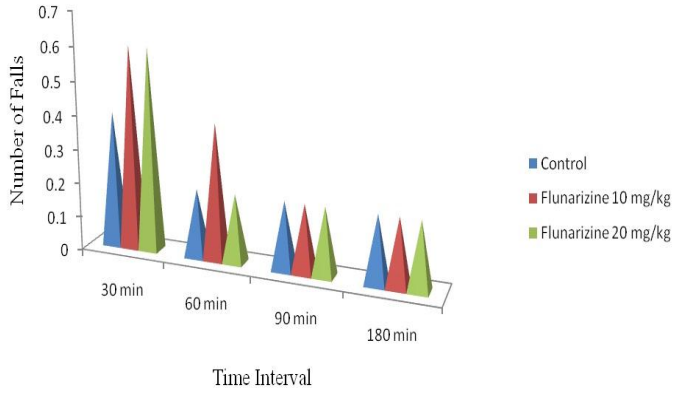
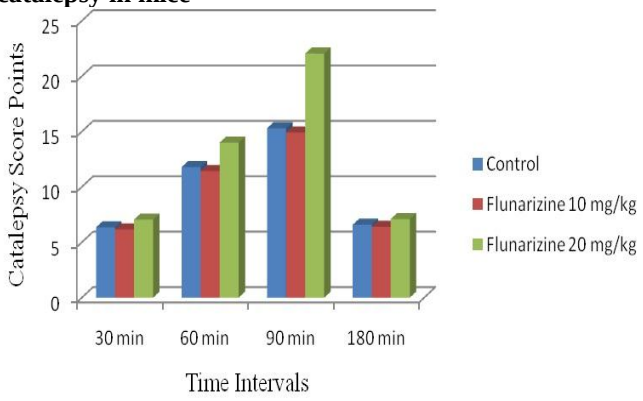



**Fig. 2. Effect of flunarizine on Haloperidol – induced catalepsy in mice**




## DISCUSSION

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 does not produce sedative effects. It was also found to produce significant reduction in spontaneous locomotor activity without producing any motor inco-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 [9]. 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 [10]. Again flunarizine is structurally similar to neuroleptic trifluoperazine.

Flunarizine exhibits a complex interaction with the dopaminergic system. The data suggest that flunarizine can act as a moderate dopamine receptor antagonist [11]. The work done by Ketaki et al. showed that flunarizine potently inhibited hyper locomotion and stereotypic behaviour induced by APM, (a model with predictive validity for antipsychotics) that produced no considerable hypo locomotion and cataleptic behaviour, a characteristic suggestive of atypical antipsychotics [12]. Therefore the results of the previous studies suggest a potential role of flunarizine as an atypical antipsychotic against schizophrenia and other psychotic disorders and devoid of extrapyramidal side effects as suggested by our results.

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. 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 [13]. Being dopamine antagonist, flunarizine is expected to inhibit hyper locomotion and stereotypy.

The Rotarod method was used to test the neurotoxicity of flunarizine. 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 (10 and 20 mg/kg; i.p.).

Therefore the experimental data suggested that flunarizine exerts multiple effects on different neurotransmitter system in the brain particularly the dopaminergic system. Flunarizine showed comparatively weak antagonistic activity towards dopamine receptors particularly D2 receptor as compared to D1 receptors which supports the concept of atypical antipsychotic profile of flunarizine.

## CONCLUSION

Since the simple interpretation of the results obtained from these different animal models indicates that flunarizine which is a potent candidate as future atypical antipsychotic and antianxiety agent, does not cause sedation, neurotoxicity and motor side effect. However, detailed studies are required to establish its benefits at molecular level.

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