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Original Article

EVALUATION OF ANTI-ANXIETY EFFECT OF NIFEDIPINE COMPARED TO DIAZEPAM IN SWISS ALBINO MICE USING BEHAVIOURAL MODELS

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ABSTRACT

Objective: The present study was undertaken to evaluate the acute and chronic anxiolytic effects of nifedipine in comparison to diazepam using in Swiss Albino mice using two behavioral models.

Methods: 30 Swiss albino mice were divided into 5 groups with 6 mice in each group. The study was conducted in two phases to evaluate acute and chronic effects. The groups consisted of diazepam (1 mg/kg), 3 doses of nifedipine (2.6 mg/kg, 5.2 mg/kg and 10.4 mg/kg) and vehicle control. The Elevated Plus Maze (EPM) and Light and Dark box were used to evaluate the anti-anxiety effects. The number of entries and time spent in the open arm of the elevated plus-maze and in the light area of light and dark box model were noted and compared among the 5 groups. Observations were analyzed using ANOVA and post hoc Tukey's test.

Results: Nifedipine (5.2 mg/kg and 10.4 mg/kg) significantly increased the number of entries and time spent in the open arm compared to vehicle control in the EPM test ($p<0.001$). Similarly, in the light and dark box test, nifedipine (5.2 mg/kg and 10.4 mg/kg) increased the number of entries and time spent in the light area compared to vehicle control ($p<0.05$). However, the low dose of nifedipine (2.6 mg/kg) did not exhibit significant findings.

Conclusion: Two doses of nifedipine (5.2 mg/kg and 10.4 mg/kg) possess anti-anxiety effects both on acute and chronic administration in both elevated plus maze and light and dark box model.

Keywords: Anxiolytic, Dihydropyridines, Behavioural model, Mice

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INTRODUCTION

Anxiety is a phenomenon characterized by uneasiness, apprehension, fear, or worry, which at times occurs without any identifiable triggering stimuli [1]. Anxiety disorders are among the most common mental, emotional, and behavioral problems and affect one-eighth of the total population worldwide [2] with a global prevalence of 7·3% and they have a substantial negative impact on the quality of life [3]. Hence treatment of anxiety is an important area of research interest in psychopharmacology.

Approximately two-thirds of the anxious patients respond to the currently available treatments, but the magnitude of improvement is still disappointing; besides, they also produce various systemic side effects and exhibit dependence and tolerance on chronic treatment which now have become a major concern about the use of currently used medicines [4].

Pharmacotherapy for anxiety disorders mainly includes the use of benzodiazepines, but regular use leads to deterioration of cognitive functioning, psychomotor impairment, amnesia, physical dependence and tolerance. Due to the lack of an ideal anxiolytic drug, the search for better anxiolytic drugs continue [5].

In the central nervous system, neurotransmitters responsible for the occurrence of symptoms of anxiety disorders include norepinephrine, serotonin, dopamine and gamma-aminobutyric acid (GABA) [6]. Neurotransmitter release involves the activation of voltage-gated calcium channels; hence it can be postulated that calcium channel antagonists (CCA) might inhibit the entry of calcium into the cells, thereby inhibiting its role as an intracellular messenger and exerting anti-anxiety [7].

Dihydropyridines (DHPs) cross the blood-brain barrier to varying extents and reach the central nervous system in sufficient quantities to bring about centrally mediated effects [8]. DHPs, namely nifedipine, nimodipine and nitrendipine are more extensively taken up into the brain from the blood [9]. Previous studies in rodents have demonstrated conflicting results for the anxiolytic activity of dihydropyridines and benzothiazepines using various behavioral models [10-12]. CCAs are devoid of the side effects seen with anxiolytic drugs like sedation, cognitive impairment and dependence [13]. Thus, the use of CCAs in patients with anxiety disorders can lead to fewer side effects as compared to anxiolytics.

Hence in this study, we aimed at determining the antianxiety property of nifedipine since it is one of the most commonly prescribed CCA in India [14, 15], using 2 behavioral models, namely Elevated Plus maze and Light and Dark box.

MATERIALS AND METHODS

The study was initiated after receiving necessary approvals from the Institutional Animal Ethics committee of our institute in accordance with CPCSEA (Approval No–IAEC/01/2017). The duration of the study was 6 mo conducted between March 2018 to August 2018.

Experimental animals

A total of 30 male Swiss Albino mice were procured from a registered breeder (Bombay Veterinary College, Parel Mumbai). The age and weight of the mice ranged from 6 to 8 w and between 20–30 grams, respectively. The mice were group-housed in steel cages with adequate bedding material and allowed to have food and water ad libitum. The temperature was maintained at 23 °C-25 °C with a 40–50% humidity and 12 h light/12 h dark cycle during the experiment. The experiment was performed between 0900 to 0200 h. screening for healthy albino mice with normal behavior and activity was done.

Chemical and drugs

1% w/v carboxymethylcellulose was procured from the experimental laboratory of our institute, diazepam (1 mg/kg) from Centaur Pharmaceuticals Ltd and nifedipine from Sun Pharma Pvt Ltd. The doses for nifedipine and diazepam were extrapolated from human doses of the drug [16, 17].

Experimental design and procedure

30 male swiss albino mice weighing between 20–30 grams were randomly divided into five groups of six mice each. Prior to the experiment, the animals were introduced in both the models to familiarize them with the model environment. Details are given in table 1.

The study was carried out in two phases. Mice were subjected to elevated plus maze followed by light and dark box to evaluate the acute anxiolytic effect of nifedipine after a single administration. This was followed by administering nifedipine for 30 d to evaluate the chronic anxiolytic effects using the same behavioural models.

Elevated plus-maze model

It is an unconditioned behavioral model used to assess the anxiolytic activity in mice [18]. Rodents generally tend to avoid open spaces and stay in darker areas. When the animal is placed on the EPM, animals with anxious behavior spend more time in enclosed arms than open arms.

The apparatus consists of two open arms and two closed arms with an open roof and elevated to a height of 50 cm above the floor. Thirty mins after oral administration of the test drug or standard, each mouse was placed in the centre of the maze facing one of the enclosed arms and was observed for 5 min. Entry into the arm was indicated by all four paws including the tail of the mouse into the arm. After 5 min of observation, the total number of entries in open arm and time spent in the open arm was noted. The maze was cleaned after each trial [19].

Light and dark box model

This is another model used to assess the anxiolytic activity in mice based on the inherent aversion to lit areas [20]. The apparatus consists of an open-top wooden box with two chambers separated by a partition wall and connected by a small opening in the center of the wall. The mouse was placed at the center of the brightly lit arena of the light and dark box. The transition between the light and dark box and the time spent in the light area were recorded for 10 min. Animals spend more time in the light chamber and show more locomotor activity after treatment with anxiolytics.

Table 1: Description of group and drugs administered during the study (n=30)

Group	Group description	No. Of animals	Dose
I	1% CMC (Vehicle Control)	6	0.5 ml (PO)
II	Diazepam (Positive Control)	6	1 mg/kg (PO)
III	Nifedipine (low dose)	6	2.6 mg/kg (PO)
IV	Nifedipine (medium dose)	6	5.2 mg/kg (PO)
V	Nifedipine (high dose)	6	10.4 mg/kg (PO)

PO–Per Oral

Statistical analysis

Data was represented as mean±SD. Data were analyzed using one-way analysis of variance (ANOVA) for between-group comparison followed by post-hoc Tukey's test. A p-value of<0.05 was considered statistically significant. GraphPad Instat version 3 was the statistical software used for analysis.

RESULTS

Two models, namely elevated plus maze and light and dark box model were used for evaluation of the anxiolytic activity of nifedipine in swiss albino mice.

Evaluation of acute anxiolytic effect

Elevated plus-maze model

In this model, efficacy was measured by an increase in the number of open arm entries and time spent in open arms between the groups.

Light and dark box model

In this model, efficacy was measured by an increase in the mean values of the 2 parameters i.e. the number of entries into the light area and the time spent in the light area of the Light-Dark box apparatus.

The results of both models are tabulated in table 2.

On performing a one-way analysis of variance (ANOVA) between groups in both models, the p-value was<0.001, which is highly significant. On post–hoc Tukey's comparison, there was a statistically significant difference between the number of open arm entries and entry into light area in the diazepam [1 mg/kg] group, medium and high doses of nifedipine [5.2 mg/kg and 10.4 mg/kg] compared to the vehicle control group (p<0.001). However, the difference between diazepam [1 mg/kg] and medium and high dose nifedipine [5.2 mg/kg and 10.4 mg/kg, respectively] was not statistically significant for time spent in open arm and light area.

Evaluation of the chronic anxiolytic effect

The animals were administered the test drug and control orally daily for 30 d and the anxiolytic activity of nifedipine was evaluated using the behavioral models. Results of the elevated plus-maze model and light and dark box model are given in table 3.

Table 2: Comparison of the number of entries and time spent in open arm and light area in elevated plus maze and light and dark box model (Acute effect)

		Elevated plus maze model		Light and dark box model	
Group no.	**Group description**	**No. of open arm entries**	**Time spent in open arm (min)**	**No. of entries in light area**	**Time spent in light area (min)**
1	Vehicle Control (0.5 ml 1% CMC p. o.)	4.35±0.35	1.26±0.63	2.33±0.81	5.14±0.37
2	Diazepam (1 mg/kg p. o.)	8.33±1.41*	3.19±0.55*	6.33±1.36*	7.34±0.48*
3	Nifedipine (2.6 mg/kg p. o.)	5.16±1.03#	1.66±0.30#	3.66±1.21#	4.06±0.70#
4	Nifedipine (5.2 mg/kg p. o.)	7.16±1.26*	3.09±0.45*$	5.16±0.75*	6.90±0.49*$
5	Nifedipine (10.4 mg/kg p. o.)	8.16±1.16*	2.75±0.49*$	5.83±0.75*	7.58±0.60*$

Values are expressed as mean±SD.*p value<0.001 as compared to control, #p value<0.01 as compared to diazepam. $p value<0.01 as compared to low dose nifedipine [2.6 mg/kg]

Table 3: Comparison of the number of entries and time spent in open arm and light area in Elevated plus maze and light and dark box model (Chronic effect)

		Elevated plus-maze model		Light and dark box model	
Group no.	**Group description**	**No. of open arm entries**	**Time spent in open arm (min)**	**No. of entries in light area**	**Time spent in light area (min)**
1	Vehicle Control (0.5 ml 1%CMC p. o)	4.33±1.03	1.29±0.19	3.33±0.81	2.40±1.02
2	Diazepam (1 mg/kg p. o)	7.83±0.98*	3.24±0.41*	6.16±0.75*	6.92±0.34*
3	Nifedipine (2.6 mg/kg p. o)	5.16±0.75#	1.39±0.38#	3.50±1.04#	3.85±0.56*#
4	Nifedipine (5.2 mg/kg p. o)	7.16±0.75*$	2.78±0.60*$	4.83±0.75*	4.71±0.41*
5	Nifedipine (10.4 mg/kg p. o)	8.16±0.75*$	3.30±0.18*$	5.83±0.98*$	6.50±0.69*$

Values are expressed as Mean+SD.*p value<0.001 compared to vehicle control. #p value<0.001 as compared to diazepam. $p value<0.01 as compared to low dose nifedipine [2.6 mg/kg].

When compared to the control group, nifedipine showed significant anxiolytic activity after 30 d of administration at medium (5.2 mg/kg) and high dose (10.4 mg/kg) by showing an increase in the number of entries in the open arm and light area. There was also an increase in the time spent in open arms and light area following drug administration. This shows that nifedipine had a chronic anxiolytic effect after 30 d of drug administration.

DISCUSSION

Anxiety, like all emotions, has cognitive, neurobiological and behavioural components. It is a negative emotion that is felt in response to perceived threats coming from internal or external sources which may or may not be real [21].

The proposed mechanism of the anxiolytic activity of calcium channel antagonist is the drugs that cross the blood-brain barrier (nifedipine, nimodipine, nitrendipine) reach at higher concentrations to the limbic system, comprising of the hippocampus and amygdala [22]. A neurocircuit that arises from the output pathways of the central nucleus of the amygdala mediates fear and anxiety responses in humans. Exaggerated output through the amygdala related circuits may be the underlying cause for the various types of anxiety disorders [23]. Binding studies reveal that the amygdala in the brain also contains binding sites for dihydropyridines, which explains its involvement in anxiolytic activity [24].

Calcium plays an important role in the release of multiple neurotransmitters such as serotonin (5-HT), acetylcholine (Ach), noradrenaline and dopamine from nerve terminals and dendrites in the CNS. Hence, it can be hypothesized that a calcium channel antagonist will block the action of these neurotransmitters by preventing their release. An imbalance in the neurotransmitters, mainly serotonin, noradrenaline and GABA, has been implicated in the pathogenesis of anxiety disorders, hence drugs that block the release of these neurotransmitters potentially possess anxiolytic activity [25].

In this study, elevated plus maze and light and dark box models were used to assess the anxiolytic activity of nifedipine. Swiss albino mice inherently show aversion to light, high and open spaces, and hence spend more time in enclosed or dark spaces. An anxiolytic drug specifically increases the number of entries and total time spent in open arm and light area. This behavioral activity forms the basis of its use as a screening model for anti-anxiety effects.

The present study indicated that both medium and high doses of nifedipine (5.2 mg/kg and 10.4 mg/kg, respectively) showed anxiolytic activity in mice when evaluated using both elevated plus maze and light and dark box models. The first part was performed where the anxiolytic effect of nifedipine was determined after a single dose oral administration of nifedipine at 3 doses. Followed by a daily administration of nifedipine for 30 d to determine a chronic anxiolytic effect. Nifedipine showed a dose-dependent anxiolytic effect in both the elevated plus maze model and the light and dark box model.

Similar findings were reported by Ganouni *et al.* who evaluated the anxiolytic effect of increasing doses of nifedipine (1.25 mg/kg, 2.5 mg/kg and 5 mg/kg) using water consumption in a novel environment model as the behavioral model. A dose-dependent increase in the anxiolytic activity was seen [10]. Another study conducted by Tanwani *et al.* evaluated the anti-anxiety, anti-depressant and sedative effects of nimodipine in swiss albino mice using the elevated plus-maze model to evaluate the anxiolytic effects, they found that a low dose of nimodipine (2.5 mg/kg) had anti-anxiety effects [12].

Anxiety disorders are closely associated with the development of hypertension and vice versa. Moreover, anxiety is one of the barriers in the treatment of hypertension [26]. ACE inhibitors and calcium channel antagonists are one of the first-line drugs used in the management of hypertension. Hence anti-hypertensive agents that have an anxiolytic effect may help in the management of anxiety along with hypertension and at the same time avoid side effects like sedation, impairment of consciousness and dependence seen with typical anxiolytics. Another advantage of nifedipine is that it does not lower the blood pressure in normotensive individuals, making it an appropriate choice for an anxiolytic calcium channel antagonist in the future.

The limitations of our study are small sample size and the combination of low dose nifedipine with diazepam was not evaluated to test the potentiation of anxiolytic activity. Further, *in vitro* studies to test for the levels of the drugs in the brain tissues are needed to add more value to the pharmacodynamic evidence obtained from the current study. The greatest opportunity for the screening of repurposed drugs like nifedipine as an anxiolytic is that the claimed mechanism has not yet been adequately addressed. The detection of an already marketed drug with a novel anxiolytic mechanism would be a significant step forward.

CONCLUSION

Our study suggests that medium (5.2 mg/kg) and high dose (10.4 mg/kg) of nifedipine has considerable anxiolytic activity comparable to diazepam and more effective than vehicle control in behavioural models on acute and chronic administration, possibly due to the presence of dihydropyridine receptors on the amygdala which is the centre responsible for anxiety. The anxiolytic property of nifedipine will enhance its therapeutic utility and improve the quality of life of patients with anxiety. Hence with further clinical studies, nifedipine can be effectively used in hypertension, which is often associated with anxiety.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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