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An experimental study to evaluate the effect of memantine in animal models of anxiety in swiss albino mice

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Abstract
The present study is done to evaluate the antianxiety effect of Memantine in swiss albino mice. They were divided into four groups containing six mice in each group. First group animals were given normal saline as a control (10ml/kg), Lorazepam (0.5mg/kg) for second group and for third group Memantine (3mg/kg) as a test drug and Memantine plus Lorazepam (3mg/kg + 0.5mg/kg) for fourth group intraperitoneally daily for 7 consecutive days. Results were analyzed by ANOVA followed by Post hoc Tukey's test. In passive avoidance test, animals treated with Memantine showed anxiolytic effect by significant decrease ($p<0.001$) in step down latency period in SFZ, significant increase ($p<0.001$) in step down error and total time spent in SZ on day '0' and day '07' when compared to control group.
In open field test, animals treated with Memantine showed anxiolytic effect by significant increase ($p<0.001$) in no. of squares crossed, time spent in central square, no. of rearing and significant decrease ($p<0.001$) in freezing time on day '0' and day '07' as compared to control group. Memantine showed synergistic antianxiety effect with Lorazepam when combined together. NMDA (N-methyl-D-aspartate) antagonist, Memantine is found to produce significant antianxiety effect in experimental models of anxiety in mice.

Keywords: memantine, NMDA-antagonist, Passive avoidance test, open field test, anti-anxiety

1. Introduction
The incidence of pathologic anxiety in the community is very high and is associated with lot of morbidity. Lifetime prevalence of anxiety in women is 30.5% and in males is 19.2% [1]. Hence, it is very important to address the problem of anxiety and find safe and effective medicines. Anxiety disorders are most often associated with dysfunction of GABAergic inhibitory neurotransmission, usually treated by Benzodiazepine class of drugs. Benzodiazepines are the standard anti-anxiety drugs but they are associated with side effects of sedation and addiction. Buspirone, the non-sedative anxiolytic agent is not effective in a high percentage of patients. It is also associated with tachycardia, palpitation, gastric discomfort etc. [2].
However, it has been suggested that glutamate deregulation may also contribute to anxiety states enormously [3]. Among them, NMDA receptor, a complex ion channel, is particularly known to play a key role in anxiety disorders [4, 5].
Memantine is a non-competitive antagonist at NMDA receptor. There are no studies in literature showing anxiolytic effect of Memantine in experimental models of anxiety in swiss albino mice. Thus, Memantine is evaluated for its anti-anxiety effect in this study.

2. Experimental section
2.1 Aims and objectives
To study the effect of Memantine on behavioral parameters of anxiety in mice.

2.2 Animals
Swiss albino mice of either sex weighing between 25 and 30 gms, were obtained and the animals were housed in cages and kept under controlled environmental condition (humidity 50–55 %, temperature 22±2°C, natural light/day cycle). The study was conducted at Department of Industrial Biotechnology, Nagarjuna college of Engineering and technology, Karnataka, from November 2010 to April 2011. All the experiments were performed in daytime between 09:30 and 15:30 hours. Care of animals was according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The permission to undertake this study was duly approved by the Institutional Animal Ethics Committee.

2.3 Drugs and Chemicals
Memantine (Sun Pharma drugs Pvt.Ltd. India), Lorazepam (Intas Pharmaceuticals Ltd. India) diluted in normal saline were used.

2.4 Experimental Groups
In the experiment mice are divided into four groups (n= 6).
Group 1- Control (Normal saline,10 ml/kg, i.p.)
Group 2- Memantine (3mg/kg, i.p.)
Group 3- Lorazepam (0.5 mg/kg, i.p.)
Group 4- Memantine (3mg/kg, i.p.) + Lorazepam (0.5 mg/kg, i.p.)

Memantine, Lorazepam, normal saline were administered intraperitoneally daily for 7 days of experimental period to observe their effects on '0' and day '07'. The mice were administered respective drugs/normal saline intraperitoneally as scheduled and behavioral assessment was conducted 30 minutes after administration.

2.5 Assessment of behavioral tests

2.5.1 Passive avoidance

This test is performed in a 34 cm x 34 cm x 20 cm chamber with a grid floor through which electric shock of 20 mV was delivered. A shock-free zone (SFZ) was provided in the center of the chamber. Mice are placed on the SFZ and when they tried to get down from the SFZ and come in contact with the grid floor, they were given an electric shock. Mice gradually learned to avoid shock by staying in the SFZ, curbing their normal exploratory behavior. This is the principle of passive avoidance. The mouse is initially trained until it avoided coming in contact with the shock zone by passively sitting in the SFZ for a minimum of 60 seconds. In the Passive avoidance test, the animal avoids punishment by refraining from making a specified response, i.e., by staying in the SFZ [6]. The parameters noted were: 1.Step-down latency (duration for which the animal stays in the SFZ) 2. Step-down error (number of attempts the animal makes to come to the shock zone) 3. Total time spent in the shock zone (SZ).

2.5.2 Open Field Test

This test is commonly used to detect anxiolytic activity. An open field apparatus suitable for mice is made comprising of a floor space of 40 cm x 40 cm with 30 cm high walls. The floor area is divided into 16 equal squares. A mouse is placed at the center [7] of the field and is left for 2 minutes for acclimatization with the apparatus. Thereafter, for the next 5 min, the parameters noted were: 1. Time spent in the central square 2.Ambulation (No. of squares crossed) 3.Rearing (No. of times the animal stands on the rear paws).

2.6 Statistical Analysis

Results were presented as Mean ± SEM. One way ANOVA is used for comparison between the groups, followed by post hoc Tukey's test. For all the tests 'P' value of 0.05 or less is considered statistically significant.

3. Results and Discussion

Table 1: Effect of single dose observation in passive avoidance test on day '0'

Groups, (dose)	Step down Latency (sec)	Step down Error (no.)	Time in shock zone (sec)
1. Control (10 ml/kg, i.p.)	280.4±8.17	1.5± 0.29	6.7±0.86
2. Lorazepam (0.5mg/kg, i.p.)	175.8±5.98*	6.4± 0.82*	34.2±1.63*
3. Memantine (3mg/kg, i.p.)	267.2±2.51††	2.1± 0.64††	8.6±0.75††
4. Memantine + Lorazepam	152.3±3.82‡‡	9.4±0.56‡‡	42.4±1.72‡‡

(n=6), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. Lorazepam), (‡p< 0.01, ‡‡p< 0.001 vs. Memantine)

Table 2: Effect of multiple dose observation in passive avoidance test on day '7'

Groups, (dose)	Step down Latency (sec)	Step down Error (no.)	Time in Shock Zone (sec)
1. Control (10 ml/kg, i.p.)	278.3± 5.49	1.4±0.19	5.6±0.6
2. Lorazepam (0.5 mg/kg, i.p.)	161.4±2.73*	8.1±0.98*	38.3±1.82*
3. Memantie (3mg/kg, i.p.)	185.4±3.87*,†	6.8±0.78*	32.1±2.22*
4. Memantine + Lorazepam	155.8±1.67‡‡	9.2± 0.63‡	41.8 ±1.81‡

(n=6), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. Lorazepam), (‡p< 0.01, ‡‡p< 0.001 vs. Memantine)

In group treated with Lorazepam as a standard, animals showed significant decrease (p<0.001) in step down latency period in SFZ, significant increase (p<0.001) in step down error and in total time spent in SZ on day '0' and day '07' as compared to control group (as shown in table 1 and table 2).

When Memantine treated group compared to control group, animals showed slight

decrease in step down latency period in SFZ, slight increase in step down error and in total time spent in SZ on day '0', but not statistically significant (as shown in table 1). Whereas, on day '07' there was significant decrease (p<0.001) in step down latency period in SFZ, and significant increase (p<0.001) in step down error and in total time spent in SZ on day '7' (as shown in table 2).

In group treated with Lorazepam when compared to Memantine treated group, mice showed significant decrease (p<0.001) in step down latency period in SFZ, significant increase (p<0.001) in step down error and in total time spent in SZ on day '0' (as shown in table 1). But on day '07' there was significant decrease (p<0.01) in step down latency period in SFZ and no statistical difference in step down errors in total time spent in SZ (shown in table 2).

On day '0' (Memantine + Lorazepam) treated group when compared to Memantine alone treated group, animals showed significant decrease (p<0.001) in step down latency period in SFZ, significant increase (p<0.001) in step down error and in total time spent in SZ (as shown in table 1). But on day '7' animals showed significant decrease (p<0.001) in step down latency period in SFZ, significant increase (p<0.01) in step down error and total time spent in SZ (as shown in table 2).

Table 3: Effect of single dose observation in open field test on day '0'

Groups, (dose)	Squares Crossed(no.)	Time spent in Central square (sec)	Rearing (no.)	Freezing time (sec)
1. Control (10ml/kg, i.p)	86.3± 1.08	2.7± 0.14	19.3±1.6	20.6±2.36
2. Lorazepam (0.5mg/kg, i.p)	104.6±0.92*	8.4 ± 0.18*	34.4±1.17*	9.6±1.35*
3. Memantine (3mg/kg, i.p.)	90.2±1.61††	3.1± 0.15††	23.5±2.39††	18.1±1.18†
4. Memantine + Lorazepam	135.6± 1.6‡‡	26.32±1.1‡‡	51.2±2.06‡‡	7.3±0.86‡‡

(n=6), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. Lorazepam), (‡‡p< 0.001 vs. Memantine)

Table 4: Effect of multiple dose observation in open field test on day '7'

Groups, (dose)	Squares Crossed (no.)	Time spent in Central square (sec)	Rearing (no.)	Freezing time (sec)
1. Control (10ml/kg, i.p)	83.2±2.96	3.4± 0.65	17± 1.81	20.2±2.29
2. Lorazepam (0.5mg/kg, i.p)	126.4±2.77*	14.3±1.53*	37.5±2.42*	8.53±0.59*
3. Memantine (3mg/kg, i.p.)	112.7±2.69*, †	11.5±1.26*	32.4±2.61*	15.2±1.12*, †
4. Memantine + Lorazepam	131.2±2.98‡‡	25.2±1.17‡‡	46.1±1.51‡‡	5.9±0.62‡‡

(n=6), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. Lorazepam), (‡‡p< 0.001 vs. Memantine)

In group treated with Lorazepam as a standard, animals showed significant increase (p<0.001) in no. of squares crossed, time spent in central square, no. of rearing and significant decrease (p<0.001) in freezing time on day '0' and day '07' as compared to control group (as shown in table 3 and table 4).

When Memantine treated group compared to control group, mice showed slight increase in no. of squares crossed, time spent in central square, no. of rearing and slight decrease in freezing time on day '0', but not statistically significant (as shown in table 3). Whereas on day '07' animals showed significant increase (p<0.001) in no. of squares crossed, time spent in central square, no. of rearing and significant decrease (p<0.001) in freezing time (as shown in table 3 and table4).

In group treated with Lorazepam as a standard when compared to Memantine treated group, animals showed significant increase (p<0.001) in no. of squares crossed, time spent in central square, no. of rearing and significant decrease (p<0.01) in freezing time on day '0' (as shown in table 3). But on day '07' there was significant increase (p<0.01) in no. of squares crossed and significant decrease (p<0.01) in freezing time and slight increase in time spent in central square, no. of rearing but not statistically not significant (as shown in table 4).

On day '0' & day '07', (Memantine + Lorazepam) treated group when compared to Memantine alone treated group, animals showed significant increase (p<0.001) in no. of squares crossed, time spent in central square, no. of rearing and significant decrease (p<0.001) in freezing time on day '0' and on day '07' (as shown in table 3 and table 4).

Fig 1: Showing the mouse staying at Shock Free Zone in Passive avoidance test

Fig 2: Showing the mouse crossing the central square in Open field test

In the management of anxiety, Benzodiazepines are mainly prescribed as first choice treatment. However chronic administration of Benzodiazepines results in side effects like sedation, ataxia, amnesia and pharmacological dependence [8].

Mainly three types of inotropic glutamate receptors are found in mammalian brain: N-methyl-D-aspartate (NMDA), 2-amino-3-hydroxy- 5-methyl-4-isoxazolepropionic acid (AMPA) and kainate [9]. Among them, NMDA receptor, a complex ion channel, plays a pivotal role in anxiety disorders [4, 5].

In addition, the anxiolytic-like effects of NMDA receptor antagonists were observed after microinjection into the dorsolateral periaqueductal gray [10]. The preclinical and clinical data provide strong evidences that diverse antagonists and partial agonists, acting at different sites of NMDA receptor complex exhibit anxiolytic-like activity. Quite often they have been compared to benzodiazepines or barbiturates [11-13].

In the passive avoidance test, the animal avoids punishment by refraining from making a specified response, by staying in the SFZ. A decrease in step-down latency and an increase in step-down errors indicate reduction of normal anxiety associated with exposure to a novel environment. All these effects are attenuated by anxiolytic compounds.

In our study, Memantine given for 7 days produced anxiolytic effect in Passive avoidance test by showing significant decrease in step down latency period in SFZ, significant increase in step down error and in total time spent in SZ on day '0' and day '07' as compared to control group.

In our study, when Memantine given for 7 days produced anxiolytic effect in open field test by showing significant increase in no. of squares crossed, time spent in central square, no. of rearing and significant decrease in freezing time on day '0' and day '07' as compared to control group. Memantine showed significant antianxiety effect in passive avoidance test and open field test parameters on day '07' when compared to day '0' indicating its anxiolytic effect.

Our study also demonstrated synergistic interaction between Memantine and Lorazepam in their antianxiety activity. Similar antianxiety effects were observed from previous studies depicting antianxiety effect of other competitive NMDA receptor antagonists [14, 15].

Indeed, there is a wealth of evidence indicating the involvement of the NMDA receptor

Complex in the modulation of anxiety. Also, it seems that NMDA glutamatergic system, nicotinic acetylcholine and serotonin (5-HT) receptors can impose an effect on the modulation of anxiolytic behaviors caused by Phencyclidine and its derivatives [16-21].

Both pre-clinical and clinical studies indicate that antagonists of glutamate N-methyl-D-aspartate (NMDA) receptors like Phencyclidine [20], Dizocilpine (MK-801) [22] have shown to produce antianxiety effects. NMDA antagonist like phencyclidine (PCP) produces hallucinations and ketamine produces drowsiness [23-25]. There is improved clinical tolerability of Memantine in comparison to other NMDA antagonists. [23-25, 26].

Recent studies also highlighted the role of hypothalamus pituitary adrenal (HPA) axis over activity in anxiety disorders. The amygdala and the hippocampus control the activity of the HPA axis in a counter-balancing way, by various neuropeptides such as corticotrophin-releasing factor, substance P, vasopressin and neuropeptide Y (NPY) [27].

The hypothesis by Olney [28] suggest that over activation of NMDA receptors leads to damage of GABA neurons and secondary damage produced by disinhibited neurons (e.g., glutamate, Ach, NPY). The previous study by Wieronska JM *et al*. [29] indicates that in the amygdala, the NMDA receptors mediated glutamatergic transmission may regulate NPY neurons. Activity of Memantine on NPY activity, which might contribute to the antianxiety activity, throws light on its further exploration as antianxiety drug.

4. Conclusion

Memantine when administered at a dose of (3mg/kg, i.p.) demonstrated antianxiety effect which was comparable to Lorazepam. Memantine could be producing its antianxiety activity by blocking NMDA receptor. However, its modulating effect on NPY which might contribute to antianxiety effect cannot be ruled out. There was synergism in antianxiety activity of Memantine and Lorazepam. Further research is required to gain closer insights into the exact mechanism of action of Memantine as antianxiety drug, which might benefit the patients of anxiety in clinical scenario.

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