EVALUATION OF THE COGNITION AND MEMORY ENHANCING EFFECT OF VENLAFAXINE IN EXPERIMENTAL ANIMALS

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Abstract: Dementia is a threatening menace to our society with no definitive treatment. The cognitive defects in dementia make it very difficult for the patient to perform day to day activities and their families to provide care. The cognitive defects are thought to be related to the neurotransmitter levels in our body. Hence the present study aims to ev aluate the ef fect of venlafaxine, a S erotonin –Norepinephrine Reuptake Inhibitor, in cognition in experimental animals.

Introduction

Cognition is the process of obtaining, organizing and using intellectual knowledge. It refers to a set of interwoven processes, such as memory, language, problem solving and developing strategies to apply to our perceptions. [1] Dementia mainly affects older people but 2 to 10% of all cases are estimated to start before the age of 65 years. After this, the prevalence doubles, with every five year increment in age. [2] . Worldwide, 44 million people live with dementia and this figure is expected to reach 135 million by 2050. Meanwhile, the cost of care reached an estimated $604 billion worldwide in 2010. [3]

Alzheimer's disease is the most common prevalent form of dementia, afflicting approximately 10% of the population over 65 years of age. [4] It affects 22 million people worldwide, out of which 3 million are from India. An estimated twofold increase by 2030 and threefold by 2050 can be expected.

Mounting evidence accumulated over the past few years indicates that the neurotransmitters serotonin and norepinephrine play a significant role in cognition. As a drug target, serotonin receptors have received notable attention due, in particular to the role of several serotonin-receptor subclasses in cognition and memory. [5] Loss of norepinephrine releasing neurons, in the locus coeruleus of the brainstem, is well documented to occur in AD. Alterations in NE have long been known to be linked to cognitive, mood and neuropsychiatric symptoms. [6]

Although theoretically, drugs which increase the serotonin and nor epinephrine levels such as the Serotonin Norepinephrine Reuptake Inhibitors (SNRIs) should help in cognition and memory, there is no consensus regarding it, as various studies have found contradicting results.

Venlafaxine is a Serotonin –Norepinephrine Reuptake Inhibitor which has been approved for the treatment of depression, anxiety disorders, fibromyalgia and neuropathic pain. It has a non tricyclic structure that inhibits the reuptake of 5-HT (serotonin) and norepinephrine. It causes enhanced serotonergic and noradrenergic neurotransmission. [7]

Keeping the above evidences in mind, the present study has been undertaken to evaluate the cognition and memory enhancing potential of Venlafaxine.

METHODOLOGY

The study was conducted in the Department of Pharmacology at Gauhati Medical College & Hospital after taking due approval from the Institutional Animal Ethics Committee.

Drugs and chemicals used in the study

(1) Venlafaxine hydrochloride obtained from Sigma Aldrich India, Bangalore.

(2) Scopolamine hydrobromide obtained from Sigma Aldrich India, Bangalore.

(3) Piracetam obtained from UCB India Pvt Ltd.Vapi, Gujarat.

(4) Vehicle: Normal Saline (0.9% NaCl).

Instruments used in the study

a) Morris Water Maze.

b) 8 Arm Radial Maze.

c) Electronic weighing machine.

d) Syringe/ needle (1ml/ 24G).

Experimental animals used in the study

The study was carried out in healthy,male swiss albino mice weighing 26 – 32 gm. The animals were procured from Animal House, Gauhati Medical College.

Mice chaw diet and water *ad libitum* during the experiment except the animals used for 8 Arm Radial Maze. Animals were maintained under controlled condition with 12 hour light and 12 hour dark cycles at a temperature of 24 ± 1^0 C and humidity of 55 ± 5 %. All the animals were acclimatized to laboratory condition for 7 days before conducting the experiment.

The animals were allowed to acclimatize to the laboratory environment for 2 weeks and were provided water and food ad libitum.

The animals were then divided into five groups each containing six animals. The groups were:

GROUP I: NORMAL CONTROL GROUP: Received Normal Saline in the dose of 0.01 ml/gm/day intra peritoneally (i.p). Animals in this group received normal diet and water. No drugs or chemicals were administered to animals in this group.

GROUP II: IMPAIRED COGNITION AND MEMORY CONTROL: Received scopolamine hydrobromide 0.4 mg/kg, i.p to impair memory and cognition.

GROUP III: IMPAIRED COGNITION AND MEMORY STANDARD: Received Piracetam 200 mg/kg i.p and scopolamine hydrobromide 0.4 mg/kg i.p.

GROUP IV: COGNITION AND MEMORY ENHANCEMENT TEST DRUG (VENLAFAXINE):

Received Venlafaxine hydrochloride 16 mg/kg body weight and scopolamine hydrobromide 0.4 mg/kg, i.p.

Piracetam and venlafaxine were administered for a period of 14 days. During the training phase, from Day 11 to 14, scopolamine was administered in the groups II to IV after 45 minutes of administration of other drugs. Training was started 30 minutes after injection of scopolamine/normal saline. On test days, i.e. 15^{th} and 18^{th} day, only normal saline was given 30 minutes prior to the retention test.

Experimental design for evaluation of cognition and memory enhancing effect:

The morris water maze consists of a circular pool filled with water, 122cm in diameter and 51cm deep with non-reflective interior surfaces (the interior was

painted black). Water was filled upto 30cm. The maze was divided into four equal quadrants with the help of two threads fixed at right angles to each other on the rim of the pool. The quadrants were marked as quadrant (Q) 1, 2, 3 and 4. External cues in the form of a doorway, overhead light, overhead fan, poster in the walls were present. A platform (10 cm × 10 cm) was submerged 1 cm below the water surface. It was kept in the middle of the quadrant 4. The position of the platform was kept unaltered throughout the training session.

The experimental animals were given an acclimatization session two days before the training period started. During the acclimatization session each rat was allowed 30 s to swim around in the maze without the platform present.

During the training period, starting from day 11, the animal was placed in the desired start position in the maze, facing the tank wall. The animal was released into the water at water-level. The mice were allowed 120 seconds to locate the submerged platform. Then, it was allowed to stay on the platform for 20 seconds. If it failed to find the platform within 120 seconds, it was gently guided onto the platform and allowed to remain there for 20 seconds. Each animal was subjected to four consecutive training trials on each day starting from Day 11 with an inter trial gap of 5 minutes. The drop location was changed for each trial during the training days and the target quadrant (Q 4) remained constant throughout the training period. After each swimming session the animal was dried with towels and then put back into the home cage.

On the 15th day, 24 hrs after the last dose was administered, the platform was removed and each mouse was allowed to explore the pool for 120 seconds. The mean

time spent by the animal in the target quadrant searching for the hidden platform, over 4 trials, was noted.

After a gap of two days, on the 18th day, again each mouse was allowed to explore the pool for 120 seconds to test for long term memory. The time spent in the training quadrant was noted.

Care was taken that the relative location of the water maze with respect to other objects in the laboratory serving, as prominent visual clues, were not disturbed during the total duration of the study. All the trials were completed between 09.00 and 17.00 hrs.

The **8- arm radial maze** is composed of a central octagonal platform 20 cm in diameter with eight arms (35cm × 5cm) extending from it. Each arm has a 5-mm deep hole 1cm from the end, which was used as a food cup to avoid visibility of the food from the central platform. Doors were placed in each arm to separate the central platform from the arms. The maze was elevated 100 cm above the floor. The maze was made of wood and was placed in a room with various external cues in the form of a doorway, overhead light, overhead fan, poster in the walls that were visible to the mice while it was on the maze. Illumination was provided from above the maze in the form of a 6 watt bulb.

Mice were placed on the maze in pairs for 4 days before the start of the trial for acclimatization. Two mice were used together to reduce the time taken for acclimatization to the maze. Food rewards were spread around the maze to encourage

exploration. On subsequent days, food was placed only on the arms, then only at the ends of the arms. Acclimatization was done in four days.

Food rewards were placed at the end of only four arms of the radial arm maze before each test session. The same arms were baited for a particular mouse during each trial. The mice were then placed in the centre of the maze. When the mice entered an arm all other arms were closed. The mice were allowed to explore the entered arm and to eat the food placed at the end of the arm. When the mice returned to the centre all arms were closed. After 10 seconds all arms were opened again and the mice was allowed to explore the maze again. The trial ended after 10 minutes or if all baits have been found. To prevent odour cues, the maze was wiped clean with spirit between animals. Each mouse was given 5 trials each day for 4 days. Five mazes were used and a particular mouse used the same maze daily. On the 15^{th} and 17^{th} day retention test was done. The following data was recorded:-

- Number of correct entries into baited arms.
- Number of entries into unbaited arms.
- Number of re-entries into baited arms.

In all cases, a visit was only counted when all four paws entered a particular arm.

The statistical analysis was carried out using Graph pad prism 5.01 software. Data were expressed as mean ± SEM. Results were analyzed by one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. p value < 0.05 was considered as statistically significant.

RESULTS

TABLE 1: EFFECT OF VENLAFAXINE IN ESCAPE LATENCY AND TIME SPENT IN TARGET QUADRANT IN MICE (MEAN ± SEM) (in seconds)

GROUPS	**DAY 11**	**DAY 12**	**DAY 13**	**DAY 14**	**DAY 15**	**DAY 18**
GROUP I NORMAL CONTROL	**111.083± 2.338**	**102.250± 0.606**	**72.167± 1.284**	**53.958± 1.567**	**64.917± 1.390**	**61.708± 1.264**
GROUP II COGNITION CONTROL	**112.875± 1.700**	**111.708± 1.868[a]**	**104.292± 1.249[a]**	**101.625± 1.211[a]**	**34.000± 0.899[a]**	**31.792± 0.672[a]**
GROUP III COGNITIONS TANDARD	**107.083± 1.839**	**102.750± 0.973[b]**	**74.042± 0.888[b]**	**56.542± 1.621[b]**	**63.458± 0.797[b]**	**59.958± 1.257[b]**
GROUP IV VENLA-	**110.167± 1.678**	**105.250± 0.829[b]**	**76.125± 1.372[b]**	**58.292± 1.713[b]**	**60.208± 1.011[ab]**	**58.167± 1.214[b]**

F	1.037	10.07	107.8	155.2	147.2	119.2
df	4,25	4,25	4,25	4,25	4,25	4,25
P value	>0.05	< 0.0001	<0.0001	<0.0001	< 0.0001	<0.0001

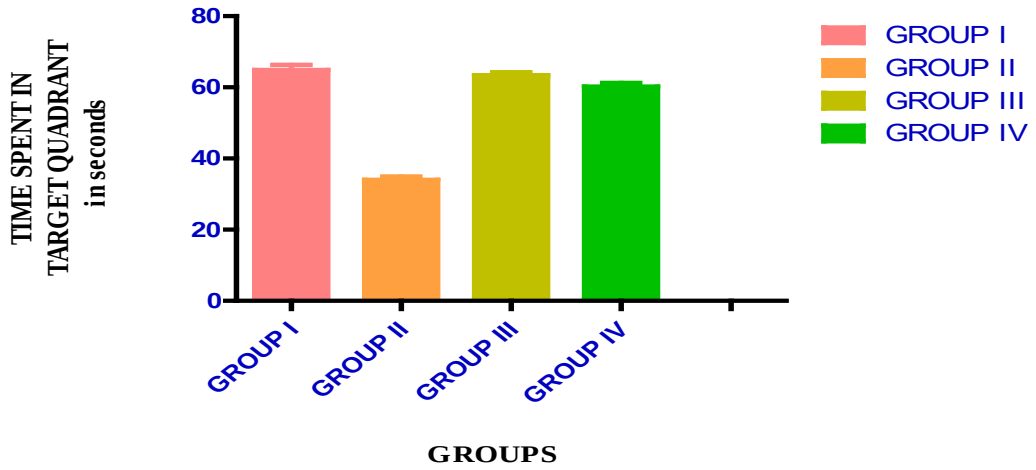
Values are expressed as Mean ± SEM (n=6);

One Way ANOVA followed by Tukey's multiple comparison test was done.

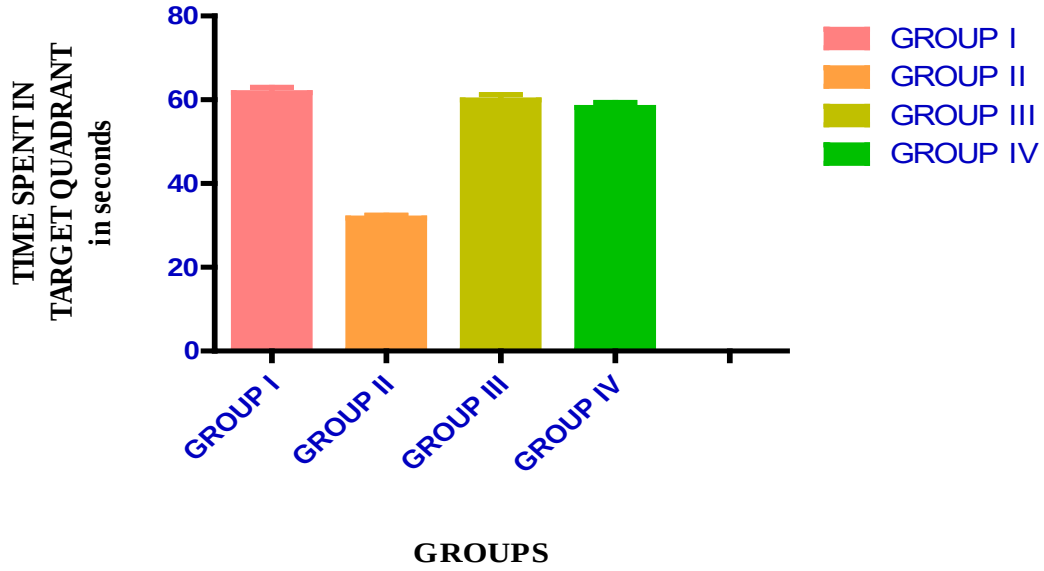
[a] p<0.05 when compared to the Normal control group.

[b] p<0.05 when compared to the Cognition control group.

GRAPH 1 TIME SPENT IN TARGET QUADRANT ON DAY 15



GRAPH 2 TIME SPENT IN TARGET QUADRANT ON DAY 18



On **Day 15 and 18**, there was a statistically significant difference (p <0.05) in the TSTQ in the venlafaxine group when the cognition control group was taken as

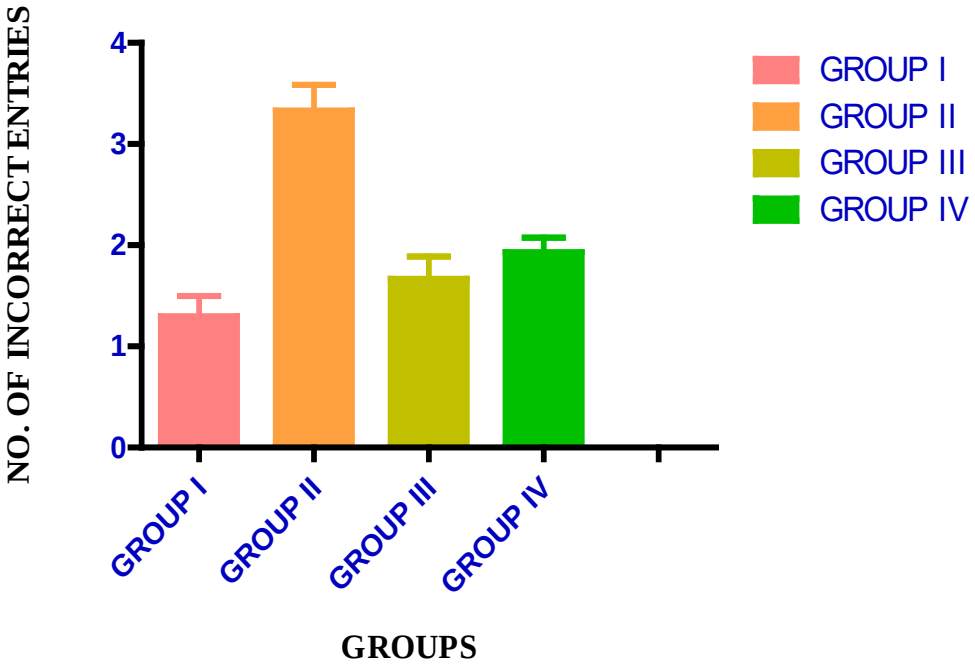
control column. The TSTQ in the piracetam group was more than venlafaxine group but this value was not significant.

TABLE 2: EFFECT OF VENLAFAXINE IN NUMBER OF CORRECT ENTRIES IN 8 ARM RADIAL MAZE IN MICE (MEAN ± SEM)

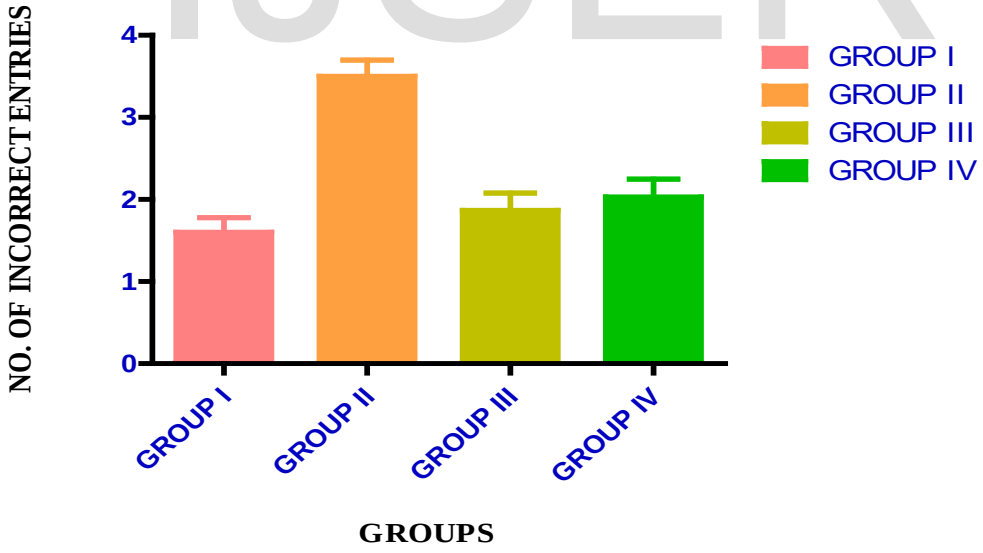
GROUPS	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	DAY 18
GROUP I NORMAL CONTROL	**1.500± 0.086**	**1.567± 0.095**	**2.500± 0.045**	**3.233± 0.095**	**3.700± 0.124**	**3.400± 0.115**
GROUP II COGNITION CONTROL	**1.500± 0.045**	**1.500± 0.112**	**1.500± 0.045[a]**	**1.500± 0.045[a]**	**1.400± 0.052[a]**	**1.367± 0.080[a]**
GROUP III COGNITION STANDARD	**1.400± 0.052**	**1.800± 0.052**	**2.467± 0.042[b]**	**2.800± 0.115[b]**	**3.500± 0.124[b]**	**3.233± 0.120[b]**
GROUP IV VENLA-FAXINE	**1.400± 0.089**	**1.700± 0.100**	**2.367± 0.108[b]**	**2.600± 0.171[ab]**	**3.333± 0.084[b]**	**3.000± 0.115[b]**
F	0.6119	1.521	36.39	22.60	76.35	36.10
df	4,25	4,25	4,25	4,25	4,25	4,25
P value	>0.05	>0.05	< 0.0001	<0.0001	< 0.0001	< 0.0001

There was a significant increase in the number of correct entries in the venlafaxine group as the training continued.

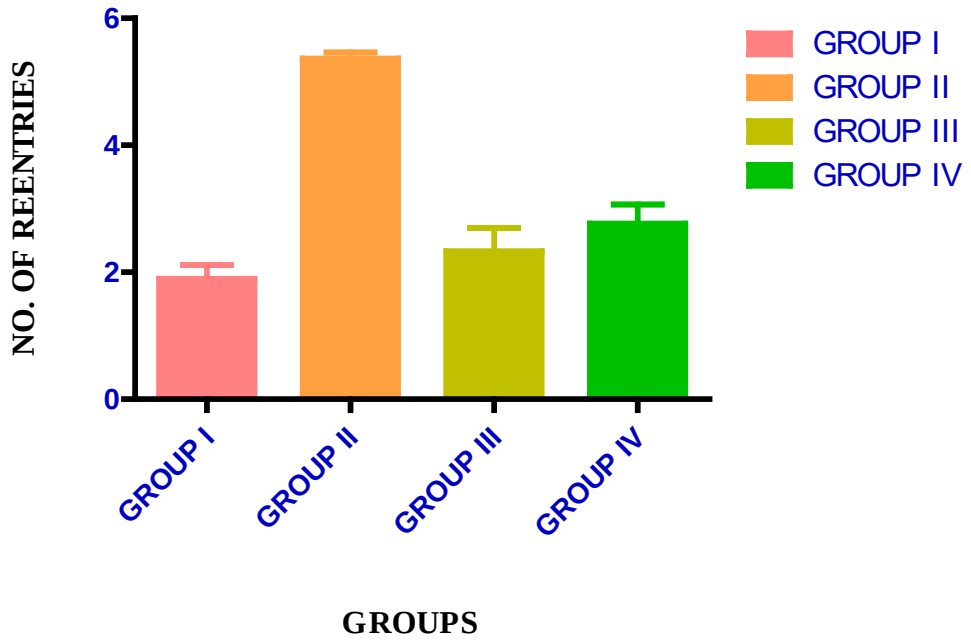
GRAPH 3 NO. OF INCORRECT ENTRIES IN 8ARM ON DAY 15



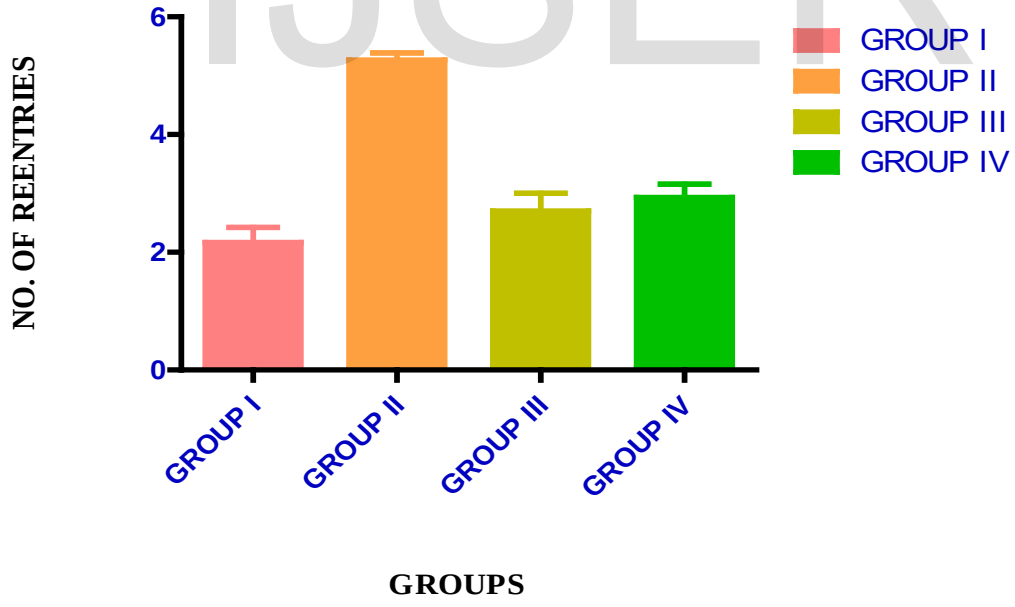
GRAPH 4 NO. OF INCORRECT ENTRIES IN 8ARM ON DAY 18



GRAPH 5 NO. OF REENTRIES IN 8ARM ON DAY 15



GRAPH 6 NO. OF REENTRIES IN 8ARM ON DAY 18



On **Day 15 and 18**, when the cognition control group was taken as control column, the venlafaxine group showed significant decrease in the number of incorrect and reentries.

DISCUSSION

The average human life expectancy has progressively prolonged over the last few decades, with a consequent rise in the prevalence of dementia and similar chronic degenerative disorders. Various studies have used different antidepressant doses of venlafaxine in animal studies. [8] [9] [10] The dose of venlafaxine taken in this study was 16 mg/kg. [11] [12]. Two mazes were used in the study namely Morris Water Maze and the 8- Arm Radial Maze. These mazes have been widely accepted as major spatial learning paradigms. The rationality behind choosing two mazes is that some of the demerits of 8- Arm Radial Maze are countered by Morris Water Maze and vice versa[13].

The results of the present study are consistent to the results of a study to evaluate the different antidepressants against 3-nitropropionic acid (3-NP)-induced cognitive impairment where it was found that venlafaxine treatments significantly improved performance in cognitive task in Morris Water Maze and elevated plus maze. [14]

Dai M *et al*. (2011) used different dosages of venlafaxine in post-stroke depression induced learning and memory dysfunction and tested the animals in Morris Water Maze. It was reported that venlafaxine improves cognition markedly. [15] This also support the results found in the present study.

Venlafaxine has been reported to cause improvement in cognition and memory in some clinical trials. [16] [17] Venlafaxine was also shown to improve cognition significantly after about 8weeks of treatment in Alzheimer patients. [18]

The positive effects of venlafaxine on cognition can be attributed to the fact that it increases the level of both serotonin and norepinephrine which plays an important role in the maintenance of cognition. However it is difficult to pinpoint the exact mechanism in cognition and memory as some other factors and mechanisms might be involved.

CONCLUSION

Dementia is a growing menace in today's world. The number of people affected worldwide is steadily increasing and so is the economic burden. Cognitive disturbances in dementia patients render them unable to perform day to day activities. In such cases Venlafaxine might offer some benefit as it has been shown to improve cognition. However, further elaborate studies are needed to evaluate the utility of these drugs in such cases.

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