

doi: <http://dx.doi.org/10.18203/2319-2003.ijbcp20150860>**Research Article****A comparative study of the effect of supplementing citicoline with fluoxetine and amitriptyline on learning and memory in albino rats****Gramle Amol^{1*}, Bhulan Prasad Loc², Y. S. Ramya³**

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Received: 08 July 2015**Accepted:** 22 July 2015***Correspondence to:**

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ABSTRACT

Background: The prevalence of psychosomatic complaints in children and adolescents has been reported to be between 10% and 25%. In addition, these problems present in a wide range of people across different age groups. Complaints of impaired learning and memory are common in patients treated with drugs acting on the central nervous system including the prototypical antidepressant drugs (AAD) such as fluoxetine and amitriptyline. Concomitant administration of the nootropic drug citicoline may help in the maintenance of cognition through the retention of memory and learning capacity.

Methods: Albino rats were used for this study as they are the most standardized of all experimental animals and divided into five groups of six rats each including the control group. The test apparatus used was the Morris water maze which is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory. The drugs used in the study were fluoxetine amitriptyline and citicoline. All the rats received respective treatment for the period of 20 days. The experiment was conducted during the last week. During this period, the rats were simultaneously trained and tested for 4 days for learning behavior (i.e. from 14th to 17th day of the study) designated as day 1, day 2, day 3, and day 4. After a gap of 2 days, i.e. on day 20, the rats were tested for the retention of memory on Morris water maze (designated as day 6).

Results: There was a statistically significant impairment in learning behavior of the rats in fluoxetine and amitriptyline group when compared to control group ($p < 0.01$ is highly significant) but no such significance was obtained when the groups containing the AADs was supplemented with citicoline. In addition, fluoxetine caused more impairment than amitriptyline and supplemental citicoline was beneficial in retaining the memory and preventing learning impairment, but the combination is more beneficial in the amitriptyline group as compared to the fluoxetine group.

Conclusions: Cognition in individuals with depression may be influenced by several factors, including basic neuropathology and the frequency and severity of depressive episodes. The major finding of the present study is that learning was impaired by both the antidepressants, i.e., fluoxetine and amitriptyline but was reversed by citicoline which has a novel mechanism of action.

Keywords: Antidepressants, Citicoline, Cognition**INTRODUCTION**

Psychosomatic disorders and depression are a worldwide problem. These problems present in a wide range of people across different age groups. The prevalence of psychosomatic complaints in children and adolescents has been reported to be between 10% and 25%.¹

Depression with anxiety is experienced by 9.7% of people in England, and depression without anxiety by 2.6%.²

Overall, depression occurs in one in 10 adults or 10% of the population.³ Around 1 in 20 people at any one time experience major or "clinical" depression.⁴

These symptoms are theorized to be a response to stress. Potential sources of stress in children, adolescents and adults include schoolwork, family problems, peer pressure, chronic disease or disability in parents, family moves, psychiatric disorder in parents and poor coping abilities. Complaints of impaired learning and memory

are common in patients treated with drugs acting on the central nervous system. Antidepressant drugs (ADDs) are commonly used in conditions such as psychosomatic illness, depression, and in depression due to dementia like Alzheimer's disease.

There have been reports of cases of impaired cognition in patients treated with fluoxetine and amitriptyline. Even in animal studies, such findings were reported in journals of repute. There are also conflicting reports on how selective serotonin reuptake inhibitors (SSRI) treatment affects performance in the Morris water maze, a typical model for spatial learning and memory.⁵ The fact that tricyclic antidepressants (TCAs) impair cognitive function has also been reported in some clinical trials.⁶ Learning and memory are most important part of cognition, so impairment of learning and memory function can also be stated in terms of impaired cognition. All commonly used ADDs have some effect on cognitive function. The effect may be substantial when crucial functions are involved. For example learning in children, driving ability in adults or when already vulnerable functions are involved, such as memory in elderly patients and especially those who suffer from Alzheimer's disease.

The commonly used ADD's belong to the class of TCAs, which include imipramine, clomipramine, amitriptyline, etc. and SSRIs such as fluoxetine, fluvoxamine, sertraline, and citalopram. Due to better tolerability and improved drug profile, SSRIs are more common used to treat depressive component in various psychiatric disorders. They are also better tolerated than TCAs especially in elderly age group. However, with the conflicting reports of impairment of cognitive functions, both the classes of drugs should be explored, systematically assessed, and studied for such adverse effects if any. The study comparing the effect between the two classes of drugs will be of particular importance since it will reveal the relative impairment caused among the drugs, so that it will be helpful in choosing the better drug for the treatment.

Citicoline, also known as cytidine diphosphate choline (CDP-choline) and cytidine 5'-diphosphocholine is a psychostimulant/nootropic. It is an intermediate in the generation of phosphatidylcholine from choline. Studies suggest that CDP-choline supplements increase dopamine receptor densities,⁷ and suggest that CDP-choline supplementation helps prevent memory impairment resulting from poor environmental conditions.⁸

Aims and objectives

1. To study the effect on learning and memory of two drugs, i.e., fluoxetine and amitriptyline
2. To compare the effect on learning and memory of antidepressant drugs, i.e., fluoxetine and amitriptyline by supplementing it with the nootropic drug, i.e. citicoline.

METHODS

This study has been done in the Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences (RIMS), Ranchi. Approval of the Institutional Animal Ethics Committee, RIMS, Ranchi was taken prior to the study.

Albino rats were used for this study as they are the most standardized of all experimental animals. They are particularly suitable for psychopharmacological study because they can be trained properly for various types of performances including the development of conditioned reflex.⁹ 30 healthy albino rats of about 150-200 g in weight were selected and were randomly divided into five groups of six animals each. Standard laboratory conditions of temperature, humidity, and feeding were maintained. In addition, they were also maintained on the natural day and night cycle. Food and water were allocated *ad-libitum*.

The drugs administered were citicoline: capsule Somazina® 100 mg from Elderr Pharmaceuticals, fluoxetine: capsule Flood® 10 mg from K.C Laboratories, Ankleshwar, Gujarat, India and amitriptyline: tablet Triwin® 10 mg from Life Care Neuro Products Ltd., India. All the drugs were procured from the local market.

Separate suspensions of each drug were prepared by mixing the drug in normal saline with 1% gum acacia. Drugs such as fluoxetine and amitriptyline were made to strength of 1 mg/ml by mixing 10 mg of the respective drugs with 10 ml of normal saline suspension of gum acacia. Citicoline was made to strength of 10 mg/ml by mixing 100 mg of the drug with 10 ml of normal saline. Only freshly prepared drugs were used each day.

All the drugs were given orally with a bent stainless steel feeding needle specially made for rats (oral gavage tube). The lumen size of the feeding tube was 18 gauges.

All the rats received respective treatment for the period of 20 days. The experiment was conducted during the last week. During this period, the rats were simultaneously trained and tested for 4 days for learning behavior (i.e., from 14th to 17th day of the study) designated as day 1, day 2, day 3, and day 4. After a gap of 2 days, i.e., on day 20, the rats were tested for the retention of memory on Morris water maze (designated as day 6).

The test apparatus used was the Morris water maze which is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory. It has also gained a position at the very core of contemporary neuroscience research.¹⁰

Rats were placed in the water at a designated starting location and the time to find the hidden platform from the starting point was defined as "Escape Latency."

Each rat was tested for four trials per day with intertrial period of 2 mins during which they were placed in their home cage.

Selection criteria: The rats for water maze are preselected. Rats that did not go to the visible platform on training and testing trials in the allotted time of 120 sec were guided to the platform through a probe for the study. Rats that could not search for the hidden platform during training and those that float on the water were removed from the study.

Statistical analysis

Data entry was done on MS Excel and “SPSS version 17” software was used for data analysis. One-way ANOVA test was used to compare the effect of the drugs on different groups. Tukey’s honestly significant difference test was used for *post hoc* analysis of significant overall differences.

RESULTS

There was a statistically significant impairment in learning behavior of the rats in fluoxetine and amitriptyline group when compared to control group ($p < 0.01$ is highly significant) but no such significance was obtained when the groups containing the antidepressant drugs was supplemented with citicoline as given in Table 1.

The overall performance of the albino rats is provided in Figure 1.

Tables 2 and 3 depict the mean of the escape latency time (ELT) in seconds and the standard deviation for learning and memory, respectively.

It can be deduced from all the Tables 1-3 that both fluoxetine and amitriptyline causes impairment in learning and memory. However, fluoxetine causes more impairment than amitriptyline and supplemental citicoline is beneficial in retaining back the memory and preventing learning impairment, but the combination is more beneficial in the amitriptyline group as compared to the fluoxetine group.

DISCUSSION

Among the two antidepressants used, the group fluoxetine caused more statistically significant disorder in learning

and memory. Fluoxetine is one of the most commonly used antidepressant. However, as per the study it causes impairment in learning and memory specially when given for shorter intervals. It thus shows relevance with the findings of the case study article published in the Annals of Pharmacotherapy (Joss JD, Burton RM, Keller CA. 2003;37(12)). This finding may prove to be a pointer in keeping the cognitive deficiencies in mind before prescribing it for patients who are already suffering from diseases like Alzheimer’s or in children suffering from psychosomatic disorders and depression where cognitive decline may prove detrimental. Amitriptyline may prove to be a safer alternative to fluoxetine in this subset of patients as the impairment is relatively less as per my study.

Determining the effects of antidepressant drugs on cognitive function in diseased subjects, both human and laboratory animals, permits assessment of the effects of these drugs on cognition without the added complexities of the disease. Cognition in individuals with depression may be influenced by several factors, including basic neuropathology and the frequency and severity of depressive episodes. The present study compared the effects of the two commonly used antidepressant drugs on learning in normal rats. The major finding of the present study is that learning was impaired by both the antidepressants, i.e., fluoxetine and amitriptyline but was reversed by citicoline, which has a novel mechanism of action. The effect of these drugs on memory also became clear from the present study as both the drugs has shown statistically significant impairment in memory which was reversed by supplementing it with citicoline and recording the ELT on Morris water maze. It is also clear from the study that fluoxetine causes more significant impairment in learning and memory as compared to the amitriptyline. Furthermore, supplemental citicoline is more beneficial for amitriptyline than fluoxetine.

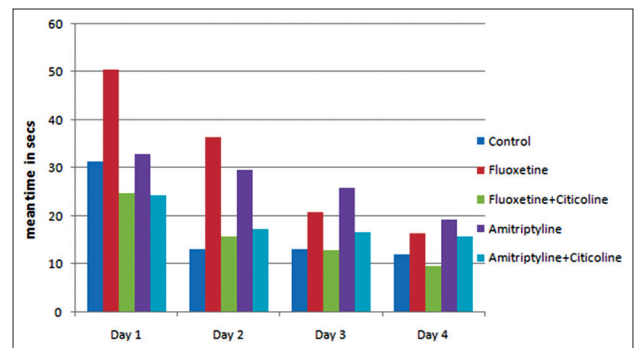


Figure 1: Overall performance.

Table 1: Impairment in learning behavior.

Group (I)	Group (J)	Mean difference (I-J)	Standard error	Significance
Control	Fluoxetine	-13.208	2.912	0.000*
Control	Fluoxetine+citicoline	1.567	2.912	0.983
Control	Amitriptyline	-9.300	2.912	0.015*
Control	Amitriptyline+citicoline	-0.833	2.912	0.999

* $p < 0.01$ is highly significant and $p < 0.05$ is significant

Table 2: Escape latency time.

Group	Mean ELT in sec±SD			
	Day 1	Day 2	Day 3	Day 4
Control	31.33±11.27	13.25±5.99	13.08±6.08	12±2.08
Fluoxetine	50.58±19.44 ^{a**}	36.38±21.10 ^{a**}	20.92±9.54 ^{a**}	16.42±5.20 ^{a**}
Fluoxetine+citicoline	24.91±9.71 ^{b**,d*}	15.75±6.53 ^{b**,d*}	12.87±5.77 ^{b**,d*}	9.66±1.33 ^{b**,d*}
Amitriptyline	32.87±9.17 ^{a*,c*,e*}	29.66±10.21 ^{a*,c*,e*}	25.91±10.47 ^{a*,c*,e*}	19.45±5.58 ^{a*,c*,e*}
Amitriptyline+citicoline	24.41±9.50 ^{a*,c*,d*}	17.29±6.17 ^{a*,c*,d*}	16.7±5.51 ^{a*,c*,d*}	15.75±4.59 ^{a*,c*,d*}

^aVersus control, ^bversus fluoxetine, ^cversus fluoxetine plus citicoline and ^dversus amitriptyline. **p<0.01 and *p<0.05. ELT: Escape latency time, SD: Standard deviation

Table 3: Escape latency time.

Group	Mean ELT in sec±SD	
	Day 4	Day 6
Control	12±2.08	11.08±2.35
Fluoxetine	16.42±5.20 ^{a*}	22.5±5.40 ^{a*}
Fluoxetine+citicoline	9.66±1.33 ^{b**,d*}	8.58±0.81 ^{b**,d*}
Amitriptyline	19.45±5.58 ^{a*,c*,e*}	22.41±5.94 ^{a*,c*,e*}
Amitriptyline+citicoline	15.75±4.59 ^{a*,c*,d*}	10.75±2.28 ^{a*,c*,d*}

^aVersus control, ^bversus fluoxetine, ^cversus fluoxetine plus citicoline and ^dversus amitriptyline. **p<0.01 and *p<0.05. ELT: Escape latency time, SD: Standard deviation

In brief, it can be suggested that though the conclusion mentioned as above are in partial correlation of the cases reported of antidepressant-induced cognitive decline, further research in this line is needed on a large number of subjects both animal and human to stamp the effect of these drugs on learning and memory.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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Cite this article as: Amol G, Loc BP, Ramya YS.

A comparative study of the effect of supplementing citicoline with fluoxetine and amitriptyline on learning and memory in albino rats. Int J Basic Clin Pharmacol 2015;4:884-7.