An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

INVESTIGATION ON MEMORY ENHANCING ACTIVITY OF DAPOXETINE HYDROCHLORIDE SEMI SYNTHETIC DRUG ON EXPERIMENTAL RATS.

Rahul Kumar*, Vrish Dhwaj Ashwlayan

Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, NH-58, Baghpat Crossing Bypass Road, Delhi Roorki Highway, Meerut (U.P.) 250005.

*Corresponding author E-mail: **krrahul7875@gmail.com**

ARTICLE INFO

Key Words

Dapoxetine hydrochloride, AChE, Piracetam, Memory enhancing activity etc.

Revised date - 04/07/2019
Acceptance date - 12/07/2019
Published date - 16/07/2019

ABSTRACT

The present study was designed for the investigation on memory enhancing activity of Dapoxetine hydrochloride a selective serotonin reuptake inhibitor against Scopolamine induced amnesia in rats. Piracetam was used as standard drug with dose of 400mg/kg, p.o and Scopolamine was used as inducible drug to induce amnesia in rats at the dose of 0.3mg/kg, i.p.The solution of Dapoxetine hydrochloride was prepared by mixing the suitable amount of drug in water to make the dose 10mg/ml for oral administration. Dapoxetine hydrochloride was studied to investigate memory enhancing activity by using Scopolamine induced amnesia as interceptive model and Morris water maze (MWM) test and Elevated plus maze (EPM) test as exteroceptive models. Two doses were selected for oral administration of Dapoxetine hydrochloride, 15mg/kg and 30mg/kg for this study. No effect of memory enhancement was recorded for test drug by MWM & EPM tests because Dapoxetine hydrochloride treated rats manifested increase in escape latency time (ELT) and decrease in time spent in target quadrant (TSTQ) in MWM test and increase in transfer latency time (TLT) through EPM test. So it was concluded that Dapoxetine hydrochloride does not possess memory enhancing effect against scopolamine induced amnesia using both models.Biochemical estimations such as AChE activity and MDA level were also examined and a non-significant increment in Acetylcholinesterase(AChE) and malondialdehyde (MDA) level was found which ultimately confirmed that Dapoxetine hydrochloride does not have memory enhancing property. The present study signalized that Dapoxetinehydrochloride does not possess memory enhancing activity.

INTRODUCTION

Memory is defined as the ability to use the past in the service of the present". It is also defined as the ability to store, encode and retrieve the past experienced information **[1]**. Alzheimer's disease (AD) is ahead invariant neurodegenerative disorder that was first distinguished and composed by Dr. Alois Alzheimer in 1907. It is generally characterized by disorientation and also results in subjective impedance, irregular conduct, character changes, an eventually demise. These days, it is the fourth driving reason for death in western nations, went before just

by coronary illness, malignant growth and stroke. Pathologically, in Alzheimer's disease beta-amyloid plaque deposits in blood vessels of cerebral cortex and neurofibrillary tangles are deposited in neurons of mesial temporal lobe of hippocampus which ultimately leads to synaptic dysfunction and neuronal degeneration. An estimated around 5.5 million peoples are affected by AD in U.S., 4.1 million in India and 50 million throughout the world by the age of 60. Studies were estimated that AD reached 30% to 50% by the age of 85 and also confirmed that women are more affected by AD than men because the population of women is large. In every 3.2 seconds a new case of dementia are registered **[2]**. The most useful category of drug has been used for the improvement of memory is Acetylcholine esterase inhibitors which show their action by blocking of an enzyme responsible for degradation of acetylcholine the main neurotransmitter responsible for memory enhancement **[3]**. Anticholinergic drugs can be used to produce amnesia in experimental animals, some common drugs are Scopolamine, Benztropine, Homotropine (anticholinergic), diazepam (sedative) etc. **[4]**. These effects can be antagonized by some drugs such as nootropics (Piracetam) and acetylcholine esterase inhibitors (Donepezil, Galantamine). Fluvoxamine maleate, Duloxetine, Sertraline, SSRI drugs are also helpful in memory improvement of rodents and Human beings. Dapoxetine hydrochloride is a SSRI which is commonly utilized for the treatment of premature ejaculation however as other SSRI it tends to be additionally utilized for memory upgrade on the grounds that a considerable lot of the medications from this classification has uncovered the property of memory improvement **[5, 6]**.

MATERIAL AND METHOD

Experimental animals: Male Wistar rats weighing from 150-200 g were used in the investigation. They were housed in separated groups of six for each and kept up under normal light and dim cycle and standard research centre conditions. They were adjusted for seven days before the investigation in the institutional animal house. The rats were sustained with standard eating routine. All the test techniques were completed as per the CPCSEA guidelines.

Drug and chemical

Dapoxetine hydrochloride (15mg/kg and 30mg/kg p.o) was bought from Tokyo chemical industry, Tokyo, Japan and Piracetam (400mg/kg p.o) and Scopolamine (0.3mg/kg i.p) were acquired from Sanofi pharmaceuticals New Delhi India. The chemicals for biochemical estimation and preparation of phosphate buffer like Na_2HPO_4, $Na_2HPO_4.2H_2O$, HCl, NaOH, DTNB, acetylcholine iodide, TCA and TBA were procured from Sigma Aldrich, Delhi.

Behavioural Animal Models

Exteroceptivebehavioural models

Morris water maze test

Morris water maze test apparatus consists of a circular pool filled with water (25±1 ^{0}C) from bottom to half of total height (approx. 30 cm). The pool was divided into four quadrants Q1, Q2, Q3, and Q4 with the help of thread or tapping. The water was made opaque by adding white water colour dye in sufficient quantity. In this pool the opaque water has a hidden platform (10 cm) in quadrant Q4 used by rats to escape from water **[7]**.

Acquisition Trials: In acquisition trial after 1 hour from drug administration each rat was allowed in water for 120 sec to find out the hidden platform from each of quadrant and escape latency time (ELT) the time taken by rats to find out the hidden platform was noted. When a rat was unable to find hidden platform within 120 sec then the ELT was noted to be 120

sec. After each trial rats were stayed at hidden platform for approx. 30 sec to memorize the location. Each day of acquisition trials rats were put in water from different quadrants.

Retrieval Trial:

At the day of retrieval trial the hidden platform was removed and after 1 hour of dosing each rat was allowed into pool for 120 sec from each quadrant and the time spent in Q4 target quadrant (TSTQ) within 120 sec was noted.

Elevated plus maze test

The elevated plus maze apparatus consists of two open arms (25×5 cm) and two enclosed arms of the same size , with 15 cm high opaque walls. The maze elevated to a height of 55 cm above the floor. In this test on 1st day rats were allowed on the edge of an open arm for 90 sec and TLT 'Time taken by the rat to enter an enclosed arm" was noted, same procedure was applied on 5th day and TLT was noted. If any rat does not enter an enclosed arm then the ELT was noted to be 90 sec**[8]**.

Interceptive behavioural model

Scopolamine induced Amnesia in Rat

Scopolamine methyl bromide (0.3mg/kg, i.p.) was administered to induce amnesia. Scopolamine and related drugs compete with ACh for common binding site on the muscarinic receptors and act as competitive antagonists. The antagonism is reversible and therefore the blockade by smaller doses of Scopolamine can be overcome by the larger concentration of cholinergic agonists or acetylcholinesterase inhibitors, so the decreased in concentration of ACh in brain produced amnesia in experimental animals by its antagonistic effect **[9]**.

Experimental protocol

Rats were divided into five groups and each group contains six rats (n = 6).

Table No. 1: Experimental protocol

S.No	Groups	Treatment	Dose
1	Control group	Normal saline 0.9% Na Cl	10ml/ kg, i.p.
2	Negative control	Scopolamine	0.3m g/kg i.p
3	Standard	Piracetam + Scopolamine	400m g/kg p.o
4	Test drug at low dose	Dapoxetine hydrochloride + scopolamine	15mg /kg p.o
5	Test drug at high dose	Dapoxetine hydrochloride + scopolamine	30mg /kg p.o

Group 1: Rats were administered normal saline (0.9% NaCl, 10 ml/kg, i.p.) for four consecutive days, 30 min prior to consecutive trials conducted on day 1 to day 4 and during retrieval trialon 5th day.

Group 2: Rats were administered scopolamine (0.3mg/kg, i.p) for four consecutive days, 30 min prior to consecutive trials conducted on day 1 to day 4 and normal saline (10ml/kg, i.p) 1 hour before retrieval trialon 5th day.

Group 3: Rats were administered Piracetam 400mg/kg, p.o and Scopolamine (0.3mg/kg, i.p) for four consecutive days, 1 hour before acquisition trials conducted on day 1 to day 4 and normal saline (0.9% NaCl, 10ml/kg, i.p.), 1 hour before retrieval trialon 5th day.

Group 4: Rats were administered Dapoxetine hydrochloride (15mg/kg, p.o) and Scopolamine (0.3mg/kg, i.p) for four consecutive days, 1 hour before acquisition trials conducted on day 1 to day 4 and normal saline (0.9% NaCl, 10ml/kg, i.p.) 1 hour before retrieval trialon 5th day.

Group 5: Rats were administered Dapoxetine hydrochloride (30mg/kg, p.o) and Scopolamine (0.3mg/kg, i.p) for four

consecutive days, 1 hour before acquisition trials conducted on day 1 to day 4 and normal saline (0.9% NaCl, 10ml/kg, i.p.) 1 hour before retrieval trialon 5th day.

Estimation of biochemical parameters

Preparation of phosphate buffer solution: 11 parts of 28.39 g of Na_2HPO_4 and 39 parts of 3.12 g of $Na_2HPO_4.2H_2O$ were mixed in 1 L distilled water. The pH (7.5) was adjusted with the help of HCl and NaOH.

Brain Homogenization

Brain was homogenized with 10% w/v, 0.1 M phosphate buffer solution (PBS) and sufficient amount of homogenate was taken and centrifuged at 10000 rpm for 10 min and the upper layer was taken for enzymatic activity and the remaining part was used for protein estimation **[10]**. Estimation of all biochemical parameters were carried out with the help of given scientific methods.

Acetylcholinesterase activity

Procedure: For Acetylcholinesterase activity test 0.1 ml of brain homogenate was taken and 2.7 ml PBS and 0.1 ml DTNB was added to them and hatched for 55 min at environmental temperature. Then freshly prepared acetyl thio choline 0.1 ml, pH 8 was added and the Abs. was observed at 422 nm for 3 min at 30 sec interval **[11]**.

Preparation of Reagent

Preparation of 5, 5'- Dithiobis (DTNB): 10 mg of DTNB was dissolved in 100 ml of Sorenson phosphate buffer.

Preparation of Acetylcholine Iodide Solution: 65 mg of Acetyl choline were disorganized in 10 ml phosphate buffer o.1 M and freeze. AChE activity was deliberated with the help of under mentioned Sutra-

$$R= (d\ O.D.XV) / (EXP)$$

Where,

R = rate of enzyme activity in 'n' mole of acetyl thio choline iodide hydrolyzed/min/mg protein.

d.O.D. = alteration in Abs. / minute

V= Volume of assay

Ex = Extinction coefficient = (13600/M/cm)

P= Protein content (mg)

Malondialdehyde (MDA) level: The supernatant layer of homogenate (1 ml) was taken. 0.5ml of 30% TCA (Trichloro acetic acid) & 0.3 ml of 0.8% TBA (Thiobarbituric acid) were added. Total solution was heated on water bath for 45 min at 80 ^{0}c. Then the solution was cooled at environment temperature for 30 min. The colour of solution was extracted with 1 ml butanol and measure the absorbance at 532 nm **[12]**.

Preparation of Reagents

Preparation of 10% Trichloro acetic Acid (TCA): 10 g of trichloro acetic + 100 ml of distilled water.

Preparation of TBA: 57.99 mg of TBA + 100 ml of glacial acetic acid.

Statistical analysis: All the results were expressed as mean ± (SEM) standard error of mean. The data were analysed using one way analysis of Variance (ANOVA) followed by Tukey's multiple comparison using graph- pad or post- hoc test. The $p<0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Impact of Normal Saline on ELT during Acquisition Trials: Rats were administered 0.9% Na Cl i.e. Normal saline (10ml/Kg, i.p.) 30 min before conducting acquisition trials from day 1 to day 4. Rats demonstrated decrease in ELT in comparison of first day of control group. Control group indicates normal learning.

Impact of Normal Saline on TSTQ during Retrieval Trial: Rats were administered normal saline (10ml/kg, i.p.) 1 hour prior to retrieval trial conducted on 5th day, spent significantly more time in target quadrant (Q4) to search missing platform in comparison of time spent in other quadrants (Q1, Q2, and Q3).

Impact of Scopolamine on ELT during Acquisition Trials: Rats were administered Scopolamine (0.3mg/kg, i.p.) 30 min prior to acquisition trials convened from day 1 to day 4. Amnesic agent significantly increased the ELT during successive acquisition trials.

Impact of Scopolamine on TSTQ during Retrieval Trial: In group II, normal saline (10ml/kg, i.p.) was administered 1 hour prior to retrieval trial convened on 5th day. Amnesic agent reduced the TSTQ (Q4) markedly in search of missing platform during the retrieval trial.

Impact of Piracetam on Scopolamine Induced Amnesia: Pre-treatment of rats with Piracetam (400mg/kg, p.o.) 60 min before acquisition trials for four days significantly decreased the ELT was conducted from day 1 to day 4 and significantly increased TSTQ. Piracetam, standard drug significantly improved the retrieval component of Scopolamine induced amnesia.

Effect of Dapoxetine hydrochloride on Acquisition Trials: Increased in ELT was noted during acquisition trials in Dapoxetine hydrochloride treated group as compared to negative group. Piracetam manifested protection against Scopolamine induced amnesia**[fig. 1]**.

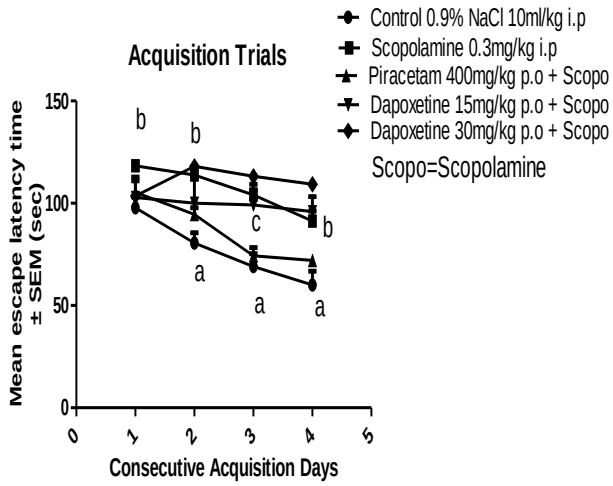

Fig. 1 Effect Dapoxetine hydrochloride on ELT during Acquisition Trials

a = p≤0.05 Vs ELT on day 1 in control group, b = p≤0.05 Vs ELT on respective days of control group and c = p≤0.05 Vs ELT on respective days of negative group.

Effect of Dapoxetine hydrochloride on TSTQ during retrieval trial: On retrieval trial scopolamine decreased the TSTQ in comparison of control group and Piracetam showed protection against Scopolamine but Dapoxetine hydrochloride in both doses 15mg/kg and 30mg/kg demonstrated insignificant reduction in TSTQ in comparison of negative group and showed no protection against scopolamine induced difference **[fig. 2]**.

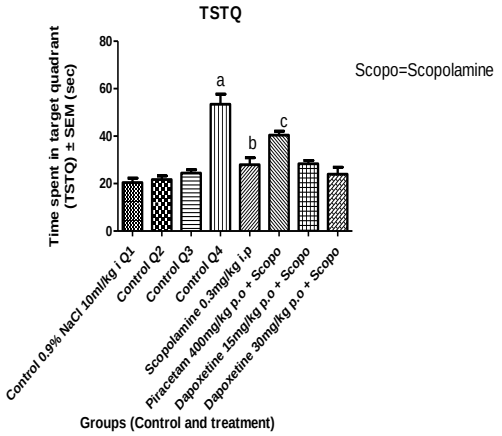

Fig. 2 Effect of Dapoxetine hydrochloride on TSTQ in Retrieval Trial.

Dapoxetine hydrochloride treated group showed an unremarkable alleviation in TSTQ as compared to amnesic group. a = p≤ 0.05 Vs time spent in other quadrants (Q1, Q2 and Q3) in control group; b = p≤ 0.05 Vs TSTQ in control group and c = p≤0.05 Vs TSTQ in scopolamine treated group.

Effect of Dapoxetine hydrochloride on TLT: The effect of Dapoxetine hydrochloride at the dose of 15 and 30 mg/kg were observed against Scopolamine using EPM test. TLT was noted and Dapoxetine hydrochloride was found to have no significant effect on TLT**[fig.3]**.

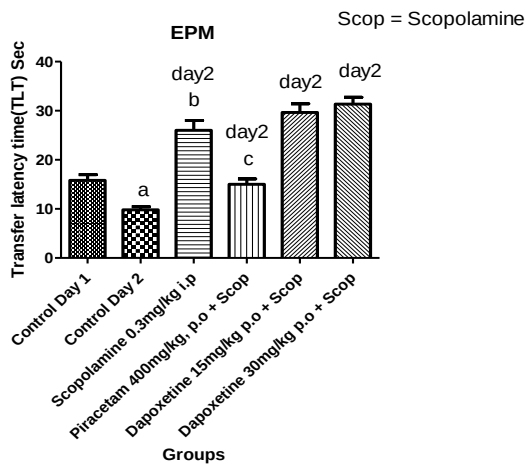

Fig. 3 Effect of Dapoxetine hydrochloride on TLT

Scopolamine treated group showed notably increment in TLT and Piracetam treated group showed notably decrement in TLT in EPM while Dapoxetine treated group demonstrated an unremarkable increment in TLT. a = p≤0.05 Vs TLT in day 1 of control group, b = p≤0.05 Vs TLT in day 2 of control group and c = p≤0.05 Vs TLT in day 2 of amnesic group.

Biochemical estimation

Effect of Dapoxetine hydrochloride on AChE level: The effect of Dapoxetine hydrochloride showed non-significant increment in AChE level while Piracetam + Scopolamine treated group notably decreased AChE activity. Negative group (Scopolamine 0.3mg/kg) significantly increased the level of AChE**[fig.4]**.

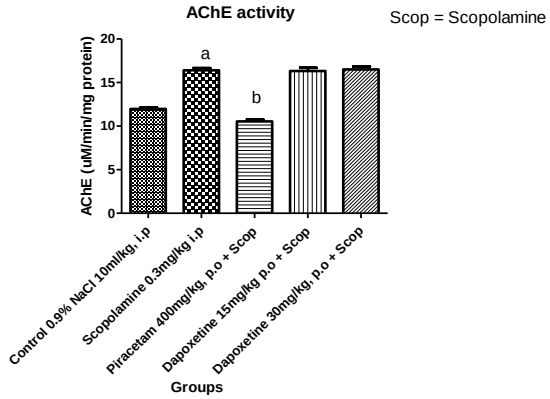

Fig. 4 Effect of Dapoxetine hydrochloride on AChE level

Dapoxetine hydrochloride treated group insignificantly increased the AChE level compared to amnesic group. a = p≤0.05 Vs AChE level in control group; b = p≤0.05 Vs AChE level in amnesic group.

Effect of Dapoxetine hydrochloride on MDA level: It was demonstrated that while negative group significantly increased the level of MDA as compared to control group and Piracetam treated group notably decreased the MDA level as compared to negative group, Dapoxetine hydrochloride treated group showed non-significant increment in MDA level**[fig.5]**.

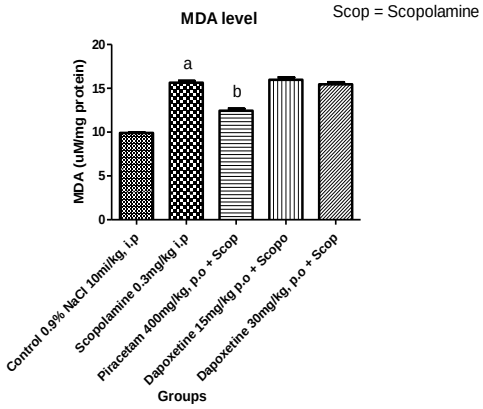

Fig. 5 Effect of Dapoxetine hydrochloride on MDA level

Dapoxetine hydrochloride treated both groups showed nonsignificant increment which ultimately displayed that Dapoxetine had no effect on memory. a = p≤ 0.05 Vs MDA in control group; b = p≤0.05 Vs MDA in amnesic group.

DISCUSSION

All the smart drugs are related to the category of psychotic drugs with specific site of action on learning and memory. Piracetam was used as Standard medicine to reverse the memory deficit by scopolamine. Piracetam is a nootropic drug commonly used as smart drug to treat amnesia and also used for the treatment of AD. Scopolamine induced amnesia test was used as an interoceptive model to induce amnesia in rats and Morris water

maze test and Elevated plus maze test were used as exteroceptive model. In MWM test's acquisition trials animal learned to find hidden plate form to escape from water (ELT) and in Retrieval trial on 5th day the highest TSTQ (Q4) shows the retrieval of memory. Scopolamine produced impairments of memory in both acquisition trials and retrieval trial by increasing the ELT and decreasing the TSTQ. The second exteroceptive model was elevated plus maze model in which the TLT was noted on first day and after 24 Hrs. The most useful category of drug has been used for the improvement of memory is Acetylcholine esterase inhibitors which show there action by blocking of an enzyme responsible for degradation of acetylcholine the main neurotransmitter responsible for memory enhancement. Anticholinergic drugs can be used to produce amnesia in experimental animals, some common drugs are Scopolamine, Benztropine, Homotropine (anticholinergic), diazepam (sedative) etc. These effects can be antagonized by some drugs such as acetylcholine esterase inhibitors (Donepezil, Galantamine). Fluvoxamine maleate, Duloxetine, Sertraline, SSRI drugs are also helpful in memory improvement of rodents and Human beings **[5, 6]**. Dapoxetine hydrochloride, a SSRI is commonly used in the treatment of premature ejaculation.

Dapoxetine hydrochloride unremarkably increased the ELT in acquisition trials and decreased the TSTQ in retrieval trial and also increased TLT as compared to scopolamine treated group. The rise in TLT & ELT was demonstrated and proved that Dapoxetine hydrochloride does not have any nootropic property.

CONCLUSION

Memory enhancing effect of Dapoxetine hydrochloride was investigated against scopolamine induced amnesia in rats applying MWM test and EPM tests. Dapoxetine hydrochloride was given at the dose of 15 and 30 mg/kg p.o. Scopolamine was given at the dose of 0.3 mg/kgi.p before test drug to induce amnesia. Increase in ELT during acquisition trials and decreasein TSTQ in retrieval trial show that Dapoxetine hydrochloride does not have memory enhancing property. Further examination of TLT by EPM test revealed that it had no effect on TLT.So both the screening models clarified that it does not have any kind of memory enhancing activity. The *in-vivo* study of Dapoxetine hydrochloride was further examined by AChE level and Malondialdehyde biochemical estimations. Both the estimations also confirmed that the test drug could not be used for memory improvement. On the roots of presented result it was educed that Dapoxetine hydrochloride had no effect on memory.

ACKNOWLEDGEMENT

The authors are Thankful to **Prof. Dr. Satish Kumar Gupta** Director, Department of pharmaceutical technology, M.I.E.T Meerut 250005 (U.P.) India.

REFERENCES:

1. The human memory at http://www.human-memory.net/sources.html
2. Wint D, Tavee J, Sweeney P. Alzheimer's Disease (2014).
3. Sharma HL, Sharma KK principle of Pharmacology 2nd edition, published by Divyesh Arvind Kothari, pp- 128-155, (2011).
4. Yashashwini Y C, Evaluation of learning and memory enhancing activity of *COCCINIA GRANDIS* fruit in rats, Journal of drug delivery and therapeutics (2018)
5. Johnson L. The use of Duloxetine for cognitive improvement in individual with mild cognitive impairment 2018

6. Falls N, Mahadevan N, Maratha S. Memory enhancing activity of Fluvoxamine maleate 2013.
7. Morris, R.G.M., Developments of water maze producer of studying spatial learning in the rats. J. Neurosci Meth, Issue, 11.pp. 47-60, 1984.
8. Kulkarni SK. Handbook of Experimental Pharmacology India: ValaabhPrakashan 1999.
9. Khatri R.S., Ashawlayan V.D., Sharma D., "Nootropic Activity of AjugaBracteosa Wall on Scopolamine Induced Memory Deficits in Swiss Albino Mice" June 2018
10. Kanval A. et al Anti-Amnesic activity of VitexNegundo in Scopolamine Induced Amnesia in rats, 2010.
11. Ellman, D. K. Courtney, V. Andres and R. M. Featherstone, "A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity," Biochemical Pharmacology, Vol.7, No. 2, pp. 88-95, (1961).
12. H. Ohkawa, N. Ohishi, K. Yagi, "Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction," Analytical Biochemistry, Vol. 95, No. 2, 1979, pp. 351- 358.
13. http://www.reboundhealth.com/cms/images/pdf/Textbooks/piracetam%20id%2017975.pdf.
14. Tortora j. Gerard, Derrickson Bryan a text book of Principle of anatomy and physiology, 12th edition, published by John Wiley and Sons Inc 2009.