ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 5, Issue, 07, pp. 5083-5090, July, 2015

Full Length Research Article

EVALUATION OF NOOTROPIC ACTIVITY OF NEWLY SYNTHESIZED N14 & N15 PHENOTHIAZINE DERIVATIVES

[1]Yuvraj Singh Surana, [1]Purnima, A, [1]Raunak Srivastava, [1]Nilesh Yadav, [2]Ramesh, P and [*2]Shiva Shanker Rai

[1]Department of Pharmacology, KLE University's College of Pharmacy, Bangalore-560010, Karnataka, India
[2]Drug safety associate, Bangalore-560050, Karnataka, India

ARTICLE INFO

Article History:
Received 10th April, 2015
Received in revised form
25th May, 2015
Accepted 09th June, 2015
Published online 30th July, 2015

Key words:
Novel Phenothiazine derivative,
Cook's pole climbing,
Elevated plus maze,
Scopolamine, Diazepam.

ABSTRACT

Objective: The present study aimed at evaluating the newly synthesized novel phenothiazine derivative (N14&N15) for memory enhancing activity in scopolamine and diazepam induced amnesic rodents using cook's pole climbing, elevated plus maze and passive avoidance paradigm
Background: N14 and N15 are newly synthesized novel phenothiazine derivatives with substitution at N-10 position, which resembles with dopamine structure, having dopaminergic receptor agonist activity. Phenothiazines are reported to have anti-psychotic, anti-convulsant activity.
Methods: Memory enhancing activity of newly synthesized novel phenothiazine derivative (at 100, 200, and 400 mg/kg b.w.p.o.) was evaluated by both exteroceptive (cook's pole climbing, elevated plus maze, and passive avoidance paradigm) and interceptive behaviour models (diazepam induced amnesia). Derivative was administered for 7 days at the dose of 100,200 and 400 mg/kg b.w.to rodents in cook's pole climbing, elevated plus maze, and passive avoidance paradigm. Scopolamine (0.3 mg/kg b.w.i.p.) and Diazepam (1mg/kg b.w.i.p.) were used to induce amnesia and Piracetam (100mg/kg b.w.i.p.) served as reference standard and different parameter were assessed.
Results: Newly synthesized novel phenothiazine derivative at all doses of (100,200 and 400 mg/kg, p.o.) produced significant memory enhancing activity when evaluated by cook's pole climbing, elevated plus maze and passive avoidance paradigm models. Treatment with novel phenothiazine derivative at all doses also improved learning and memory in normal rodents.
Conclusion: The memory enhancing activity of the derivative attributed to dopaminergic receptor agonist activity, which would have been afforded by the active constituents present in the synthesized derivative. Thus, the findings of the present study reveal the nootropic activity of newly synthesized novel phenothiazine derivative. It can be utilized in treating diseases with memory deficits like senile dementia, schizophrenia and Alzheimer's disease.

INTRODUCTION

Dementia is a mental disorder, and it is a clinical syndrome characterized by cluster of signs and symptoms, manifested by difficulties in memory, disturbances in language, psychological and psychiatric changes, and loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. About 12 million people worldwide have dementia, and this is likely to excepted increase by 2040 to 25 million (Vijaylakshmi *et al*., 2012).

Corresponding author: Shiva Shanker Rai,
Drug Safety Associate, Bangalore-560050, Karnataka, India.

The most common cause of dementia, Alzheimer's disease, which is progressive neurodegenerative disorder is associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (Dhingra *et al*., 2004). The disorders that causes dementia include; Alzheimer's disease, Vascular dementia, Parkinson's disease, dementia with Lewy Bodies and Frontotemporal dementia (Vijaylakshmi *et al*., 2012). Among all Alzheimer's disease (AD) is a leading cause of dementia in developed countries and primarily affects the elderly persons and currently about 18 million people in the worldwide are suffering from dementia. The incidence increases with age and may reach nearly 30 to 50% in those with age more than 85 years old. Epidemiological studies on

Indian population show that dementia is largely hidden problem and the number is increasing. Especially in rapidly developing and heavily populated countries such as India, China and Latin America (Farooq *et al*., 2007). Oxygen free radicals, the harmful by products of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of AD in elderly (Joshi *et al*., 2006). As it is characterized by low levels of the neurotransmitter, acetylcholine (Ach) in brain, it can be treated by enhancing cholinergic function by stimulation of cholinergic receptors or prolonging the availability of ACh released into the neuronal synaptic cleft by use of agents which restore or improve the levels of Ach.

Inhibition of acetylcholinestrase (AChE), which causes breakdown of Ach, is considered to be a promising strategy for the treatment of AD. (Adewusi *et al*., 2010). Nootropic represents a new class of psychotropic agents with selective facilitatory effect on itegrative functions of the central nervous system, particularly in intellectual performance, learning capability and memory (Joshi *et al*., 2006). Nootropic agents such as *Piracetam, Pramiracetam, Aniracetam, Oxiracetam* and Choline esterase inhibitors like *Donepezil, Metrifonate, Physostigmine, Tacrine, Rivastigmine* and *Galanthamine* are being primarily used to improve memory, mood and behavior. But they have many side-effects like loss of appetite, nausea, vomiting, diarrhea, stomach cramps, headache, dizziness, fatigue and insomnia (Dubey *et al*., 2004). So, there are drugs like Antipsychotics, that ameliorate mental aberrations that are characteristic of the psychoses. Derivatives like Phenothiazine are most focusing on people with seizures, dementia, concussion or other neurological problem (Beale John, 2011). Phenothiazines belong to the oldest, synthetic antipsychotic drugs, which do not have their precursor in the world of natural compounds. They have fundamental neuroleptic action connected with the dopaminergic receptor blockade (Jaszczyszyn *et al*., 2012). Phenothiazines are a group of chemical agents with neuroleptic, antiemetic, antihistaminic, antichoinergic and sedative effects. Their main pharmacological response is determined by varying in the chemical structure at the phenothiazine of the side-chain at position 10 of the ring. These compounds have decreased anatomic activity but the greatest extrapyramidal effects of all the phenothiazine subgroups (Wampler, 1983). Many potentially useful phenothiazine derivatives have been synthesized and evaluated pharmacologically. Consequently, the large body of information permits accurate statements about the structural features associated with activity (Katzung , 2009). Dopaminergic receptor agonist are having anticonvulsant and antipsychotic activity (Morrison *et al*., 2008). Taking into consideration that newly synthesized novel phenothiazine derivatives have structure similarity with dopamine, the present study aims at evaluating for their memory enhancing activity.

MATERIALS AND METHODS

Procurement of newly synthesized novel phenothiazine derivatives

The test compounds N14 and N15 are novel phenothiazine derivatives procured from Department of Chemistry, K.L.E.University's, College of Pharmacy, Bangalore. Dose selection was done after the acute toxicity studies according to OECD test guidelines (www.oecd.org/ehs).

Drugs and Chemicals

- Piracetam (Nootropil, by UCB India Pvt.Ltd.)
- Scopolamine (Buscopan, by Cadila healthcare Ltd.)
- Diazepam (Calmpose, by Ranbaxy laboratories Ltd.)
- Dimethyl sulfoxide 50%
- 5% Sodium bi carbonate

Animals

Healthy, Albino Swiss mice weighing between 18-25g were used for Elevated plus maze and Passive avoidance paradigm and albino Wistar rats weighing between 180-250g (not exceeding 10% difference within the group) were employed for Cook's pole climbing paradigm which were purchased from licensed laboratory animal breeder Clearance from Institutional Animals Ethics Committee of this institution was obtained prior to the experimentation (IAEC /03/PA/2012-14).

Spectral studies: The identification and characterization of the compounds were carried out by the following procedure to ascertain chemistry and structure of newly, synthesized compounds.

1. Melting point
2. Solubility
3. Thin layer chromatography
4. I.R (Infra red spectroscopy)
5. N.M.R
6. LCMS-6410

All the melting points and boiling points were determined by capillary method in paraffin and are uncorrected. All the solvents were used after distillation. Silica gel used for the preparation of TLC was purchased from Sd. fine chemicals, Mumbai. FT I.R spectra were recorded in KBR on JASCO V460PLUS IR spectrometer by diffuse reflectance technique. ^{1}HNMR spectra were taken in DMSO on a bruker ultraspec AMX 400 MHZ spectrometer. The chemical shifts are expressed as δ ppm and tetra methyl silane was used as internal standard. Mass spectra were recorded in triple quadrapole LCMS-6410 from Agilant technologies.

Acute toxicity studies

Acute oral toxicity study for newly synthesized novel phenothiazine derivatives (N14 and N15) were conducted as per OECD guideline 425 using female Swiss albino mice weighing between 18-25g. Each animal was administered doses upto2000 mg/kg b.w. by oral route. The animals were observed for any changes continuously for first 4h and upto 24h for mortality or any behavioural change and daily thereafter for a total of 14 days.

Grouping of animals for memory enhancing activity in rodents

Female albino Wistar rats and Swiss albino mice were randomly divided into IX groups of 5 animals each. I,II and III groups served as normal,control and standard treatment.

Animals of groups IV to IX were treated groups and they were administered derivatives at three different doses (100,200,400 mg/kg b.w.p.o.) for 7days and on the last day (7^{th} day) of treatment scopolamine (0.3 mg/kg,i.p) and Diazepam(1mg/kg,i.p) were administered for their respective paradigm after 60 min. of regular dosing.

Experimental schedule

Female albino Wistar rats and Swiss albino mice were divided into IX groups of 5 each. Group I,II and III served as normal (distilled water at 1ml/100g), control group (scopolamine at 0.3mg/kg,i.p) and standard treatment group (Piracetam at 100mg/kg,i.p) respectively. Animals of groups IV to IX were the treated groups and they were administered derivatives at three different doses (100,200,400 mg/kg b.w.p.o.) for 7days and on the last day (7^{th} day) of treatment scopolamine (0.3 mg/kg,i.p) was administered for respective paradigm and Diazepam(1mg/kg,i.p) was administered for its respective paradigm after 60 min. of regular dosing.

Cook's pole climbing

Groups I to IX were trained for ten days to attained acquisition transition (95-99%). Then all groups treated with respective doses and groups were challenged with scopolamine (0.3 mg/kg b.w) except normal group for 7 days and conditioned avoidance response was assessed.

Elevated Plus Maze

Group I, II,III served as normal (distilled water at 1ml/100g), control group (scopolamine at 0.3mg/kg,i.p) and standard treatment group (Piracetam at 100mg/kg,i.p) respectively. Animals of groups IV to IX were the treated groups and they were administered derivatives at three different doses (100,200,400 mg/kg b.w.p.o.) for a period of 6 day and on 6^{th} day, 60 min after administration of water/standard/compound, each mouse was placed at the end of the open arm, facing away from the central platform. TL was recorded on first day (training session) for each animal. Retention of learned task was recorded after 24 h. Animals of group II were administered scopolamine i.p. 45 min prior to recording TL. Whereas for animals of groups IV to IX, scopolamine was administered i.p. after 60 min of administration of drugs. TL was recroded after 45 min of scopolamine injection and again after 24h.

Inflexion ratio (IR)

The TL was expressed as retention after 24h by calculating the IR using the formula,

$IR=L_1-L_0/L_0$

Where, L_O= transfer latency after 24 h and

L_1= initial transfer latency in sec

Passive avoidance paradigm

Groups IV to IX were treated for a period of 6 days. On 6^{th} day, 60 min administration of water/standard/compound, each mouse was gently placed on the wooden platform set in the centre of the grid floor and shocks were delivered for 15 sec when the mouse stepped down placing all its paws on the grid floor. SDL was recorded during both the sessions of training. Retention (memory) was examined after 24 h (i.e., seventh day, 24 h after last dose). Animals of group II were administered scopolamine i.p. 45 min prior to recording SDL. Whereas for animals of groups IV to IX, scopolamine was administered i.p. after 60 min of administration of drugs. SDL was recroded after 45 min of scopolamine injection and again after 24h.

Diazepam-induced amnesia

Diazepam (1mg/kg,i.p) was administered to young mice (3 months) and TL and SDL was noted after 45 min. of injection on 6^{th} day and after 24h. Test drug and standard drug (200 mg/kg,i.p.) were administered for 6 successive days. After 60 min. of administration of the last dose on the 6^{th} day, diazepam was administered, TL and SDL were noted after 45 min. of administration of diazepam and after 24 h.

Statistical analysis

The interpretation of the results was done after subjecting the data obtained from various studies to statistical analysis which include t-test and One way ANOVA followed by post –test Dunnett's 's Multiple Comparision Test. $P<0.05$ was considered as statistically significant.

RESULTS

Infra red spectra

Table 1. Infra red spectral study of the synthesized N14 and N15 compounds

Compound name	Spectral peaks (cm^{-1})	Molecular nature
N 14	2918.73-2857.02	Al. C-H (stretching)
	3028.66	Ar. H
	1527.35	NO^2 (stretching)
	1617.98-1451.17	C=C (stretching)
	3419.17-3278.39	NH (stretching)
N 15	2918.73-2857.02	Al. C-H (stretching)
	3028.66	Ar. H
	1527.35	NO^2 (stretching)
	1617.98-1451.17	C=C (stretching)
	3419.17-3278.39	NH (stretching)
	1176.36-1066.44	Cl (stretching)

Acute toxicity studies

Phenothiazine derivatives (N14 and N15) were conducted as per OECD guidelines 425 using female Swiss albino mice. There was no change in normal behavioural pattern of animals. No sign and symptoms of toxicity were observed during the observations which was done continuously for the first 4 h and then observed up to 24 h for mortality and continued after each dose. The derivatives were safe upto a dose of 2000 mg/kg b.w. The in-vivo studies were carried out for both derivatives at doses of 100,200,400 mg/kg b.w.

Memory enhancing activity

Elevated Plus Maze: Elevated plus maze serves as the exteroceptive behavioural model to evaluate memory in mice.

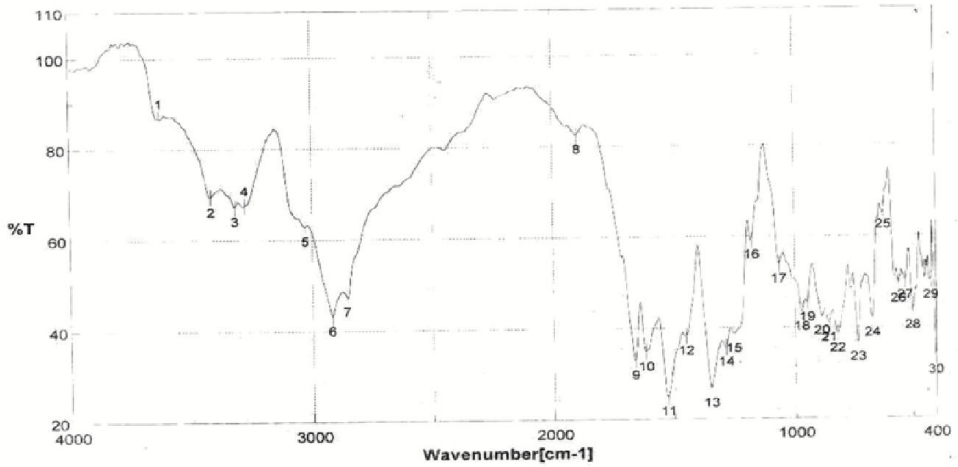

Fig.1. Infra red spectral of N 15 Phenothiazine derivative

^{1}H NMR Spectra

Table 2. ^{1}H NMR spectral data of synthesized compounds

Compound name	Chemical shift value (δ) in ppm	Proton nature
N 15	3.380	Ar-NH
	5.320	CH_2
	3.380	CH_3
	7.002-7.96	Ar-H

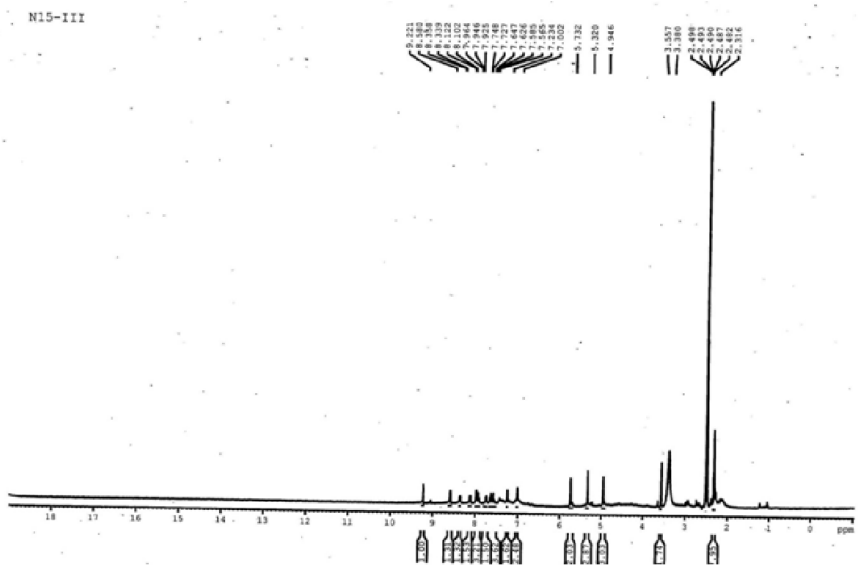

Fig. 2.^{1}H NMR spectra of N 15 compound

Mass spectra

Table 3. Mass spectral study of synthesized compounds

Compound name	m/z value
N 15	399.4

Inflexion ratio (IR)

IR was calculated after recording TL on 6th day and after 24 h i.e. on 7th day. Increase in IR after 24 h indicates improved retention of learned task. Control group showed a significant ($p<0.05$) decrease in IR as compared to normal animals. Maximum IR was observed in mice with higher doses (200mg/kg b.w. and 400mg/kg b.w.) of derivatives N14 and N15 Piracetam (100mg/kg b.w,i.p.) and N14 and N15(200 and 400mg/kg b.w, p.o.) administration for 6th days significantly ($p<0.001$) protected the animals from scopolamine induced learning and memory impairement and increased the IR as compared to the scopolamine treated group. Maximum protection was afforded by N14 (200 mg/kg) and N15(400mg/kg), as evident by significant ($p<0.001$) increase in IR in scopolamine induced memory impaired mice. Inflexion Ratio shown in the Table 4

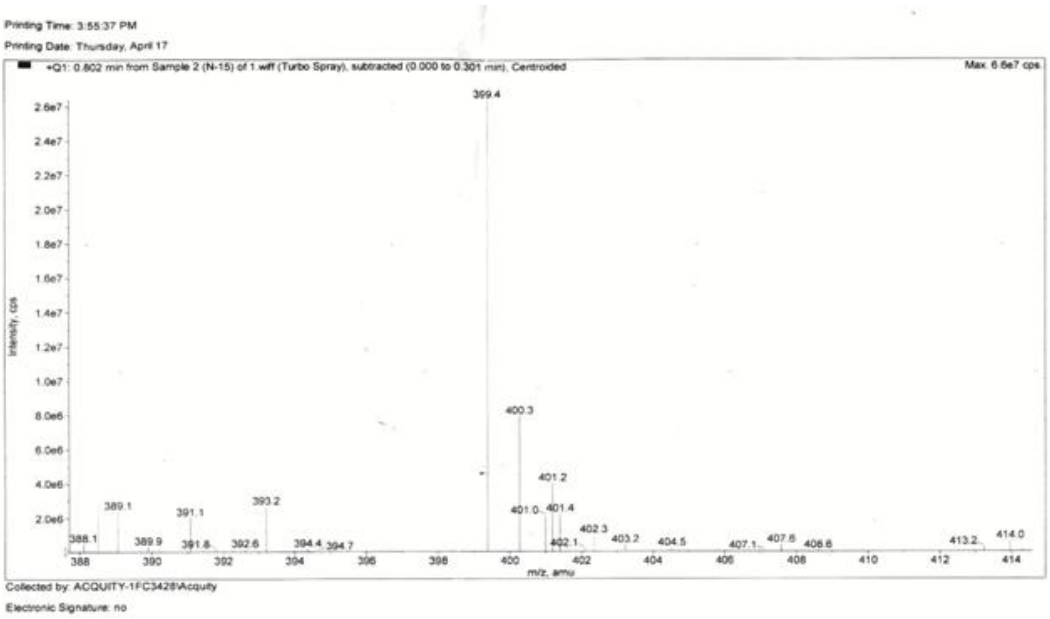

Fig. 3. Mass spectra of N15 compound

Table 4. Effect of newly synthesized novel phenothiazine derivative on IR in Scopolamine induced animals

Treatment	TL -I DAY(L_1)	TL - II DAY (L_0)	IR(L_1-L0/L_0)
Normal	21.40±5.564	14.60±4.261	0.498±0.051
Positive control (scopolamine)	8.00±0.7071	10.20±0.800	0.144±0.016***
Standard treatment+scopolamine	12.60±2.400	9.200±1.685	0.308±0.033**
N14(100mg/kg b.w.)+ scopolamine	19.60±2.657	16.60±2.676	0.218±0.049***
N14(200mg/kg b.w.)+ scopolamine	29.00±6.504	23.60±5.671	0.228±0.040***
N14(400mg/kg b.w.)+ scopolamine	32.40±3.600	28.80±5.671	0.178±0.310***
N15(100mg/kg b.w.)+ scopolamine	14.80±0.9695	12.20±0.7348	0.210±0.020***
N15(200mg/kg b.w.)+ scopolamine	42.40±5.418	35.60±5.36	0.202±0.0315***
N15(400mg/kg b.w.)+ scopolamine	25.00±7.162	20.00±5.657	0.234±0.276***

n=5, values are expressed as mean± S.E.M, One-way ANOVA followed by Dunnett's Multiple Comparison test. Indicate phenothiazine derivatives at varying doses*** p<*0.001,* ** p<0.01 comparison with control.

Table 5. Effect of newly synthesized novel phenothiazine derivative on SDL in Scopolamine induced animals

Groups	Step down latency after 24 hrs (second)
Normal	95.20±1.530
Positive control (scopolamine)	32.40±1.691
Standard treatment+ scopolamine	115.8±4.212***
N14(100mg/kg)+ scopolamine	82.00±1.000***
N14(200mg/kg)+ scopolamine	84.60±0.7483**
N14(400mg/kg)+ scopolamine	83.40±0.5099***
N15(100mg/kg)+ scopolamine	84.60±0.8124**
N15(200mg/kg)+ scopolamine	85.20±1.068**
N15(400mg/kg)+ scopolamine	82.20±1.562***

n=5, values are expressed as mean± S.E.M, One-way ANOVA followed by Dunnett's Multiple Comparison test. Indicate phenothiazine derivatives at varying doses*** p<*0.001,* ** p<0.01 comparison with control.

Passive avoidance paradigm

Piracetam (100mg/kg b.w,i.p.) and N14 and N15 (100,200 and 400mg/kg b.w,p.o.) treatment for 6^{th} days. On 6^{th} day all groups except normal group all groups were treated with Scopolamine (0.3mg/kg b.w,i.p.) after 60 min. after last dose. Scopolamine (0.3mg/kg b.w., i.p.) treated animals showed a significant decrease in SDL as compared with the normal. This indicating impairment of memory. Piracetam (i.p.) and all the doses, administered orally for 6^{th} days significantly ($p<0.001$) reversed amnesia induced by scopolamine when compared to the scopolamine treated The Step down latency value shown in Table 5

Cook's pole climbing apparatus

All groups of animals treated with standard Piracetam (100mg/kg b.w.,i.p.), newly synthesized novel phenothiazine derivatives (N14 and N15) all doses orally and scopolamine (0.3mg/kg b.w.,i.p.) After 60 min of doses on each day rats

were placed in apparatus to avoid electric stimuli or to avoid condition response. On 7th day the same procedure were repeated. Scopolamine (0.3mg/kg b.w.,i.p.) treated groups show decrease in condition avoidance response blocked compare to normal groups indicating impairment of memory in rats. Piracetam (i.p.) and all the doses (100,200,400 mg/kg b.w.,p.o) administered orally for 6th days significantly ($p<0.001$) reversed amnesia induced by scopolamine when compared to the scopolamine treated animals

Table 6. Effect of newly synthesized novel phenothiazine derivative on CAR in Scopolamine induced animals

Groups	CAR BLOCKED
Normal	10.00±0.00
Positive control (scopolamine)	4.429±1.066
Standard treatment+ scopolamine	9.571±0.202***
N14 (100mg/kg)+ scopolamine	5.143±0.911***
N14 (200mg/kg)+ scopolamine	5.857±0.799***
N14 (400mg/kg) + scopolamine	7.000±0.534*
N15 (100mg/kg) + scopolamine	5.143±0.986***
N15 (200mg/kg) + scopolamine	7.000±0.577*
N15 (400mg/kg) + scopolamine	7.143±0.508*

n=5, values are expressed as mean±S.E.M, One-way ANOVA followed by Dunnett's Multiple Comparison test. Indicate phenothiazine derivatives at varying doses*** $p<0.001$, * $p<0.05$ comparison with control.

Diazepam-induced amnesia

Diazepam (1mg/kg) was administered to young mice (3 months) and Total-latency was noted after 45 min. of injection on 6th day and after 24 h. Test drug and standard drug (200mg/kg,i.p.) were administered for 6 successive days. After 60 min. of administration of the last dose on the 6th day, diazepam was administered; TL and SDL were noted after 45min. of administration of diazepam and after 24 h.

Transfer latency

Inflexion ratio (IR) IR was calculated after recording TL on 6th day and after 24 h i.e. on 7th day. Increase in IR after 24 h indicates improved retention of learned task. Control group showed a significant ($p<0.05$) decrease in IR as compared to normal animals. Maximum IR was observed in mice with higher doses (200mg/kg b.w. and 400mg/kg b.w.) of derivatives N14 and N15. Piracetam (200mg/kg b.w,i.p.) and N14 and N15(200 and 400mg/kg b.w, p.o.) administration for 6th days significantly ($p<0.001$) protected the animals from diazepam induced learning and memory impairement and increased the IR as compared to the diazepam treated group. N14 (200 mg/kg) and N15(400mg/kg), as evident by significant ($p<0.001$) increase in IR in diazepam induced memory impaired mice. Inflexion ratio value shown in Table 7

b] Step-down latency

In the set of experiment, effect of newly synthesized novel phenothiazine derivative on memory was studied on normal animals. Piracetam (200mg/kg b.w,i.p.) and N14 and N15 (100,200 and 400mg/kg b.w,p.o.) treatment for 6th days. On 6th day all groups except normal group all groups were treated with Diazepam (1mg/kg b.w,i.p.) after 60 min. after last dose. Diazepam (1mg/kg b.w., i.p.) treated animals showed a significant decrease in SDL as compared with the normal group. Piracetam (i.p.) and all the doses, administered orally for 6th days significantly ($p<0.001$) reversed amnesia induced by Diazepam. The step down latency value shown in Table 8

DISCUSSION

Dementia is a clinical syndrome characterized by the development of multiple cognitive defects that are severe

Table 7. Effect of newly synthesized novel phenothiazine derivative in Diazepam induced animals.

Groups	TL - I DAY(L_1)	TL - II DAY (L_0)	Inflexion ratio (IR)
Normal	57.00±11.28	27.40±7.639	1.950±0.6471
Positive control(Diazepam 1mg/kg)	80.20±3.85	74.40±3.544	0.0740±0.02821***
Standardtreatment+diazepam	52.60±5.212	31.60±2.337	0.6670±0.1360**
N 14(100mg/kg) + diazepam	69.80±6.224	46.00±3.450	0.4800±0.08837***
N 14(200mg/kg) + diazepam	42.20±7.921	28.40±6.361	0.5920±0.07473**
N 14(400mg/kg) + diazepam	63.00±9.110	47.00±7.810	0.3660±0.07257***
N15(100mg/kg) + diazepam	51.60±9.511	32.40±5.183	0.5120±0.1554**
N15(200mg/kg)+ diazepam	49.40±12.07	32.20±5.765	0.4820±0.01507***
N15(400mg/kg)+ diazepam	37.80±8.777	22.80±4.259	0.5980±0.09447**

n=5, values are expressed as mean± S.E.M, One-way ANOVA followed by Dunnett's Multiple Comparison test. Indicate phenothiazine derivatives at varying doses*** $p<0.001$, ** $p<0.01$ comparison with control.

Table 8. Effect of newly synthesized novel phenothiazine derivative on SDL in Diazepam induced animals

Groups	Step down latency after 24 hrs(second)
Normal	95.20±1.530
"Diazepam treated"	32.80±0.8602
"Positive control+ diazepam"	114.4±2.713***
N14(100mg/kg)diazepam	82.00±1.000***
N14(200mg/kg)+diazepam	81.40±0.5099***
N14(400mg/kg)+diazepam	82.40±0.7483***
N15(100mg/kg)+diazepam	84.40±0.6782***
N15(200mg/kg)+diazepam	80.6782±0.8602***
N15(400mg/kg)+diazepam	81.40±0.5099***

n=5, values are expressed as mean± S.E.M, One-way ANOVA followed by Dunnett's Multiple Comparison test. Indicate phenothiazine derivatives at varying doses*** $p<0.001$, comparison with control.

enough to interfere with day to day social and professional functioning. Presently, there are no satisfactory diagnostic procedures and therapeutic regimens available for the management of cognitive dysfunctions. Unlike dementia due to stroke, the onset of Alzheimer's disease is almost imperceptible, without abrupt changes in cognition or function. Loss of memory is typically the presenting patient complaint. Memory is a non-specific term representing many diverse areas of cognitive function. In early Alzheimer's disease the ability to lay down new memory (learn) and recall events is impaired, whereas recall for remote events is spared until later in the disease process. Anomie is a problem, with difficulty recalling names of familiar objects or people. Persons with Alzheimer's disease often conceal their memory problem well at first, and may deny or "forget" that they have a memory problem (Joseph *et al* 1990).

Alzheimer's disease (AD), the most common form of dementia, was first reported in 1906. In 2006, there were about 26.6 million. Although this disease has been identified for a long time, most research progress was made in the recent 30 years. However, no definitive cure is available for this disease and eventually it leads to death. Therefore, the drug discovery for Alzheimer's disease remains challenging (Cheng *et al*., 2014). Based on experimental and clinical evidences, acetylcholine is considered to be the most important neurotransmitter involved in regulation of cognitive functions and dopamine receptor are also important in various vital central nervous system functions including voluntary movement, feeding, sleep, working memory and learning. The cholinergic receptor agonists (muscarinic and nicotininc) and enhancers of endogenous level of Ach (synthesis promoters and inhibitors of its metabolizing enzyme) have been used for treating dementia and several numerous advances have occurred in understanding the properties of dopamine receptors, that have led to the development of multiple pharmacologically active compounds that directly target dopamine receptors such as antiparkinsons drug and antipsychotics.

Administration of selective D_1 receptor agonists in low doses enhanced memory in aged mankeys. Dopamine receptor agonist pergolide improved memory in human beings. Microinjection of D_1 receptor antagonist into prefrontal cortex region of monkeys and rats impaired spatial working memory (Parle *et al*., 2004). N14 & N15 derivatives are newly synthesized phenothiazine derivatives with substitution at N-10 position, which resembles with the dopamine receptor agonist. The elevated plus maze is used to measure the anxiety state in animals, however transfer latency i.e., the time elapsed between the movement of the animal from an open to an enclosed arm will markedly shorten if the animal has previous experience of entering into open and closed arm and this shortened transfer latency has been showed to be related with memory process and the increase in inflexion ratio indicates nootropic activity.

Recent studies of several nootropics and amnestic agents on elevated plus maze made this model a widely accepted paradigm to study learning and memory processes in rodents. Passive avoidance behaviour based on negative reinforcement was used to examine the long term memory. A sequence of stimuli is presented during which the animal has freedom to transfer to a shock free area indicates the passive behaviour, which is used as a short term memory task. Cook's pole climbing apparatus is used to evaluate condition avoidance response. In this animals were train to attained acquisition transition i.e. to avoid shock free zone or blockade of condition avoidance response. Diazepam, a GABA mimetic agent induces memory impairment and the subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory. In the present study, administration of three doses of newly synthesized novel phenothiazine derivatives N14 & N15 (100,200 and 400 mg/kg,b.w.p.o.) for 6 days improved learning and memory of animals significantly in both the exteroceptive behavioural models as evident by the decrease in TL and increase in IR in elevated plus maze model, increase in SDL in passive avoidance paradigm and increase in SDL in passive avoidance paradigm and increase in CAR blocked in cook's pole climbing paradigm.

The stimulus lies outside the body in exteroceptive behaviour models, whereas, it lies within the body in the case of interoceptive models. Furthermore, pre-treatment with three doses of novel phenothiazine derivatives N14 & N15 for 6 days protected the animals from learning and memory impairment produced by interoceptive stimulus (scopolamine and diazepam). The importance of central cholinergic system in memory function is well established. Among the various approaches attempted to increase cholinergic activity, the inhibition of acetylcholinesterase (AChE) is the most successful one. Cholinesterase inhibitors (Tacrine and Rivastigmine) are the only class of compounds consistently proven to be efficacious in treating the cognitive and functional symptoms of alzhimer's disease. Nootropics represents a new class of psychotropic agents with selective facilitatory effect on integrated functions of the central nervous system, particularly on intellectual performance, learning capacity and memory. Piracetam, the established nootropic agent was used in the present study for comparison because; it improves memory by facilitation of synaptic transmission (increase choline uptake in cholinergic nerve endings, thereby facilitating cholinergic transmission) in brain.

Piracetam elevates the density of frontal cortex acetylcholine receptors by 30-40% restoring the levels of acetylcholine in the brain. The importance of central cholinergic system in memory function is well established. Among the various approaches attempted to increase cholinergic activity, the inhibition of acetylcholinesterase (AChE) is the most successful one. Cholinesterase inhibitors (Tacrine & Rivastigmine) are the only class of compounds consistently proven to be efficacious in treating the cognitive and functional symptoms of AD. Scopolamine induced amnesia is a widely used model for evaluating the nootropic drugs. It is an anticholinergic drug, by the virtue of which it produces memory impairment. Other than this, various other mechanisms are also involved in causing memory impairement. From, the present study we can conclude that the novel phenothiazine derivatives N14 & N15 possess memory enhancing activity when administered alone and on pre-treatment, it was able to reverse the scopolamine and diazepam induced amnesia. Thus, N14 &N15 may be value for treating patients with impaired learning and memory.

Conclusion

It concluded that the newly synthesized novel phenothiazine derivatives (N14&N15) has shown promising memory enhancing activity by offering protection against scopolamine and diazepam induced memory impairement in rodents.

Acknowledgements

Authors are thankful to the management of KLE University's College of Pharmacy, Bangalore for providing necessary research facility to carry out this research project.

REFERENCES

"Guidence document on acute oral toxicity testing" series on testing and assessment NO. 24, organization for economic co-operation and development, OECD Environment, health and safety publication, Paris 2001 (www.oecd.org/ehs).

Adewusi, E.A, Moodely, N., Steenkamp, V. 2010. Medicinal plants with cholinesterase inhibitory activity: A revies. *Afr J Biotechnol* 9(49): 8257-76.

Beale John, M, Block Jhon, H. Wilson and Gisvold's of Pharmaceutical chemistry 12th edition, Lippincott Williams and Wikins,.458-65.

Cheng, H.Y., Chnang, L.Y., Chang, H.W., Yang, C.H. 2014. *Biomed Res Int.* 2014:897653.

Dhingra, D., Parle, M., Kulkarni, S.K., 2004. Memory enhancing activity of *Glycyrrhiza glabra* in mice. *J Ethanopharmacol;* 91: 361-5.

Dubey, N.K., Kumar, R., Tripathi, P. 2004. Global promotion of herbal medicine: India's opportunity. *Curr Sci* :86:37-41.

Farooq, S.M, Alla, T.R., Roa, V.N., Prasad, K., Shalam, Nandkumar, K., Gouda, T.S., Satyanarayana S. 2007. A study on CNS effects of milk extracts of nuts of *Semecarpus anacardium*.Linn. Pharmacologynoline;1: 49-63.

Jaszczyszyn A, Gasiorowski K, Swiatek P, Malinka W, Cieslik-Buczula K, Petrus J, Czarnik-Matusewicz B. Chemical structure of phenothiazines and their biological activity. Pharmacological Reports 2012;64:16-23.

Joshi, H., Parle, M. 2006. Evaluation of nootropic potential of *Ocimum sanctum* Linn. in mice. *Indian J of Experimental Biology* ;44:133-6.

Joseph, T., Robert, R.S. 1999. Pharmacotherapy A Pathophysiological Approch, Chapter No. 61, Alzheimer's Diseaase Fourth edition. 1065-82.

Katzung, B.G, 2009. Basic and clinical pharmacology 9th edition, Mc Graw Hill,462-3.

Morrison, R.T, Boyd, R.N., Bhattacharya, S.K., 2008. Organic chemistry,7th edition 1387-89.

Parle, M., Dhingra, D., Kulkarni, S.K. 2004. Neurochemical basis of learning and memory. *Indian J Pharm.scie* ;66:371-6.

Vijaylakshmi, Adiga S, Bhat P, Chaturvedi A, Bairy KL, Kamath S. 2012. Evaluation of the effect of *Ferula asafoetida* Linn.gum extract on learning and memory in wistar rats. *Indian J Pharmacol* ;44:82-7.

Wampler, G. 1983. The pharmacology and clinical effectiveness of phenothiazines and related drugs for managing chemotherapy induced emesis. Issue 1 supplement ;25:35-51.

****