IJPSR (2017), Vol. 8, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 August, 2016; received in revised form, 16 November, 2016; accepted, 01 December, 2016; published 01 March, 2017

PROTECTIVE EFFECT OF *NYCTHANTHES ARBOR-TRISTIS* ON SCOPOLAMINE INDUCED AMNESIA IN RATS "A BEHAVIOURAL AND BIOCHEMICAL APPROACH"

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Keywords:

Nycthanthes arbour-, acetylcholine, scopolamine, piracetam, Morris water maze, Elevated plus maze, transfer latency time.

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ABSTRACT: The hydroalcoholic extract of *Nyctanthes arbor-tristis* (HeNAT) leaf was prepared using Soxhlet apparatus. Memory impairment (amnesia) in male albino rats induced by scopolamine hydrochloride was recorded as transfer latency time (TLT) in EPM model; escape latency time (ELT) and time spent in Q4 target quadrant (TSTQ) in MWM model. Scopolamine markedly decreased the TLT over 2 min, ELT, TSTQ over 90 sec and consecutively impaired learning and memory. HeNAT leaf extract (20 and 40 mg/kg, p.o.) increased the TLT, ELT, TSTQ and improved memory. Higher levels of brain AChE, MDA and NO and lower level of GSH activity were observed in scopolamine treated rats, which were significantly attenuated by HeNAT leaf extract. It is concluded that the leaf extract exhibited a significant nootropic effect in scopolamine induced impairment of memory in rats. Nootropic effect of the plant leaf extract may be mediated through the inhibition of oxidative stress and due to presence of iridoid and phenylpropanoid glycosides which was reported to possess anti-cholinesterase activity.

INTRODUCTION: The word nootropic was coined in 1972 by a romanian psychologist and chemist, corneliu e. Giurgea, from the greek words noos or "mind", and tropein meaning to bend or turn [1]. Nootropics also called smart drugs, memory enhancers, neuro enhancers, cognitive enhancers and intelligence enhancers are drugs, supplements, nutraceuticals, and functional foods that improve one or more aspects of mental function [2]. Epidemiological studies of indian population reveal that dementia is largely a hidden problem. The memory loss can be caused by traumatic emotional event. Today's memory impairment is major problem due to dementia in elder people.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(3).1346-55

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (3).1346-55

Memory is the most common cognitive ability lost with dementia; 10% of persons over age 70 and 20 to 40% of individuals over age 85 have clinically identifiable memory loss. Scopolamine, centrally acting anti-muscaranic drug impairs learning and memory both in rat and human beings. Loss of the acetylcholine, neurotransmitter in the brain of patient with Alzheimer's diseases appears to be a critical element to produce dementia. Acetylcholine was considered one of the neuro-chemical transmitters responsible for memory retention. In human, acetylcholine (Ach), a key neurotransmitter for brain function is synthesized locally in the cholinergic nerve endings.

Choline is actively taken by axonal membrane by a na^+ choline acetyl transferase present in the axoplasm. Choline and its metabolites are needed for three main physiological purpose: structural integrity and signalling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and a major source for methyl groups

via its metabolite, trimethylglycine (betaine), which participates in the s- adenosylmethionine (same) synthesis pathways [3]. Choline is a precursor to trimethylamine, which some persons are not able to break down due to a genetic disorder called trimethylamine. Persons suffering from this disorder may also suffer from a strong fishy or otherwise unpleasant body odour even on a normal diet- i.e., one that is not particularly high in choline. Persons with trimetylaminuria are advised to restrict the intake of foods high in choline; this may help to reduce the sufferer's body odour. Studies on a number of different populations have found that the average intake of choline was below the adequate intake.

The adequate intake (ai) of choline is 425 milligrams per day for adult women, and higher for pregnant and breast feeding women. The ai for adult men is 550 mg/day. There are also ais for men (550mcg), women (425mcg), pregnant females of any age (450 mg), lactating females of any age (550 mg), infants 0-6 months (125 mg), babies 6-12 months (150 mg), toddlers 1-3 years (200 mg), children 4-8 years (250 mg), young males 9-13 years (375 mg), young females 9-13 years (375 mg), teen and adult males 14 years and older (550 mg), teen females 14-18 years (400 mg), endurance athletes and people who drink a lot of alcohol may be at risk for choline deficiency and may benefit from choline supplements.

***Nyctanthes arbor-tristis* (Night jasmine):** *Nyctanthes arbor-tristis* is one of the most useful traditional medicinal plants in India. It is distributed widely in sub-Himalayan regions and southwards to Godavari. Each part of plant has some medicinal value and is thus commercially exploitable. *Nyctanthes arbor-tristis* is a shrub or a small tree growing to 10 m tall, with flaky grey bark. The leaves are opposite, simple, 6–12 cm long, with an entire margin. The flowers are fragrant, with a five- to eight lobed white corolla with an orange-red centre [4, 5].

Parts used: Leaves, roots, flowers, stem, and bark are the parts used in medicine.

Chemical Constituents:

Leaves: The leaves contain D-mannitol, β-sitosterol, flavanol glycosides, astragalin, nicotiflorin, oleanolic acid, nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside, an amorphous resin, trace of volatile oil, carotene, friedeline, lupeol, mannitol, glucose, fructose, iridoid glycosides, phenylpropanoid glycosides and benzoic acid [6, 7, 8].

Flowers: The flowers contain essential oils, nyctanthin, D-mannitol, tannins, glucose, carotenoids, glycosides including β-monogentiobioside ester of α-crocetin (or crocin-3), β-monogentiobioside-β-D monoglucoside ester of α-crocetin, and β-digentiobioside ester of α-crocetin.

Seeds: The seeds contain arbortristosides A and B; glycerides of linoleic, oleic, lignoceric, stearic, palmitic and myristic acids; nyctanthic acid; 3,4-secotriterpene acid; and a water-soluble polysaccharide composed of D-glucose and D-mannose.

Bark: The bark contains glycosides and alkaloids.

Stem: The stems contain the glycoside naringenin-4'-0-β-glucapyranosyl-α-xylopyranoside and β-sitosterol.

Flower oil: The flower oil contains α-pinene, p-cymene, 1-hexanol, methylheptanone, phenyl acetaldehyde, 1-decenol and anisaldehyde [9].

Medicinal uses: The leaves of *Nyctanthes arbor-tristis* are used as antibacterial, anthelmintic, anti-inflammatory, hepato-protective, immune-potential, anti-pyretic, antioxidant and anti-fungal. The Flowers are used as diuretic, anti-bilious, antioxidant, anti-inflammatory. Seeds are used as anti-bacterial, anti-fungal, immuno-modulatory and anti-leishmanial. Bark is used as anti-microbial. Stem is used antipyretic and antioxidant [10]. From ancient civilization various parts of different plants were used to eliminate pain, control suffering and counteract disease.

Choline supplements can increase efficiency in the frontal lobe, the main area of the brain responsible for reasoning, decision-making and working memory. Choline can empower the frontal lobe [11]. Drugs which mimic the effect of ACh chemical have been found to boost memory [12]. Consuming a choline rich diet will relieve signs of choline

deficiency: the fatty liver and haemorrhagic kidney necrosis. In present age herbal medicines are not only providing traditional and ethinic medicines but also promising for highly efficient novel bioactive molecules [13]. *Nycthanthes arbor-tristis* leaves contain iridoid glycosides & phenylpropanoid glycosides which possessed anti-cholinesterase activity. It was speculated that *Nycthanthes arbor-tristis* leaves may have nootropic activity. Therefore, the aim and objective of present research work is to investigate nootropic activity of *Nyctanthes arbor-tristis* leaves using interoceptive (scopolamine induced amnesia) and exteroceptive (elevated plus maze and Morris water maze) behavioural models in Swiss albino male rats and provide scientific basis for the same.

MATERIAL AND METHODOLOGY:

Experimental Animals: Albino Swiss male rats in equal numbers per group (n=6) were taken. Normal weight of male rat was 150-200 gm. The temperature of the experimental animal room was maintained to be 22ºC (±3ºC). Relative humidity was maintained between 50-60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets were used with drinking water supplied. Healthy male rats were assigned to the control, standard, and treatment groups.

Drugs and chemicals:

Drug: Piracetam, scopolamine, were purchased from Sigma Aldrich.

Chemicals: Petroleum ether, ethanol. Analytical reagent grade chemicals were used in study.

Method of collection of the data (including sampling procedure): In the first phase of the study, it was planned to prepare petroleum ether extract, chloroform, methanol extract of *Nyctanthes arbor-tristis* leaves using standard extraction procedures. Then these extracts were subjected for qualitative phytochemical test. Later on, in the second phase it was planned to investigate nootropic activity of methanol extract of *Nyctanthes arbor-tristis* using introceptive (scopolamine and ageing induced amnesia) and exteroceptive (elevated plus maze and Morris water maze) behavioural models and provide scientific basis for the same.

Extroceptive behavioural models:

Elevated plus-maze model: The elevated plus-maze served as the exteroceptive (where stimulus existed outside the body) behavioural model to evaluate learning and memory in rats [13]. The apparatus consists of two open arms (16cm × 5cm) and two covered arms (16cm ×5cm× 12cm). The arms extended from a central platform (5cm × 5cm), and maze is elevated to a height of 25 cm from the floor. On the first day, each mouse will be placed at the end of an open arm, facing away from the central platform. Transfer latency time (TLT) will be the time taken by the rat to move into any one of the covered arms with all its four legs. TLT is recorded on the first day. If the rats do not enter into one of the covered arms within 90 sec, they will be gently pushed into one of the two covered arms and the TLT will be assigned as 90 sec.

The rat will be allowed to explore the maze for 10 sec and then return to its home cage. Memory retention will be calculated 24 hrs of acquisition trial on second day as inflation ratio using the following formula described by inflation ratio = L1- L0/L0 where L0 is transfer latency time after 24hrs and L1 is the initial transfer latency time in second [14].

Morris water-maze model: Morris water maze model is employed to evaluate learning and memory. It consists of a circular water tank (diameter 150cm and height 45cm), filled with water maintained at 25 ºC. The water is made opaque with a white coloured dye. The tank is divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10cm) of 29cm height is located in the centre of one of these four quadrants. The position of platform and clues are kept consistent throughout the training session.

In the present study, target quadrant will be Q4. Escape latency time (ELT) to locate the hidden platform will be noted as index of acquisition and learning during acquisition trials conducted on four consecutive days. The mean time spent by the rat in target quadrant for searching the hidden platform will be noted as an index of retrieval during retrieval trial conducted on day 5 [15].

Introceptive behavioural models:

Scopolamine- induced amnesia in rat: Scopolamine hydro bromide (0.3mg/kg, i.p.) was administered to induce amnesia. Scopolamine is muscarinic receptor antagonist with amnestic properties that have been used for decades in experimental rats to induce impairment in their performance of a variety of tasks requiring intact working and reference memory [16]. Scopolamine has also been used clinically as an adjunct to surgical or obstetric procedure to induce sedation and post-procedural amnesia. This reversible amnestic effect was induced by centrally acting muscarinic cholinergic antagonists. Indeed, blockade of central muscarinic receptors could induce a pattern of cognitive impairment. Scopolamine actions are limited to the blockade of brain function mediated via cholinergic (muscarinic) receptors. Scopolamine, however, is relatively nonselective pharmacologically with respect to receptor subtypes, and the drug does not discriminate very much with respect to receptor subtypes, and the drug does not discriminate very much with respect to brain region although the scopolamine reversal model is in wide use in preclinical stages of drug development, in clinical trials [17].

Extraction of *Nyctanthes arbor-tristis* leaves: Extraction of *Nycthantes arbor- tristis* leaf was done by successive solvent extraction. Soxhlet apparatus was used for the successive solvent extraction. Leaves were grinded to coarse powder and the powder was divided into three different batches of 20g, each to standardize the extraction procedure in three consequent steps using soxhlet apparatus. Each batch was first extracted with petroleum ether (BP 40-60ºC) for defatting of crude drug. Finally each batch was extracted with ethanol of HPLC grade (BP 78.37ºC).

Estimation of oxidative stress marker: Drug induced lipid per-oxidation was measured by estimation of the content of malondialdehyde (MDA), acetyl cholinesterase (AChE), glutathione (GSH), and nitric oxide (NO) in brain blood sample of the rats. The determination was done by precipitating the protein substance using trichloroacetic acid (10% w/v), the protein free sample used for estimation of lipid per-oxidation parameters as follows.

Protocol for the estimation of Malondialdehyde (MDA) in brain homogenate:

Principle: It is heat- induced reaction; where in 1 mole of MDA reacts with 2 moles of TBA in an acid solution. It results in the formation of trimethene coloured complex.

Procedure: MDA were performed by the Ohkawa *et al.,* method. MDA was formed as an end product of lipid per-oxidation, which reacts with thiobarbituric acid and form faint pink coloured product. Mda was expressed as µM per mg protein 1 ml of supernatant was taken. 0.5ml of 30% of TCA (Trichloro acetic acid) and 0.3ml of 0.8% of thiobarbituric acid (TBA) was added in test tubes. The tubes were covered by the aluminium foil and kept in water bath for 30 min at 800 ºC. Then the mixture was kept in ice cool water for 30 min. After cooling, the colour was extracted in 1 ml butanol and the intensity was read at 540 nm using UV spectrophotometer [18].

Protocol for the estimation of Glutathione (GSH) in brain homogenate:

Principle: Reduced glutathione on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) produces a yellow coloured product that absorbs at 412 nm.

Procedure: Reduced glutathione (GSH) was estimated by the method which is described by Ellman. The assay contained 1 ml supernatant and 1 ml of 10% of TCA. This mixture was centrifuged for 10 min at 3000-4000 gyrations. Again the supernatant was collected and 10µl of supernatant, 2ml of 0.1 M phosphate buffer (pH-7.4), 0.5ml of DTNB (5,5'-dithio-bis(2- nitro benzoic acid) and 0.4 ml of distilled water were added and mixed. Absorbance of this mixture was measured at 412 nm within 15 minutes [19].

Protocol for the estimation of Nitric oxide (NO):

Procedure: The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined by a colorimetric assay with the Griess reagent [0.1% N-(1-napththyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 5% phosphoric acid]. Equal volumes of the supernatant and the Griess reagent were mixed and incubated for 10 min at room temperature in the dark. The absorbance was taken

at 540 nm using a spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve [20].

Estimation of Brain Acetyl cholinesterase (AChE) activity:

Principle of Acetyl cholinesterase: Acetyl cholinesterase efficiently catalyzes the hydrolysis of acetylthiocholine (ATCh) – sulfer analog of the natural substrate of these enzymes. Upon hydrolysis, this substrate analog produces acetate and thiocholine. Thiocholine, in the presence of the highly reactive DTNB ion generates a yellow colour, which is visible and can be quantitatively monitored by spectrophotometric absorption at 420 nm.

Procedure: On the fifth day rat was euthanized by cervical dislocation carefully to avoid any injury to tissues. The whole brain AChE activity was measured using the Ellman method. The end point was the formation of the yellow colour because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The resulting yellow colour was due to reduction of DTNB by certain substances in the brain homogenate and due to non enzymatic hydrolysis of substrate. After having calibrated the instrument, the change in absorbance per min of sample was read at 420nm [21].

Experimental protocol: Experimental protocol: Rats were allocated into eight experimental groups and each group contained six rats.

Group 1: Control group: Rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.) 60 minutes before the acquisition trial for four consecutive days and 60 minutes before the retrieval trial on day 5.

Group 2: Negative control group (Scopolamine induced amnesia): Rats were administered scopolamine (0.3mg/kg; i.p.) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 3: Positive control group (Piracetam treated group): Rats were administered piracetam (200mg/kg, i.p.) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 4: Positive control group (Extract low dose treated rats): Rats were administered extract (20 mg/kg, p.o.) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 5: Positive control group (Extract high dose treated rats): Rats were administered extract (40 mg/kg, p.o.) during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 6: Positive control group (Piracetam and Scopolamine treated rats): Rats were administered Piracetam (200mg/kg; i.p.) 60 minutes before and scopolamine (0.3mg/kg, i.p.) 30 minutes before during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 7: Positive control group (Extract low dose and Scopolamine treated rats): Rats were administered extract low dose (20mg/kg; i.p.) 60 minutes before and scopolamine (0.3mg/kg, i.p.) 30 minutes before during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

Group 8: Positive control group (Extract high dose and Scopolamine treated rats): Rats were administered extract high dose (40mg/kg; i.p.) 60 minutes before and scopolamine (0.3mg/kg, i.p.) 30

minutes before during acquisition trials conducted from day 1 to day 4 and then subjected to Morris water maze test. On day 5 rats were administered normal saline (0.9% sodium chloride solution; 10ml/kg, i.p.), and then subjected to retrieval trial after 60 minutes.

RESULTS AND DISCUSSIONS:

Pharmacological screening using Morris water maze model:

Effect of HeNAT on scopolamine induced changes in ELT during acquisition trials: HeNAT was investigated for its effect on scopolamine induced amnesia using Morris Water Maze test. Hydro alcoholic extract of the plant was administered at 20mg/kg, p. o. and 40mg/kg, p. o. doses in rats. The ELT of hydro alcoholic extract of *Nycthantes arbor –tritis* leaves (20mg/kg and 40mg/kg), conducted on four consecutive days are shown. It is noted that the scopolamine have a significantly increasing effect on ELT. Hydro alcoholic extract of the plant drug and piracitam have a decreasing effect.

TABLE 1: EFFECT OF HENAT ON SCOPOLAMINE INDUCED CHANGES IN ELT DURING ACQUISITION TRIALS

Groups	Treatments	Dose	ELT(sec) on Acquisition days			
			Day 1	Day 2	Day 3	Day4
1	Control (Normal saline)	10 ml/kg	119± 0.8	112.25± 4.47	95±4.2	76.50±7.66
2	Scopol-amine	0.4 mg/kg	119.25± 0.9	116.25± 3.12	102± 5.33	93±4.96
3	Piracetam	400 mg/kg	112.5± 1.2	103.5±2.23	87.25± 5.75	67±4.26
4	HeNAT	20 mg/kg	110.75±5.24	100.75±6.21	89.25± 5.32	71.25±3.97
5	HeNAT	40 mg/kg	101.25±4.8	97.5± 3.21	73±5.2	63.5± 4.32
6	Piracetam + Scopolamine	400+0.4 mg/kg	102.5±7.54	92± 3.42	76.25± 4.12	75±2.12
7	HeNAT + Scopolamine	20+ 0.4 mg/kg	72±4.8	67.5± 1.12	50.75± 1.24	46.5± 1.32
8	HeNAT + Scopolamine	40+ 0.4 mg /kg	74.75±3.23	63.5± 4.21	57.75± 2.13	52.25± 7.12

Effect of HeNAT on scopolamine induced changes in Time spent in target Quadrant (TSTQ) during retrieval trial using Morris water maze model: HeNAT was investigated for its effect on scopolamine induced amnesia using Morris Water Maze test. Hydroalcoholic extract of the plant was administered at 20mg/kg, p. o. and 40mg/kg, p. o. doses in rats. The TSTQ of hydro alcoholic extract of *Nycthantes arbor –tritis* leaves (20mg/kg and 40mg/kg), conducted on fifth day are shown. Note that while the scopolamine has a significantly decreasing effect on TSTQ, hydroalcoholic extract of the plant drug and piracetam has an increasing effect.

Scopolamine treated rats decreased time spent in target quadrant as compared to target quadrant (Q4) of control group. But HeNAT increased time spent in target quadrant and showed protection against scopolamine induced amnesia. Results were expressed as mean ± S.E.M. with n=6 in each group.

a = p≤0.05 Vs time spent in target quadrant (TSTQ) in control group; b = p≤0.05 Vs time spent in target quadrant (TSTQ) in Scopolamine treated group.

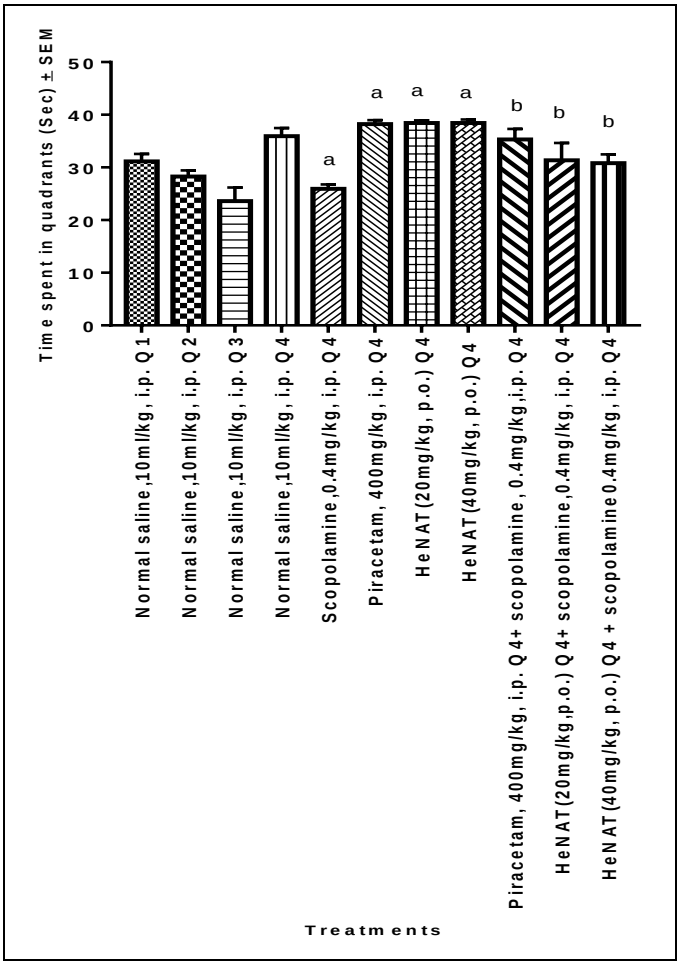

FIG. 1: EFFECT OF HENAT IN TIME SPENT IN TARGET QUADRENT (TSTQ) DURING RETRIEVAL TRIAL

Effect of HeNAT on scopolamine induced changes in Transfer Latency Time (TLT) of rats using Elevated plus-maze model: The effect of hydro alcoholic extract of *Nycthantes arbor –tristis* leaves on TLT at doses of 20 mg/kg, p.o. and 40 mg/kg, p.o. was observed against scopolamine induced amnesia in rats using Elevated plus maze test. HeNAT and piracetam was found to increase the TLT and restore memory function at higher dose.

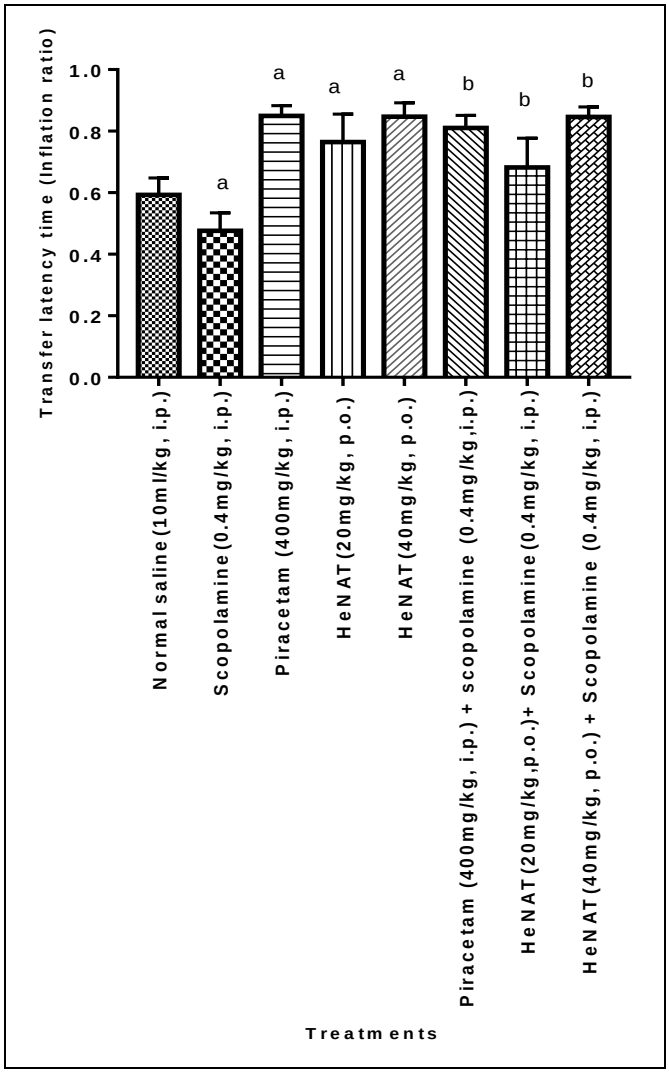

FIG. 2: EFFECT OF HENAT IN TRANSFER LATENCY TIME (TLT) OF RATS USING ELEVATED PLUS-MAZE MODEL

In case of scopolamine treated rats, significant decrease in Transfer Latency Time (TLT) was noted. But HeNAT and piracetam treated group increased TLT in Elevated plus maze. Results were expressed as mean ± S.E.M. with n=6 in each group. a = p≤0.05 Vs TLT in control group; b = p≤0.05 Vs TLT in scopolamine treated group.

Biochemical parameters:

Effect of HeNAT on MDA level: The MDA level for control (normal saline), standard drug (Piracetam), amnestic agent (scopolamine) and plant drug (HeNAT) are shown. It is noted that while the amnestic agent has a significantly increasing effect on MDA level, the normal saline and plant drug have a decreasing effect. Each value represents mean MDA level ± S.E.M.

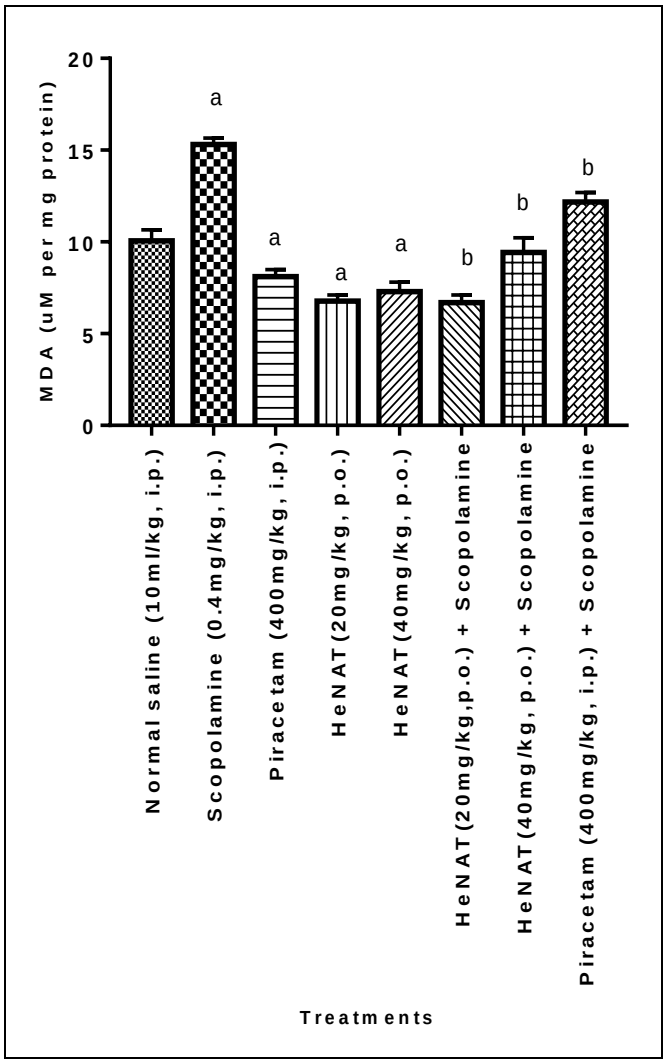

FIG. 3: EFFECT OF HENAT ON MDA LEVEL

Bar graph representing effect of HeNAT on Brain MDA level: As compared to control group, significant increase in MDA level in scopolamine treated group was noted. Piracetam, *Nyctanthes arbor-tristis* (20mg/kg, p.o. and 40mg/kg, p.o.) treated group decreased MDA level. Results were expressed as mean ± S.E.M. with n=6 in each group. a = p≤0.05 Vs Brain MDA level in control group; b = p≤0.05 Vs MDA level in Scopolamine treated group.

Effect of HeNAT on GSH level: The GSH level for control (normal saline), standard drug (Piracetam), amnestic agent (scopolamine) and plant drug (HeNAT) are shown. It is noted that while the amnestic agent has a significantly decreasing effect on GSH level, the normal saline and plant drug have an increasing effect. Each value represents mean GSH level ± S.E.M.

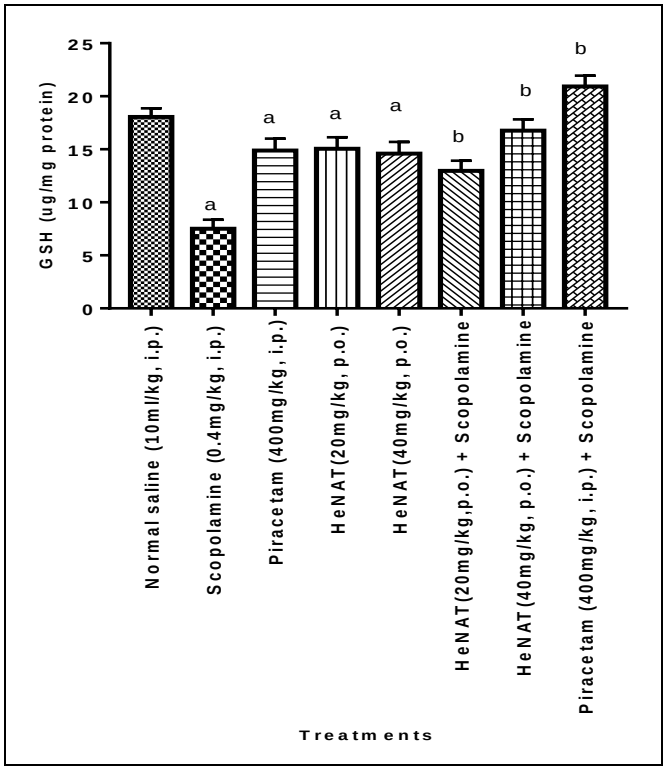

FIG. 4: EFFECT OF HENAT ON GSH LEVEL

Bar graph representing effect of *Nyctanthes arbor-tristis* (hydroalcoholic leaf extract) on GSH level: As compared to control group, significant decrease in GSH level in scopolamine treated group was noted. Piracetam, *Nyctanthes arbor-tristis* (20mg/kg, p.o. and 40mg/kg, p.o.) treated group increased GSH level. Results were expressed as mean ± S.E.M. with n=6 in each group. a = p≤0.05 Vs GSH level in control group; b = p≤0.05 Vs GSH level in Scopolamine treated group

Effect of HeNAT on Nitric oxide level: The nitric oxide level for control (normal saline), standard drug (Piracetam), amnestic agent (scopolamine) and plant drug are shown. It is noted that while the amnestic agent has a significantly increasing effect on nitric oxide level, the normal saline and plant drug have a decreasing effect. Each value represents mean nitric oxide level ± S.E.M.

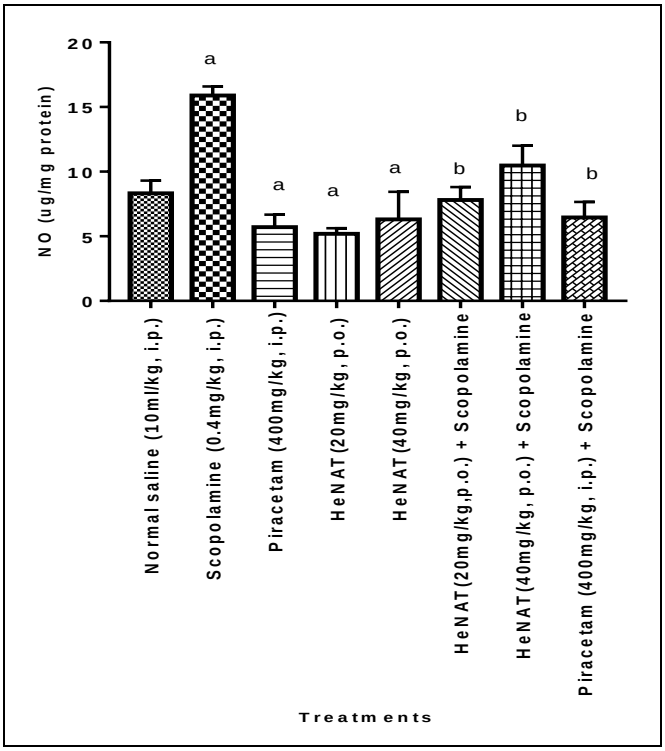

FIG. 5: EFFECT OF HENAT ON NITRIC OXIDE LEVEL

Bar graph representing effect of HeNAT on NO level: As compared to control group, significant increase in NO level in scopolamine treated group was noted. Piracetam, *Nyctanthes arbor-tristis* (20mg/kg, p.o. and 40mg/kg, p.o.) treated group decreased NO level. Results were expressed as mean ± S.E.M. with n=6 in each group. a = p≤0.05 Vs NO level in control group; b = p≤0.05 Vs NO level in Scopolamine treated group.

Effect of HeNAT on AChE level: The AChE level for control (normal saline), standard drug (Piracetam), amnestic agent (scopolamine) and plant drug (HeNAT) are shown. It is noted that while the amnestic agent has a significantly increasing effect on nitric oxide level, the normal saline and plant drug have a decreasing effect. Each value represents mean AChE level ± S.E.M. Bar graph representing effect of HeNAT on AChE level: As compared to control group, significant increase in AChE level in scopolamine treated group was noted. Piracetam, *Nyctanthes arbor-tristis* (20mg/kg, p.o. and 40mg/kg, p.o.) treated group decreased AChE level. Results were expressed as mean ± S.E.M. with n=6 in each group. a = p≤0.05 Vs AChE level in control group; b = p≤0.05 Vs AChE level in Scopolamine treated group

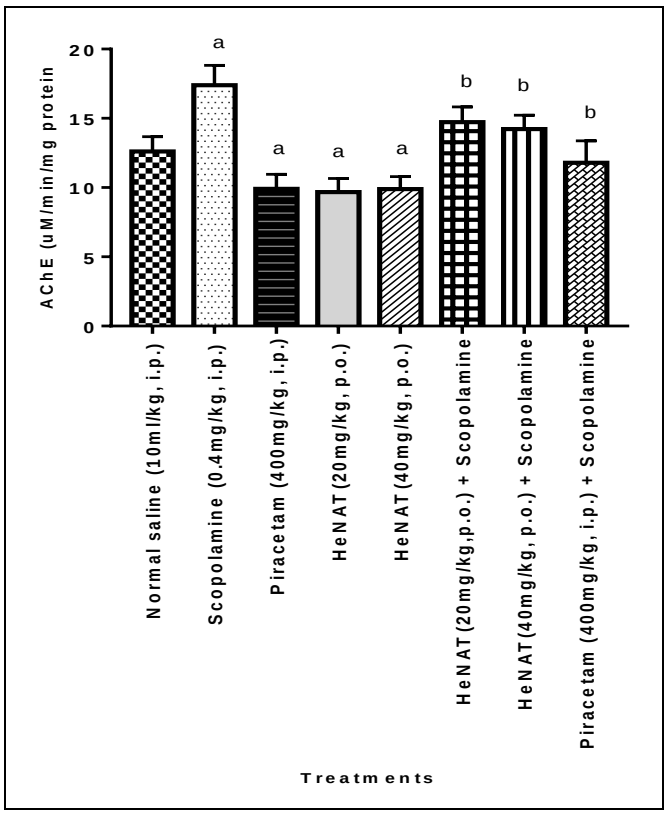

FIG. 6: EFFECT OF HENAT ON ACHE LEVEL

CONCLUSION: The plant extract was investigated for its effect on scopolamine induced amnesia using morris water maze test. After injection of scopolamine (0.4mg/kg, i.p.), rats showed impairment of memory compared to that of control group in which the little change in escape latency time was noted to find the hidden platform. Plant extract was administered different doses (20, 40 mg/kg, i.p.), decrease in escape latency time to find the hidden platform and the time spent by the rats in the target quadrant was taken as the index of retrieval. The HeNAT significantly the amnesia induced by the scopolamine. Further estimation of TLT was done with the help of elevated plus maze. TLT was measured on 4 and 5 day of study. Plant drug extract was found to increase the TLT. Thus the plant was found to have a nootropic effect.

The *in-vivo* study of *Nycthanthes arbor-tristis* was further supported by the estimation of biochemical parameter like brain AChE activity, GSH estimation, MDA estimation and NO estimation. On the basis of results it can be conducted that HeNAT leaves improve learning and memory.

ACKNOWLEDGEMENTS: I take this privilege and pleasure to acknowledge Prof. Dr. Satish Kumar Gupta, Director, Department of Pharmaceutical Technology, M.I.E.T., Meerut who has been inspirational and supportive throughout my work undertaken and endowed me with the most precious knowledge to see success in my endeavour. I consider myself most lucky to place on record my heartfelt gratitude to Dr. Vipin Kumar Garg H.O.D. Department of Pharmaceutical Technology, M.I.E.T, Meerut for providing me a very cordial and conductive environment for the work and also for his timely help and constant encouragement.

REFERENCES:

1. Sibi, P. I. and Delphia, P. G., Nootropic Studies of Ethanolic Extract of *Mimosa pudica* Linn. in Albino Wistar rats. American Journal of Phytomedicine Clinical Therapy, 2014; 1:266-275.
2. Grottick AJ.; Higgins GA.; Assessing a vigilance decrement in aged rats: effects of pre-feeding, task manipulation, and psychostimulants. An article PubMed of psychopharmacology, 2002, 164: 33-41.
3. Jo YS; Park EH; Kim IH; Park SK, Kim H, Kim HT *et al.* the medial prefrontal cortex is involved in spatial memory reterival under partial-cue conditions. An aricle PubMed of J Neuroscience, 2007, 27: 13567-13578.
4. Lee AC; Robbins TW, Pickard JD; Owen AM. Asymmetric frontal activation during episodic memory: the effects of stimulus type on encoding and reterival. An aricle PubMed of neuropsycologia, 2000**,** 38: 677-692.
5. Nutt DJ. The neuropharmacology of serotonin ana noradrenaline in depression, 2002**,** 17:S1-12.
6. Saxena R.S, Gupta B, Saxena K.K, Srivastava V.K, Prasad D.N, *et al*., Analgesic, anthipyretic and ulcerogenic activity of *Nyctanthes arbor-tristis* leaf extract Journal of ethanopharmacology, 1987, 19(2): 192-200.
7. Rathor A, Juneja R.K, Tandon J.S. An iridoid glycoside from Nyctanthes arbor-tristis. Phytochemistry. 1987**,** 28(7):1913-17.
8. Rathor A, Rivastava V, Srivatava K.C, Tondon J.S. Iridoid glucoside from Nyctanthes arbor-tristis. Phytochemistry, 1990**,** 29(6):1917-20.
9. Ratnasooriya WD, Jayakody JRAC, Hettiarachchi ADI and Dharmasiri MG. Sedative Effect of Hot Infusion of *Nyctanthes arbor-tristis* on Rats. Pharmaceutical Biology. 2015; 43(2):140-146.
10. Paul BN and Saxena AK. Depletion of tumornecrosis factor-c-in mice by Nyctanthes arbortristis. J Ethanopharmacol. 1997; 56:153-158.
11. Khatune N.A, Mosaddik A.M, Haque EM., Antibacterial activity and cytotoxicity of *Nyctanthes arbor-tristis* flowers. Fitoterapia, 2001**;** 72(4): 17-20.
12. Saxena RS, Gupta B, Lata S., Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor-tristis* leaf extract. Journal of ethanopharmacology, 2002**;** 81(3): 321-25.
13. Parle M, Singh N. Animal models for testing memory. Asia Pac J Pharmacol 2014; 16: 101-120.
14. Hampson RE, Rogers G, Lynch G, Deadwyler SA. Facilitative effects of the ampakine CX516 on short- term memory in rats: correlations with hippocampal neuronal activity. An Article of J Neurosci, 1998a, 18: 2748-2763.

15. Morris, R.G.M., Developments of water maze producer of studying spatial learning in the rats. J. Neurosci Meth, 1984**,** 11: 47-60.
16. Safer DJ, Allen RP. The central effects of scopolamine in man. An Article PubMed of Biol Psychiatry, 1971, 3: 347-355.
17. Sharma AC, Kulkarni SK. Reversal of scopolamine and diazocliping induced memory dysfunction by angiotensin converting enzymes inhibitor in rats and mice, Indian J. Pharmacol, 1992, 24: 147-53.
18. Okhawa, H., Ohishi, N., Yagi, K., Assay for lipid per-oxidation in animal tissue by thiobarbituric acid reaction. Analytical Biochemistry, 1979, 95(2): 351-358.
19. Nickerson M. Drugs. In: Goodman LS, Gilman (editors). The Pharmacological Basis of Therapeutics. New-York: Macmillan; 2013, p 727-743.
20. Beutler E., Duron O., Kelly B., Reduced glutathione estimation. Journal of Lab Clinical Medicin, 1963, 61, 82.
21. Ellman G.L., Courtney, K.D., Andres, J.V., Feather-stone, R.M. A new and colorimetric determination of acetyl cholinesterase activity. Biochem Pharmacology, 1961, 7: 81-95.
22. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 2001; 36:60–90.
23. Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacol 1984; 101 (1):27-33.
24. Banerjee A, Paddar A, Ghanta S, Chakraborty A, Chattopadhyay S., *Nyctanthes arbor-tristis*. Spectrum of its bioactivity potential. Planta medica 2007; 73(9), 78-82.

How to cite this article:
Antal S, Ashwlayan VD and Kumar A: Protective effect of *nycthanthes arbor-tristis* on scopolamine induced amnesia in rats "a behavioural and biochemical approach". Int J Pharm Sci Res 2017; 8(3): 1346-55.doi: 10.13040/IJPSR.0975-8232.8(3).1346-55.