Cognition Enhancing and Antioxidant Activities of *Nepeta hindostana* (Roth) Haines flowers extract against Scopolamine-induced amnesia in Sprague Dawley rats

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ABSTRACT

The effects of hydro-alcoholic extract of *Nepeta hindostana* flowers on neurobehavioral and cognitive functions in rats with Scopolamine-induced amnesia were investigated here. Rats were randomly divided into five groups each containing five animals (n=5). Test groups, rats were pre-treated orally with hydroalcoholic extract of *Nepeta hindostana* flowers at a dose of 100 and 200 mg/kg, b. wt., for 28 days. On the 28^{th} day, a single i.p. injection of Scopolamine (1.0 mg/kg, b. wt.) was given in each rat. Standard group, Rivastigmine (1.5 mg/kg, orally), an anti-cholinesterase drug was given. Behavioral responses locomotor activity and step down latency (SDL) in passive avoidance response were observed. Acetylcholinesterase (AChE) activity and other relevant biochemical markers including antioxidant status in hippocampus of rats were assessed. Administration of *Nepeta hindostana* flowers extract at two different doses (100 and 200 mg/kg, body weight) for 28 days significantly ($P<0.05$) restored memory and learning impairments in rats induced by Scopolamine. Step-down latency period (acquisition SDL/retention SDL), and locomotor activity, were significantly ($P<0.01$) decreased whereas the brain regions. AChE activity was increased significantly ($P<0.05$) in Scopolamine-treated rats as compared to normal control rats. Current study demonstrates cognition enhancing properties of the hydro-alcoholic extract of Nepeta hindostana flowers.

Keywords: Amnesia, Antioxidant, *Nepeta hindostana*, Rivastigmine, Scopolamine

1. Introduction

Traditional medicinal plants are utilized for the preservation of good health and better quality of life. A large percentage of the population in developing countries still depends on traditional medicinal plants to meet their primary health care needs.[1] Medicinal plants have provided a variety of potent compounds that are extremely useful in the prevention of several ailments.[2] Many plants have also been used in the prevention of several neurodegenerative diseases including Alzheimer's disease. Medicinal plants such as *Withania somnifera*, *Bacopa monnieri*, *Convolvulus pluricaulis*, *Ginkgo biloba*, *Panax ginseng*, *Curcuma longa*, *Glycyrrhiza glabra*, *Coffea Arabica*, *Camellia sinensis*, *Rosa damascene*, *Calendula officinalis* and several other plant extracts alone or in combinations have been used for the neuroprotective effects.[3,4] Many polyherbal formulations comprising the above-mentioned plant ingredients were widely used in traditional systems of medicine.[5] These formulations based on medicinal plants were known to be safe and effective in the treatment of several forms of brain disorders including memory impairments.[6] Memory impairment (amnesia) is caused by damage to the brain that affects the hippocampus along with the other structures associated with long term memory.[7] Amnesia refers to a partial or complete loss of memory depending upon the extent of damage to the brain.[8] There are many types of amnesia and different types of amnesia can have a diverse impact on learning and memory patterns.[9] The brain's cholinergic system is implicated in the cognitive processes of memory and attention.[10] Scopolamine is a muscarinic cholinergic receptor antagonist which impairs learning and memory process in mammals.[11] The animal model of Scopolamine-induced amnesia is a well-established model and this model can be used to study underlying mechanisms and management of cognitive dysfunctions in neurodegenerative diseases such as Alzheimer's disease. Manipulations of Acetylcholine in the cortex, hippocampus, amygdala, and striatum region of the brain regulate learning and memory processes.[12] The cholinesterase inhibitor, Rivastigmine is a widely recommended drug for cognitive dysfunctions in mild to moderate form of Alzheimer's disease.[13] In traditional systems of medicine, different parts of the plant *Nepeta hindostana* of family Lamiaceae are utilized as a component of various polyherbal formulations. The plant is known as "Badrang-e-Boya" and its decoction is useful as a gargle for sore throat.[14] In the Unani system of medicine, it is used as a sedative, resolvent, tonic, as antipyretic as well as in the treatment of cardiac asthma and syncope.[15,16] Alcoholic extract of *the Nepeta hindostana* plant has been reported to possess hypocholesterolemic activity.[17] Essential oils from leaf, stem, flower and aerial part of *Nepeta hindostana* also represented a potential reservoir of molecules with potent antioxidant and antimicrobial potentials.[18] The main chemical constituents of the plant are a triterpenoid aldehyde nepehinal whereas other terpenoids include nepetidone, nepedinol, nepeticin and triterpenic acid.[17,19] Abana®, an Ayurvedic herbomineral preparation that contains *Nepeta hindostana* as one of its ingredients.[20] Vasudevan and Parle 2008 have examined the anti-dementia potential of Abana® on Scopolamine and diazepam-induced memory impairments in young and aged rats.[21] Above formulation also effectively produced a dose-dependent

improvement in memory and reversed the amnesia in rats.[20,21] Several reports indicate that the selected medicinal plant *Nepeta hindostana* is traditionally utilized in the treatment of memory disorders. However, there is partial scientific evidence is available concerning neuroprotective effects of a hydro-alcoholic extract obtained from *Nepata hindostana* flowers. Hence, the present study was aimed to investigate the effect of *Nepata hindostana* flower extract on memory and brain cholinesterase activity in impaired rats.

2. Materials and methods

2.1. Reagents and instruments

All the reagents and chemicals used in this study were of analytical grade. Folin-Coicalteau Phenol reagent, Ethanol, and Methanol were purchased from Fisher Scientific Ltd. (Mumbai), India. Ethyl Acetate, Bovine Serum Albumin (BSA), and DTNB [5, 5-dithiobis (2-nitrobenzoic acid)] were procured from HiMedia Laboratories (P) Ltd., Mumbai, India. Diethyl ether and Acetic Acid were obtained from S.D. Fine Chemical Limited (Mumbai), India. Formic Acid (Rankem), Sodium Nitrite (Sigma-Aldrich), and Rivastigmine from Dr. Reddy's Laboratories, India. Rotary evaporator (Buchi Rotavapor-R; Labco, India), and UV-Spectrophotometer (Model UV-1700) was from Shimadzu scientific instruments, Shimadzu Corporation (Tokyo), Japan.

2.1. Procurement and authentication of the plant materials

The plant material was obtained from Hamdard Dawakhana, Aminabad, Lucknow, India. The plant sample was authenticated and a voucher specimen (IU/PHAR/HRB/15/21) of *Nepeta hindostana* flowers was deposited in the herbarium of the department.

2.2. Preparation of flowers extract and calculation of an extractive value

The procured flowers of *Nepeta hindostana* were dried in shade and further subjected to a coarse powder with the help of a mechanical grinder. Extraction was carried out using a 50% hydroalcoholic solvent through cold maceration for 72 hours with intermittent agitation. Finally, the extract was concentrated to dryness using vacuum rotary evaporator. The extractive value in terms of % yield was calculated.

2.3. Experimental animals

Animals used were adult male *Sprague Dawley* rats (*Rattus norvegicus*) weighing between 150-200 g. These rats were procured from the animal house facility of CSIR-CDRI (Central Drug Research Institute), Lucknow, India. The rats were housed separately in polypropylene cages under standard laboratory conditions (i.e. 12 hours light/12 hours dark cycle, 23±2°C and relative humidity of 50-60%) maintained throughout the experimental period. Throughout the experimental periods, rats were kept on a standard pellet diet and provided drinking water ad libitum. All the experiments on the animals were performed according to the guidelines of the CPCSEA after approval of this study protocol (Approval#IU/Pharm/M.Pharm/IAEC/15/12).

2.4. Acute oral toxicity study

Acute oral toxicity test of the hydro-alcoholic extract of *Nepeta hindostana* was evaluated and all the methods were followed as per the OECD TG423 guidelines. The hydro-alcoholic extract at the doses of 5, 50, 300, and 2,000 mg/kg body weight, orally administered to different groups. The animals were observed for about 30 minutes for any signs of toxicity, mortality or morbidity and further observations were made every eight hours for the first 24 hours after administration of extract and followed by two weeks for any signs of delayed toxicity.[22]

2.5. Experimental design

Experimental design of Scopolamine-induced amnesia was adopted to study the possible effects of the hydro-alcoholic extract of N. hindostana flowers on cognitive functions in rats. After the acclimatization period, the rats were divided into 5 groups consisting of five rats in each group (n=5). Group, I rats served as vehicle control and received Saline (10 mL/kg, body weight, per oral) once daily for 28 days. Group II rats served as untreated control and administered the vehicle followed by Scopolamine (1.0 mg/kg, body weight i.p.) on the 28th day. Group III served as the standard group and received standard drug Rivastigmine (1.5 mg/kg, body weight) for 28 days. Group IV and V represented the treatment groups and orally received the hydro-alcoholic extract of *N. hindostana* flowers at the dose of 100 and 200 mg/kg, body weight respectively throughout the study duration. A few hours after the final dosing on day 28th day, rats of all groups except group-I were subjected to Scopolamine (1.0 mg/kg, i.p.). All the neurobehavioral activities were evaluated 45 minutes after the Scopolamine administration. The locomotor activity was assessed using an actophotometer whereas the acquisition trail that corresponds to learning and memory was performed by the passive avoidance paradigm. After 24 hours of Scopolamine administration, the retention trial was carried out and again the acquisition and retention trials were performed to assess as an index of memory. Then the rats were euthanized through cervical delocalization followed by decapitation and brains were removed for evaluation of Nitric oxide in brain tissues in addition to the brain Acetylcholinesterase (AChE) activity.[23]

2.6. Passive shock avoidance paradigm in rats

A passive shock avoidance test was used to examine long term memory (Kulkarni et al. 2010). This apparatus is comprised of a box having three walls of wood (27×27×27 cm3). One wall of Plexiglas and a grid floor made up of a 3 mm stainless steel rod set that was 8 mm apart from the wooden platform (10×7×1.7 cm3) kept in the center which was illuminated with a 15W bulb. During the training session, each rat was placed on the above wooden platform. Electric shock (50 Hz, 1.5 mA) for 1 second was given to the grid floor when the rat stepped down and placed its paw on the grid floor. The step-down latency (SDL, the time taken by the rat to step down and place all four paws on grid floor) was documented and the rats showing it in the range of 2-15 seconds were selected for the acquisition and retention tasks. After 90 min of the training session, the acquisition task was carried out and the rats were removed from the shock-free zone

if they did not step down within 60 seconds. Following 24 hours, the retention task was tested similarly except with an upper cut-off time of 180 seconds.[23]

2.7. Locomotor activity

Evaluation of locomotor activity (horizontal activity) was done using an actophotometer.[23,24] Each rat was placed in the activity cage for 5 minutes and basal activity was recorded. Thereafter, the individual rat from each group was given their respective treatment as per the experimental protocol. After 60 minutes of treatments, the rats were placed in the activity cage for recording the activity score as taken earlier.

2.8. Brain Acetylcholinesterase activity

After sacrificing the animals through instant decapitation, the whole brain was quickly removed and kept in an ice bath. Rats hippocampus were separated and a known amount of the tissues were homogenized in 0.03M sodium phosphate buffer (pH 7.4) to get a 10% (w/v) homogenate that was centrifuged at 10,000 rpm for 15 minutes using a cooling centrifuge at 4°C. Following centrifugation, 1.0 ml of the supernatant was mixed with 9.0 ml of sucrose solution to get a 1% post-mitochondrial supernatant (PMS). AChE activity in hippocampus tissues were measured using the above 1% PMS. This assay was based on the principle that the rate of formation of yellow-colored thiocholine from Acetylthiocholine iodide in the presence of DTNB [dithio-bis (2-nitrobenzoic acid)] increases with the increase in tissue cholinesterase. As per the Ellman‘s method (1961), the processed tissue sample (0.1 ml of 1% PMS) was mixed in a test tube with 0.1 mM, sodium phosphate buffer (pH 8.0) and pre-incubated at 37°C for 5 minutes. The reaction was initiated by adding 0.1 ml of the substrate (30 mM acetylthiocholine iodide) in the above reaction mixture and the absorbance was recorded at the wavelength of 412 nm against reagent blank at the 1-minute interval for 3 minutes. The specific activity of AChE was calculated using a suitable formula. AChE activity was expressed as moles/min/mg of protein.[25]

2.9. Measurement of hippocampus tissue nitrite

The Griess reagent system was used for the estimation of nitrite in the hippocampus of the rat brain. This nitrate reductase method is based on the chemical reaction, which uses Sulphanilamide and N-1- naphthyl ethylenediamine dihydrochloride (NED) under acidic conditions govern by phosphoric acid. This assay is based upon the two-step diazotization reaction where acidified nitrite produces a nitrosating agent that reacts with Sulfanilic acid to produce the diazonium ion. This ion is coupled with NED to form the chromophoric azo-derivative which absorbs light at 540-570 nm.[26] Identical volumes of Griess reagent and brain homogenate were treated with reagents 1mM NADPH, Nitrate reductase 1.0 U/mL and 1 M Zinc acetate and incubated at 37°C for 30 minutes. The absorbance was recorded spectrophotometrically at a wavelength of 542 nm using Elisa plate reader and values were expressed as µg/mg protein.

2.10. Assessment of oxidative stress parameters:

2.10.1. Assay of lipid peroxidation in hippocampus of rat

Lipid peroxidation level was determined by measuring the amount of malondialdehyde (MDA) in brain tissues. Thiobarbituric acid reactive substances (TBARS) assays were used to detect the amount of MDA formed as a byproduct of lipid peroxidation. In triplicate 0.1 ml of brain tissues, the homogenate was mixed in 0.45 ml of 5%TCA and 0.45 ml of 0.67% TBA in a test-tube and centrifuged at 10,000 rpm for 10 minutes. All the sample tubes were then covered with aluminum foils and kept in a shaking water bath at 80°C temperature for 30 minutes followed by cooling under ice-cold water for 10 minutes. These test tubes were again centrifuged at 3,000 rpm for 15 minutes and the supernatants were collected. The absorbance of the generated pink color chromophore was read against the blank at 535 nm using a spectrophotometer. The level of lipid peroxides was expressed as in µmoles of MDA formed/mg of protein.[27]

2.10.2. Assay of reduced glutathione (GSH) in hippocampus of rat

Reduced glutathione (GSH) in brain tissues was estimated using the method as described by Ellman, (1959). In this colorimetric procedure, equal quantities of brain tissue homogenate and 10% trichloroacetic acid (TCA) solution were mixed and centrifuged at 3,000 rpm for 15 minutes. In 0.01 ml of supernatant from the above mixture was mixed with 0.5 mL 5,5-dithiobisnitro benzoic acid (DTNB), 2 mL of phosphate buffer (pH 7.4), and 0.4 mL of double-distilled water. Finally, the absorbance was recorded against reagent blank at a wavelength of 412 nm within 5 minutes of the addition of DTNB. The GSH content was calculated and presented as µg/mg of protein.[28]

2.11. Statistical analysis

The data are presented as mean ± SEM (standard error of the mean). All the measurements were carried out in triplicates. Statistical analyses were performed through ANOVA followed by Dunnett's test using Graph-Pad InStat 3 software. Values of the $p<0.05$, $p<0.01$ and $p<0.001$ were considered as statistically significant.

3. Results

The extractive value of the hydro-alcoholic extract of N. hindostana flowers (% yield of extract) was found to be 9.48±45% w/w. Oral administration of hydro-alcoholic extracts of *N. hindostana* flowers in rats at 2000 mg/kg, body weight during acute toxicity study was found to be devoid of any toxicity or mortality in rats.

3.1. Effect on the step-through latency with passive shock avoidance paradigm in rats

The results of acquisition and retention performance are given in Table 1. In Scopolamine treated group (Group-II), the step-down latency period (acquisition SDL and retention SDL) as well as locomotor activity significantly decreased ($p<0.01$) when compared with the normal control

group (Group-I). Whereas, these were increased significantly ($p<0.01$) with all the doses of hydroalcoholic extract of N. hindostana flowers (100 and 200 mg/kg, body weight) of hydro-alcoholic extract of N. hindostana flowers and Rivastigmine-treated groups (i.e. Group III, IV & V) respectively in comparison to Scopolamine-treated control (Group II) as depicted from Fig. 1.

3.2. Effect of Nepeta hindostana flowers extract on the locomotor activity

The locomotor activity was significantly decreased ($p<0.01$) in the Scopolamine-treated group (Group II) as compared with the Normal control (Group I) as presented in Table 1 and Fig. 1B. Although the oral administration of *Nepeta hindostana* flowers extract (100 and 200 mg/kg, body weight) and Rivastigmine (1.5 mg/kg, body weight) significantly increased ($p<0.01$) the locomotor activities (Fig. 1), when compared with the Scopolamine-treated rats (Group-II).

3.3. Effect of Nepeta hindostana flowers extract on brain AChE activity

As depicted in Table 2, the brain AChE activity was significantly increased ($p<0.01$) in Scopolamine-treated rats (Group II), as compared with the Normal control (Group I). While the AChE activity (Fig. 1C) of N. hindostana flower extract and Rivastigmine treated group was significantly decreased ($p<0.01$) when compared with the Scopolamine-treated group (Group II).

3.4. Effect of Nepeta hindostana flowers extract on tissue Nitrite levels in rats brain

In Fig. 1, the brain tissues Nitric oxide levels were significantly increased ($p<0.01$) in Scopolamine-treated rats (Group II), when compared with the normal control rats (Group I). However, the tissue Nitrite levels were significantly decreased ($p<0.01$) after treatment with N. hindostana flower extract and Rivastigmine respectively (Table 2), when compared with the Scopolamine-treated rats (Group II).

Table 1 Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers extract on specific learning and memory functions

Treatment groups ⇨ / **Specific learning and memory functions** ⇩		**Group I** (Normal control) Normal saline, 10 ml/kg, *p.o.*	**Group II** (Untreated) Normal saline, 10 ml/kg, *p.o.*± Scopolamine, 1 mg/kg, *i.p.*	**Group III** (Standard drug) Rivastigmine 1.5 mg/kg, *p.o* ± Scopolamine, 1.0 mg/kg, *i.p.*	**Group IV** (Test drug dose-I) 100 mg/kg, *p.o.*± Scopolamine, 1 mg/kg, *i.p.*	**Group V** (Test drug dose-II) 200 mg/kg, *p.o.*± Scopolamine, 1 mg/kg, *i.p.*
Passive shock avoidance paradigm	Acquisition step-down latency (sec)	4.83±0.47	2.17±0.17**	3.5±0.72$^{\#\#}$	3.00±0.58$^{\#\#}$	3.16±0.87$^{\#\#}$
	Retention step-down latency (sec)	9.16±0.60	4.83±0.30**	7.00±2.13$^{\#\#}$	6.00±1,5$^{\#\#}$	6.67±2.35$^{\#\#}$
Locomotor activity	Activity score on 28th day	157.75±6.22	100.5±4.5**	165.25±4.09$^{\#\#}$	163.5±3.62$^{\#\#}$	160.75±2.87$^{\#\#}$

All the measurements were carried out in triplicates. The data are presented as mean ± SEM (*n=5*).
$^{*}P<0.05$ and $^{***}P<0.001$ Vs Normal control (Group I), $^{\#}P<0.05$, and $^{\#\#}P<0.001$ Vs Scopolamine treated (Group II)

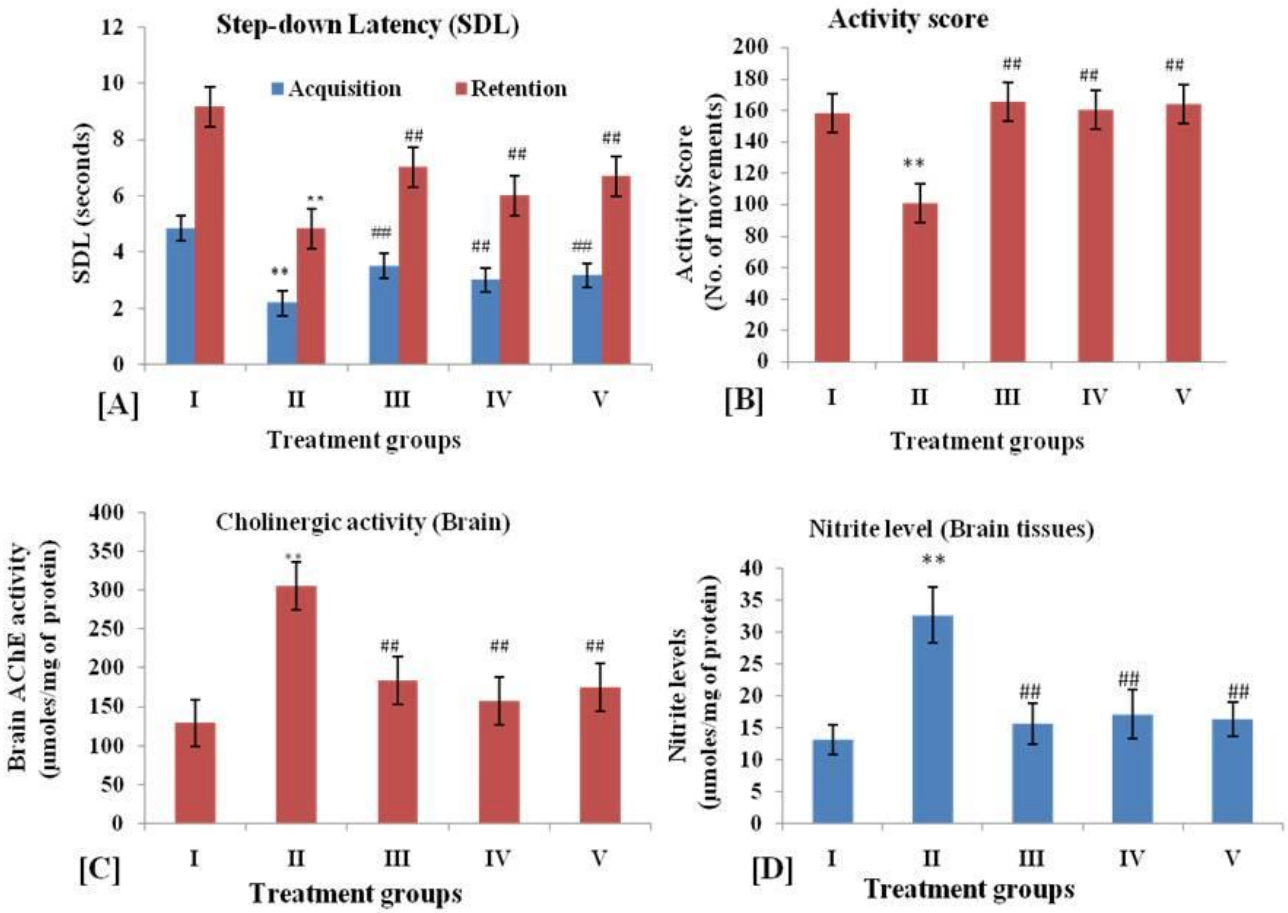

Fig. 1 Effect of different treatments on specific learning and memory functions in rats: [A] Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers on Step-down latency (SDL) in passive shock avoidance response in rats, **[B]** Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers on Locomotor activity, **[C]** Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers on Brain tissues AChE activity in rats, and **[D]** Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers on tissues Nitrite levels in rats brain [Data are expressed as mean±SEM *(n=5).* $^{**}p<0.01$ vs. Normal control and $^{\#\#}p<0.01$ vs. Untreated control].

3.5. Effect of Nepeta hindostana flowers extract on lipid peroxidation (LPO) in brain tissues

Results for lipid peroxidation in brain tissues in terms of MDA contents are shown in Table 2. Exposure to Scopolamine significantly increased ($p<0.01$) the MDA contents in brain tissues as compared to normal rats (Group I). It was observed that the administration of the hydroalcoholic extract of *Nepeta hindostana* flowers at two different doses (100 and 200 mg/kg, body weight)

for 28 days, significantly reduced the MDA contents in rat's brains. Moreover, Rivastigmine treatment at a dose of 1.5 mg/kg, body weight also reduced the MDA contents when compared to Scopolamine treated rats (Group II).

3.6. Effect of Nepeta hindostana flowers extract on GSH contents in brain tissues

The GSH content was significantly decreased ($p<0.001$) in the brain tissue of Scopolamine treated rats (Group II) from 4.27 ± 0.14 to 1.64 ± 0.06 µmoles/mg protein as compared with normal rats (Group I). Supplementation of the hydro-alcoholic extract of *Nepeta hindostana* flowers at two different doses (100 and 200 mg/kg, body weight) for 28 days, resulted in a significant increase ($p<0.001$) in GSH contents in rats brain (Table 2). Similarly, Rivastigmine (standard drug) treatment to the Scopolamine-induced amnesic rats (Group III) resulted in a significant increase ($p<0.001$) in GSH contents from 1.64 ± 0. 06 to 3.93 ± 0.15 (µmoles/mg, of protein). Moreover, remarkable improvements in non-enzymatic antioxidant glutathione (GSH) levels in brain tissues were observed after treatment with test drugs.

4. Discussion

Amnesia is a neurodegenerative disorder associated with a decline in cognitive abilities including learning, and memory. Despite the severity and high prevalence rate of amnesia, there is no established treatment in the allopathic system of medicine. Many therapeutic interventions in this area have failed either due to serious adverse effects or non-significant benefits. Thus, it is highly desired to investigate and employ different traditional medicinal plants that can be utilized for memory enhancing (nootropic) activity. Traditionally, *Nepeta hindostana* plant is known to be a very effective memory enhancer and various parts of this plant are used as brain tonic both in Unani and Ayurvedic systems of medicine.

Table 2 Effect of hydro-alcoholic extract of *Nepeta hindostana* flowers on brain AChE, Nitrite, MDA, and GSH levels in rats

Treatment groups	AChE activity in brain tissues (µmoles/min/mg protein)	Brain tissue Nitrite (µmol/mg of protein)	MDA (µmol/mg of protein)	GSH (µmol/mg of protein)
Group I (Control) Normal saline, 10 ml/kg, *per oral*	129.19 ± 5.49	13.11 ± 2.28	0.73 ± 0.06	4.27 ± 0.14
Group II (Untreated) Scopolamine, 1 mg/kg, *intraperitoneal*	305.62 ± 20.99**	32.58 ± 4.36**	4.2 ± 0.34**	1.64 ± 0. 06**
Group III (Standard) Rivastigmine, 1.5 mg/kg, *per oral* ± Scopolamine, 1 mg/kg, *intraperitoneal*	183.85 ± 5.33$^{\#\#}$	15.65 ± 3.16$^{\#\#}$	1.23 ± 0.10$^{\#\#}$	3.93 ± 0.15$^{\#\#}$
Group IV (Test drug dose-I) *Nh*FE=100 mg/kg, *p.o.*± Scopolamine, 1 mg/kg, *intraperitoneal*	157.21 ± 2.70$^{\#\#}$	17.10 ± 3.82$^{\#\#}$	2.83 ± 0.23$^{\#}$	2.78 ± 0.09$^{\#}$
Group V (Test drug dose-II) *Nh*FE=200 mg/kg, *p.o.*± Scopolamine, 1 mg/kg, *intraperitoneal*	175.62± 1.21$^{\#\#}$	16.32± 2.68$^{\#\#}$	2.41 ± 0.19$^{\#\#}$	3.43 ± 0.11$^{\#\#}$

All the measurements were carried out in triplicates. The data are presented as mean ± SEM (*n=5*). $^{*}P<0.05$ and $^{***}P<0.001$, Vs Normal control (Group I), $^{\#}P<0.05$, and $^{\#\#}P<0.001$, Vs Scopolamine treated (Group II)

A herbomineral preparation known as Abana contains *the Nepeta hindostana* plant as an ingredient of the formulation is an Ayurveda system of medicine. Oral supplementation of "Abana®" in memory-impaired rats effectively improved the memory and also reversed the neurobehavioral features of amnesia.[20,21] Our study was intended to validate the traditionally used medicinal plants *Nepeta hindostana* for its memory enhancing and neuroprotective properties. Scopolamine-induced amnesia a model in rodents is a highly studied model in the field of neurodegenerative diseases that harm memory and cognitive functions. After exposure to Scopolamine, both the acquisition, retention (SDL) and locomotor activities were significantly

decreased whereas the AChE activity was increased significantly. It is well known that cholinergic neuronal systems play an important role in the cognitive deficits associated with AD, aging and neurodegenerative diseases.[22]

Studies in experimental animals have also documented the synthesis of Nitric oxide in the brain tissues. Nitric oxide (NO) have a key role in a variety of neuronal functions including learning and memory processes, cortical arousal, nociception, including dilatation of blood vessels and immune processes. An increase in the brain tissue Nitrite levels in the Scopolamine treated rats indicates an increase in the production of nitrous free radicals in the brain tissues. Additionally, free radicals have been associated with neurodegeneration and memory dysfunctions.[29,30] Increased production of reactive oxygen species (ROS) can severely alter the status of anti-oxidants defense in brain tissues leading to significant oxidative damage. There was a significant increase in lipid peroxidation and a decrease in the level of reduced glutathione in brain tissues after exposure to Scopolamine.

The extractive value in the hydro-alcoholic solvent of N. hindostana flowers indicated the nature and amount of chemical constituents in the above extract. The percentage yield value was found to be 9.48±45% w/w indicating the polar constituents in the extract. In acute toxicity study, the oral administration of hydro-alcoholic extracts of N. hindostana flowers in rats at 2000 mg/kg, body weight was found to be devoid of mortality in any animals. Hence, optimal oral doses of 100 and 200 mg/kg, body weight were selected for the current study. In our study, the anti-amnesic activity of the hydro-alcoholic extract of *Nepeta hindostana* flowers is assessed and compared with the clinically approved memory-enhancing drug Rivastigmine. Administration of hydro-alcoholic extract of *Nepeta hindostana* flower extracts at two different doses significantly improved the learning and memory as revealed by the retention of spatial memory. In the passive-shock avoidance paradigm, both the acquisition and retention SDL were significantly increased with all the dose of N. hindostana extract. This suggests that the animal has retention of memory of the shock once entered in the shock-free zone. In the case of the locomotor activity, the number of movements was increased after the administration of N. hindostana flower extract. Pretreatment of N. hindostana flowers extracts and standard drug Rivastigmine for 28 days, significantly reduced brain AChE activity. The AChE activity was significantly ($p<0.01$) decreased with all doses of N. hindostana flowers extract and in Rivastigmine-treated group when compared to the scopolamine-treated group. On the other hand, oral administration of hydro-alcoholic extract of N. hindostana flowers at doses i.e. 100 and 200 mg/kg, p.o. and Rivastigmine (1.5 mg/kg, p.o.) significantly ($P<0.01$) decreases the lipid peroxidation and tissue nitrite levels in rat brain and also restored the level of reduced glutathione (GSH) in brain tissues as compared to the Scopolamine-treated group. Moreover, long term treatment with N. hindostana flowers extracts showed a pronounced effect in the reversal of the amnesia.

Our result indicates that the hydro-alcoholic extract of N. hindostana flowers influences the cholinergic system through inhibition of AChE enzyme activity in rat brain. This memory-enhancing effect of N. hindostana flowers extract could be attributed to its anti-Acetylcholinesterase activity. Therefore, the *Nepeta hindostana* flowers extract may be used in

delaying the onset and reducing the severity of certain neurodegenerative diseases due to its antioxidant and cognition enhancing properties.

5. Conclusions

Supplementation with *Nepeta hindostana* flower extract (hydro-alcoholic) for four weeks duration also improves the tissue's antioxidant status in experimental rat hippocampus. The study results suggest the therapeutic potential of the *Nepeta hindostana* flowers extract in the management of memory dysfunction. Hence, the study strengthens the uses of this medicinal plant (flowers) as an ingredient of traditional formulations for its memory-enhancing properties.
Limitations of the study: In this study, we explored the antioxidant and cognition enhancing potential of the hydro-alcoholic extract of *Nepeta hindostana* flowers. However, further work is desired to isolate the major active constituents from the above extracts and standardization is desired for further study of major components responsible for possible memory-enhancing properties.

Conflict of interests

The author(s) declare that they have no conflicts of interest.

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