Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Ameliorative Activity of Ethanolic Flower Extract of *Nyctanthes arbor-tristis* (L.) against Scopolamine-Induced Amnestic Effect and Profiling of Active Compounds Using Gas Chromatography–Mass Spectrometry and Ultra-Performance Liquid Chromatography-Quadrupole-Time-of-Flight Mass Spectrometry

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Submitted: 29-08-2018 Revised: 01-11-2018 Published: 17-01-2019

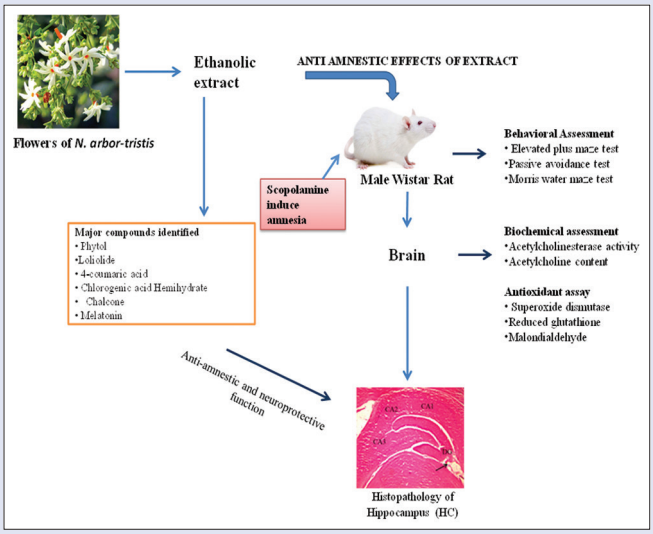
ABSTRACT

Background: Amnesia state damages the hippocampus and leads to the loss of short-term memory. *Nyctanthes arbor-tristis* (L.) is used in traditional medicines to treat various ailments. **Objective:** In the present investigation, we evaluated the efficacy of ethanolic extract of flowers of *N. arbor-tristis* against scopolamine-induced amnestic effect in male Wister rats and intended to identify the major compounds present in the extract. **Materials and Methods:** The anti-amnestic profile of flower extract was screened by elevated plus maze (EPM), passive avoidance (PA), and Morris water maze (MWM) tests. **Results:** EPM test confirmed the anxiolytic effect of the extract in rats and decreased the transfer latency in the protected arm of the EPM. During PA test, the extract resulted significant increase in step-down latencies during both the acquisition and retention sessions. In MWM task, the scopolamine injection significantly prolonged the escape latency time, whereas this time was shortened in flower extract-treated group. For the confirmation of anti-amnestic effect of extract, acetylcholine (Ach) content, acetylcholinesterase (AChE) activity, superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels in hippocampus brain were evaluated. The extract significantly increased ACh content and decreased the activity of AChE in the hippocampus of the brain. Similarly, the extract declined the MDA and increased the GSH and SOD levels in brain tissues. The phytol (RT 19.69) and loliolide (RT 23.50) were identified in the extract through gas chromatography–mass spectrometry analysis. The four major compounds such as 4-coumaric acid, chlorogenic acid hemihydrate, chalcone, and melatonin were identified using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. **Conclusion:** The anti-amnestic effect of ethanolic extract of the flower was confirmed. It contains several compounds which might be useful in the treatment and to control several neurodegenerative diseases.

Key words: Anti-amnestic effect, neurological disorder, *Nyctanthes arbor-tristis* (L.), oxidative stress, scopolamine

SUMMARY

- *Nyctanthes arbor-tristis* (L.) ethanolic flower extract showed the anti-amnestic effect against scopolamine-induced cognitive impairment in rats
- It improves the memory by inhibiting the acetylcholinesterase activity and reducing the oxidative stress level in the brain
- Ethanolic extract of flower shows the presence of neuroprotective compound phytol and loliolide which was identified by gas chromatography–mass spectrometry study.



Abbreviations used: ACh: Acetylcholine; AChE: Acetylcholinesterase; EPM: Elevated plus maze; MWM: Morris water maze; PA: Passive avoidance.

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DOI: 10.4103/pm.pm_448_18

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Mishra AK, Upadhyay P, Dixit J, Kumar P, Tiwari KN, Mishra SK, *et al.* Ameliorative activity of ethanolic flower extract of *Nyctanthes arbor-tristis* (L.) against scopolamine-induced amnestic effect and profiling of active compounds using gas chromatography–Mass spectrometry and ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. Phcog Mag 2018;14:S596-604.

INTRODUCTION

Neurological disorders are more common in the population among people aged between 18 and 60 years.[1] It is characterized by several psychiatric disorders such as depression, panic attacks, phobias, generalized anxiety, and obsessive-compulsive and post-traumatic stress disorders. Among the brain disorders, Alzheimer's disease (AD) is the most common degenerative disease, characterized by neuronal loss, inflammation, memory loss, and age-related decline in cognitive and learning disabilities.[2] Dysfunction of the cholinergic system and increased oxidative stress in the hippocampal part of the brain result in memory impairment and pathogenesis of AD.

Nyctanthes arbor-tristis (L.) is a vital medicinal plant which belongs to family Oleaceae. It is widely distributed in tropical and subtropical regions of the world. All parts of the plant are used in traditional medicinal system such as Ayurveda, Unani, and Sidha. The plant possesses various pharmacological properties such as anti-cancer,[3] anti-arthritic,[4] anti-diabetic, anxiolytic,[5] and anti-inflammatory[6] activities.

The flower of the plant is small, denty, and snowy white, being orange-red in color at the center. It contains important phytochemicals such as cyclohexylethanoid rengyolone, iridoid glucosides (6-o-trans-cinnamoyl-7-o-acetyl-6 β-hydroxyloganin),[7] carotenoid aglycone (crocetin),[8] β-digentibioside ester of α-crocetin,[9] sugar, and carotenoids. Arborside C and nyctanthoside are also important chemical constituents of the flower. The medicinal property of the flower is due to its phytochemical component. The ethanolic extract of the flower is rich with polyphenols and possesses good antioxidant properties.[10] The flower extract also exhibits anti-proliferative effect on different human cancer cell lines.[11]

The objective of the study was to evaluate the protective effect of the ethanolic extract of flower of *N. arbor-tristis* against scopolamine-induced cognitive deficits in rats as well as to identify the major compounds present in the extract using gas chromatography–mass spectrometry (GC-MS) and ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS). Till date, there is no report of detailed investigation of phytoconstituents present in the flower of *N. arbor-tristis* and its effect on memory and cognition.

MATERIALS AND METHODS

Plant material and preparation of extract

Fresh flowers of *N. arbor-tristis* were collected from Ayurvedic garden, Banaras Hindu University, Varanasi. Flowers were dried at room temperature (25°C) for a week and grind. 30.50 g of the powdered sample was extracted with 300 ml of ethanol (95%) using a Soxhlet extractor. The extract was evaporated to dryness at 45°C with a rotary evaporator. The dried extract obtained was stored at 4°C for further use.

Animals and their care

All experiments were performed according to the institutional guidelines for animal care. The experimental procedure was approved by the Ethical Committee of the Banaras Hindu University, Varanasi (F. Sc./88/IAEC/2016-17/22). Adult male Wistar rats (150 ± 25 g) were maintained under controlled conditions (28°C ± 2°C, relative humidity 75%, and 12 h light/dark cycle). Rats were fed with standard pellet diet and water *ad libitum*.

Experimental design

Rats were categorized (Groups 1–5 and $n = 6$ rats per group) as follows: Group 1 received normal saline (0.9% NaCl) and Group 2 received scopolamine (1 mg/kg body weight [b.w.]) via intra-peritoneal (i.p.) injection. Group 3 received scopolamine (1 mg/kg b.w., i. p.) and donepezil (5 mg/kg, p.o.). Group 4 received scopolamine (1 mg/kg b.w., i.p.) and flower extract (250 mg/kg, p.o.). Group 5 received scopolamine (1 mg/kg b.w., i.p.) and flower extract (500 mg/kg, p.o.). Animals received flower extract dissolved in saline prior to the i.p. dose of scopolamine. Donepezil in normal saline was administered orally to the rats (reference control). Toxic dose of the water-soluble portion of the ethanolic extract of the flower was >2.0 g/kg b.w.[12] The experimental schedule is depicted in Figure 1. After the completion of the treatment period (15 days), the rats were anesthetized and sacrificed by decapitation after euthanizing by CO_2 (flow rate was adjusted at 3 L/min in rat cage and continued until 1 min after breathing stopped).

Behavioral analysis

To investigate the anti-memory impairment activity, conventional behavioral animal test was performed. Behavioral patterns in rats were studied by Elevated plus maze test (EPM) according to the method reported by Lister.[13] Examination of aggravated learning and memory ability in rats was studied by passive avoidance (PA) test using the method of Park *et al.*[14] Morris water maze (MWM) test was conducted to evaluate the hippocampal depend spatial learning and memory of rats by using the method of Morris.[15]

Biochemical analysis

After sacrifice, the rat's blood was collected immediately and centrifuged for serum isolation. Further, the brain was carefully dissected. The hippocampus was excised, washed with saline, and transferred into 10% formalin (pH 7) for histopathology. Fourth, for biochemical and antioxidant assays, brain tissues were stored in phosphate-buffered saline (PBS) at −80°C.

Acetylcholinesterase assay and acetylcholine content

Acetylcholinesterase (AChE) activity in the brain tissue was analyzed using the method described by Ellman *et al.*[16] Enzyme activity was

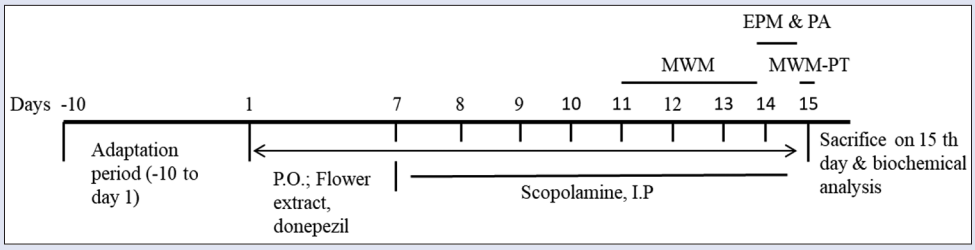


Figure 1: Schematic schedule of experimental design to study the anti-amnestic effect of *Nyctanthes arbor-tristis* flower. I.P.; intraperitoneal, P.O; per os (orally), probe trial of Morris water maze test

expressed as nmoles of acetylthiocholine hydrolyzed per mg of protein per hour. Quantification of acetylcholine (Ach) content was performed by following the method of Hestrin.[17] The ACh content was expressed as nmoles of Ach per gram wet tissue.

Antioxidant assay

Brain tissue was homogenized in ice-cold PBS (0.1 M, pH 7.4) and centrifuged (2800 g; 4°C; 30 min). The supernatant was collected and used for antioxidant assay. Superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined in brain homogenates using methods of McCord and Fridovich,[18] Ellman,[19] and Devasagayam *et al.*,[20] respectively.

Biochemical serum analysis

Basic liver function test and renal function test of serum were performed with commercially available kits of ARKRAY Health care Pvt. Ltd., Surat, Gujarat, India.

Histology of hippocampus

The excised hippocampus was fixed with graded alcohols. A paraffin block was made and cut into thin sections (5 μm). Hematoxylin and eosin (H and E) was used to stain it. Each section was observed under light microscope and photographed.

Statistical analysis

Experimental results were expressed as mean ± standard error of the mean (SEM) ($n = 6$). The data from all tests were analyzed statistically through analysis of variance. Statistical significance was accepted at $P < 0.05$.

Gas chromatography–mass spectrometry analysis

The identification of volatile compounds present in the ethanolic extract was analyzed using GC–MS (Thermo GC-Trace Ultra Ver: 5.0 Thermo MS DSQ II). GC-MS experimental conditions were as follows: DB 35-MS capillary standard column, dimension: 30 m × 0.25 mm, film thickness 0.25 μm. Oven temperature was increased from 70°C to 260°C at 6°C/min. Injector and detector temperatures were programmed at 220 and 260°C, respectively. 1 μl of the sample diluted with ethanol was injected. The flow rate of mobile phase (He, carrier gas) was 1 ml/min. The total GC run time was 37.50 min. Mass spectra were taken for 50–650 Da fragment. Results obtained were matched to National Institute of Standards and Technology (NIST) and Wiley 9 library database.

Ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry analysis

The analysis of ethanolic extract was performed using ACQUITY UPLC-Q-TOF-MS/MS system (Waters Corp., Milford, MA, USA). The chromatography was carried out with Acquity BEH C_{18} column (dimension: 100 mm × 2.1 mm, 1.7 μm; temperature: 25°C). The mobile phase consisted of 0.1% formic acid (a), acetonitrile (b), and methanol (c), and its flow rate was 300 μl/min. Sample injection volume was 5 μl. Gradient elution program was optimized as follows: initial 90:10% B: C and increased to 80:20% in 2 min, 50%–60% B: C for 1–3 min, 30%–70% B: C for 3–6 min, then 10:90% B: C for 1 min, and finally increased quickly to 90%–10% in 7–10 min. MS analysis was performed in positive ion modes. Centroid mode data were collected over the m/z range 100–1000 Da with a scan time of 1 s. Accurate mass and molecular formula denomination was acquired with Waters MassLynx 4.1 software (Waters MS Technologies).

RESULTS AND DISCUSSION

The problem of neurological disorders and dementia has increased significantly in the aging population. The change in social behavior is a characteristic of chronic neuro-degenerative disease such as AD, and it is associated with decline in cognitive abilities and mental functions.[21] Hippocampus is an important part of the brain which is damaged in such type of diseases. It causes short-term memory loss and disorientation. The present study was designed to explore the anti-amnestic properties of *N. arbor-tristis* flower extract using scopolamine-induced amnesia in rats. Scopolamine is a nonselective muscarinic receptor antagonist which disrupts the cholinergic system and induces amnesia and short-term memory loss in animals.[22]

Behavioral assessment

Three different behavioral tests (EPM, PA, and MWM) were employed to assess the learning and memory in this study. Scopolamine administration disrupted the learning and memory as determined by an increase in transfer latencies both in acquisition and retention sessions in the EPM task [Figure 2a]. A decrease in step-down latencies (acquisition and retention) in the PA task was observed [Figure 2b]. Scopolamine treatment resulted significant increase in the escape latencies in the MWM task [Figure 2c]. Scopolamine-treated rats spent less time in the target quadrant during the MWM probe trial when compared to that of controls [Figure 2d]. MWM was performed widely because it evaluates spatial memory and detects the effective changes in central cholinergic systems.[23] Co-administration of the *N. arbor-tristis* flower extract in scopolamine-treated rats improved the learning and memory deficiencies in all the three tests. Hence, it confirmed the positive effect of the floral extract on cognitive deficits. Scopolamine-induced amnesia was mediated by the disruption of cholinergic signaling due to its muscarinic receptor antagonist activity.[24]

Biochemical assessment

Effect of flower extract against amnesia in rat brain

The ACh is a neurotransmitter synthesized in the cholinergic neurons, which controls many cognitive processes including attention, learning, and memory. Defects in the cholinergic neurons result in the inhibition of release of ACh in the synaptic cleft and cause memory dysfunction.[25] The duration of ACh action is dependent on the activity of AChE, which hydrolyzes ACh after its release.[26] The inhibition of AChE activity is the proper approach for the cure and prevention of AD and different types of dementia.[27] In addition, in several studies, it was observed that the function of AChE was increased after the administration of scopolamine. The AChE inhibitor reverses the amnesia effects of scopolamine.[28] Similarly, in the present study, in scopolamine-treated rats, AChE activity was significantly ($P < 0.001$) increased, whereas ACh content was significantly decreased as compared with control and donepezil-treated groups. The flower extract (250 and 500 mg/kg, p.o.) significantly ($P < 0.001$) decreased the AChE activity in scopolamine-treated rats [Figure 3a]. Similarly, in extract-treated group, ACh content was significantly ($P < 0.001$) increased in the brain [Figure 3b], which counteracts the effect of scopolamine. Enhanced ACh level in the brain and reduced AChE activity in the scopolamine-treated rats showed positive effect of the flower extract on learning memory, which is partially mediated by the cholinergic system in brain. The anti-amnestic drug, donepezil, reverses the effect of scopolamine-induced social recognition memory deficit.[29] Donepezil is an AChE inhibitor and used as positive control in the scopolamine-treated rats. It significantly improved the learning and memory function in behavioral tests and showed antioxidant activity.

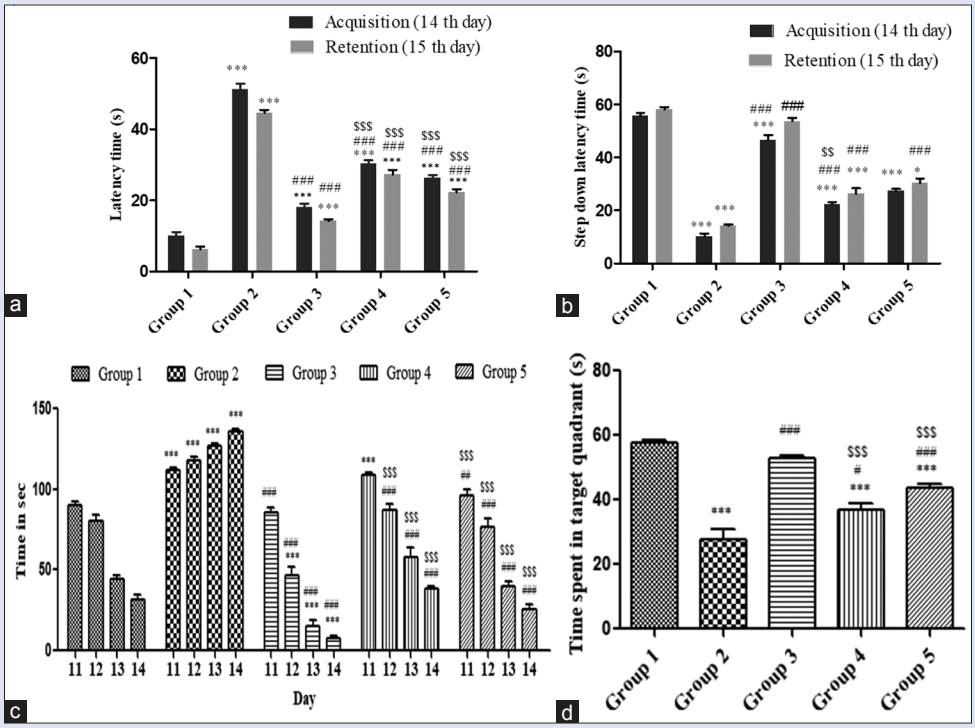


Figure 2: Effect of ethanolic flower extract of *Nyctanthes arbor-tristis* (L.) on learning and memory in scopolamine-induced rats (a) elevated plus maze test, (b) passive avoidance test, and (c and d) Morris water maze test. Data were presented as mean ± standard error of the mean; $n = 6$. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus corresponding value in the control group. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ versus corresponding values in the scopolamine group and $^{\$\$\$}P < 0.001$, $^{\$\$}P < 0.01$, $^{\$}P < 0.05$ versus corresponding values in scopolamine plus standard group

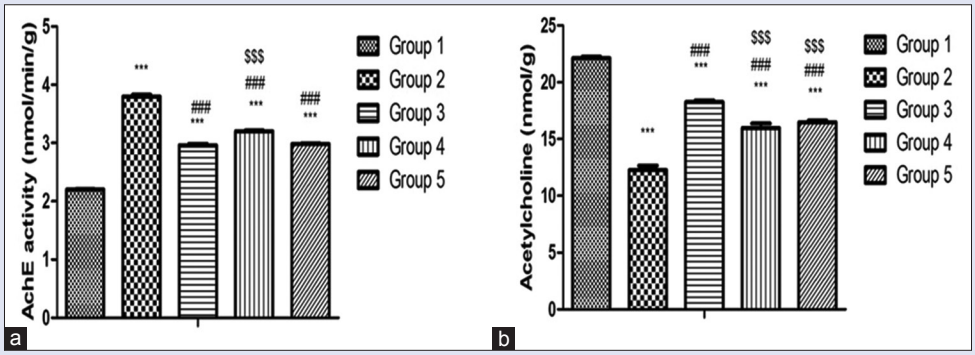


Figure 3: Effect of ethanolic flower extract of *Nyctanthes arbor-tristis* (L.) on acetylcholine esterase (a) and acetylcholine content (b). Data were presented as mean ± standard error of the mean; $n = 6$. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus corresponding values in the control group. $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ versus corresponding values in the scopolamine group and $^{\$\$\$}P < 0.001$, $^{\$\$}P < 0.01$, $^{\$}P < 0.05$ versus corresponding values in scopolamine plus standard group

Antioxidant effect of the flower in rat brain

Oxidative stress plays an important role in neurological and psychiatric disorders. The central nervous system of the brain needs proper availability of oxygen for normal functioning. Oxidative stress in the brain induces the production of free radical as by-products.[30] These free radicals cause damage in the brain tissue. Thus, this damage induces the impairment of learning and memory. It is caused by enhanced level of lipid peroxides and reduction of antioxidant level in the brain.[31] The hippocampus part of the brain plays a crucial role in the short-term, long-term, and spatial memory. Hippocampus and amygdala part are more sensitive to oxidative injury.[32] The SOD activity [Figure 4a] and GSH level [Figure 4b] were significantly ($P < 0.001$) reduced, whereas MDA content ($P < 0.001$) was increased [Figure 4c] in the brain of scopolamine-treated rats as compared to control and donepezil-treated groups. The scopolamine injection induced the oxidative stress in hippocampus region of the brain, which caused the enhancement in the level of reactive oxygen species and MDA. In this situation, the level of antioxidant enzyme (SOD and GSH) in the brain tissue reduced drastically. The neuroprotective effect of flower extract and donepezil declined the level of MDA and increased the level of GSH and SOD. These results revealed that the antioxidant potential of the flower extract contributes in enhancing the learning-memory function.

Effect of the flower extract on liver and kidney

The level of liver functions (serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase) in the blood serum of scopolamine-treated rats elevated than that of the controls [Table 1]. Similarly, renal functions (level of urea, blood urea nitrogen, and creatinine) also increased significantly ($P < 0.05$) in the similar groups [Table 1]. All liver functions (level of parameters) drastically reduced in scopolamine- and flower extract-treated groups. Similarly, level of all renal parameters in scopolamine- and flower extract-treated groups also decreased.

Histopathology

Histopathological observation of rat hippocampus brain revealed that, in the control group, there were no significant changes in the cornuammonis (CA) pyramidal cell layers (CA1, CA2, CA3) of the brain [Figure 5a]. The scopolamine-treated group showed the major alteration in the form of constriction in the brain tissue and development of hemorrhagic patches near the CA3 and dentate gyrus (DG) regions. Later, there was marked inflammation and ultimately degeneration of CA layer of the pyramidal cells (CA1, CA2, and CA3) of the hippocampus [Figure 5b]. The standard drug donepezil combined with scopolamine-treated group showed a marked reappearance of the CA pyramidal cell layer as well as reduction of hemorrhagic patches near the CA3 and DG regions. It also reduced the neuronal loss and degeneration in the pyramidal cell layer of the hippocampus [Figure 5c]. The flower extract (250 and 500 mg/kg)-treated groups showed marked reappearance of the pyramidal cell layers. It also improved the blood circulation and minimized neuronal loss in the CA1, CA2, and CA3 regions [Figure 5d and e]. The flower extract (500 mg/kg) was found effective in reducing the alteration in the pyramidal cell layers and also reduced hemorrhagic patches near CA3 and DG regions of hippocampus when compared with that of control group [Figure 5e]. The proper connection between CA (CA1, CA2, and CA3) and DG in hippocampus is essential for normal behavior. The DG is responsible for the separation of episodic memory. CA3 pyramidal neurons received excitatory input signal and stored auto-associative memories in their associative network.[33] Hippocampus in the brain is involved in the formation of episodic memory as well as spatial memory processing which is used in navigation.

Identification of major compounds

The GC-MS facilitates the analysis of semi-volatile and volatile compounds. The compounds were identified by comparing with the retention time, molecular weight, and peak area percent, with the NIST and Wiley 9 library. The GC–MS chromatogram is shown in Figure 6a. The analysis of extract revealed the presence of two important compounds, namely phytol and loliolide,

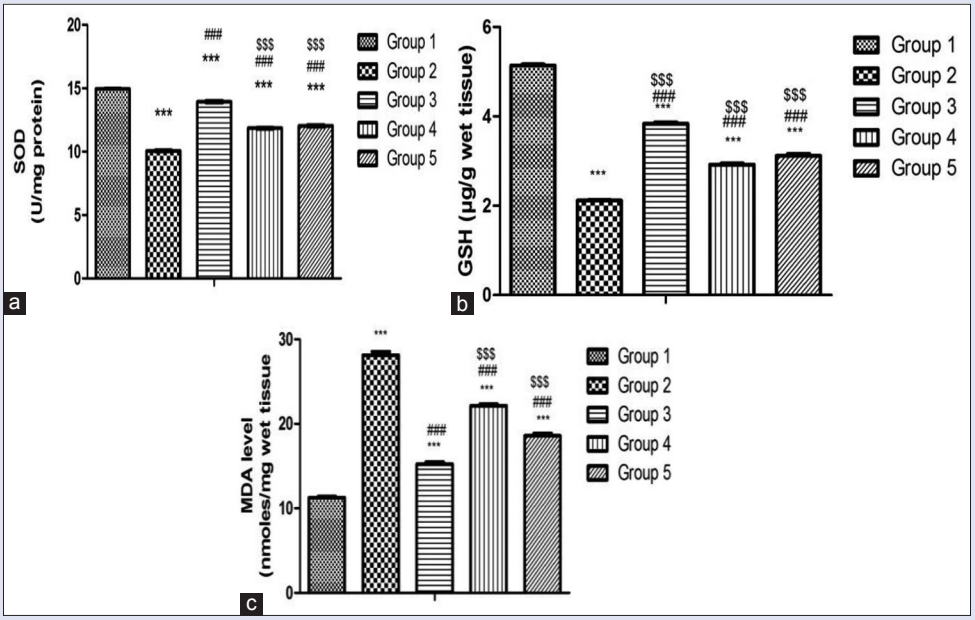


Figure 4: Effect of ethanolic extract of flower of *Nyctanthes arbor-tristis* (L.) on the (a) superoxide dismutase, (b) reduced glutathione, and (c) malondialdehyde levels. Data were presented as mean ± standard error of the mean; $n = 6$. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ versus corresponding values in the control group. ###$P < 0.001$, ##$P < 0.01$, #$P < 0.05$ versus corresponding values in the scopolamine group and $^{\$\$\$}P < 0.001$, $^{\$\$}P < 0.01$, $^{\$}P < 0.05$ versus corresponding values in scopolamine plus standard group

Table 1: Effect of extract on liver and renal functions in scopolamine-induced memory-impaired rats

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Urea (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)
Group 1	164.50±2.41*	48.66±9.21*	94.00±4.19*	61.16±7.76*	23.66±2.30*	0.40±0.05*
Group 2	181.50±4.52*	50.33±6.21*	97.33±6.76*	64.33±5.93*	29.27±3.44*	0.50±0.05*
Group 3	165.67±6.73*	48.33±7.74*	96.50±7.15*	60.66±8.05*	24.51±2.77*	0.39±0.06*
Group 4	172.67±4.94*	45.67±2.59*	96.58±7.78*	62.67±7.37*	28.33±3.74*	0.31±0.07*
Group 5	166.83±9.23*	39.33±3.77*	95.33±8.70*	61.67±8.05*	24.94±3.76*	0.27±0.04*

*Significant at $P<0.05$. BUN: Blood urea nitrogen, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase

showing a retention time of 19.69 and 23.50, respectively. The mass fragment of phytol and loliolide is shown in Figure 6b and c. The neuroprotective function of phytol as an anticonvulsive agent has already been reported.[34] Similarly, loliolide is a monoterpene lactone, which possesses anti-depressant activity and shows affinity to serotonin.[35] The chemical structure and bioactivity of these compounds are summarized in Table 2.

UPLC-Q-TOF-MS/MS analysis of ethanolic flower extract was carried out in positive ion modes, and mass spectra chromatogram is shown in Figure 7a. The identification of compounds was based on their retention time and m/z ratio. The identification of compounds was confirmed by the Riken Tandem Mass Spectral Data Base library. The identified compounds belong to phenylpropanoid (4-coumaric acid and chlorogenic acid hemihydrate), flavonoid (chalcone), and alkaloid (melatonin) groups. Mass spectra and nature of identified compounds and their structures and biological activities are summarized in Table 3 and Figure 7b-d. Among the major possible component, 4-coumaric acid was also identified, and it reduced oxidative stress and improved electrophysiological cognitive functions.[36] It has regulatory effect on central cholinergic synapses and improved cholinergic system.[36] The 4-coumaric acid possesses anti-oxidant, anxiolytic, and analgesic activities.[37] Chlorogenic acid hemihydrate also belongs to phenylpropanoid category and possesses anti-oxidant and neuroprotective activities. It is reported to reduce AChE function and MDA level, which helps in the protection of the neurons in hippocampus and frontal cortex of the brain.[38] Chalcone is a flavonoid, possess anti-oxidant, anti-convulsant, anti-hypertensive, and memory improvement activities.[39,40] The alkaloid melatonin possesses neuroprotective effect and ameliorates the memory impairment disorders.[41] Melatonin also reduces the oxidative stress and decreases the level of MDA in hippocampus brain.[42] The presence of these phytoconstituents in the flower extract would be responsible for neuroprotective and memory improvement activities against scopolamine-induced amnesia. Hence, the constituents of flower can be used for the enhancement of memory function via regulating neurotrophins and protecting neurons against oxidative and metabolic stress. These bioactive compounds were not reported earlier in the flower extract of *N. arbor-tristis*. This investigation revealed that *N. arbor-tristis* flower may be a potential source for various neuroprotective bioactive compounds. The exact mechanism by which the flower extract suppresses AChE activity requires further investigation.

CONCLUSION

Ethanolic extract of the flower improves memory by inhibiting the AChE activity and reducing the oxidative stress in the brain tissue. Anti-memory impairment activity of the extract was confirmed using EPM, PA, and MWM tests. GC-MS and UPLC-Q-TOF-MS/MS were used for the identification of important compounds in the extract, which regulate the brain function. Phytol and loliolide were identified in the extract through GC-MS analysis. Four compounds, namely 4-coumaric acid, chlorogenic acid hemihydrate, chalcone, and melatonin, were identified in the extract using UPLC-Q-TOF-MS/MS. These compounds were reported for the first time in the flower

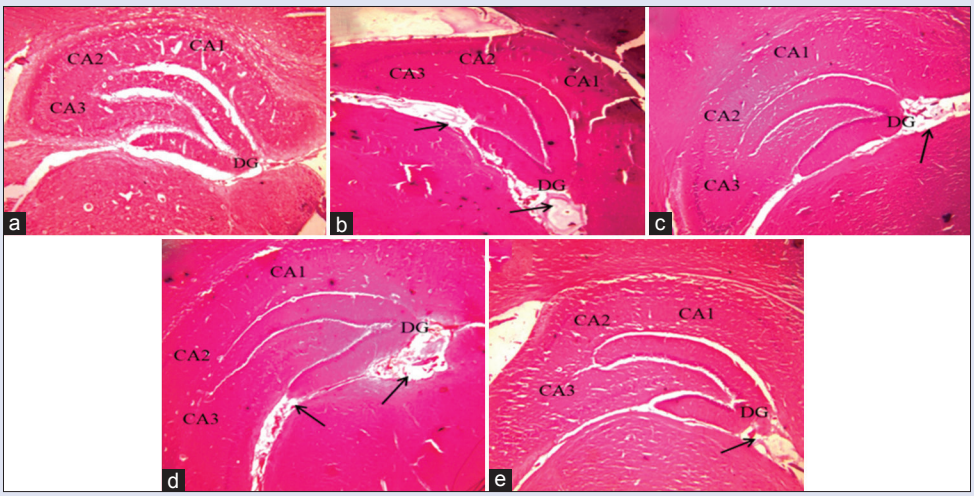
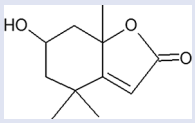


Figure 5: Histopathology of scopolamine-treated rat brain (hippocampus region) (H and E, ×10). Control group (a), scopolamine-treated group (b), scopolamine and donepezil-treated group, and (c), flower extract-treated group (d and e). Arrow represents the hemorrhagic patches developed by scopolamine treatment

Table 2: Major compounds identified by gas chromatography–mass spectrometry analysis in ethanolic flower extract of *Nyctanthes arbor-tristis* (L.)

Retention time (min)	Identified compound	Molecular formula	Molecular weight	Area %	Structure	Reported activity	References
19.69	Phytol	$C_{22}H_{42}O_2$	338	0.87		Antinociceptive, anticonvulsant, antioxidant	34
23.50	Loliolide	$C_{11}H_{16}O_3$	196	1.01		Antidepressant, immunosuppressive	35

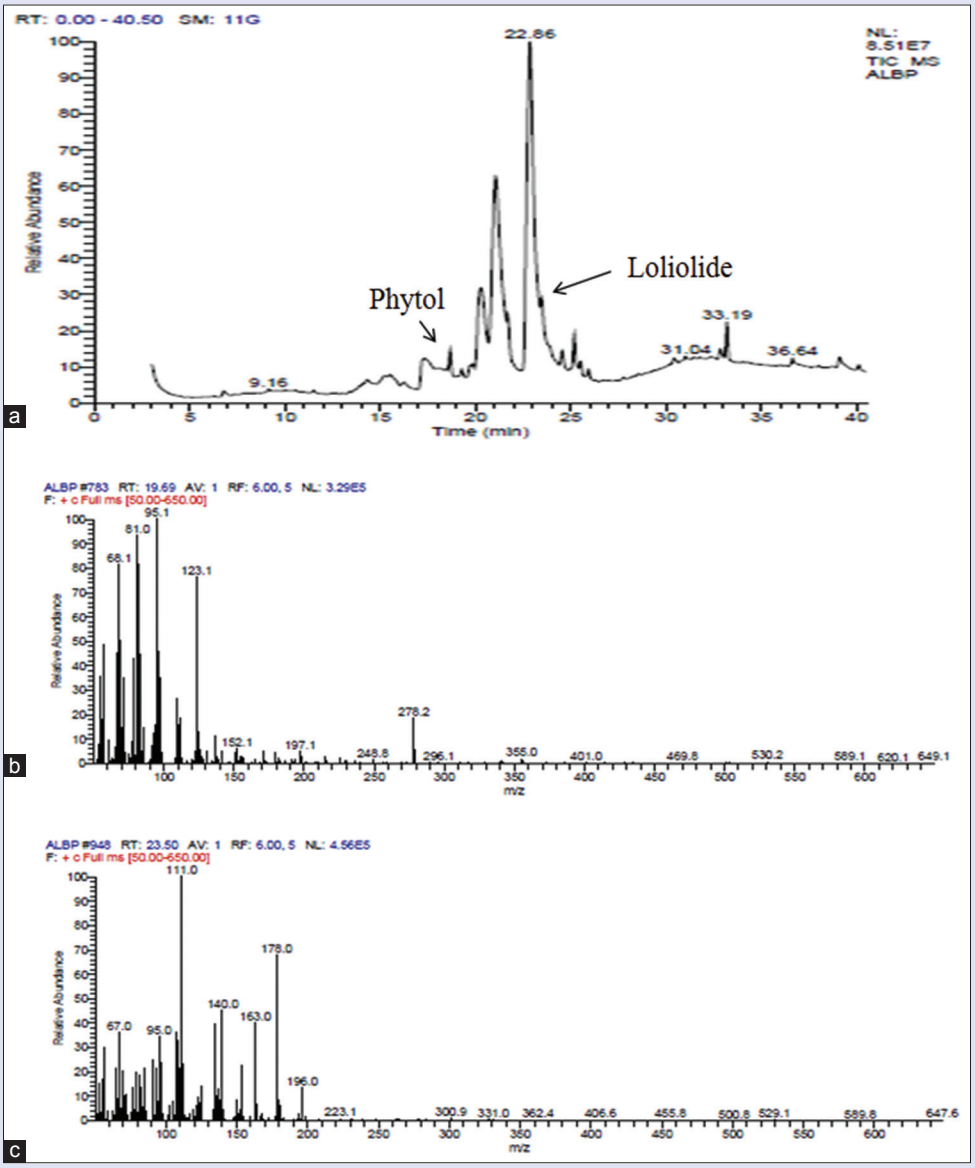
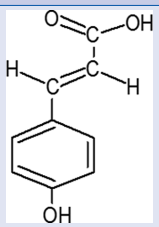
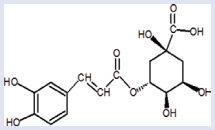


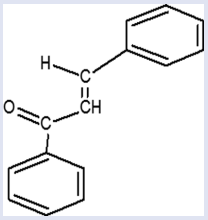
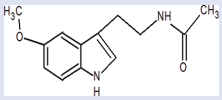
Figure 6: Gas chromatography–mass spectrometry profile of ethanolic flower extract of *Nyctanthes arbor-tristis* (L.) (a), mass spectra of phytol (b) and loliolide (c)

Table 3: Compounds identified in ethanolic flower extract of *Nyctanthes arbor-tristis* (L.) using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry

Retention time (min)	Mass-positive mode	Identified compound	Molecular formula	Molecular weight	Nature of the compound	MS-MS spectra	Structure	Reported activity	References
0.884	165.0542	4-coumaric acid	$C_9H_8O_3$	164.16	Phenylpropanoid	165, 164, 147		Antioxidant, anxiolytic, antipyretic, analgesic	36,37
0.884	355.0904	Chlorogenic acid hemihydrate	$C_{16}H_{18}O_9$	354.31	Phenylpropanoid	355, 163		Antioxidant, neuroprotective	38

Contd...

Table 3: Contd...

Retention time (min)	Mass-positive mode	Identified compound	Molecular formula	Molecular weight	Nature of the compound	MS-MS spectra	Structure	Reported activity	References
6.244	105.0302	Chalcone	$C_{15}H_{12}O$	208.26	Flavonoid	209.0966, 105.0363		Antioxidant, anticonvulsant, anti-hypertensive,	39
8.550	131.0473	Melatonin	$C_{13}H_{16}N_2O_2$	232.28	Alkaloid	131, 115		Neuroprotective	40

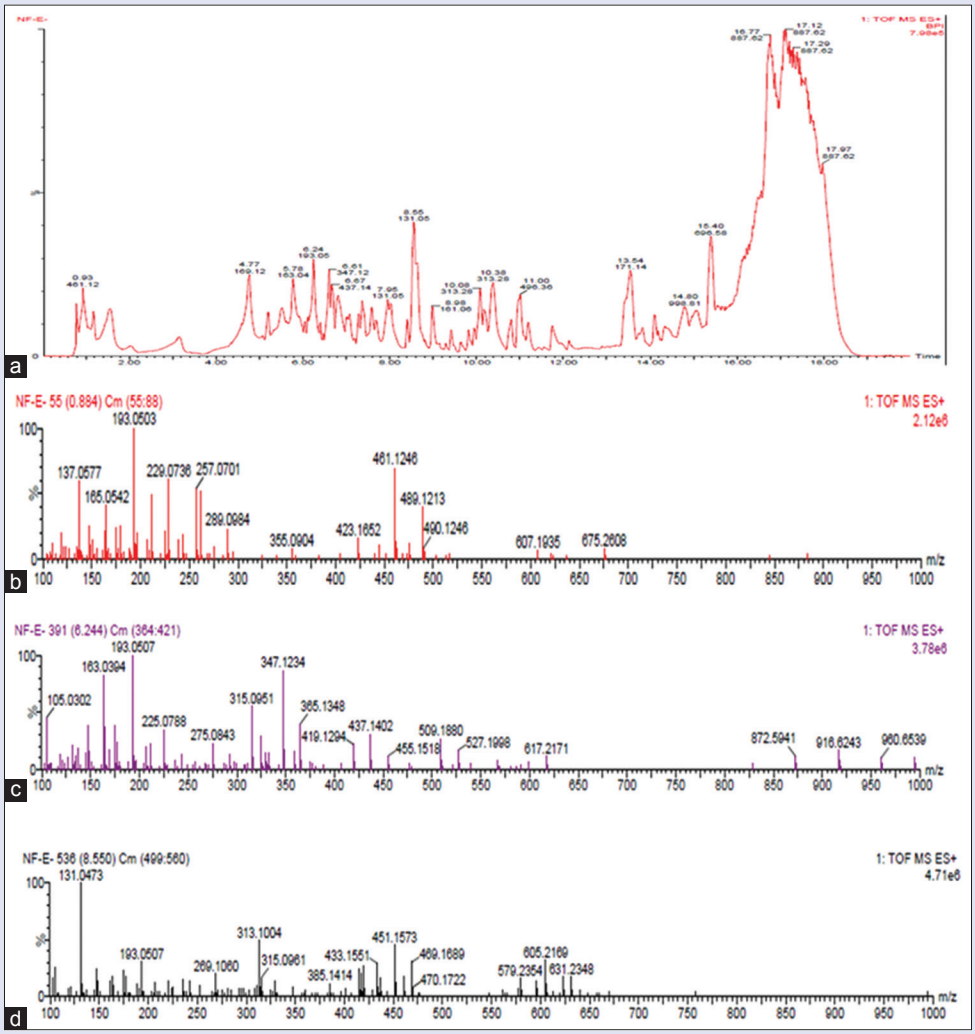


Figure 7: Ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry-positive mode total ion chromatogram (a) and mass spectra of identified compounds present in ethanolic flower extract based on retention time and m/z ratio; 4-coumaric acid (0.884, 165.0542) and chlorogenic acid hemihydrate (0.884, 355.0904) (b), chalcone (6.244, 105.0302) (c), melatonin (8.550, 131.0473) (d)

extract of *N. arbor-tristis* (L.). The neuroprotective activity of all these compounds was reported in different studies. Therefore, this extract might be useful in the treatment and control of neurodegenerative diseases.

Acknowledgements

The author (AKM) would like to thank AIRF-JNU, New Delhi, for technical support in GC-MS and UPLC–Q-TOF-MS/MS and gratefully acknowledge for the fellowship support from UGC, New Delhi.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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