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Protective effect of fruits of *Morinda citrifolia* L. on scopolamine induced memory impairment in mice: A behavioral, biochemical and cerebral blood flow study

Shakti Deep Pachauri[a], Santoshkumar Tota[b], Kiran Khandelwal[a], P.R.P. Verma[e], Chandishwar Nath[c], Kashif Hanif[b], Rakesh Shukla[b], J.K. Saxena[d], Anil Kumar Dwivedi[a,*]

[a] Division of Pharmaceutics, CSIR-Central Drug Research Institute, Lucknow, India
[b] Division of Pharmacology, CSIR-Central Drug Research Institute, Lucknow, India
[c] Division of Toxicology, CSIR-Central Drug Research Institute, Lucknow, India
[d] Division of Biochemistry, CSIR-Central Drug Research Institute, Lucknow, India
[e] Department of Pharmaceutical Sciences, Birla Institute of Technology (BIT), Mesra, Ranchi, India

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ABSTRACT

Ethnopharmacological relsevance: Noni (*Morinda citrifolia* L.) is widely used for different illnesses including CNS disorders. Recently Noni has been reported to prevent amyloid beta induced memory impairment in mice. However, the influence of Noni on cholinergic system has not been explored so far. Therefore, present study was designed to investigate effect of Noni fruit on memory, cerebral blood flow (CBF), oxidative stress and acetylcholinesterase (AChE) activity in scopolamine induced amnesia model.
Materials and methods: Mice were orally treated with ethanolic extract of Noni fruit and chloroform, ethyl acetate and butanol fractions of ethanolic extract for three days. Scopolamine was administered 5 min prior to acquisition trial and memory function was evaluated by passive avoidance test. CBF was measured by laser doppler flowmetry. AChE activity and oxidative stress parameters were estimated in mice brain at the end of behavioral studies. Further, effect of ethanolic extract and its fractions (5–400 μg/ml) on AChE activity was measured *in vitro*.
Results: Scopolamine caused memory impairment along with reduced CBF, increased AChE activity and oxidative stress in mice brain. Ethanolic extract of Noni fruits and its chloroform and ethyl acetate fractions significantly improved memory and CBF. However, butanol fraction had no effect. Further, increased oxidative stress and AChE activity following scopolamine was significantly attenuated by ethanolic extract of Noni and its fractions. Moreover ethanolic extract and its fractions showed dose dependent inhibition of AChE activity *in vitro*.
Conclusion: These observations suggest that Noni may be useful in memory impairment due to its effect on CBF, AChE and oxidative stress.

1. Introduction

Alzheimer's disease (AD) is clinically characterized by a progressive loss of cognitive abilities, which particularly affects elder population in their daily activities such as memory, speaking, and problem solving. The pathophysiology of AD is complex and involves several different biochemical pathways (Kang et al., 2005). The key symptoms of AD are primarily caused by cholinergic dysfunction (Wang et al., 2006). It is known that acetylcholine (ACh) is an important neurotransmitter related to learning and memory. Scopolamine, a muscarinic receptor antagonist, interferes with memory in animals and humans, particularly the processes of learning acquisition and short-term memory (Jones et al., 1991; Jeong et al., 2008). Scopolamine significantly increases acetylcholinesterase (AChE) and malondialdehyde (MDA) levels in the cortex and hippocampus (Sakurai et al., 1998; Jeong et al., 2008). Emerging evidences indicate that cerebral circulation plays an important role in learning and memory function in animals and humans (Wyper et al., 1993; Tota et al., 2010, 2011). Scopolamine has been shown to abolish cerebral blood flow (CBF) which was recovered by administration of physostigmine or tacrine suggesting important role of cholinergic neurotransmission in regulation of CBF (Honer et al., 1988; Ogawa et al., 1994; Tsukada et al., 1997).

Morinda citrifolia commonly known as Noni is widely used as a food in tropical regions from Indonesia to Hawaiian Island (Kirtikar and Basu, 1981). It is a small tree with a straight trunk and cultivated in the wild state in various parts of India (McClatchey, 2002). As a

* Corresponding author Tel.: +91 0522 2614302; fax: +91 0522 2623405.
E-mail address: anilcdri@gmail.com (A.K. Dwivedi).
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popular herb, the Noni fruit juice has been used as an alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, menstrual difficulties, heart disease, cancers, gastric ulcers, mental depression and atherosclerosis (Wang et al., 2002).

The fruit and leaf extracts of Noni exhibited analgesic (Younos et al., 1990), anti inflammatory (Basar et al., 2010), antioxidant (Su et al., 2005) and immunomodulatory effects (Palu et al., 2008). A polysaccharide rich substance of fruit juice of *Morinda citrifolia* has been reported to have anti tumor activity in the lewis lung peritoneal carcinoma model (Hirazumi and Furusawa, 1999). Noni fruit juice was also reported to have hepatoprotective effect against CCl4-induced chronic liver damage in rats (Wang et al., 2008). There are also studies reporting its blood pressure lowering and vasodilatory properties (Runnie et al., 2004; Gilani et al., 2010). Further, Noni contains number of phytochemicals that have been studied for anxiolytic and sedative effects (Deng et al., 2007). Moreover, Noni fruit juice has been reported to prevent neuronal damage induced by focal ischemia (Harada et al., 2009). Recently, Muralidharan et al. (2010) reported that ethyl acetate extract of Noni prevented memory impairment and oxidative stress induced by amyloid beta peptide in mice.

Previously we have reported the beneficial effects of natural products like guggulipid, quercetin and curcumin using different animal models of memory impairment (Saxena et al., 2007; Awasthi et al., 2010; Tota et al., 2010). In the present study, we investigated effect of Noni fruit on scopolamine induced memory impairment in mice.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice weighing 25–30 g were used. The animals were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. The animals were kept in polyacrylic cage (22.5 cm × 37.5 cm) and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65%) with a 12 h light and dark cycle. Food and water were available ad libitum but food was not allowed from 1 h prior to and till completion of behavioral study. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Central Drug Research Institute, India.

2.2. Materials and methods

The biochemicals, i.e. scopolamine, chloral hydrate, sodium chloride (NaCl), sodium nitrate ($NaNO_2$), bovine serum albumin (BSA), acetylthiocholine iodide, 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB), 2-thiobabituric acid (TBA), quercetin, rutin, scopoletin and tacrine were purchased from Sigma–Aldrich, USA. Acetonitrile was purchased from Merck, Germany. Donepezil was purchased from Hetero Drugs Limited, India,

2.2.1. Plant material

Noni fruits were collected in the months of October to November from Port Blair (Andaman and Nicobar islands) and were authenticated in Central Agricultural Research Institute (CARI), Port Blair, India.

2.2.2. Preparation of extracts

Noni fruits were shade dried at room temperature. The shade dried plant material (2.8 kg) was coarsely powdered and defatted with hexane. The defatted marc was subjected to ethanol (90%) extraction and evaporated under reduced pressure. The solvent free ethanol extract was suspended in distilled water and fractionated with chloroform, ethyl acetate and butanol.

2.2.3. Quantification of scopoletin, rutin and quercetin in the extract

2.2.3.1. Chromatographic condition. Chromatographic separation was performed on a HPLC system (Class-VP, Shimadzu Corporation, Kyoto, Japan) coupled with a photodiode array (PDA) detector and equipped with C18 column (250 mm, 4 mm, 5 μm, Purospher® STAR, Merck, Germany). The HPLC system consisted of binary gradient pump (LC-10AT), columnoven, and system controller (SCL-10A). The mobile phase consisted of acetonitrile: potassium dihydrogen phosphate (0.05 M, pH 2.7 containing 0.1% triethyl amine) (70:30) (v/v) was degassed prior to analysis using a Millipore vacuum pump. The detector was set at 257 nm for rutin, 345 nm for scopoletin and 370 nm for quercetin with a total runtime of 20 min. The flow rate was maintained 1.0 ml/min. The column oven temperature was maintained to 30 °C. The injection volume was 20 μl. CLASS-VP workstation was used for data acquisition.

2.2.3.2. Standard solutions and sample preparation for quantification. For simultaneous estimation of scopoletin, rutin and quercetin the combined stock solution was prepared in methanol (1 mg each/ml). Further dilutions were made by methanol to get different working stock solutions. The calibration curve was used in the range 0.5 μg–50 μg/ml. Triplicate 20 μl injections of each solution were chromatographed under the conditions described above. Peak areas were plotted against the corresponding concentrations to obtain calibration plot.

Samples were prepared by dissolving 5 mg of dried ethanolic extract of Noni fruit and its fractions in 1 ml methanol and 20 μl of this solution was injected in triplicate and peak areas were quantified by using calibration curve for each constituent. The concentration of each constituent was expressed in μg/mg of dry extract.

2.3. Experimental protocol and drug administration

Sic mice were used in each group. The ethanolic extract of Noni fruit and its ethyl acetate, butanol and chloroform fractions were administered at a dose of 50 and 100 mg/kg orally for three days. Donepezil (5 mg/kg), a positive control, was administered 1 h before the acquisition trial (Agrawal et al., 2008). Scopolamine (3 mg/kg, IP) was administered 5 min prior to acquisition trial to induce memory impairment (Saxena et al., 2007). Ethanolic extract, ethyl acetate and butanol fractions were dissolved in normal saline. The chloroform fraction was suspended in 1.0% (w/v) gum acacia immediately before administration in a constant volume of 10 ml/kg body weight. A group received normal saline 1 h prior to 1st trial served as control.

2.4. Passive avoidance test

The mice were subjected to the passive avoidance test by placing in a light compartment of computerized shuttle box with a software programme PACS 30 (Columbus Instruments, OH, USA). The light compartment was isolated from the dark compartment by an automated guillotine door. After an acclimatization period of 30 s, the guillotine door was opened and closed automatically after entry of the mouse into the dark compartment. The subject received a low-intensity foot shock (0.5 mA; 10 s) in the dark compartment. Infrared sensors monitored the transfer of the animal from one compartment to another, which was recorded as transfer latency time (TLT) in seconds. The duration of a trial was 270 s. The 1st trial was for acquisition and retention was tested in a 2nd trial given 24 h after the 1st trial. The shock was not delivered in the retention

trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention trials as compared to acquisition trial (Saxena et al., 2007; Tota et al., 2009; Awasthi et al., 2010).

2.5. Spontaneous locomotor activity

Each animal was observed for 10 min after a period of 30 min acclimatization in Optovarimax activity meter (Columbus Inc., USA).

2.6. Measurement of cerebral blood flow

Cerebral blood flow (CBF) was measured by laser doppler flowmetry (LDF 100, BIOPAC, USA) on 3rd day in a separate set of all animal groups. LDF qualitatively measures CBF in arbitrary blood perfusion units (BPU) (Tota et al., 2010; Awasthi et al., 2010). 1 h after administration of drugs on third day, mice were anesthetized with chloral hydrate (300 mg/kg, IP) and a 0.5 mm diameter microfiber laser doppler probe was fixed on the skull (6 mm lateral and 1 mm posterior of bregma) and CBF was monitored within cortical region (Tota et al., 2010; Awasthi et al., 2010). Baseline CBF was recorded for 5 min and animals were injected with scopolamine. CBF was again monitored for 20 min taking reading at every 5 min.

2.7. Estimation of biochemical parameters

2.7.1. Brain tissue preparation

The mice were decapitated under ether anesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples (0.03 M sodium phosphate buffer, pH 7.4) was prepared by using an Ultra-Turrax T25 (USA) homogenizer at a speed of 9500 rpm. The homogenized tissue preparation was used to measure acetylcholinesterase, MDA and GSH.

2.7.2. Acetylcholinesterase assay in brain

The brain homogenate in volume of 500 μl was mixed with 1% Triton X-100 and centrifuged at 100,000 × *g* at 4 °C in a Beckman Ultracentrifuge (LE 80, USA) for 60 min. Supernatant was collected and stored at 4 °C for acetylcholinesterase estimation by method of Ellman et al. (1961). The kinetic profile of enzyme activity was measured spectrophotometrically (Shimadzu, USA) at 412 nm with an interval of 15 s. One unit of acetylcholinesterase activity was defined as the number of micromoles (μmol) of acetylthiocholine iodide hydrolyzed per minute (min) per milligram (mg) of protein. The specific activity of acetylcholinesterase is expressed in micromoles/min/mg of protein.

To estimate *in vitro* AChE inhibitory effect, different concentrations of ethanolic extract of Noni and its chloroform, ethyl acetate and butanol fractions (5–400 μg/ml) and standard drug, tacrine (0.1–100 μmol), were prepared in phosphate buffer and incubated with supernatant of mice brain homogenate in 96 well plate. A kinetic profile of the enzyme activity was measured at the interval of 15 s at 412 nm.

2.7.3. Measurement of MDA

Malondialdehyde (MDA), a marker of lipid peroxidation, was estimated in the brain tissues, according to the method of Colado et al. (1997). After homogenization, tissue homogenate was mixed with 30% trichloroacetic acid, 5 N HCl followed by the addition of 2% thiobarbituric acid in 0.5 N NaOH. The mixture was heated for 15 min at 90 °C and centrifuged (Remi cold centrifuge) at 12,000 × *g* for 10 min. The pink colour of the supernatant was measured at 532 nm using ELISA plate reader (BIOTEK, USA). MDA concentration was calculated by using standard curve prepared with Tetra ethoxy propane and expressed as nmol/mg protein.

2.7.4. Measurement of GSH

GSH was determined by its reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to yield a yellow chromophore which was measured spectrophotometrically (Ellman, 1959). The brain homogenate was mixed with an equal amount of 10% TCA and centrifuged at 2000 × *g* for 10 min at 4 °C. The supernatant was used for GSH estimation. To 0.1 ml of processed tissue sample, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of DTNB and 0.4 ml of double-distilled water were added and the mixture was shaken vigorously on vortex. The absorbance was read at 412 nm using ELISA plate reader (BIOTEK, USA). GSH concentration was calculated by using standard curve prepared with reduced glutathione and expressed as μg/mg protein.

2.7.5. Protein estimation

Protein was measured in all brain samples for GSH and MDA by the method of Lowry et al. (1951) and protein for acetylcholinesterase activity by the method of Wang and Smith (1975). Bovine serum albumin (BSA) (1 mg/ml) was used as standard and measured in the range of 0.01–0.1 mg/ml.

2.8. Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis of passive avoidance data was performed by Student's *t*-test and analysis of biochemical and locomotor activity data was done by one way analysis of variance (ANOVA) followed by Tukey's test.

2.9. Estimation of IC_{50} values

The concentrations of test samples that inhibited hydrolysis of the substrate (acetylthiocholine) by 50% of (IC_{50}) were determined by monitoring the inhibitory effect of increasing concentrations of extracts with in the assays. The IC_{50} values were then calculated using GraphPhad prism computer software.

3. Results

3.1. Quantification of scopoletin, rutin and quercetin in the extract

The concentration of different constituents in various Noni extracts is shown in Table 1. Ethanolic extract and ethyl acetate fraction contain rutin as a major constituent followed by scopoletin and quercetin. However, butanol fraction contains only rutin whereas chloroform fraction showed rutin and scopoletin. Further, we found traces of quercetin in chloroform fraction and scopoletin in butanol fraction which were below the level of quantification.

3.2. Passive avoidance test

The transfer latency time (TLT) was significantly increased [$P < 0.01$] on 2nd trial (retention trial) as compared to 1st trial (acquisition trial) in control group. However, there was no significant [$P > 0.05$] change in TLT of retention trial in scopolamine treated group as compared to acquisition trial indicating impairment of memory. As shown in Fig. 1 administration of donepezil prevented scopolamine induced memory impairment in mice as revealed by significant [$P < 0.01$] increase in TLT during retention trial as compared to acquisition trial. Treatment with ethanolic extract [$P < 0.05$], ethyl acetate [$P < 0.05$] and chloroform [$P < 0.05$] fraction of Noni prevented scopolamine induced memory impairment as revealed by significant increase in TLT of retention trial.

Table 1
Concentration of different constituents (μg/mg dry extract) in ethanolic extract and its chloroform, ethyl acetate and butanol fractions of Noni fruit.

Constituent	Rutin (μg/mg dry extract) ± S.E.M.	Scopoletin (μg/mg dry extract) ± S.E.M.	Quercetin (μg/mg dry extract) ± S.E.M.
Ethanolic extract	5.5 ± 0.15	1.27 ± 0.04	0.11 ± 0.005
Chloroform fraction	2.93 ± 0.03	4.47 ± 0.23	Below quantification limit
Ethyl acetate fraction	15.49 ± 1.21	1.07 ± 0.08	0.50 ± 0.01
Butanol fraction	8.59 ± 1.36	Below quantification limit	Not detected

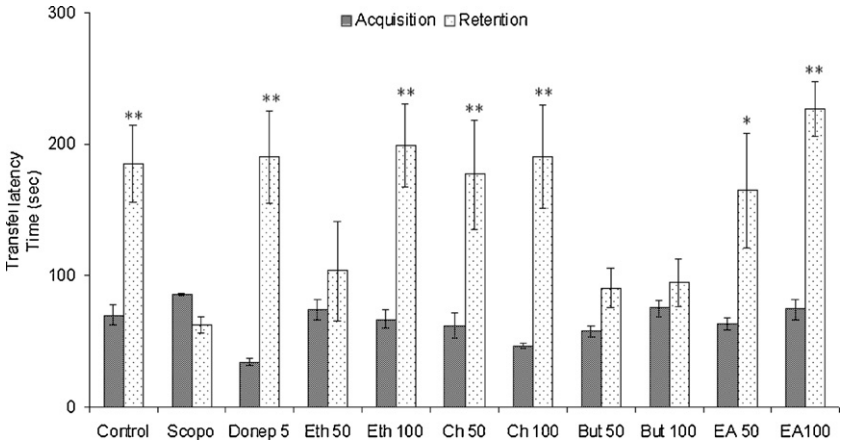

Fig. 1. Effect of Noni on scopolamine induced memory impairment in passive avoidance test. Data values are expressed as mean transfer latency time (s) ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$ vs acquisition trial.

However, ethanolic fraction at lower dose and butanol fraction at both doses did not increase TLT of retention trial significantly [$P>0.05$] as compared to acquisition trial.

3.3. Locomotor activity

The spontaneous locomotor activity remained unaltered among different groups [Total: $F(10, 44)=0.24$, $P>0.05$, Ambulatory: $F(10, 44)=0.1812$, $P>0.05$ and Vertical: $F(10, 44)=1.244$, $P>0.05$].

3.4. Measurement of cerebral blood flow (CBF)

The separate animal groups were used for measuring cerebral blood flow (CBF). Noni extracts were administered for three days. On the last day, 1 h after drug administration, baseline CBF was measured. Following this, scopolamine (3 mg/kg, IP) was administered and then CBF was measured at each 5 min continuously for 20 min. In comparison to baseline values, there was no significant change in CBF of control [one way ANOVA followed by *Tukey's* multiple comparison tests, $F(4, 25)=0.51$, $P>0.05$] group. However, a significant [$F(4, 25)=98.65$, $P<0.01$] reduction in CBF after scopolamine administration, in comparison to baseline values, was observed from 5 min onwards and the effect lasted throughout the experimental duration (20 min). Further, three days treatment with ethanolic extract and chloroform and ethyl acetate fractions of Noni prevented scopolamine induced reduction in CBF. However, butanol fraction failed to reverse effect of scopolamine on CBF as there was a significant reduction in CBF in comparison to baseline value [$F(4, 25)=5.67$, $P<0.05$] (Fig. 2).

3.5. Estimation of biochemical parameters of oxidative stress

3.5.1. MDA level

The MDA level (nmol/mg protein) was estimated in brain after the completion of behavioral studies. The MDA level rose significantly in scopolamine treated mice [$P<0.05$] as compared to control group. On the other hand, donepezil significantly decreased [$P<0.05$] MDA level in comparison to scopolamine group. Preventive treatment with 100 mg/kg [$P<0.05$] ethanolic extract significantly decreased MDA level in scopolamine injected mice brain whereas lower dose [$P>0.05$] was not effective. Moreover, treatment with chloroform [$P<0.01$], butanol [$P<0.05$] and ethyl acetate fractions [$P<0.01$] of Noni dose dependently decreased MDA level in scopolamine injected mice brain (Fig. 3).

3.5.2. GSH level

GSH (μg/mg protein) was estimated in brain after the completion of behavioral studies. As shown in Fig. 4, a significant fall in the levels of GSH was observed in the scopolamine group [$P<0.01$] as compared to the control. There was significant rise in level of GSH in the group treated with 100 mg/kg ethanolic extract [$P<0.01$] in comparison to scopolamine group. However, lower dose [$P>0.05$] of ethanolic extract had no significant effect on GSH level in scopolamine injected mice brain. Further, chloroform [$P<0.01$], ethyl acetate [$P<0.05$] and butanol [$P<0.05$] fractions dose dependently

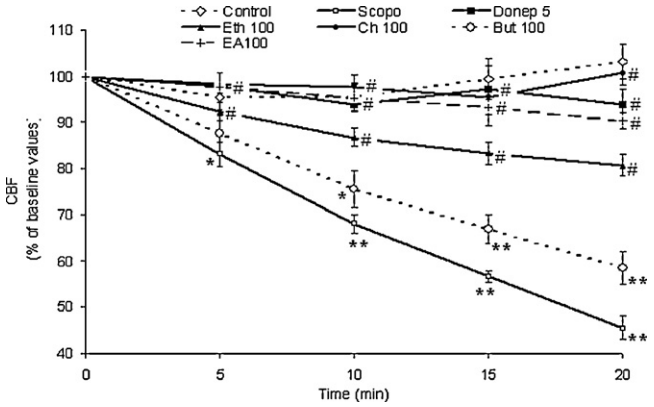

Fig. 2. Effect of Noni on cerebral blood flow in scopolamine induced amnesic mice. Data values are expressed as % of baseline CBF (blood perfusion units) ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$ vs control group and $^{\#}P<0.01$ vs scopolamine group.

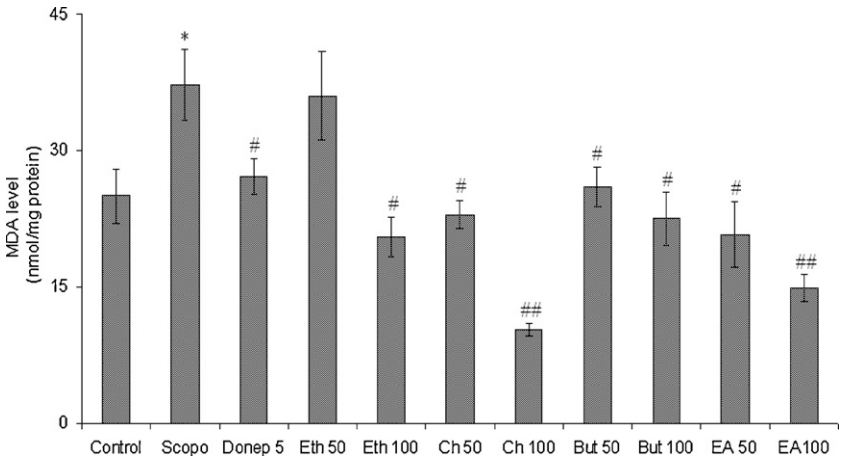

Fig. 3. Effect of Noni on MDA level in mice brain. Data values are expressed as mean MDA level (nmol/mg protein) ± S.E.M. $^{*}P < 0.01$ vs Control group and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs scopolamine group.

increased GSH level in mice brain. Administration of donepezil [$P < 0.01$] 1 h prior acquisition trial significantly increased GSH level in comparison to scopolamine group.

3.5.3. AChE activity

As shown in Fig. 5, there was significant ($P < 0.01$) increase in AChE activity in scopolamine group [$P < 0.01$] as compared to control. Donepezil significantly decreased [$P < 0.01$] AChE activity in scopolamine injected mice brain. Preventive treatment with ethanolic extract of Noni significantly decreased [$P < 0.01$] AChE activity in scopolamine injected mice brain. Moreover, treatment with chloroform [$P < 0.01$], ethyl acetate [$P < 0.01$] and butanol [$P < 0.05$] fractions of Noni dose dependently decreased AChE activity in scopolamine injected mice brain.

3.6. In vitro AChE activity

To study inhibitory effect on AChE activity *in vitro*, different Noni extracts (5–200 μg/ml) were incubated with supernatant of mice brain homogenate for 30 min. The enzyme activity was estimated by Ellman's method and % inhibition was calculated. As shown in Fig. 6, there was a concentration dependent decrease in AChE activity by Noni extracts. The IC_{50} values of ethanol, chloroform, ethyl acetate and butanol extracts were found to be 138.4, 78.11, 486.2 and 486.9 μg/ml respectively. The IC_{50} value of standard drug tacrine was found to be 0.18 μmol.

4. Discussion

Present study investigated the effect of ethanolic extract of Noni fruit along with its chloroform, ethyl acetate and butanol fractions on the scopolamine induced memory impairment in mice using step through passive avoidance test. It is well known that scopolamine, a non selective centrally acting muscarinic receptor antagonist, impairs learning and memory in rodents and humans. This experimental model of memory impairment has been extensively used in research to screen for drugs with potential therapeutic value in dementia (El-Sherbiny et al., 2003; Saxena et al., 2007; Sharma et al., 2010).

In the present study, systemic administration of scopolamine 5 min before acquisition trial induced memory impairment as tested by step through passive avoidance test. Further, scopolamine induced amnesia was associated with reduced CBF, increased oxidative stress and acetylcholinesterase (AChE) activity in mice brain. The clinically used antidementic drug donepezil ameliorated scopolamine induced memory impairment by reducing AChE activity and oxidative stress and restoring cerebral circulation (Agrawal et al., 2008).

Treatment with ethanolic extract of Noni fruit reversed scopolamine induced memory impairment in mice as shown by significant increase in transfer latency time during retention trials. Further, the effect of various fractions of ethanolic extract of Noni was investigated on scopolamine induced memory impairment. The chloroform and ethyl acetate fractions showed dose dependent

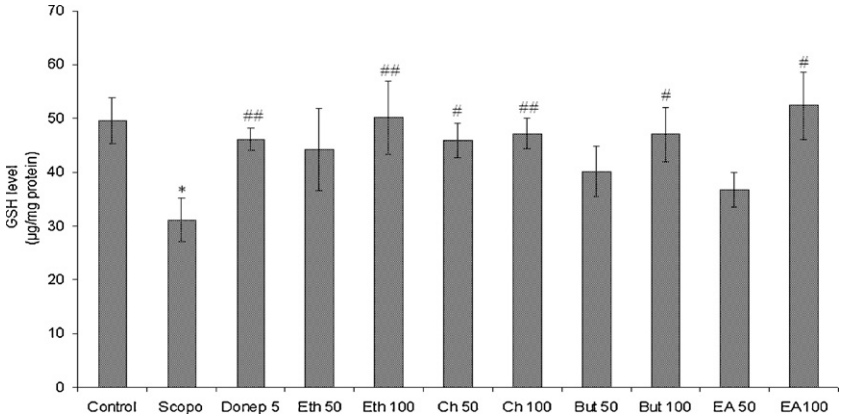

Fig. 4. Effect of Noni on GSH level in mice brain. Data values are expressed as mean GSH level (μg/mg protein) ± S.E.M. $^{*}P < 0.05$ vs control group and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs scopolamine group.

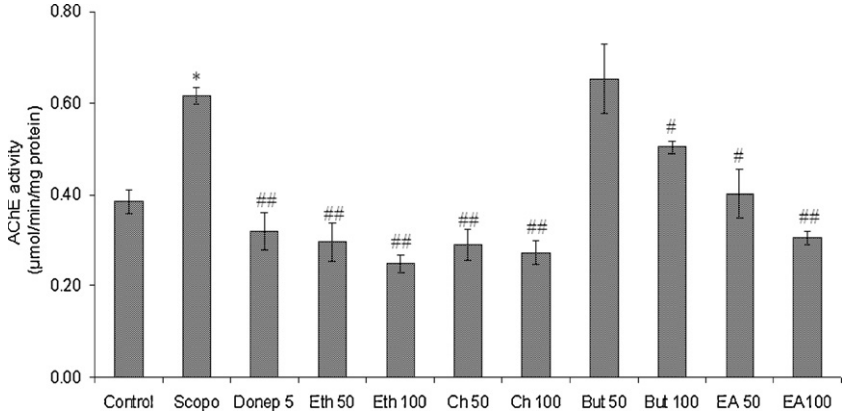

Fig. 5. Effect of Noni on AChE activity in mice brain. Data values are expressed as mean AChE activity (µmol/min/mg protein) ± S.E.M. $^{*}P < 0.01$ vs control group and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs scopolamine group.

reversal of scopolamine induced memory impairment. However, butanol fraction was found ineffective in this model. No significant difference in locomotor activity was found among different groups. This excludes the possibility that the locomotor activity *per se* may have contributed to the changes in passive avoidance behavior. Recently, Muralidharan et al. (2010), reported that ethyl acetate extract of Noni prevents amyloid beta induced memory dysfunction by reducing oxidative stress in mice brain. However, this is the first study reporting beneficial effects of Noni fruit in scopolamine induced memory dysfunction indicating its anti acetylcholinesterase activity. The Noni fruit is reported to contain number of constituents having antioxidant and neuroprotective effect (Wang et al., 2002; Pawlus and Kinghorn, 2007). We have standardized the Noni fruit extract by using scopoletin, rutin and quercetin as markers, as these are the major constituents of Noni fruit (Wang et al., 2002; Su et al., 2005; Samoylenko et al., 2006). Studies showed that these compounds exhibited neuroprotective effect *in vitro* and *in vivo* (Rollinger et al., 2004; Lee et al., 2004; Pu et al., 2007; Tota et al., 2010; Bhutada et al., 2010; Richetti et al., 2010; Hernandez et al., 2010). Therefore, the antiamnesic effect of different Noni extracts observed in the present study may be attributed to the presence of various phytoconstituents with neuroprotective property. Further, we found a linear correlation between concentration of active constituents and memory improving effect. In passive avoidance test, ethanolic extract and its chloroform and ethyl acetate fraction were found effective against scopolamine induced amnesia. Phytochemical investigation revealed presence of scopoletin, rutin and quercetin in ethanolic extract and its ethyl acetate fraction while chloroform fraction showed presence of rutin and scopoletin with traces of quercetin. However, butanol fraction,

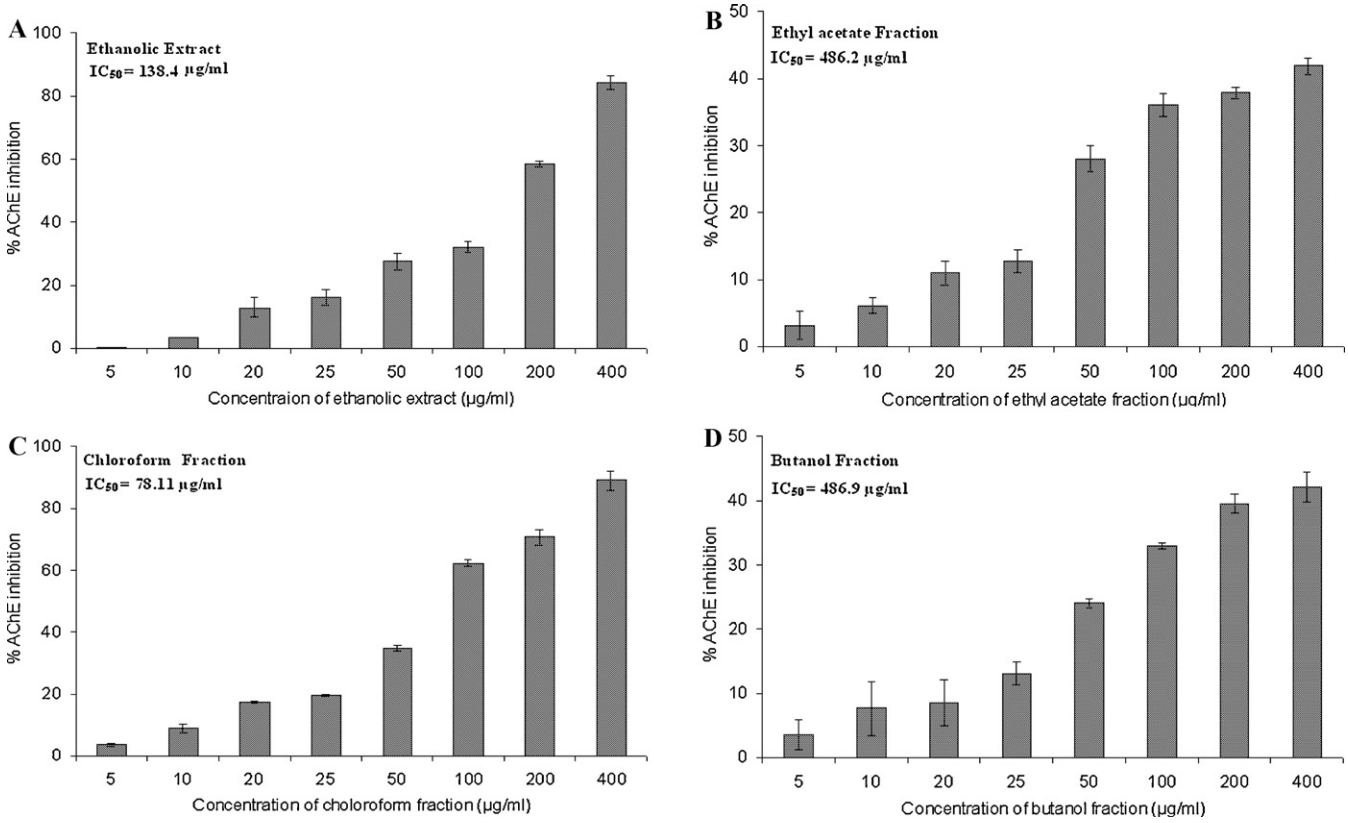

Fig. 6. *In vitro* AChE inhibitory effect of (A) ethanolic extract, (B) chloroform fraction, (C) ethyl acetate fraction and (D) butanol fraction.

which was ineffective, contained rutin with traces of scopoletin and quercetin.

Previously it has been reported that scopolamine induced memory impairment in animals and humans is associated with reduced cerebral blood flow (Honer et al., 1988; Tsukada et al., 1997; Ogawa et al., 1994). In agreement with previous reports, in this study also we found a significant reduction in CBF following scopolamine injection. The scopolamine induced reduction in CBF is due to cholinergic hypofunction as treatment with cholinergic drugs normalized the CBF suggesting important role of cholinergic system in the regulation of cerebral circulation (Honer et al., 1988; Tsukada et al., 1997; Ogawa et al., 1994). In this study also donepezil prevented scopolamine induced decrease in CBF in mice. Further, treatment with ethanolic extract of Noni and its chloroform and ethyl acetate fractions ameliorated scopolamine induced reduction in CBF. However, butanol fraction failed to prevent scopolamine induced cerebral hypoperfusion in mice.

The exact mechanism responsible for beneficial effects of Noni against scopolamine induced memory impairment and reduction in CBF is not known but it may be due to improvement in central cholinergic function by Noni. Because it is reported that scopolamine induced amnesia and cerebral hypoperfusion is due to cholinergic hypofunction which was ameliorated by improving central cholinergic function. The most important strategy to increase cholinergic function is inhibition of AChE. Therefore, we evaluated the effect of Noni on AChE activity *in vitro* and *in vivo*. In agreement with previous studies, we found a significant increase in AChE activity in scopolamine injected mice brain (Agrawal et al., 2008; Sharma et al., 2010). Preventive treatment of ethanolic extract and its different fractions decreased AChE activity in scopolamine injected mice brain. The chloroform and ethyl acetate fractions were found most effective in inhibiting AChE activity in mice brain while ethanolic extract and its butanol fraction showed antiAChE effect at higher dose only. Further, *in vitro* study showed that ethanolic extract of Noni and its three fractions exhibited dose dependent inhibition of AChE activity in mice brain homogenate. The IC_{50} value of ethanolic extract of Noni fruit was found to be 138.4 μg/ml. Among different fractions of ethanolic extract, the chloroform fraction was found to be the most active with an IC_{50} value 78.11 μg/ml whereas the IC_{50} value of ethyl acetate and butanol fraction were 486.2 and 486.9 μg/ml respectively. Our *in vivo* study revealed that both chloroform and ethyl acetate fractions were equally effective against scopolamine induced changes. But *in vitro*, the ethyl acetate fraction was found much less effective in inhibiting AChE activity than chloroform fraction. The exact reason for this type of observation is not known but it can be speculated that one or more components of ethyl acetate fraction are undergoing *in vivo* activation which are otherwise inactive. Previous studies revealed presence of compounds like rutin and scopoletin in Noni which are reported to inhibit AChE activity (Chung et al., 2001; Lee et al., 2004; Rollinger et al., 2004; Orhan et al., 2008; Hernandez et al., 2010). Our study showed presence of scopoletin and rutin in ethanol, ethyl acetate and chloroform extract while rutin was found in butanol fraction. The difference in the concentration of these constituents may be responsible for different potency of these extract against AChE activity.

As reported earlier, we also found significant increase in MDA and decrease in GSH level in mice brain following scopolamine administration (El-Sherbiny et al., 2003; Sharma et al., 2010). Preventive treatment with ethanolic extract and its three fractions dose dependently reversed scopolamine induced oxidative stress in mice. This effect is due to the antioxidant compounds like quercetin and rutin present in Noni (Rastogi, 1990; Su et al., 2005).

In conclusion, the beneficial effect of the *Morinda citrifolia* fruit on scopolamine induced memory impairment is due to its antioxidant property and inhibition of AChE activity. The improvement in cerebral circulation may also play an important role in the beneficial effects of Noni. Therefore, the use of Noni as dietary supplement should be encouraged to ward off age-associated memory disorders like AD.

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