Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

NEUROPROTECTIVE EFFECTS OF POLYHERBAL FORMULATION (INDIAN NONI) ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN MICE

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Received: 06 Oct 2013, Revised and Accepted: 31 Oct 2013

ABSTRACT

The aim of the study is to evaluate the Neuroprotective effect of Indian NONI juice. The objective of the study is to carry out *in vivo* tests to evaluate the cognitive enhancing effects of Indian NONI juice against scopolamine-induced amnesia in mice. The effect on NONI of acetylcholine esterase activity was screened by in vitro method. The effect of NONI against scopolamine induced cognitive dysfunction in mice was studied and the effect of NONI against scopolamine induced oxidative stress in mice brain was measured. All the animals were treated with their respective extracts / drug twice a day orally for 14 days and the control animals will receive vehicle. On day 8, 9 and 10 scopolamine was injected intraperitonially to all the animals after one hour of extract/drug treatment. Then the animals were subjected to behavioral analysis and then sacrificed for the biochemical and histopathological analysis. Results were express in mean± SEM. Biochemical and behavioral paradigms were analysed by one way ANOVA followed by Dunnett tes. P value <0.05 was fixed as significant criterion. NONI produced a dose depend decrease in AChE activity with an IC_{50} value of 152.90±1.90µg/0.1ml. Administration of NONI significantly reversed the scopolamine induced alteration in arm time spent in mice. The effect of NONI was comparable with that of the reference drug Piracetam. The results of this study clearly indicate that oral administration of NONI to mice reverse impairment of retention in step down latency inhibitory test. From the above results, it could be posulated that NONI exerts a protective effect against oxidative damage induced by scopolamine by maintaining the activities of SOD. These results suggested that NONI might offer a useful therapeutic choice in either the prevention or the treatment of Alzheimer's disease.

Keywords: Oxidative Stress, Antioxidant, Alzheimer, NONI

INTRODUCTION

Atropine and scopolamine are muscarinic receptor antagonists with amnesic properties that have been used for decades in experimental animals to induce impairment in their performance of a variety of tasks requiring intact working and reference[1-3]memory. As long as 30 years ago, scopolamine had been used in clinical research studies. Scopolamine has also been used clinically (though less frequently than in past years) as an adjunct to surgical or obstetric procedures to induce sedation and post-procedural amnesia. Since the first reports of a central cholinergic deficit associated with Alzheimer's disease, the connection had been made between the cognitive and memory deficits associated with this disease and the reversible amnesic effects induced by centrally acting muscarinic cholinergic antagonists. Indeed, blockade of central muscarinic receptors could induce a pattern of cognitive impairment even in young subjects reminiscent of that observed in the aged, or in individuals with Alzheimer's disease. For many years, the amnesic action produced in animals by the administration of centrally acting muscarinic cholinergic antagonists, particularly scopolamine, has been a widely used model for the characterization of potential cognition-enhancing[4] drugs.

Herbal system of medicine has proved to play a key role in treatment of cognitive impairment and related disorders. Present study aims at the neuroprotective effect of NONI juice in dementia & memory impairment in mice. NONI is a polyherbal formulation of *Morinda citrifolia* extract and *Garcinia cambogi.* Apart from studying a single herb or an active compound of an herb, currently researchers focus more on polyherbal formulation which has proved to be successful throughout for the treatment[5,6] of dementia.

A strong relationship between learning and memory functions and the cholinergic system in experimental animals have been suggested in earlier[7]studies. Cholinergic neurons originating in the medial septum project to areas such as the cortex and hippocampus, which play a role in Ach-associated[8,9]cognition. Behavioral and psychological symptoms of dementia such as aggression, agitation, irritability, and hallucinations are displayed at some point of illness in majority of patients with Alzheimer's disease (AD) and other forms of senile dementia (Naoki *et al*).

In preclinical studies, perhaps the two most widely used rodent tasks for studying or screening cognition-enhancing drugs are the inhibitory avoidance and the water maze tasks. However, clinical versions of these tasks are not well established. Computer-presented operant tasks designed for assessing the cognitive deficits associated with Alzheimer's disease are available such as the CogState™ product (Collie *et al*)the CANTAB™ product(Blackwell *et al* and Beglinger *et al*). Computer presented cognitive test procedures are becoming more prevalent in the clinical cognitive testing domain. Therefore, there would be some advantage to having available preclinical models that are more relevant than those often used for preclinical drug screening.

MATERIALS AND METHODS

Chemicals

Scopalamine (Sigma chemical Co, USA), Piracetam (Registered pharmacy store, Chennai), Carboxy methyl cellulose (CDH lab, India). All the chemicals used for the biochemical analysis were procured from Sisco research laboratory, India.

Preparation of Indian NONI

An ayurvedic proprietory formulation, Indian **NONI** drink was obtained from Health India Laboratories the concentrated **NONI** drink was diluted in honey.

Invitro Acetyl Choline Esterase Inhibitory Activity

To the extracts at various concentrations, 10% homogenate and 0.025M of sodium phosphate buffer (pH-8.0) were added. The reaction was triggered by the addition of the substrate (50mM acetyl thiocholine iodide). After incubation at 37°C for 15min, the reaction was terminated by addition of 10% trichloroacetic acid. The mixture was stirred well and centrifuged for 10min at 3500rpm. The supernatant was collected and allowed to react with the coloring reagent 0.1mM 5,5'-Dithiobis(2-Nitro benzoic acid). The intensity of color developed was measured at 415nm in Thermo Scientific multiskan spectrophotometer, USA (Ellmans *et al.,* 1984.). The percentage inhibiton of the enzyme activity was calculated using the formula

Percentage inhibition = [(test – control)/test]*100

Experimental Animals

Male swiss albino mice weighting 22-24g were used in the pharmacological studies. The inbred animals were taken and

maintained in CEFT, of Sri Ramachandra University, Porur, Chennai-116. The animals were maintained in day and night rhythm (12h light: 12h dark, temp: 20±2°C, humidity: 40-60%) in a well-ventilated room. Animals were given with normal diet regularly.

Treatment

All the animals were treated with their respective extracts / drug twice a day orally for 14 days and the control animals will receive vehicle. On day 8, 9 and 10 scopolamine was injected intraperitonially to all the animals after one hour of extract/drug treatment. Then the animals were subjected to behavioral analysis and then sacrificed for the biochemical and histopathological analysis.

Groups

Group I: Control (0.5% CMC) ;Group II: Scopolamine (0.3mg/kg body weight, i.p.) + vehicle;Group III: Piracetam (250mg/kg body weight, P.O.) + Scopolamine (0.3mg/kg body weight, i.p.);Group IV: NONI juice (0.13ml/kg body weight, p.o) + scopolamine (0.3mg/kg body weight, i.p.);Group V: NONI juice (0.26ml/kg body weight, p.o) + scopolamine (0.3mg/kg body weight, i.p.).

Behavioral Analysis

Y-Maze

The y-maze test was performed according to Aggleton *et al*., **1986**. The mice memory function was tested under different conditions, like under the influence of drugs that may impair or enhance memory. The maze was carefully cleaned with a wet tissue paper (10% ethanol solution) between each experiment.

Transfer Latency

This test has been widely validated to measure anxiety in rodents (pillow *et al*., **1985**; lister, **1985**). After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

Step Down Latency

The memory retention deficit was estimated in the continuous avoidance apparatus (Gemini, sanfrancisco, USA) (kang *et al*., **2005**). The chamber was cleaned with 10% alcohol each time and the influence of drugs that may impair or enhance memory was analyzed.

Biochemical Estimations

Superoxide Dismutase Activity (SOD)

Superoxide dismutase was assayed (Kakkar P. *et al.,* **1984**). 10% brain homogenate, 0.025M of sodium pyrophosphate buffer (pH 8.3), 186µM of PMS and 300µM of NBT (pH 8.3) were used. The reaction was initiated by addition 780µM of NADH (pH 8.3). After incubation at 30[0]C for 90 seconds, the reaction was stopped by addition of 0.25ml glacial acetic acid. Then the reaction mixture was stirred vigorously and shaken with 2.0ml of n-Butanol. The mixture was allowed to stand for 10 minutes and centrifuged. The colour intensity of the chromogen was read at 560nm in Spectrophotomer (Multiskan spectrum, v1.2, USA).

Lipid Peroxidation (TBARS), Reduced Glutathione (GSH)

Lipid peroxidation was determined according to procedure of Ohkawa H.*et al.,* **1979**. The

Glutathione content present in brain homogenate was estimated was estimated (Moren *et al.,* **1979**).

Total Protein by Biuret Method

The total protein was determined by the method of (Robinson *et al* **1940***)*. Estimation of protein was assayed by taking 0.2ml saline, 10% homogenate followed by addition of 1.25ml of working biuret reagent and incubated at room temperature for 15 min. The colour intensity was read at 540nm in spectrophotometer (Multiskan spectrum, v1.2, USA)

RESULTS AND DISCUSSION

Results were express in mean± SEM. Mean difference between the experimental animal body weight was analysed by Student 't' test and biochemical and behavioral paradigms were analysed by one way ANOVA followed by Dunnett test as post hoc. P value <0.05 was fixed as significant criterion.

Effect of NONI on acetylcholine esterase assay – in vitro

The effect on NONI of acetylcholine esterase activity was screened by in vitro method. NONI produced a dose depend decrease in AChE activity with an IC_{50} value of 152.90±1.90µg/0.1ml. Fig.1. It has been demonstrated that impairments in learning, memory and behavior observed in patients with dementia are caused, at least in part, by changes within the cholinergic system. It has been demonstrated by previous animal and human studies that learning and memory can be modifided by drugs affecting the central cholinergic system. Cholinergic transmission is terminated mainly by acetylcholine hydrolysis via the enzyme acetylcholinesterase. This enzyme is essential in maintaining the normal function of the nervous system, since it rapidly terminates the action acetylcholine released in to the synapse. This is why we evaluated the effect of indian NONI on acetylcholinesterase activity and correlated this activity with its anti-amnesic activites. Indian NONI inhibited 49.5% of acetylcholinesterase

Body Weight

Effect of NONI on the body weight experimental animals is shown in the Figure 2. No change body weight was observed in the scopolamine and Piracetam treated groups in comparison to the vehicle treated animals during the experimental period. But, a non-significant decrease in body weight was observed with NONI treated group. As Garcinia had shown the antiobesity properties.

Y-maze

Time spent

CMC treated group spent significant time in novel arm at 1, 2 inter trail interval (ITI) when compared to the start and other arm. There was no significant difference between the time spent between the arms at 4h ITI. A significant (P<0.05) decrease in time spent in novel arm was observed with CMC+SCOP group when compared to decreased start and other arm and also with the novel are time spent in vehicle group. Administration of NONI significantly reversed the scopolamine induced alteration in arm time spent in mice. The effect of NONI was comparable with that of the reference drug Piracetam.

Number of entries

CMC treated group showed increased entries in novel arm when compared to start arm and other arm (p<0.05). In CMC+SCOP treated group, a non-significant decrease in novel arm entry was observed when compared to the vehicle treated novel arm entry. Administration of NONI did not produce any remarkable change in novel arm entries at 2 and 4H ITI. The effect of NONI was comparable with that of the reference drug Piracetam. Our findings demonstrated that NONI and its with-drawl impaired mice spatial recognition memory in the Y-maze. Our data support this view by showing that there were always significant arm effects on either absolute measures or percentage measures of total duration of entries and time spent in each arm. Moreover, after 4H ITI, most animals showed no significant arm differences which suggesting that mice could not distinguish the novel arm from the other two familiar arms after a longer interval.

Transfer Latency

In toxication of scopolamine decreased the inflexion ratio significantly (p<0.01) when compared with vehicle treated group in the transfer latency. Administration of NONI for a period of 14 days significantly (p<0.01) increased the inflexion ratio in the experimental animals when compared with scopolamine treated group. Piracetam increased the inflexion ratio significantly (p<0.01) when scopolamine group. Cognitive dysfunction such as learning impairment and delayed amnesia are the most striking age-related changes observed in human beings and animals. These types of deficits probably are due to the vulnerability of the brain cells to increased oxidative stress during aging process. The results of this

study clearly indicate that oral administration of NONI to mice reverse impairment of retention in transfer latency.

Step down latency inhibitory test

The effect of NONI on inflexion ratio in Step down latency inhibitory test. Administration of scopolamine significantly ($p<0.05$) decreased the inflexion ratio in CMC treated group when compared to the vehicle treated animals on day 2 and a highly significant ($p<0.01$) decrease was observed on day 9. Administration of NONI significantly increased the inflexion ratio on day 2 ($p<0.05$) and day 9 ($p<0.01$). The effect of NONI was comparable with that of the reference drug Piracetam. Cognitive dysfunction such as learning impairment and delayed amnesia are the most striking age-related changes observed in human beings and animals. These types of deficits probably are due to the vulnerability of the brain cells to increased oxidative stress during aging process. The results of this study clearly indicate that oral administration of NONI to mice reverse impairment of retention in step down latency inhibitory test.

Lipid Peroxidation (Tbars)

The effect of NONI on lipid peroxidation content in scopolamine treated mice brain. Administration of scopolamine increased the brain lipid peroxidation content significantly ($p<0.01$) in CMC+SCO group in comparison to the vehicle treated group. Treatment with NONI significantly ($p<0.05$) decreased the lipid peroxidation content in mouse brain when compared to the CMC+SCO group. The effect was comparable to the reference drug, Piracetam. The amount of lipid peroxides estimated by this methowas in good agreement with the values of conjugated dienes. However, the values estimated for TBARS given as malondialdehyde equivalents were much lower in the first **24** hr of oxidation. This simply reflects that lipid peroxides are at first the major products formed during oxidation of LDL. Their breakdown to malondialdehyde in order to be measurable by the TBA assay might have been impaired by the presence of EDTA (which was used

together with BHT to stop the oxidation process in the sample.

Superoxide Dismutase (Sod)

The effect of NONI on superoxide dismutase activity in mouse brain. Administration of scopolamine decreased the brain SOD activity significantly ($p<0.01$) in CMC+SCO group when compared to the vehicle treated group. Treatment with NONI significantly ($p<0.01$) increased the SOD activity in mouse brain when compared to the CMC+SCO group. The effect was comparable to the reference drug, Piracetam. The most remarkable effect of NONI is the increased activity of SOD in mice brain. Although there are still conflicting reports associated with the SOD activity in Alzheimer's disease, most recently, SOD mimetics have come to the forefront of antioxidantive therapeutics of neurodegenerative diseases. From the above results, it could be posulated that NONI exerts a protective effect against oxidative damage induced by scopolamine by maintaining the activities of SOD.

Reduced Glutathione (Gsh)

The effect of NONI on reduced glutathione content in mouse brain. Administration of scopolamine decreased the brain GSH content significantly ($p<0.05$) in CMC+SCO group when compared to the vehicle treated group. Treatment with NONI significantly ($p<0.05$) increased the GSH content in mouse brain when compared to the CMC+SCO group. Piracetam produced a non-significant increase in GSH content in comparsion to the CMC+SCO group. Many clinical studies have reported strong evidences that oxidative stress is involved in the pathogenesis of Alzheimer's disease. Although the reduced activity of glutathione peroxidase induced by scopolamine was little changed by the acute treatment with NONI, it was increased by the prolonged treatment.

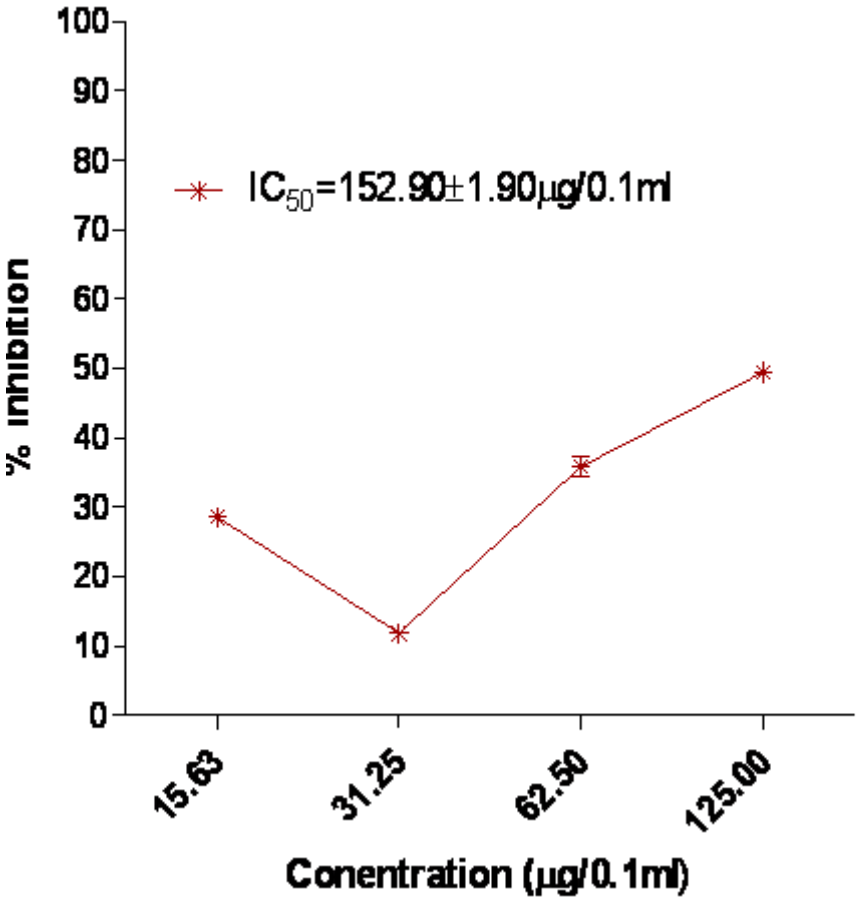

Fig. 1: Acetylcholine esterase inhibitory assay

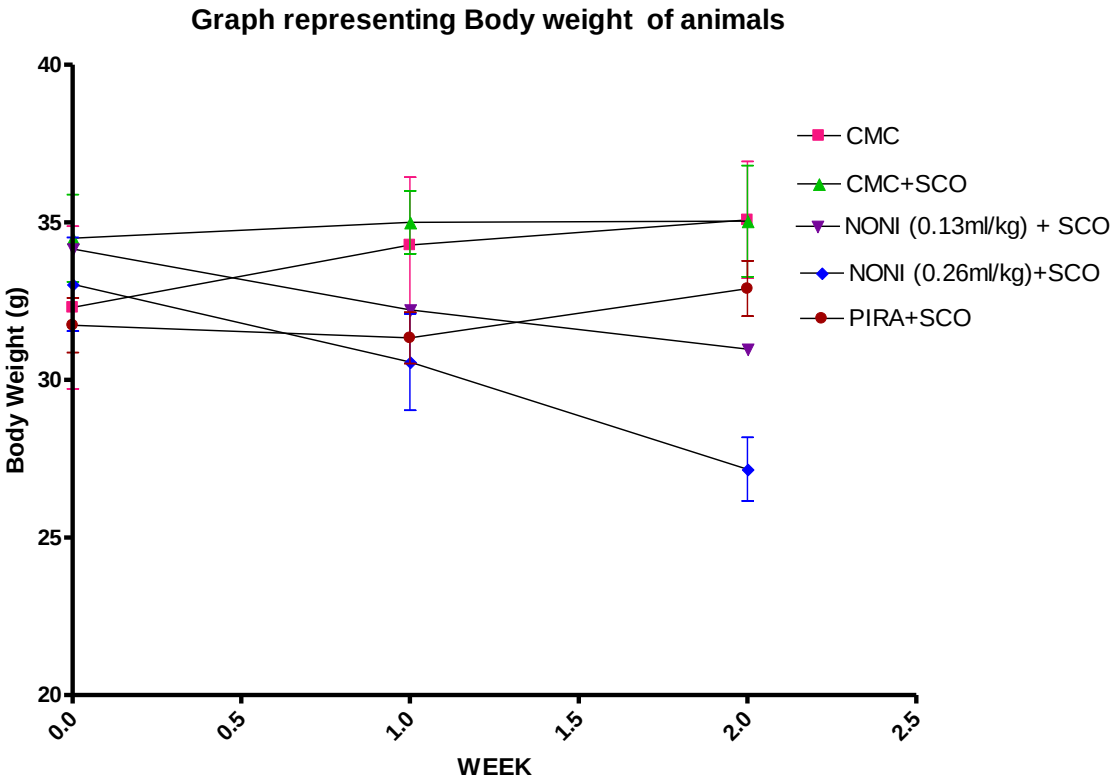

Fig. 2: Body Weight

CONCLUSION

These results suggested that NONI might offer a useful therapeutic choice in either the prevention or the treatment of Alzheimer's disease.

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