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Neuroprotective Effect of *vitex negundo* Against Scopolamine Induced Cognitive Impairment and Oxidative Stress in *Wistar albino* Rats

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The present study was undertaken to investigate the neuroprotective effect of *Vitex negundo* on cognitive impairment induced by scopolamine, a muscarinic antagonist, in *Wistar albino* rats. Memory impairment was induced by administration of scopolamine intraperitoneally. Aqueous extract of *Vitex negundo* was fed to the rats at a dosage of 1000 mg per kilogram body weight of the animal for a period of 15 days. Learning and memory were evaluated using the classical T-maze test. Oxidative stress parameters like Malondialdehyde, glutathione-S-transferase and superoxide dismutase were assessed and acetylcholinesterase activity was estimated. We conclude that the aqueous extract of *Vitex negundo* leaf has potential therapeutic effects on improving the cognitive impairment in *Wistar albino* rats through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes and decreasing acetylcholinesterase activity in the brain. We suggest further detailed study in this area to separate the exact compound in the extract responsible for the effect for therapeutic purpose.

Key words: Donepezil, Neuro-protective agent, Scopolamine, *Vitex negundo*, *Wistar albino*, T-maze.

It is generally accepted that memory is one of the most vital functions of the brain. Memory is the process by which organisms are able to record their experiences and use this information to adapt their responses to the environment (Dunning and During, 2003). Central cholinergic system is considered as the most important neurotransmitter involved in the regulation of cognitive functions (Blokland, 1995). Progressive decline in memory and impaired cognitive functions are the major features of Alzheimer's disease (Siddiqui and Levey, 1999).

Memory formation is a very complex process involving a number of neuronal pathways and neurotransmitters. It is well known that the cholinergic neuronal system plays an important role in memory in humans and animals (Morris, 1989, Sutherland *et al.*, 1982, Winkler *et al.*, 1995). Dementia is a progressive neurodegenerative disorder that is slow in its onset but ultimately leads to memory impairment. Based on the cholinergic hypothesis, many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity via acetylcholinesterase (AChE) inhibitors ((Blokland, 1995, Ramakrishna *et al.*, 1998). There is substantial evidence that oxidative stress is a causative factor in the pathogenesis of major neurodegenerative diseases like dementia, Alzheimer's disease, Parkinson's disease etc (Mariani *et al.*, 2005). Both endogenous and dietary antioxidants can protect the nerve tissue from oxidative stress (Grant, 1997). Where there is an imbalance in the oxidant and anti-oxidant level oxidative stress will be expressed (Chaturvedi *et al.*, 2015).

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It was suggested that a variety of herbs are used to reduce dementia and the concerned neurological problems like memory loss, lack of concentration, learning etc. They have anti-acetylcholinesterase property and may be useful as a nootropic agent in delaying the onset and reducing the severity of dementia when compared to that of reference drugs. A study conducted by Anitha *et al.,* (2011) showed that the extracts of leaves and plants of some medicinal plants have good potential for use in deprived memory (Anita *et al.,* 2011). *Vitex negundo* (Verbenaceae) is a large aromatic shrub found throughout India, east Africa, the Philippine islands, Malaysia and also in the warm zones of Taiwan. It has been extensively studied for its various pharmacological properties including antinociceptive (Gupta *et al.,* 2015), anticonvulsant (Li Yu-Jie *et al.,* 2015) and anti-inflammatory (Tandon and Gupta, 2005) activities. The leaf extract is used in Ayurvedic and Unani system of medicine (Malik, 2004). Literature survey of *Vitex negundo* revealed that the leaves possess antioxidant activity and it was found to be correlated with its polar compounds, such as total phenolic content, catechin, epicatechin, quercetin and myricetin (Devi *et al.,* 2007; Tiwari *et al.,* 2007; Zargar *et al.,* 2011). Lignans showed anti-cholinesterase activity in *in-vitro*, which is a natural compound present in *Vitex negundo* (Malik, 2004). However, very less number of studies was conducted to investigate the effect of *Vitex negundo* extract against cognitive impairment *in-vivo***.** Hence the present study was undertaken to evaluate the neuroprotective effect of *Vitex negundo* against scopolamine induced cognitive impairment and oxidative stress in rats.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments were conducted according to the CPCSEA guidelines on the use and care of experimental animals. 42 Adult male *Wistar albino* rats weighing around 180 + 20 grams were used for the study. The animals were housed in polypropylene cages (22.5 X 35.5 X 15 cm) with controlled temperature (25 + 2^{o}C), humidity (50-55 %), light (12 hr light and dark cycle) with food and water *ad libitum*.

Vitex negundo was collected from Kondotty, Malappuram district of Kerala state, India. The taxonomic identity of the plant was confirmed by comparing collected voucher specimen with those of known identity, which are located in the herbarium (Acc. No. 88441) of the Dept. of Botany, University of Calicut, Kerala with the help of a pteridophyte taxonomist. Fresh extract was made by grinding the leaves using a mortar and pestle, with a known amount of water. This was fed to the rats by orogastric feeding tube at a dosage of 1000 mg of *Vitex negundo* per kilogram body weight of the animal for 15 days.

Scopolamine was procured from Kemwell Biopharma, Bengaluru, India. It was administered to the rats as intraperitoneal injection at a dosage of 1 mg / kg body weight for 15 days, 30 minutes prior to the behavioral experiments. The model of scopolamine induced dementia test in rodents is one of the well established animal models of memory dysfunction and is widely used as a primary screening test for anti-Alzheimer drugs (El-Sherbiny *et al.,* 2003; Fan *et al.,* 2005; Jeong *et al.,* 2008; Aswathy *et al.,* 2015).

Donepezil was obtained from Alkem laboratories, Mumbai, India and it was administered to the rat through oro-gastric feeding tube at a dosage of 3 mg/kg body weight for 15 days and two hours prior to the behavioral experiments. The other chemical reagents used in the study were obtained from Hi Media, Mumbai, India.

The experimental animals were divided into six groups as follows

Group I (n=7): neither scopolamine nor Vitex was administered

Group II (n=7) : only scopolamine was administered

Group III (n=7): only Vitex was administered

Group IV (n=7): Scopolamine+ Vitex was administered

Group V (n=7): only Donepezil was administered

Group VI (n=7): scopolamine + Donepezil were administered

Design of the behavioral experiments

T-Maze

T-maze is used largely in preference and spatial learning tasks (Jissa *et al.,* 2014). Animals learn to alternate between arms based on their memory of the previously visited arms or choose an arm based on the reward presented. The T-maze test was carried out on the 1st, 8th and 15th day

of treatment. The whole experiment was performed in a silent dark room under a dim red light and after 9.00 pm. Before performing this experiment, the animals were left without food for 24 hours with only water to drink. This makes the animal more active to find food during the experiment. The wooden T-maze apparatus consisted of a stem (35 X 12 cm), a choice area (15 X 12 cm), which is one of the two arms. The animal was left at the tail end. The presentation of the food in one of the arms is in such a way that it is concealed from the sight of the rat. The animal has to move forward and turn left or right of the T-maze. The move was considered to be a positive response if the rat reached near the concealed food, and if not it is considered as a negative response. A total of 10 trials were given in each set with a gap of five minutes per set. The trials were continuous till a set gets 90 % of positive response.

Biochemical estimation

Preparation of brain homogenate

On day 15th following the behavioral testing, animals were sacrificed and the brain tissues were quickly removed, cleaned in ice cold saline. After washing, the tissue samples were homogenized with 10 times (W/V) 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from the rat brains were separated and used to measure MDA (Niehaus and Samuelsson, 1968). The remaining homogenates were centrifuged using Sigma Aldrich cooling centrifuge at 5400 rpm for 10 minutes at 4^{o}C to separate the supernatant. The supernatant was used for the quantification of acetylcholine (Ellmann *et al.*, 1961), SOD (Kono, 1978) and GST (Habig *et al.*, 1974). A portion of the supernatant was mixed with 10 % TCA, shaken well and centrifuged at 4000 rpm for 10 minutes to estimate total protein.

Data analysis

Data was analyzed by SPSS 16.0. The values obtained as percentage of positive response in different animal groups were statistically analyzed for significance using ANOVA followed by post-hoc test.

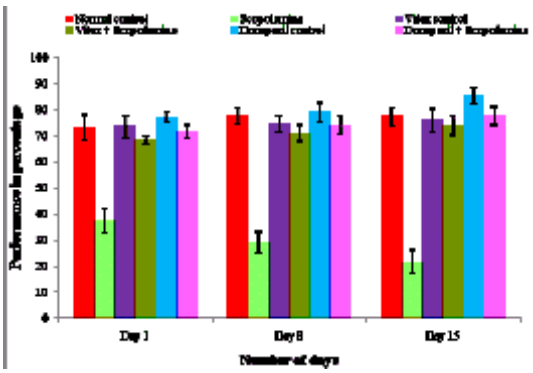
RESULTS AND DISCUSSION

In this study, the effect of *Vitex negundo* and the modern medicine donepezil were compared in scopolamine induced cognitive impairment in rats. This study also focused on different parameters like memory test and enzyme activity related with oxidative stress, including estimation of superoxide dismutase, glutathione-S-transferase and lipid peroxidation and acetylcholinesterase. Figure 1 shows the comparison of the performance in T-maze in percentage of different groups of animals. The normal rats fed with *Vitex negundo* showed an increase in the performance from 1st to 15th day than the normal control and it was found to be significant at 5 % level. *Vitex negundo* possesses antinociceptive, anticonvulsant, anti-inflammatory and antioxidant properties (Huang *et al.,* 2012). The leaves of *Vitex negundo* possess acetylcholinesterase inhibiting activity and this tends to allow more retention of brain acetylcholine level, which is important for learning and memory (Kanwal *et al.,* 2010).

Scopolamine induced group showed a decline in memory performance. The administration of antimuscarinic agent scopolamine confers impairment in learning and memory and reduces acetylcholine level in brain (Izquierdo, 1989; Kumar *et al.,* 2013). Earlier studies reported that memory impairment in the scopolamine induced animal model is associated with increased oxidative stress within the brain (Tsa *et al.,* 2015; Du *et al.,* 2015). In case of normal rats fed with donepezil, it showed an increase in memory performance than *Vitex negundo* fed group. Donepezil improved learning and memory in multiple sclerosis patients with initial cognitive difficulties in a single-centre clinical trial (Christodoulou *et al.*, 2006). From the classical T-maze task, it was clearly seen that *Vitex negundo* extract has a protective effect, indicating that these extracts ameliorated memory impairment. The long term administration of these extracts (15-day administration) exhibited pronounced effect in the reversal of scopolamine induced cognitive impairment.

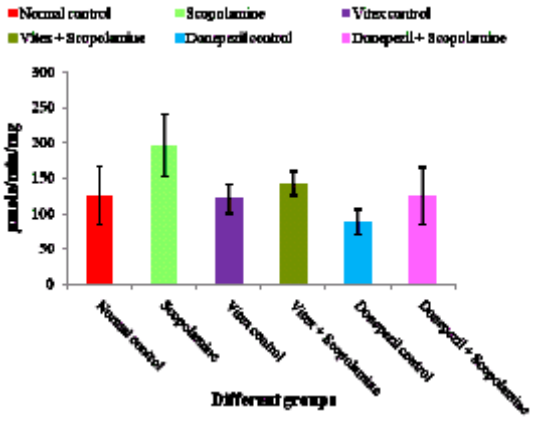
Cholinergic neuronal pathway plays an important role in cognitive deficits associated with dementia, ageing and neurodegenerative disorders (Ellis, 2005). Estimation of acetylcholinesterase (AChE) activity provides a relatively easy and better understanding of cholinergic functions (Lekha *et al.,* 2010). Acetylcholinesterase activity was significantly increased in scopolamine induced group (Figure 2). Scopolamine is a muscarinic receptor antagonist that inhibits central cholinergic

neuronal activity and impairs learning and short-term memory (Kumar *et al.*, 2013) and reduces acetylcholine level in the hippocampus (Seifhosseini *et al.*, 2011). When the normal control group was compared with the control group of *Vitex negundo*, a significant change in the level of AChE was observed. Literature survey of *Vitex negundo* revealed the presence of lignans derivative, which is responsible for anticholinesterase activity (Malik *et al.*, 2004; Huang *et al.*, 2012). Earlier studies of this plant showed that it has anti-oxidant, antimicrobial, hepatoprotective, cytotoxic, atherothrombolytic and anti-inflammatory activities (Chattopadhyay



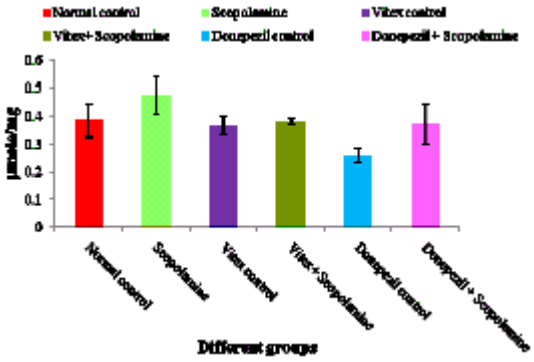
*Comparison between normal control and Scopolamine
*Comparison between normal control and vitex control
*Comparison between vitex control and vitex + Scopolamine
*Comparison between Donepezil control & Donepezil + Scopolamine
* Significant at 5 % level.
** Significant at 1 % level.

Fig. 1. T-maze performance on dementia induced *Wistar albino* rats on different groups (mean + SD).



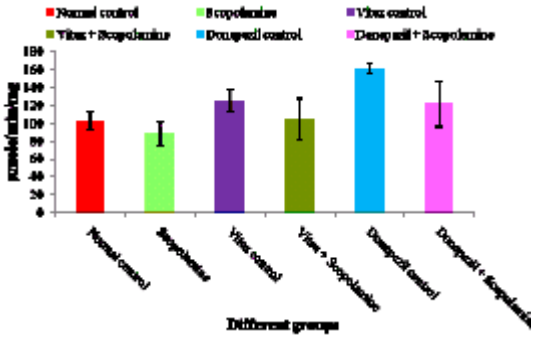
*Comparison between normal control and Scopolamine
*Comparison between normal control and vitex control
*Comparison between vitex control and vitex + Scopolamine
*Comparison between Donepezil control & Donepezil + Scopolamine
** Significant at 1 % level

Fig. 2. AChE level in brain in dementia induced *Wistar albino* rats in different groups (mean + SD).



*Comparison between normal control and Scopolamine
*Comparison between normal control and vitex control
*Comparison between vitex control and vitex + Scopolamine
*Comparison between Donepezil control & Donepezil + Scopolamine
** Significant at 1 % level

Fig. 3. MDA level in brain in dementia induced *Wistar albino* rats in different groups (mean + SD).



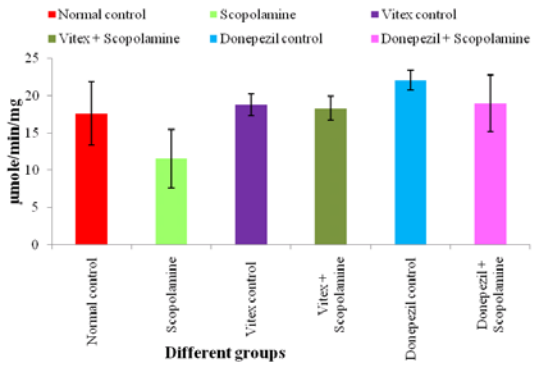
*Comparison between normal control and Scopolamine
*Comparison between normal control and vitex control
*Comparison between vitex control and vitex + Scopolamine
*Comparison between Donepezil control & Donepezil + Scopolamine
** Significant at 1 % level.

Fig. 4. SOD level in brain in dementia induced *Wistar albino* rats in different groups (mean + SD).

et al., 2012; Kadir *et al.*, 2013; Kadir *et al.*, 2013; Khan *et al.*, 2013). Co-administration of scopolamine and *Vitex negundo* showed a significant reduction in AChE level in the brain. In comparison with Donepezil, the extract treated group showed a better result which indicates therapeutic efficacy of *Vitex negundo* against cognitive impairment.

Oxidative stress in brain generates oxygen radicals like superoxide anion, hydroxyl radical and hydrogen peroxide, which act on polyunsaturated fatty acids in the brain, thereby propagating the lipid peroxidation (Coyle and Puttfarcken, 1993). The major antioxidant and oxidative free radical scavenging enzymes like SOD and GST plays an important role to reduce oxidative stress in brain. In the present study rats after scopolamine treatment showed a significant increase in MDA levels in the brain (Figure 3), which is a marker of lipid peroxidation and free radical generation. Co-administration of scopolamine and *Vitex negundo* showed a significant reduction in the MDA levels in the brain. Antioxidant property of *Vitex negundo* leaves may be the attributing factor for this reduction in MDA.

Scopolamine reduced the SOD and GST activity in brain (Figure 4 & 5). SOD is the only enzyme that uses the superoxide anion as a substrate and converts hydrogen peroxide as a metabolite (Akoyl *et al.*, 2002). Pretreatment with *Vitex negundo* increased the SOD and GST activity in the brain and it significantly prevented the reduction of SOD activity during scopolamine treatment. Antioxidants are essential to destroy the free radicals that form in our body during metabolism (Thapa and Walia, 2007) Several phytochemical studies revealed the presence of volatile oils, lignans, flavonoids like flavones, luteolin-7glucoside, glycosides and phenols in *Vitex negundo(*Gautam *et al.*, 2008). Moreover, the ethanolic extract of *Vitex negundo* possesses free radical scavenging activity probably due to its higher concentration of flavonoids and phenols (Janakiraman *et al.*, 2015). These findings are in agreement with the present results. In comparison with donepezil, the extract treated group showed almost an equal result, which indicates therapeutic efficacy of *Vitex negundo* against scopolamine induced cognitive impairment and oxidative stress.



*Comparison between normal control and Scopolamine
*Comparison between normal control and vitex control
*Comparison between vitex control and vitex + Scopolamine
*Comparison between Donepezil control & Donepezil + Scopolamine
** Significant at 1 % level.

Fig. 5. GST level in brain in dementia induced *Wistar albino* rats in different groups (mean + SD).

CONCLUSION

We conclude that aqueous extract of *Vitex negundo* leaf has potential therapeutic effects on improving the cognitive impairment in *Wistar albino* rats through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes and decreasing acetylcholinesterase activity in brain. We suggest further detailed study in this area to separate the exact compound in the extract responsible for the effect for therapeutic purpose.

Limitations

We have conducted the study only in male rats.

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