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Short Communication

Neuroprotective Effect of Curcumin and Vitamin D3 on Scopolamine Hydro-Bromide Treated Rat Model of Alzheimer's Disease

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Abstract

Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder which characterized by gradual memory loss and shrinkage of neuronal cells particularly in the hippocampus and basal for brain regions. Loss of central cholinergic neurotransmitter and accumulation of ubiquitinated proteins in the neurons and sign of inflammation are considered important hallmarks of AD. The development of drug effective for AD widely anticipated because of the increase in the elderly population and progressively increasing number of AD patients worldwide. The aim of this study is to determine neuroprotective effect of Curcumin/Vitamin D3 for improving AD care to initiate appropriate strategy for treatments and prevention of AD.

Methods: Curcumin/VitaminD3 has taken as neuroprotective agents against scopolamine induced dementia in male Sprague Dawely rats of 200 ± 25g. Donepezil was used as Standard drug. The rats were divided into 5 groups; untreated control group, treated with scopolamine (2.5 mg/kg) group, and treated with scopolamine along with Curcumin (oral, 80 mg/kg) group. Treated with scopolamine along with VitaminD3 (oral, 0.0179 mg/kg) group. Donepezil was given at dose (oral, 2.5 mg/kg). Animals were tested for behavioral tasks: rectangular maze test and locomotor activity. Histology of brain tissue slice from treated groups and control was done focusing on hippocampal area.

Results/Discussion: In drug treated animals, the response rate during acquisition and retention period was significantly lower than control group, revealed that scopolamine successfully induced neurodegeneration. On the other hand, in animal treated with scopolamine and Curcumin/VitaminD3 performed better rate for both tasks, almost equal to the control group. Histological studies show no significant difference in all treated groups. Behavioral improvement in treated, we can conclude that Curcumin/VitaminD3 may have potential therapeutic effect on improving anti dementia and anti-amnesic activity in rats through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes, and decreasing acetyl cholinesterase (AChE) activity in brain.

Keywords: *Alzheimer's disease; Memory impairment; Scopolamine; Curcumin; VitaminD3*

Introduction

AD is a neurodegenerative disorder, which is highly prevalent form of dementia reported to be found more frequently among elderly population [1]. AD affected over 35 million people all over the world, the prevalence is expected to be triple by 2050 [2].

It is characterized by multiple cognitive impairment including memory, impairment in daily living and progression of physical deterioration. Primary neuro-pathological features are due to the presence of (intracellular) neurofibrillary tangles result from hyper phosphorylation of tau [2,3,4]. Similarly, deficiency of neurotransmitter acetylcholine is linked to the pathology of AD and loss of cholinergic

function in the central nervous system contributes to cognitive impairment [5]. Acetylcholine receptors play essential role in the encoding of new memories [6]. The blockade of muscarinic acetylcholine receptor using muscarinic receptors antagonist can reduce the acetylcholine level in hippocampus [7]. Oxidative stress is reported to be the event in the pathogenesis of AD [7,8].

Scopolamine is used to induce memory impairment in animal model. It can produce the similar memory impairment seen in the elderly population [9,10]. Scopolamine acts by reducing the effective action at synapse of given concentration of acetylcholine without actually changing the concentration of acetylcholine. Scopolamine has the ability to occupy the muscarinic acetylcholine receptors post synoptically without having effect on depolarization [11]. It can interfere with other neurotransmitters system [12]. Disturb the regional cerebral blood flow was observed during performance of memory tasks [13]. There are many of pharmacotherapies and anti-dementia drugs against AD are used for decades which are although capable of enhancing cholinergic function but many of them are observed can cause sleep disturbances, and have gastrointestinal, cardiorespiratory, extrapyramidal, genitourinary, and musculoskeletal side effects [14].

Curcumin has various beneficial properties including antioxidant, anti-inflammatory, and antitumor therefore can be used as affective drug against AD. Animal model of AD proved improvement in the disease pathologies such as inhibition of Aβ deposition, Aβ oligomerization and tau phosphorylation in the brains of AD animal models. Curcumin can cross blood brain barrier because of its lipophilic nature and bind to plaques, and shows improvement in behavioral impairment [15]. The Curcumin act as antioxidant by activation of microphages to remove reactive oxygen (ROS) species like, superoxide anions, H_2O_2 and nitrite radicals. Its anti-inflammatory property proves on animal, *in vivo* and *in vitro* to decrease acute and chronic inflammation [16,17].

Material and Methods

Animals

Sprague dawely rats of weight 200 ± 25g are obtained from animal house (University Brunei Darussalam). They are housed individually in cage (at an ambient temperature of 25 ± 2°C) and 45-55% relative humidity with 12 hrs light/dark cycles. Rats are free to access food and water *ad libitum*. All rats are handled daily for a minimum of 1 week prior to behavioral testing. One week prior to testing in the "Rectangular Maze" and throughout testing in the rectangular Maze and for locomotor activity using "Actophotometer", the food is restricted to a daily feeding of approximately 80% of their *ad libitum* consumption to maintain the weight of each rat at approximately its freely fed weight. Experiments are performed between 09:00 to 17:00 hours to reduce the stress effect of noise and other variants.

Experimental Design

a. All experiments were conducted during the day time between 9:00 am and 17 pm
b. Procedure of drug treatment was carried out for 27 days.
c. All experiments were conducted in accordance with institutional guideline for animal care and use.

Group I	Saline-control	(0.9% saline) + behavioral
Group II	Disease control	Scopolamine (2.5 mg/kg) i. p. + behavioral test
Group III	Experimental	Scopolamine (2.5 mg/kg) i. p. Curcumin (80 mg/kg) oral + behavioral test
Group IV	Experimental	Scopolamine (2.5 mg/kg) i. p., Donepezil (2.5 mg/kg) oral
Group V	Experimental	Scopolamine (2.5 mg/kg) i. p. Vitamin D3 (0.0179 mg/kg) oral +behavioral test

Drugs

1. Saline 0.9% was given (i.p.) injection for each day for 27 days.
2. Scopolamine 2.5 mg/kg was given (i.p.) injection for each day for 27 days.
3. Curcumin Sigma (Turmeric) (80 mg/kg) was given oral dose for each day for 27 days

4. Donepezil (2.5 mg/kg) (sigma, USA) was used as anti-dementia drug in this study.
5. Vitamin D3 (0.0179 mg/kg) in vegetable oil was given oral to experimental groups for 27 days.

Drug treatment

There were 30 animals divided into 5 groups of six animals in each group. All groups except vehicle group received scopolamine (2.5 mg/kg body wt.) i.p. injection for each day for 27 days to induce excitotoxicity. Donepezil (2.5 mg/kg) (serves as standard drug) oral dose (by using oral gavage) was given to experimental groups for each day for 27 days. Group 5 received vitamin D3 (0.0179 mg/kg) oral for days for 27 days. Curcumin (80 mg/kg) oral dose was given to experimental groups except vehicle group. Vehicle group was given 0.9% saline (i.p. Injection).

Behavioral Tests

All the animals were trained for behavioral tasks for 1 week before drugs administration.

Rectangular Maze Test

To assess the learning and memory of the animals after drugs treatment rectangular maze test was performed. It is rectangular box with an entry and reward chamber appended at opposite ends. Animals were trained prior to the experiment with the rectangular maze. Time was recorded to reach the reward. For each animal five reading was taken and average was calculated as learning score. The time taken by the animals to reach the reward chamber from the entry chamber was noted [18].

Locomotor Activity

Animal performance for locomotor activity was recorded. Animals were placed individually in the (actophotometer) activity cage for 2 minutes and activity was monitored. The photo cell count was noted and decrease or increase in locomotor activity was calculated [19].

Histology

After behavioral study on animals, they were anaesthetized, and decapitated. Brains were removed from skull and are stored in paraformaldehyde (4%), and then brains were embedded in paraffin and kept in refrigerator. 5 µm coronal sections were prepared using rotary microtome stained with Hematoxylin and Eosin. Photographs were taken for each section.

Results

The activity of Curcumin was evaluated using rectangular maze. The rats in Curcumin group except scopolamine treated group showed lower transfer latency which was given in Figure 1.

Locomotor Activity

The activity of Curcumin was evaluated using actophotoactometer. The rats showed significant transfer latency in treatment groups except scopolamine treated group which is given in Figure 2 below.

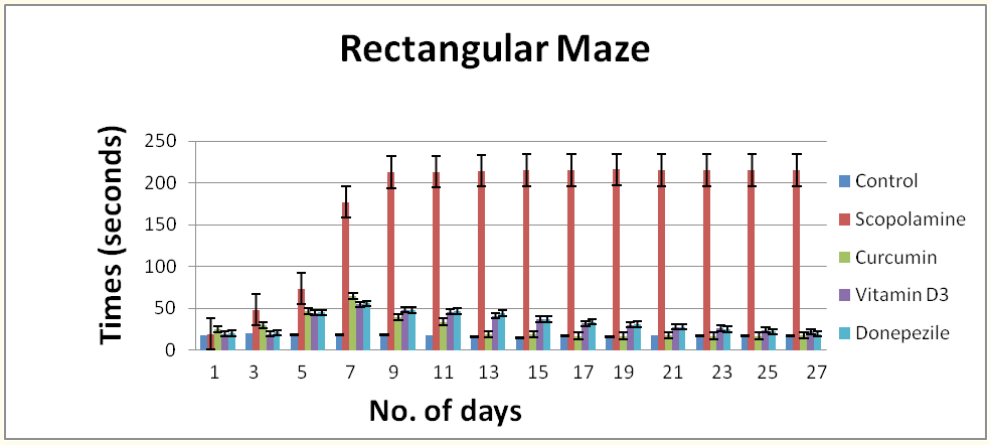

Figure 1: *Rectangular maze test. Effect of Curcumin, Vitamin D3 and Donepezil on latency time compared to the disease control group (Mean ± SD, n = 6). Graph showing mean ±SD of latency time in second.*

No. of Days	Control	Scopolamine	Curcumin	Vitamin D3	Donepezil
1	17.53	19.5	25.053	19.666	20
3	20.27	48.33	29.611	19.3	21.06
5	18.6	73.9	47	45	45.22
7	18.32	177.16	64.75	55	56
9	18.15	212.91	39.53	48.333	47.666
11	17.755	213.25	34.2	46	46.666
13	16.31	214.48	18.88	41	44.3
15	14.96	214.83	18.84	37	36.966
17	16.9	215.166	17.4	32	34.02
19	16.17	215.75	17.21	30.06	31.074
21	17.8	215.5	17.64	28.12	28.128
23	17.3	215	17	26.18	25.182
25	17	214.8	17.4	24.24	22.236
27	16.9	214.9	17.57	22.3	19.29

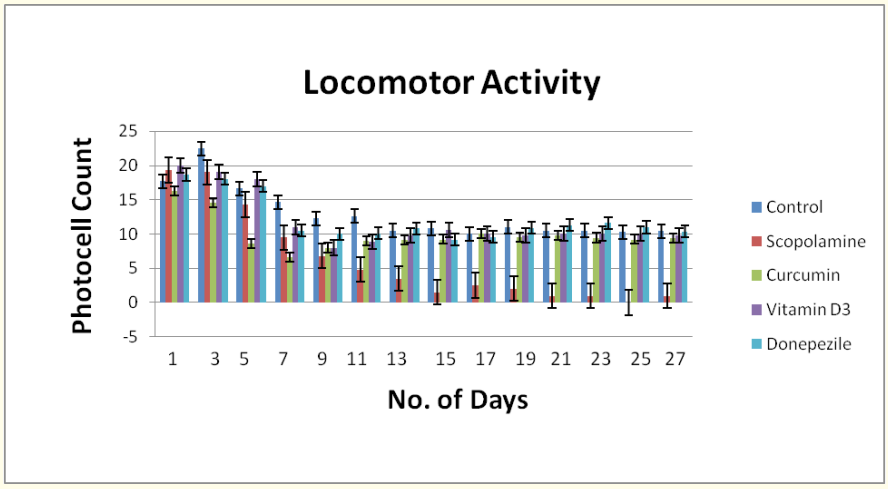

Figure 2: *Locomotor Activity. Effect of Curcumin, Vitamin D3 and Donepezil on latency time compared to disease control group (Mean ± SD, n = 6). Graph showing mean ± SD latency time in second.*

No. of Days	Control	Scopolamine	Curcumin	Vitamin D3	Donepezile
1	17.666	19.33	16.33	19.99	18.66
3	22.5	19	14.5	19	18.02
5	16.66	14.3	8.56	18	17
7	14.66	9.5	6.66	11	10.5
9	12.3	6.8	8	8	10
11	12.6	4.833	9	8.88	10.11
13	10.5	3.5	9.1	9.76	10.82
15	10.83	1.5	9.2	10.64	9.18
17	10	2.5	10	10	9.54
19	11	2	9.5	9.8	10.9
21	10.5	1	9.83	10	11.26
23	10.5	1	9.45	10	11.62
25	10.3	0	9.2	10.02	10.98
27	10.4	1	9.43	9.82	10.34

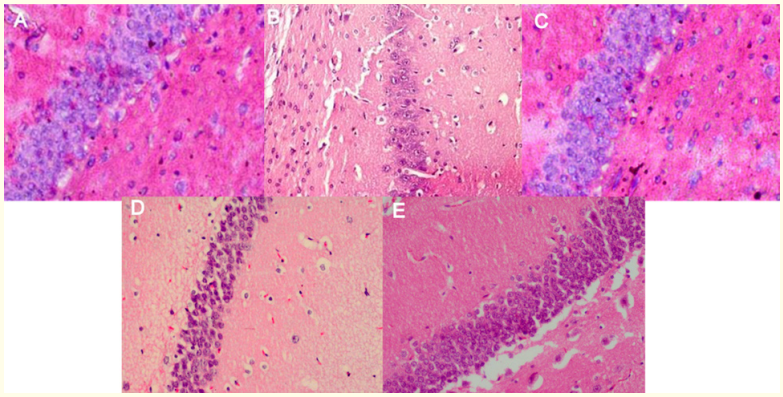

Figure 3: *Hematoxylin and Eosin staining (A = control, B = Scopolamine, C = Curcumin, D = Vitamin D3 and E = Donepezil) shows no significant difference in hippocampal area found in experimental groups (Curcumin, Vitamin D3 and Donepezil) with scopolamine group. Coronal sections (5 µm) at magnification 40X.*

Discussion

Scopolamine is widely used as animal AD model for screening anti-Alzheimer's disease [20]. Scopolamine acts as muscarinic cholinergic antagonist cause impairment in cognition and learning [21]. Scopolamine amnesia in animal model of Alzheimer's is associated with increased level oxidative stress observed in the brain [22]. It is clearly seen that there was general decrease in the transfer latency in all treated groups as compared to the scopolamine-treated group from behavioral study. The memory loss effect of scopolamine is more prominent compared to the control group. The Curcumin treated group had almost equal performance which indicates neuroprotective effect of Curcumin, against memory loss. Meanwhile locomotor activity test is done which also indicate the leaning ability (Figure 2). On the other hand histological studies show no significant difference in all treated groups. From behavioral study it may conclude that Curcumin has potential therapeutic effect on improving the anti-amnesic activity in rats through mechanism of inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes, and decreasing acetyl cholinesterase (AChE) activity in brain.

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