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The effect of equeous extract of Centella asiatica on learning and spatial memory in Alzheimer's disease animal model

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ABSTRACT

This study aimed to evaluate the effects of Centella asiatica (CeA) on AD-like cognitive deficiency in NBM-lesioned rats induced by ibotenic acid. The learning and memory functions were examined by the Morris water maze performance. Twenty four male Wistar rats weighing 380±30 gr at the experiment were used that were divided randomly into three groups (n=8). Control; Lesion and CeA+lesion. NBM was bilaterally lesioned by stereotaxic microinjections of ibotenic acid. The results indicated that the rats with NBM lesioned showed significant reduction in spatial learning and memory in the water maze performance. The aqueous extract of CeA dieting (6 weeks, 100 mg/kg, orally) followed by NBM lesioning in aged rats could be alleviating pre-lesion memory impairment. The percent of time spent in goal quarter was decreased significantly ($p<0.01$) in lesion group when compared with control group and was also increased significantly ($p<0.05$) in CeA+Lesion group when compared with lesion group. The present study demonstrated that Centella asiatica extract prevents memory deficits induced with NBM lesion in rats. The finding suggested that the aqueous extract of Centella asiatica had potential uses of the neuroprotective action in NBM lesioned rats induced dementia and an antioxidant mechanism is involved.

KEYWORDS

NBM;
Centella asiatica;
Morris water maze;
Rat;
Neuroprotection.

INTRODUCTION

Alzheimer's disease is an age-related disease characterized by dementia and the loss of neuronal cells in the brain[1]. Alzheimer's disease is a progressive neurodegenerative disease, age-related disease that is associated with neurobehavioral destruction in brain[2]. Hippocampus has been recognized as an important integration center for learning and memory[3,4]. Hippocampal neurons are especially vulnerable to injury induced by Alzheimer's disease[5]. Damage to the hippocampal formation has been shown to impair explicit memory functioning as well as spatial and contextual learning ability[6]. The decline in cognitive functions can be largely related to cholinergic dysfunction arising from disruption of basal forebrain cholinergic pathways (cholinergic hypothesis)[7,8].

The correlation between the progressive and irre-

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versible decline of memory and the loss of cholinergic neurones in the forebrain cholinergic projection system (FCPS) led to the cholinergic hypothesis of learning and memory decline in Alzheimer's disease[9] excitotoxic lesions of the NBM induce specific memory deficits in rats as evaluated in several tasks[10-14]. The cholinergic projection neurons of the basal forebrain and upper brain stem, contain six groups which were named Ch1-Ch6 on the basis of cytoarchitetonic criteria and patterns of connectivity[15]. In the basal forebrain are found cholinergic neurons within the medial septal area (Ch1), vertical limb nucleus of the diagonal band of Broca (Ch2). they are found also within lateral part of horizontal limb nucleus of the diagonal band of Broca (Ch3) and in the nucleus basalis magnocellularis, substantia innominata, nucleus praeopticus magnocellularis (Ch4). The cholinergic neurons of the upper brain stem are found within the nucleus pedunculopontinus (Ch5) and laterodorsal tegmental nucleus (Ch6)[15,16]. Three of these sectors were shown to be involved in learning and memory functions, contain Ch1, Ch2 and Ch4 which Lesions to these areas produced severe mnemonic deficits in rodents[17,18].

Centella asiatica is a small herbaceous plant growing predominantly in the southern hemisphere that is closely related to Hydrcotyle species and produces a characteristic essential oil and flavonoids[19]. The plant of Centella asiatica has been shown to be beneficial in improving memory and learning[20]. Preliminary studies on the central nervous system effect of Centella asiatica suggest that extracts of this herb are well tolerated and may have pro-cognitive effects in humans and rodents. Centella asiatica improves memory retention in rodents[21-23] and increases performance and behavior in mentally retarded children[24]. CeA has been indicated in vivo to prevent the cognitive deficits that occur following treatment with streptozotocin and to protect cholinergic neurons from the toxic effects of aluminium[25].

The present study aimed to evaluate the effects of CeA on improving learning and spatial memory deficits in NBM-lesioned rat induced by ibotenic acid to mimic the Alzheimers disease.

MATERIALS AND METHODS

Plant material and preparation of the extract

Centella asiatica plant was collected during spring from Anzali wetland region, Iran. The samples were then authenticated for their correct botanical identity by the Chief Botanist, Department of Biology, in the Faculty of Science, Yasouj University, Iran.

The whole plant was dried and coarsely ground with a grinder. For the preparation of aqueous extract, the coarse powder of the plant (5g) was extracted with 8 parts of double- Distilled water under boiling for 5h and cooled. The supernatant was then filtered through a 400-mesh cloth to Collect the extract and rotary evaporated at 40 °C for 30 min. Later the product after flash evaporation was Lyophilized to yield a greenish brown powder (total yield _ 1.5 g).

Animals

Healthy male Wistar rats used in this study were fed food and water ad libitum and maintained in a 12:12 h dark and light cycle. The room temperature was kept constant at 25 °C. All behavioral tests were performed between 9:00 and 13:00 h. All experiments were carried out with prior approval from the institutional animal ethical committee. Only the minimum required number of rats were used and they were handled in a humane way.

Grouping of animals

Animals were randomly divided into three groups of eight animals each, such as:

Group I: Aged control rats (above 24-months-old weighing 380±30 g, intact rats).

Group II: aged rats with NBM lesion + normal diet. The lesion was induced bilaterally by using the ibotenic acid (0.5 µg/site/5 min) infusion into nucleus basalis of magnocellularis (meynert) (NBM).

Group III: aqueous extract of CeA dieting (6 weeks, 100 mg/kg, orally) followed by NBM lesioning in aged rats.

Extract of Centella asiatica (100 mg/kg body weight/day) was dissolved in 0.89% physiological saline and administrated orally. Control animals received physiological saline alone. On completion of experimental period, animals were killed by decapitation.

NBM lesioning

Animals were anesthetized with intraperitoneal (ip) injections of Ketamine (100 mg.kg^{-1} body weight) and Xylazine (10 mg.kg^{-1} body weight) (Alfasan, Woerden-

Holland). NBM lesion was induced by the method of Wang et al., [2005][26] with some modifications[27]. After fixing the head of animals in a steriotaxic instrument (Narishige, Tokyo, Japan), the lesion was induced by injection of ibotenic acid (0.5 μg.0.1μl^{-1} for 5 min in each side, Sigma-Aldrich Chemical Co., USA) dissolved in distillated water into NBM bilaterally (AP; -1.3, L; ±2.3, V; -6.6). coordinates were chosen based on a rat brain atlas[28]. Injection was made through 2 μl Hamilton syringe connected to a short piece of polyethylene tube and a injection needle (gage 27). All animale were allowed to recovery period (7-10 days).

Training appratus

To investigate the effects of the above agents on the memory and spatial learning, Morris water maze test was used (29). The maze consists of a circular metal pool with black inner lining and a diameter of 145 cm and 80cm height. The pool was filled with tap water (22±2 ˚C) with a depth 60 cm. The maze was divided geographically into four equal size quadrants and release points were designed in each quadrant as north Q1, Q2, Q3 and Q4. A metal escape platform with dark color and 12 cm diameter was placed in a fixed location in the tank, 2 cm below the water surface. The platform was not visible from just above the water level. On the training trials, the platform remained in a constant location in the center of one quadrant (Q2) equidistant from the center and the edge of the pool. Some fixed visual cues including computer, desk, shelves, posters and illumination lights were placed on the walls around the pool. A camera was positioned above the center of the pool which was connected to a computer to record the animal motions. An automated tracking system (Radiab ver. 2, Tehran, Iran) was used to measure the escape latency, swimming distance and speed.

Training procedure

From 24 hours after the last receiving of aqueous extract of CeA dieting (43th day) animals were subjected to the training procedure of one session of four trials (block) daily for four consecutive days in the water maze. In each trial, the animals were allowed 60 s to find the platform, after then were allowed to remain there for 30 s, If did not find the platform within 60 s animals were gently guided to the platform by the experimenter. After the completion of a trial, animals was returned to a holding cage for an intertrial interval of 60 s. 24 hours after the last training trial on the probe day, the platform was removed from the pool, the animals were allowed to swim for 60s in the pool and the time spent in the target quadrant Q2 (the quadrant in which the platform was placed during training) was recorded. The percentage of time spent in the previous training quadrant Q2 was used as an index of memory[30].

Statistical analysis

Data were expressed as mean ±SEM. Data analyzed by SPSS version 15.0. The statistical test include one-way ANOVA to compare groups for total sessions. Post-hoc Tukey's test was performed for inter-groups comparisons. Values of $p \leq 0.05$ were considered significant.

RESULTS

Escape latency

The mean Escape Latency to find the hidden platform in four consective days was increased significantly (**p<0.01) in lesion group (rats with NBM lesion) when compared to control group in water maze and the latency was shorter significantly (**p<0.01) in CeA+Lesion group than lesion group (Figure 1).

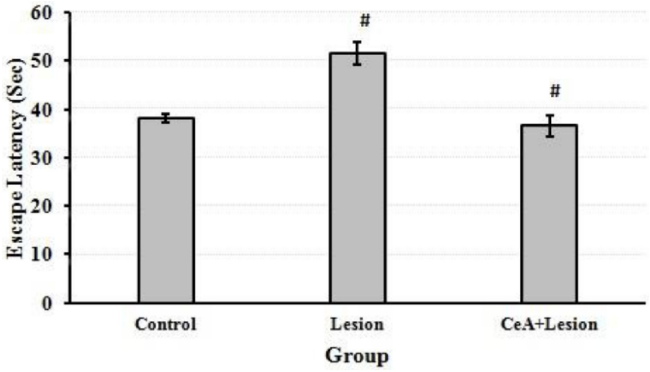

Figure 1 : Effect of Centella asiatica extract on scape latency.

Path length

Comparing the mean swimming path length between the various groups indicate significant increase in lesion group when compared to control (**p<0.01), but it was decreased significantly (**p<0.01) in CeA+Lesion group comparing to lesion group (Figure 2).

Suimming speed

The mean speed of lesion rats was decreased sig-

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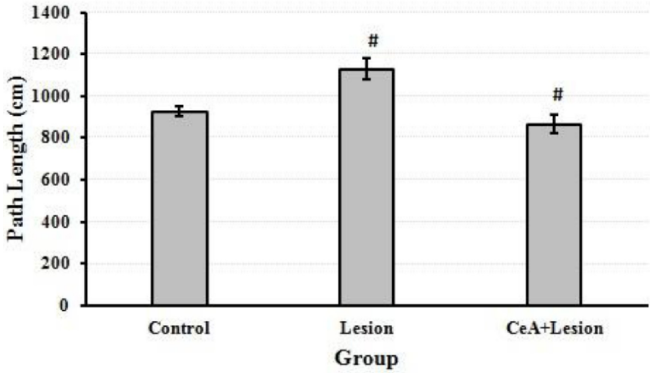

Figure 2 : Effect of Centella asiatica extract on path length.

nificantly for total acquisition trials into water maze during 4 consecutive training sessions comparing to control group (*p<0.05), while it indicated significant increase (*p<0.05) in CeA+Lesion when compared with lesion group (Figure 3).

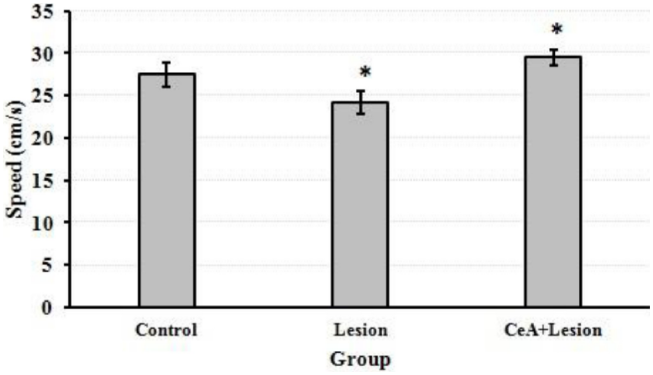

Figure 3 : Effect of Centella asiatica extract on swimming speed.

Probe trial

The percent of time spent in goal quarter while escape platform removed during probe trial 24h after 4th training session was decreased significantly (**p<0.01) in lesion group when compared with control group (Figure 4) and was also increased significantly (*p<0.05) in CeA+Lesion group when compared with lesion group (Figure 4).

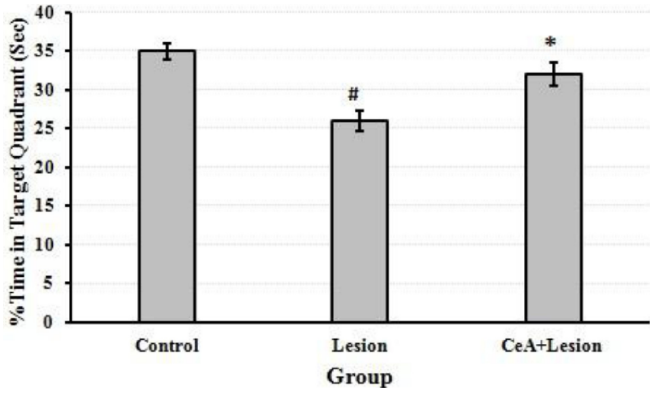

Figure 4 : The percentage of time spent swimming within the training quadrant during the probe trial.

DISCUSSION

The present study demonstrates that Centella asiatica extract prevents memory deficits induced with NBM lesion in rats. In this work, learning and spatial memory among rats with NBM lesion have decreased significantly. An impairment of cholinergic and somatostatinergic neurotransmission have been reported in dementia[31]. The concentration and function of few brain transmitters, particularly acetylcholine and somatostatin, reduce in AD in the cerebral cortex, as well as in subcortical structures, e.g., the hippocampus and the hypothalamus[32].

The results of this study showed that 100 $mg.kg^{-1}$ daily by oral administration of Centella asiatica (C. asiatica) for 6 weeks enhanced the learning and spatial memory in aged male rats with NBM lesioned as assessed by the Morris water maze test. Morris water maze test is extensively used to test spatial memory.

Kumar and Gupta (2002) have reported that C. asiatica has memory improving effects in normal rats[22]. Sulochana et al., (2005) also reported that C. asiatica treatment during postnatal period enhances learning and memory in mice[33]. The result of the present study confirm this effect C. asiatica in aged male rats with NBM lesioned. In the present study, rats administered with CeA (Centella asiatica) showed a significane decrease to find and locate the hidden platform and the swimming path length was also decreased in CeA+Lesion. The speed was decreased in lesioned rats compare to control group. But it was increased significantly in CeA+Lesion group to compare with lesion group. As well as the percent of time that rats spent in goal quarter during probe trial was decreased significantly in lesion group compare to control group, but increased significantly in CeA+Lesion group with compare to lesion group.

The whole plant of C. asiatica has been shown to be beneficial in improving memory[20,34] and has also been reported to improve the general mental ability of intellectually disabled children[24,35]. Nalini et al., (1992b) have shown that fresh leaf juice improves the passive avoidance task in rats[36]. Recently, the Kumar and Gupta (2003) have demonstrated that an aqueous ex-

tract of C. asiatica has cognitive enhancing properties in different paradigms, such as the shuttle box, step through, step down and elevated plus-maze, with associated decreases in brain oxidative stress parameters, in normal rats[25].

Centella asiatica has recently been shown to improve the mental capabilities/function of rodents as well as humans[24].

The Kumar and Gupta (2003) demonstrates that C. asiatica significantly prevented cognitive impairment and attenuated the oxidative stress induced by brain glucose metabolism impairment in i.c.v. STZ-treated rats by its neuroprotective property[25]. However, the possibility of an effect of C. asiatica on neurotransmitters in improving cognitive deficits cannot be ruled out.

Centella asiatica has been shown in vivo to prevent the cognitive deficits that occur following treatment with streptozotocin and to protect cholinergic neurons from the toxic effects of aluminium[25]. In addition, Centella asiatica treatment decreased protein carbonyl production in the brains of aged rats[37]. These data suggest that Centella asiatica may reduce Alzheimer's disease neuropathology.

Alzheimer's disease is one of the most prevalent neurodegenerative disorders in the United States[38]. Several studies provide evidence that Centella asiatica has mechanisms of action relevant to Alzheimer's disease therapeutics. A neuroprotective effect of Centella asiatica has been demonstrated following exposure of cultured neurons to glutamate[39]. In addition, Centella asiatica treatment decreased protein carbonyl production in the brains of aged rats [2005]. These data suggest that Centella asiatica may reduce Alzheimer's disease neuropathology.

There is another interesting finding that chronic treatment with CA extract reversed the AChE activity of D-galactoseinduced aging mice which reflects that this compound may improve dysfunction of the cholinergic system long term exposed to oxidative stress[40].

Central administration of colchicine produces marked destruction of hippocampal granule cells and septohippocampal pathways resulting in loss of cholinergic neurons and decreased activities of acetylcholinesterase and choline acetyltransferase[41]. In one of study, colchicine caused a significant increase in the acetytlcholinesterase activity thereby leading to learning and memory deficits. CA was able to ameliorate the colchicine induced decrease in AChE activity. Chronic administration of CA prevents colchicine-induced cognitive impairment and associated oxidative stress[42].

Cognitive effects of the aqueous extract of CA (100-300 mg/kg/day) have been evaluated in several rodent studies using standard tests including shuttle box, step-through paradigm, elevated plus maze and passive avoidance tests. CA extract markedly improved learning and memory of wild-type rats[22], rats subjected to CNS toxicity (intracerebroventricular streptozotocin)[25] and pentylenetetrazole (PTZ) kindled rats[23]. When administered to neonatal mice from day 15 to 30 postpartum, the extract caused significant enhancement in learning efficiency and spatial memory with no effects on locomotor function[43]. Direct neurotropic effects of CA have also been reported. CA aqueous extract caused significant increases in dendritic arborization of apical and basal dendrites in hippocampal neurons of neonatal mice[43] and both adult[44] and neonatal[45] rats. These studies, performed in diverse settings, show that CA water extract has biological effects of relevance to memory, learning and aging and potentially to disease progression in Alzheimer's disease (AD).

CONCLUSION

Therefore, the present findings suggest that the aqueous extract of C. asiatica has potential uses of the neuroprotective action in NBM lesionee rats induced dementia and an antioxidant mechanism is involved.

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