The Effect of Chronic Administration of *Apium Graveolens* Aqueous Extract on Learning and Memory in Normal and Diabetic Rats

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Article info:
Received: 25 June 2009
First Revision: 10 August 2009
Accepted: 5 September 2009

Key Words:
Apium graveolens,
Learning,
Memory, Passive
Avoidance Test,
Alternation behavior,
Diabetes Mellitus

ABSTRACT

Introduction: Diabetes mellitus accompanies with disturbances in learning, memory, and cognitive skills in the human society and experimental animals. Considering the beneficial antidiabetic potential of Apium graveolens (AG), this research study was conducted to evaluate the effect of chronic i.p. administration of AG on learning and memory in diabetic rats using passive avoidance and Y-maze tests.

Methods: Female Wistar rats were randomly divided into control, AG-treated control, diabetic, and AG-treated diabetic groups. AG treatment continued for 4 weeks. For induction of diabetes, streptozotocin was injected i.p. at a single dose of 60 mg/kg. For evaluation of learning and memory, initial latency (IL) and step-through latency (STL) were determined at the end of study using passive avoidance test. Meanwhile, alternation behavior percentage was determined using Y maze.

Results: There was a significant increase ($p<0.05$) in IL in diabetic and AG-treated diabetic groups after 4 weeks as compared to control group. In this respect, there was no significant difference between diabetic and AG-treated diabetic groups. On the other hand, STL significantly decreased ($p<0.05$) in diabetic group and significantly increased ($p<0.05$) in AG-treated diabetic group as compared to control group at the end of study. In addition, STL did not significantly change in AG-treated control group in comparison with control group. In addition, results of Y-maze test showed that there is no significant difference between diabetic and Ag-treated diabetic groups and between control and Ag-treated control group regarding alternation behavior.

Discussion: In summary, chronic oral administration of AG could enhance the consolidation and recall capability of stored information only in diabetic animals and did not affect spatial memory of diabetic animals.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder, characterised by disturbed glucose metabolism due to an absolute or relative insulin deficiency. In the long term diabetes leads to complications in a number of organ systems. As these complications can be only partially prevented by maintaining strict glycaemic control, additional therapeutic interventions are in demand. It is increasingly recognised that the brain is another site of diabetic end-organ damage (1). Diabetic patients may express cognitive deficits, which are generally modest in young adults and, but can be quite marked in the elderly (2).

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Cognitive deficits are also reported in animal models of diabetes. Streptozotocin (STZ)-diabetic rats develop learning deficits which can be prevented, but not fully reversed, with insulin treatment. Moreover, STZ-diabetes affects long-term potentiation (LTP) in the hippocampus. LTP is a form of activity-dependent synaptic plasticity, which is widely accepted as a model for the neuronal substrate of learning and memory. Like in diabetic patients, cognitive deficits in STZ-diabetic rats develop in association with other functional and structural deficits in the brain, among which increased auditory and visual evoked potential latencies (3-4).

The pathogenesis of cerebral dysfunction in diabetes has not been fully elucidated. It appears to be a multifactorial process, which, analogous to the pathogenesis of diabetic neuropathy, involves both vascular and metabolic disturbances. Previous studies have demonstrated reduced cerebral blood flow, enhanced non-enzymatic glycation of proteins and oxidative damage in the brain of STZ-diabetic rats. Links may also exist with the pathogenesis of brain ageing, which, like diabetes, is associated with microvascular disturbances, non-enzymatic glycation of proteins and oxidative damage (5-6). As the effects of diabetes on the brain are most pronounced in the elderly, diabetes and ageing may even interact at the pathogenetic level.

Considering the beneficial antidiabetic potential of Apium graveolens (AG), this research study was conducted to evaluate the effect of chronic i.p. administration of AG on learning and memory in diabetic rats using passive avoidance and Y-maze tests.

2. Methods

Adult female Wistar rats (Pasteur's Institute, Tehran), weighing 180-210 g at the start of the experiment were housed three to four per cage in a temperature-controlled colony room under light/dark cycle. Animals were given free access to water and food. This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH).

Rats (n = 24) were randomly divided into control, AG-treated control, diabetic, and AG-treated diabetic groups. AG treatment continued for 4 weeks. For induction of diabetes, streptozotocin was injected i.p. at a single dose of 60 mg/kg. For evaluation of learning and memory, initial latency (IL) and step-through latency (STL) were determined at the end of study using passive avoidance test. Meanwhile, alternation behavior percentage was determined using Y maze.

2.1. Y-maze Task

Working short-term memory performance was assessed by recording spontaneous alternation behavior in a single session in Y-maze. The maze was made of black-painted Plexiglas. Each arm was 40 cm long, 30 cm high, and 15 cm wide. The arm converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was basically the same as that described previously as follows: each rat, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The alternation percentage was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two).

2.2. Single Trial Passive Avoidance Test

This test was always conducted 2-3 days after Y-maze task. The apparatus (BPT Co., Tehran) consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (2 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 2 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded form the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL up to a maximum of 600 s as cut-off).

3. Results

There was a significant increase ($p<0.05$) in IL in diabetic and AG-treated diabetic groups after 4 weeks as compared to control group. In this respect, there was no significant difference between diabetic and AG-treated diabetic groups. On the other hand, STL significantly decreased ($p<0.05$) in diabetic group and significantly increased ($p<0.05$) in AG-treated diabetic group as

compared to control group at the end of study (Fig. 1). In addition, results of Y-maze test showed that there is no significant difference between diabetic and AG-treated diabetic groups and between control and AG-treated control group regarding alternation behavior (Fig. 1).

4. Discussion

Although severe peripheral neuropathy has been reported in diabetic patients, but the diabetic brain has not been studied so much and its possible dysfunctions have remained to be clarified. According to existing data, Alzheimer's patients have a relatively high frequency of diabetes mellitus (7). However, no significant differences between diabetic and control subjects with respect to severity of Alzheimer-type pathologies including both senile plaques and neurofibrillary tangles have been observed (8). In addition, diabetics show impaired cognitive performance relative to age-matched control subjects (9). It seems that diabetes induces impairment of cognitive performance; however, the decrease of learning ability may not be restricted to only Alzheimer's disease (2). In this research study, body weight, intake of food or water, and spontaneous motor activity of diabetic rats were significantly different from those of the control rats.

In summary, chronic oral administration of AG could enhance the consolidation and recall capability of stored information only in diabetic animals and did not affect spatial memory of diabetic animals.

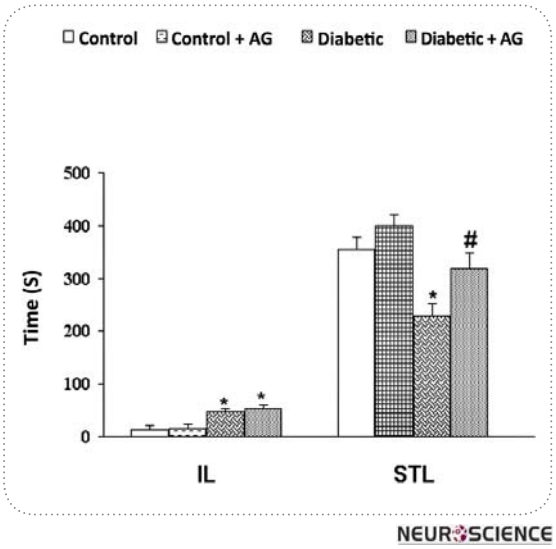

Figure 1. The effect of AG administration on learning and memory of diabetic rats

* p<0.05 (relative to control)

# p<0.05 (Relative to diabetic)

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