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The effect of chlorogenic acid on learning and memory and acetylchoinesterase activity in rats with cognitive deficit induced by intracerebroventricular streptozotocin

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Abstract

Background and Objective: Chlorogenic acid (CGA) is a major polyphenolic component of coffee. Reduction in the risk of a variety of diseases following CGA consumption has been mentioned in recent studies. The effect of CGA on learning and memory in rats with cognitive deficit induced by intracerebroventricular streptozotocin (STZ) and acetylchoinesterase (AChE) activity was evaluated in this study.

Materials and Methods: For this purpose, 32 male Wistar rats (250-290 g) were divided into four different groups as: control, control plus CGA, STZ treated group, and CGA-treated STZ group. STZ was injected (bilaterally, 3 mg/kg body weight, on days 1 and 3). CGA was administered through intraperitoneal route at a dose of 50 mg/kg for 14 days started one week after STZ injection. To evaluate the spatial learning and memory, Y maze (alternation behavior) and passive avoidance tests were used. Finally, AChE activity was measured via specific kits in hippocampal homogenate.

Results: CGA-treated STZ group did not show significant improvement in spontaneous alternation behavior as compared to STZ group. In passive avoidance test, there was significant difference between STZ and CGA-treated STZ groups. In the latter group, learning and memory was improved. In STZ group, AChE activity as compared to control group significantly increased and treatment with CGA significantly decreased the levels of AChE.

Conclusion: Administration of CGA can improve learning and memory in passive avoidance test with apparently no improvement of spatial memory. Also, CGA can modulate the AChE activity in the hippocampus. Therefore, these results demonstrate the effectiveness of CGA in preventing part of cognitive deficits caused by ICV STZ in rats and also show its potential in the treatment of neurodegenerative diseases such as Alzheimer's disease.

Key words: Chlorogenic acid, Streptozotocin, Learning and memory, Alzheimer's disease.

1. Introduction

The global burden of Alzheimer's disease (AD), already the most common type of dementia, is expected to increase still further due to population ageing. AD not only causes severe distress for patients and caregivers, but also results in a large economic burden on society. AD pathogenesis is complex, involving abnormal amyloid-β (Aβ) metabolism, tau hyperphosphorylation, oxidative stress, and other pathological events (1).Since the discoveries of amyloid β (Aβ) and tau, the main components of plaques and tangles respectively, research has provided detailed information about molecular pathogenic events, yet little is known about the cause of AD and no curative treatments are available (2). AD is characterized by a general and progressive loss of mental, behavioral, functional decline and ability to learn. Cognitive impairment is associated pathologically with extracellular deposition of amyloid-β peptide and intracellular aggregation of tau protein, mainly in hippocampus and cerebral cortex.

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These lead to cholinergic dysfunction, generation of free radicals, and finally neuronal loss. Evidences suggest connection of impaired memory function with increase in cholinesterase enzyme [acetylchoinesterase (AChE)] (3). AchE play a principal role in maintenance of acetylcholine levels which is essential for learning and memory (3-5).

Streptozotocin (STZ) is a glucosamine-nitrosourea compound (C8H15N3O7) derived from soil bacteria (6, 7). Intracerebroventricular streptozotocin (ICV-STZ) is one of the most commonly used model of AD as it represents metabolic changes very similar to those found in the sporadic form of AD. It is posited that the impairment of glucose and energy metabolism caused by STZ may be a potential source of oxidative stress, cholinergic damage and neuronal cell death. These finally cause progressive loss of memory (3, 7). The cholinergic system, which is important for learning and memory, is one of the most widely studied neurochemical alterations noted in ICV-STZ-injected animals. ICV-STZ-treated rats showed impaired learning and memory performance, possibly as a result of cholinergic dysfunction (8).

Chlorogenic acid (CGA), an important biologically active dietary polyphenol, is produced by certain plant species such as *Crataegus monogyna*, Eucalyptus globules, Eupatorium perfoliatum, and Vaccinium angustifolium and is a major component of coffee. This polyphenol possesses many health-promoting properties, most of them related to the treatment of metabolic syndrome, including anti-oxidant, anti-inflammatory, antilipidemic, anti-diabetic, and antihypertensive activities. CGA acts on the CNS via the blood–brain barrier (BBB) either in its intact form or as a metabolite (9-11). Numerous epidemiological studies have shown strong correlations between the consumption of polyphenols in the diet and reduced risk of developing neurodegenerative conditions like Alzheimer's.

With the increasing incidence of degenerative diseases, the general public is now turning to natural herbal supplements. As one of these agents, CGAs have been biologically and medically emphasized and can be expected to be addressed as a topic for future studies, medical trends and pharmacology (12). The present study evaluated the effect of CGA on learning and memory and AChE activity in rats with cognitive deficit induced by intracerebroventricular STZ**.**

2. Materials and Methods

2.1. Animals

In this study, 32 healthy male Wistar rats (250-290 g) at about 12 weeks of age were allocated for the pharmacological screening. The animals were housed at a temperature-controlled room with 12 h light/dark cycle. Rats were fed with standard diet and water. All behavioral experiments were carried out between 11:00 a.m. and 04:00 p.m. Protocols of the present investigation for all the animal studies were approved by the Ethical Committee of Shahed University and carried out in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (13).

2.2 Experimental groups and treatments

Rats were randomly divided to four different groups having 8 rats in each group as: control, Control plus Chlorogenic acid (CGA), Streptozotocin treated group (STZ), and Chlorogenic acid-treated STZ group (STZ + CGA). STZ and CGA-treated STZ groups rats were anesthetized by IP injection of a combination of ketamine and xylazine (100 and 5 mg/kg, respectively) and then the rats were operated by using stereotaxic apparatus (NARISHIGE, Japan). Paxinos and Watson stereotaxic atlas was used for the surgery, rats scalp washed by using iodine solution, incised on midline and a hole was drilled through the skull 0.8 mm post bregma, 1.4 mm lateral from midsagittal line and 3.4 mm below the dura. The STZ and CGA-treated STZ groups received bilateral ICV injection of STZ (3 mg/kg body weight). STZ was freshly dissolved in cold artificial CSF and at a volume of 10 µl on each side. The injection was repeated on day 3. (4, 13). CGA was administered through intraperitoneal route at a dose of 50 mg/kg for 14 days started one week after STZ injection (13).

2.3. Behavioral tests

Behavioral experiments were carried out 2 weeks after STZ injection.

2.3.1 Spontaneous alternation behavior Y-maze test

Y-maze test as a reliable and non-invasive short-time memory test and as a behavioral marker for spontaneous alternation was used to determine the cognitive changes in rats (14). The apparatus employed in the present investigation consisted of a wooden Y-maze comprising three arms, forming the Y shape. Each arm was 40 cm long, 30 cm high, and 15 cm wide and was positioned at 120° extending from a central platform. Normal rats typically prefer to investigate a new arm of the maze rather than the familiar one. The test was carried out on two successive days. On the first day, which was designated for training, each rat was placed at the central platform and allowed to move freely through the maze for 8 min. On the test day, the sequence of arms entered by each rat was recorded during the 8-min session. After every rat tested during each session, the maze cleaned with 70% ethanol to remove any olfactory cues that may introduce errors into the observations. An actual alternation was defined as

successive entries into all three arms, known as overlapping triplet sets. Possible alternations were defined as the total number of arm entries. The percentage of spontaneous alternation behavior was calculated as the ratio of actual alternations to possible alternations multiplied by 100 (8).

2.3.2 Single trial passive avoidance test

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, 1 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 from 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 280 s). This test was conducted after 16 days post-surgery (13).

2.4. Hippocampal acetylchoinesterase assessment

After behavioral tests, at the end of week 3 post-STZ, animals were decapitated, under overdose of diethyl ether and the brains were quickly removed. Hippocampal tissue (n=6 from each group) was separately dissected out and 10% homogenate was prepared in ice-cold normal saline containing 0.1% Triton X100 and protease inhibitor cocktail (containing AEBSF, aprotinin, bestatin, E-64, leupeptin and EDTA; SigmaAldrich, USA) and the obtained supernatant was aliquoted and stored at −70°C for the following experiments. The acetylchoinesterase activity was determined on the basis of degradation of acetylthiocholine iodide into a product that binds to 5, 5- dithiobis-2-nitrobenzoic acid and turns yellow (15). The kinetics of the reaction was followed spectrophotometrically over 5 min at 412 nm. Acetylchoinesterase activity was expressed as mM of substrate hydrolyzed/min/g protein.

2.5 Statistical Analysis

Statistical analysis and graphical representation were performed using SigmaStat software (version 3.5, 2006). Results were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA) method and if a difference was found to be significant, followed by Tukey test with a p value <0.05 considered statistically significant.

3. Results

3.1. Spatial recognition memory in Y-maze

Results of performance of rats in Y-maze task indicating short-term spatial recognition memory has been shown in Fig. 1. Alternation behavior score of STZ group and CGA-treated STZ group significantly decreased relative to control group (P<0.01) and chlorogenic acid treatment of STZ group did not improve alternation score as compared to STZ group.

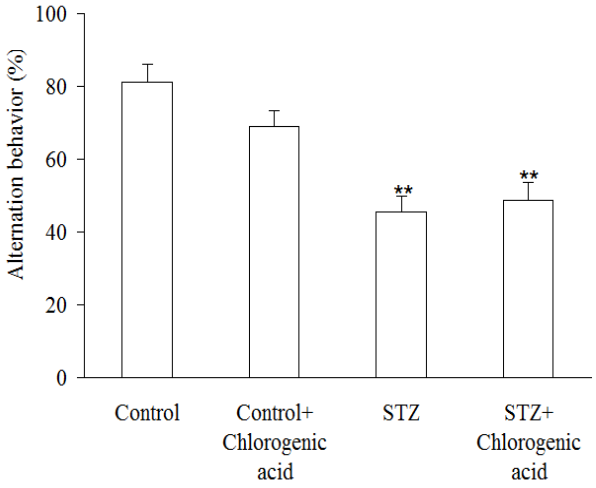

Fig. 1. Alternation behavior in Y-maze task.

Animals were divided into four groups, which were designated as the normal control group, control plus CGA group, STZ group, CGA-treated STZ group. The last group was injected first with STZ (3 mg/kg, ICV) followed by injection with chlorogenic acid (50 mg/kg, i.p. for 14 days). Animals (n = 6-8) in each group were subjected to the Y-maze test. Statistical analyses of the mean percentage of spontaneous alternation were performed using one-way analysis of variance (ANOVA) followed by the Tukey test, whereby each value was presented as mean ± standard error of the mean (SEM). All animals were subjected to Y-maze testing, and the percentage of spontaneous alternation for each rat was carefully recorded for 8 min. The mean percentage of spontaneous alternation recorded for all rats in the same group was calculated. **p<0.01 (as compared to control)

3.2. Passive avoidance test

Fig. 2 displays the performance of rats in passive avoidance paradigm as shown by initial (IL) and step-through (STL) latencies. With respect to IL, no significant difference among the groups was observed. Furthermore, STZ group exhibited a significant disturbance of retention and recall in this test (P<0.01), as it was apparent by a lower STL and

chlorogenic acid- treated STZ group significantly improved STL (P<0.05). Also there was significant decline in STL time in CGA plus control group compared with control group (p<0.05).

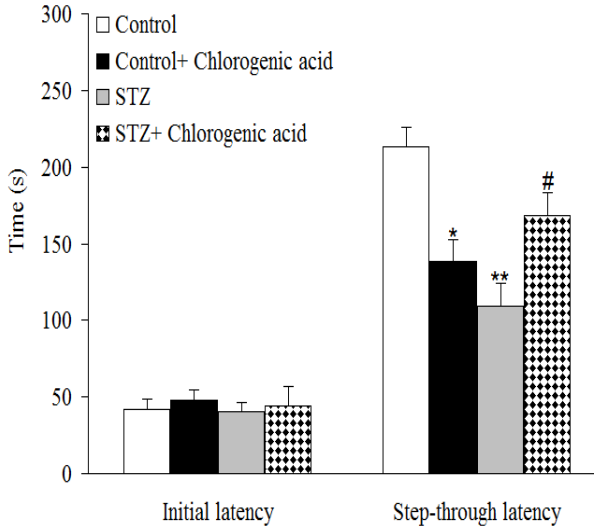

Fig. 2. Initial (IL) and step-through (STL) latencies in single-trial passive avoidance task.

Animals were divided into four groups, which were designated as the normal control group, control plus CGA group, STZ group, CGA-treated STZ group. The last group was injected first with STZ (3 mg/kg, ICV) followed by injection with Chlorogenic acid (50 mg/kg, i.p. for 14 days). Animals (n = 6-8) in each group were subjected to the passive avoidance test. Statistical analyses of the mean percentage of data were performed using one-way analysis of variance (ANOVA) followed by the Tukey test, whereby each value was presented as mean ± standard error of the mean (SEM). For each group, * P<0.05, ** P<0.01 (as compared to control); # P<0.05 (as compared to STZ group)).

3.3. Hippocampal acetylchoinesterase activity

Fig. 3 shows acetylchoinesterase activity in hippocampal lysate. This activity was significantly greater in STZ group compared with control group (P<0.05) and it was significantly lower in CGA-treated STZ group as compared to STZ group (P<0.05). In addition, there was a slight rise in control plus CGA group in relation to control group.

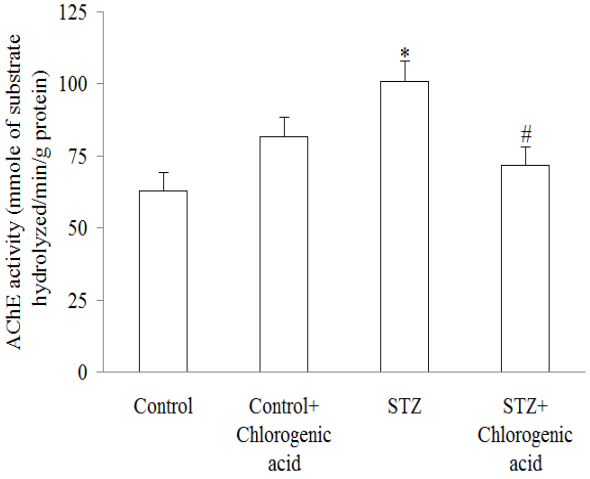

Fig. 3. Animals were divided into four groups, one of which was designated as the normal control group.

The second group received STZ (3 mg/kg) and was thus denoted the STZ group. The third group was normal group which received Chlorogenic acid (CGA). The last group, CGA-treated STZ group was injected first with STZ (3 mg/kg, ICV) followed by injection with CGA (50 mg/kg, i.p. for 14 days). Animals brains (n = 6) in each group were obtained and homogenized as 10% homogenate in 0.1% Triton X100, then centrifuged, and supernatants were used in the AChE Assay kit. Statistical analyses of mean AChE activity were performed using one-way analysis of variance (ANOVA) followed by the Tukey test, whereby each value was presented as mean ± standard error of the mean (SEM). For each group, *p<0.05 (as compared to control), #p<0.05 (as compared to STZ group)

4. Discussion

This study was designed to evaluate the effect of chlorogenic acid, a major polyphenolic component of many plants and beverages, on ICV-STZ induced sporadic model of AD in rats (3, 5, 9). STZ, a glucosamine-nitrosourea compound derived from soil bacteria, when given through parenteral route, preferentially damages pancreatic β cells and produces diabetes. However, when its sub-diabetogenic dose is administered through ICV route, it induces a progressive dysfunction of learning and memory by impairing cerebral glucose and energy metabolism, increasing oxidative stress, cholinergic imbalance and other pathological changes similar to characteristic features of sporadic Alzheimer's disease (7, 13). In this study, STZ at a dose of 3 mg/kg was used. This dose has been shown not to cause any change in the peripheral blood glucose level, however this dose induces a significant cognitive impairment in all of the animals (5, 7, 13).

Spontaneous alternation behavior in the Y-maze is an indication of short-term and working memory. CGA administration (50 mg/kg) did not ameliorate impairment of working memory induced by ICV-STZ according to Y-maze test. The passive avoidance tests is a useful tool for the evaluation of standard learning and long-term memory. Here, CGA administration (50 mg/kg) inhibited reductions in step-through latency induced by STZ, but did not change latencies during the training trials. The results from the passive avoidance test showed that the STZ-injected rats have significantly reduced retention latencies (STLs), suggesting an impairment in learning and memory processes. It is suggested that impairment in passive avoidance behavior may reflect poorer acquisition and/or retention of memory after ICV STZ injection. (50 mg/kg/day) CGA, starting 7 day after surgery for 2 weeks caused a significant improvement in learning and memory (9, 13, 16, 17)

Cholinesterase degrades acetylcholine, a major neurotransmitter that plays an important role in learning and memory. We examined the activity of AChE in the hippocampus. The activities were significantly increased in hippocampus in STZ group in accordance with previous findings. Treatment with CGA attenuated STZ-induced increased activity of AchE in hippocampus. These data indicate that this decrease in cholinesterase activity after CGA treatment may be responsible for reversal of cognitive functions. The beneficial effect of CGA in this study could be connected to the following mechanism: AD is characterized by alterations at the level of various neurotransmitters and related markers and receptors. By the whole of these, the most severely affected by far is the cholinergic system. The cholinergic system is responsible for the storage and retrieval of items in memory and its degradation correlates well with the severity of cognitive and memory impairment. It has been suggested that elevation of the acetylcholine (AChE) level might be helpful in attempts to improve the symptoms of cognitive deficits in AD. Loss of cholinergic innervation, as demonstrated by elevated AChE activity is well correlated with the degree of dementia and severity of the neuropathological hallmarks of AD. Therefore, CGA through its inhibitory effect on acetylchoinesterase activity could exert an alleviating effect on amnesia in experimental models of SAD (9, 10, 13).

In conclusion, results obtained demonstrated that CGA treatment attenuates ICV-STZ induced cognitive impairment as evidenced by improvement of some of the behavioral parameters and cholinesterase activity. Thus, our study demonstrated the potential of CGA as an adjuvant in the treatment of age-related neurodegenerative disorders.

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