The effect of saffranal on intracerebroventricular streptozotocin-induced cognitive deficits in rat

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Background and Objective: Intracerebroventricular (ICV) injection of streptozotocin (STZ) causes cognitive impairment in rats. The beneficial effect of saffron main component, safranal was investigated on ICV STZ-induced learning, memory, and cognitive impairment in male rats.

Materials and Methods: For this purpose, rats were injected with ICV STZ bilaterally, on days 1 and 3 (3 mg/kg). The STZ-injected rats received safranal (60 mg/kg; i.p.) one other day starting one day pre-surgery for three weeks. The learning and memory performance was assessed using passive avoidance paradigm, and for spatial cognition evaluation, Y maze task was used.

Results: It was found out that safranal-treated STZ-injected rats show higher correct choices and lower errors in Y maze than vehicle-treated STZ-injected rats. In addition, safranal administration significantly attenuated learning and memory impairment in treated STZ-injected group in passive avoidance test.

Conclusion: Therefore, these results demonstrate the effectiveness of safranal in preventing the cognitive deficits caused by ICV STZ in rats and its potential in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD).

1. Introduction

Injection of STZ in ICV form in rats cause a long-term and progressive deficits in learning, memory, and cognitive performance in rats that is similar to Alzhimer's disease (AD) (1). AD has been known as a chronic neurodegenerative disorder characterized by progressive cognitive impairment, memory loss, and behavioral disturbances (2) and is considered as the most common cause of dementia in elderly patients (3). Interventions that could delay SAD onset would have a major public health impact (2). Free radical generation has been associated with cognitive impairment in ICV STZ model of SAD in rats (1, 4) and ICV STZ has also been known to impair cholinergic neurotransmission (5).

There is also experimental evidence that saffron constituents are involved in cognition. However, chemical analysis of this plant has shown the presence of water-soluble carotenoids, and its glucoside (safranal and picrocrocin) and flavonoids (quercetin and kaempferol) (6, 7). Its crude extract and purified chemicals have been demonstrated to prevent tumour formation (8-10), atherosclerosis or hepatic damage (11, 12). It has been shown that administration of saffron and its constituents crocins could antagonized ethanol-induced memory impairment in the passive

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avoidance in mouse (13, 14). Also, recently, it has demonstrated that saffron extracts counteracted recognition memory deficits and persist against scopolamine-induced, performance impairments in the passive avoidance task in rat (15). Rgarding to mentioned reports the aim of the present study was to investigate the role of a main saffron constituent safranal in learning and memory processes. For this purpose, So, there was evaluated the effect of safranal in antagonizing extinction of recognition memory in STZ–induced model of AD in the rats using Passive avoidance and Y maze tasks.

2. Materials and Methods

2.1. Materials

Streptozotocin (STZ) was purchased from Sigma Chemicals Co (St. Louis, MO, USA). Ketamone (10 %) and xylazine (2 %) were purchased from Alfasan Co (Holland). Chemical constitutes ACSF to make ready from Merck Co (Germany). Saffron were provided from local market and then scientifically identified by the department of botany of Shaheed Beheshti University (SBU).

2.2. Methods

2.2.1. Animals

Male adult Wistar rats (310-350 g) (Pasteur's Institute, Tehran), at the start of the experiment were housed three to four per cage in a temperature-controlled colony room under 12 h light/dark cycle. Animals were given free access to water and kept at 80–85% of their free feeding body weight throughout the experiment. All behavioral experiments were carried out between 11 a.m. and 4 p.m. This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH).

2.2.2. Experimental procedure

Rats (n = 60) were randomly divided into the following groups: 1. Control group, 2. Sham-operated group (SH) that received bilateral ICV injection of artificial CSF (ACSF) (10 µl on each side) as the solvent of STZ. 3. Safranal-treated control group (safranal), 4.STZ-injected group (STZ) which received ICV injection of STZ. and 5. safranal-treated STZ group (STZ + safranal), which also received safranal (60 mg/Kg; i.p.) once for 2 day. STZ and safranal-treated STZ groups were given a bilateral ICV injection of STZ (Sigma, St. Louis, USA) (3 mg/kg). 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. In the sham group, only artificial CSF (120 mM NaCl; 3 mM KCl; 1.15 mM CaCl2; 0.8 mM MgCl2; 27 mM NaHCO3; and 0.33 mM NaH2PO4 adjusted to pH 7.2) (Merck Chemical, Germany) was ICV injected. For stereotaxic surgery, rats were anesthetized with a combination of ketamin (100 mg/Kg, i.p.) and xylazine (5mg/Kg, i.p.), placed in a stereotaxic apparatus (Stoelting, USA). The scalp was cleaned with iodine solution, incised on the midline and a burr hole was drilled through the skull (A, -0.8 mm from bregma; L, 1.4 mm; 3.4 mm below the dura) according to the stereotaxic atlas (16).

2.3. Behavioral tests

2.3.1. Spatial Y-maze memory

The experimental apparatus for Y-maze consisted of a black-painted maze made of Plexiglas. Each arm of the Y-maze was 40 cm long, 30 cm high and 15 cm wide (17, 18) and positioned at an equal angle (labeled A, B and C) and converged in an equilateral triangular central area with 15 cm at its longest axis. Each rat, naïve 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 sequence of each arm entry recorded manually (i.e., ACBABACACBCACAC , etc.).

A spontaneous alternation behavior, which is regarded as a measure of spatial memory, was defined as the entry into all three arms on consecutive choices in overlapping triplet sets (i.e., ACB,ABA,CAC,BCA,CAC,) (18). The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations. Percent Alternation = [Actual Alternation (i.e., ACB, BCA = 6)/Maximum Alternation.

*(i.e., ACBABACACBCACAC = 15 – 2 = 13)] ×100 = (6/13) ×100 = 46.15 %.

* Total Number of arms entered minus 2 (18). Each test was done once on each animal.

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 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 150 s). This test was conducted after 3 weeks post-surgery.

2.4. Statistical analysis

All results were expressed as mean S.E.M. For the passive avoidance test, nonparametric Kruskal-Wallis test was used, which if significant, was followed by Mann-Whitney U-test for pair-wise comparisons. Data for the Y maze task were evaluated by Wilcoxon's rank sum test. In all calculations, a difference at $p<0.05$ was regarded as significant.

3. Results

3.1. Effect of safranal on memory retention deficit in passive avoidance test

The initial latency was 33.11, 29.89, 30.25, 37.57 and 26.66 s in Control, SH, Safranal, STZ, and STZ+Safranal groups respectively. There was no significant difference between experimental groups. Step through latency in STZ (20.28) and STZ+CSE (33.95) groups reduced markedly in comparison to control (53.18) animals. On the other hand, the STZ+Safranal group exhibited significant reversal of STL ($P<0.05$) as compared to vehicle-treated STZ group, indicating improved acquisition or retention of memory (Fig. 1).

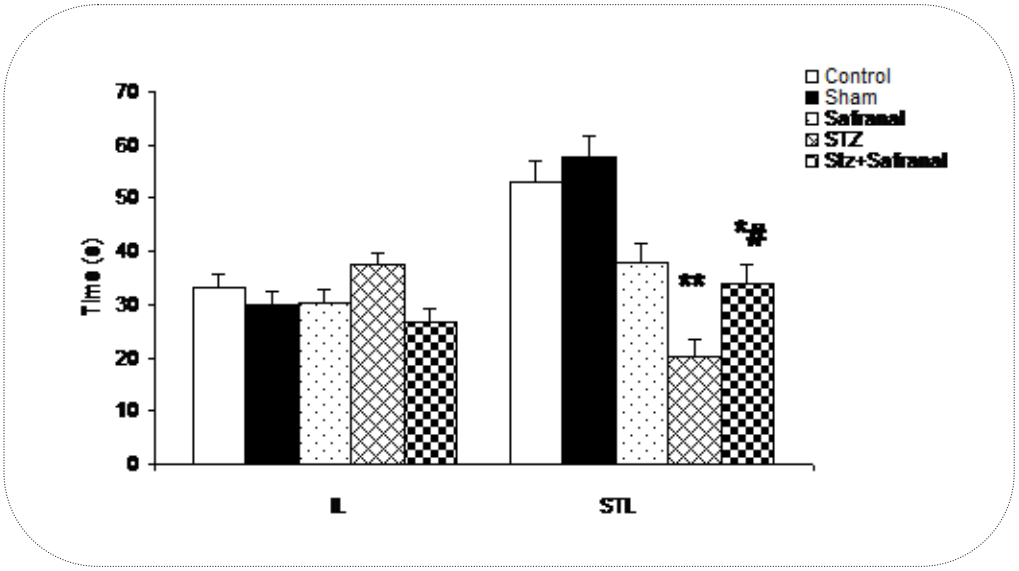

Fig. 1: The effect of Safranal treatment (60 mg/Kg) on passive avoidance performance after ICV injection of STZ in rats as indicated by initial and step-through latencies after 3 weeks. Values are expressed as means ± S.E.M. * $P<0.05$, ** $p<0.01$ (in comparison with control group); # $p<0.05$ (STZ+Safranal vs. STZ) (nonparametric Kruskal-Wallis and Mann-Whitney U-tests)

3.2. Effect of Safranal on spatial cognition deficit in Y maze task

As shown in Fig. 2, the mean scores of alternation behavior for control, SH, Safranal, STZ and STZ+Safranal were 79.23, 75.89, 85.23, 39.91 and 65.68 % respectively. There was found a marked reduction in alternation score in STZ and STZ+Safranal groups regarding to control animals. However in STZ+Safranal group the treatment could significantly modified the STZ memory impairment effect ($P < 0.05$).

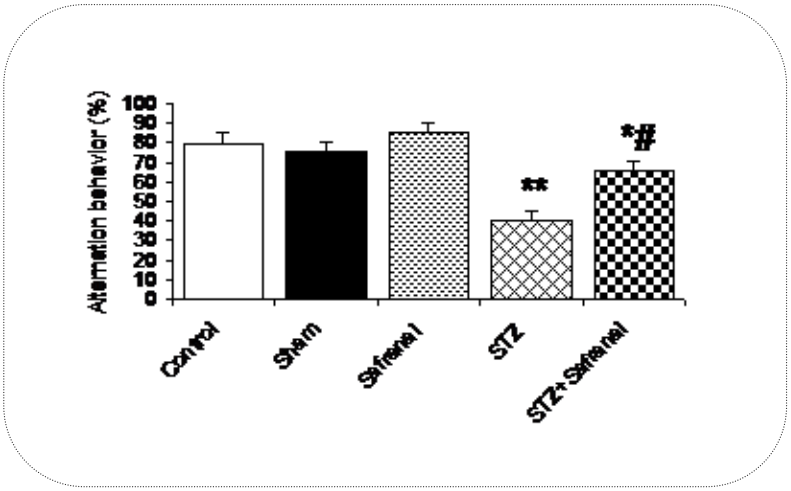

Fig. 2: Percentage of alternation behavior in Y-maze task on the third week after treatment (mean ± SEM). * and # $P < 0.001$ as compared to control and STZ groups respectively.

4. Discussion

The results of the present study demonstrated that ICV STZ injection in rats induces a significant learning and memory disturbance in passive avoidance paradigm and a spatial cognitive deficit in Y maze task and treatment of rats with safranal for 3 weeks could significantly attenuate these abnormalities.

It is a well-established fact that ICV injection of STZ is characterized by a progressive deterioration of learning, memory, and cerebral glucose and energy metabolism and this may provide an appropriate and relevant experimental model of AD (4, 19). In the present 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, although this dose induces a significant cognitive impairment in all of the animals (4). The possibility of the effect of increased CSF pressure due to ICV injection was rejected in this study as no behavioral changes reflecting significant increase in intracranial pressure e.g. bulging of eyes were observed. Also, in the sham-operated rats, no apparent signs of raised intracranial pressure were observed. The results from the passive avoidance test showed that the STZ-injected rats reveal significantly reduced retention latencies (STLs), suggesting an impairment in learning and memory processes. In conformity with this, the results from Y maze task for the first time showed that ICV STZ animals also exhibit a higher score of errors and lower correct choices, indicating an abnormality in spatial cognitive processes. On the basis of the obtained results, it is suggested that impairment in passive avoidance behavior may reflect poorer acquisition and/or retention of memory after ICV STZ injection. The results from the Y maze task may also indicate a spatial cognition deficit in ICV STZ rats.

In this study, treatment of ICV STZ rats with safranal starting 1 day before surgery for three weeks caused a significant improvement in learning, memory, and spatial cognitive skills. The beneficial effect of CSE in this study could be attributed to the following potential mechanisms: first, it has been verified that brain damage due to oxidative stress induces the impairment of

learning and memory abilities and the development of disturbance in spatial cognitive functions as evaluated by water maze and RAM tasks (20), so the neuroprotective (20, 21), antioxidant (21,22) and free radical scavengering of safranal (23) could describe the antagonized extinction recognition memory action of the CSE. Secondly, AD is characterized by alterations at the level of various neurotransmitters and related markers and receptors. Out of these, the most severely affected by far is the cholinergic system (24). 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. Hence it has been suggested that elevation of the acetylcholine (ACh) level might be helpful in attempts to improve the symptoms of cognitive deficits in AD (25). Reports on antagonization action scopolamine-induced memory deficits by safranal, potentiate the direct or indirect hypotesis of cholinergic mimicking action of the safranal. Third, there is strong evidence for the fact that inflammatory processes are associated with the pathophysiology of Alzheimer's disease and that treatment with NSAIDS and natural phenolic compounds could reduce the risk for AD (26, 27). Third, there is scant evidence however, for other behavioral effects of safranal. The mechanism(s) of action underlying safranal role on memory is still under investigation. Among the potential mechanisms, the promotion of hippocampal long-term potentiation (LTP), a form of activity-dependent synaptic plasticity that may underlie learning and memory (28).

In conclusion, the present study clearly demonstrated that safranal treatment could significantly prevent the cognitive impairments following ICV STZ and this suggests the therapeutic potential of this extract in aging and age-related neurodegenerative disorders where cognitive impairment are involved. However, for other behavioral effects of safranal and underling mechanism(s) of action, further preclinical investigation and the use of genuine models of memory deficits (e.g., old rats) are mandatory for assessing safranal potential on cognition.

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