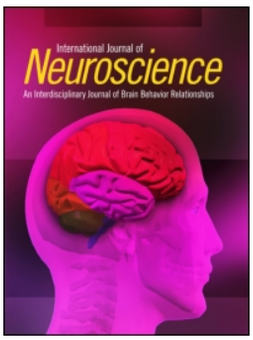

International Journal of Neuroscience

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ines20

Investigating the effects of *Citrullus colocynthis* on cognitive performance and anxiety-like behaviors in STZ-induced diabetic rats

Mohammad Amin Rajizadeh, Amir Hashem Aminizadeh, Khadijeh Esmaeilpour, Mohammad Abbas Bejeshk, Asie Sadeghi & Fouzieh Salimi

To cite this article: Mohammad Amin Rajizadeh, Amir Hashem Aminizadeh, Khadijeh Esmaeilpour, Mohammad Abbas Bejeshk, Asie Sadeghi & Fouzieh Salimi (2021): Investigating the effects of *Citrullus colocynthis* on cognitive performance and anxiety-like behaviors in STZ-induced diabetic rats, International Journal of Neuroscience, DOI: 10.1080/00207454.2021.1916743

To link to this article: https://doi.org/10.1080/00207454.2021.1916743

Published online: 26 May 2021.

INTERNATIONAL JOURNAL OF NEUROSCIENCE
https://doi.org/10.1080/00207454.2021.1916743

Routledge
Taylor & Francis Group

ORIGINAL ARTICLE

Investigating the effects of *Citrullus colocynthis* on cognitive performance and anxiety-like behaviors in STZ-induced diabetic rats

Mohammad Amin Rajizadeh[a,b,c,d], Amir Hashem Aminizadeh[a], Khadijeh Esmaeilpour[b], Mohammad Abbas Bejeshk[c,d], Asie Sadeghi[e] and Fouzieh Salimi[a,e]

[a]Student Research Committee, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran; [b]Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; [c]Department of Physiology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran; [d]Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; [e]Department of Clinical Biochemistry, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

ABSTRACT

Background: Diabetes can impair cognitive performance and lead to dementia. Patients with type 1 diabetes mellitus (T1DM) are reported with different levels of cognitive dysfunctions in various cognitive domains ranging from general intellectual testing to specific deficits with visuospatial abilities, motor speed, writing, attention, reading, and psychomotor efficiency. The present study aimed to investigate the effect of *Citrullus colocynthis on* cognitive functions.

Methods: A total of 42 male Wistar rats (3-4 months old and weighing 200-250 g) were tested in the current study. Rats were randomly allocated into 3 groups of control, Diabetes, and Diabetes + Drug. The diabetic rats received Citrullus colocynthis extraction orally. The behavioral tests included the open field, elevated plus maze (EPM), novel object recognition (NOR), passive avoidance tests, and Morris Water Maze (MWM) tests. Data were analyzed using student and paired *t*-tests *via* SPSS software version 16.

Results: Our results showed the protective effects of Citrullus colocynthis administration against cognitive impairments. This is followed by STZ-induced diabetes in the MWM, novel object recognition, and passive avoidance tasks. Also, it was found that Citrullus colocynthis improved anxiety in diabetic rats.

Conclusion According to the findings of this study, the administration of 200 mg/kg *C. colocynthis* once per day for 40 days can lead to ameliorated cognitive impairments and antidiabetic effects such as increasing body weight and decreasing FBS.

ARTICLE HISTORY
Received 5 August 2020
Revised 21 December 2020
Accepted 30 December 2020

KEYWORDS
Citrullus colocynthis; STZ induced diabetes; behavioral functions; anxiety like behaviors

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by widespread complications. It is the world's largest endocrine disease associated with increased morbidity and mortality rates [1]. In Iran, among the adults' population (25-64 years old), 2 million people had diabetes, of which 16.8% had also impaired fasting glycemia [2]. Type 1 diabetes typically is diagnosed in childhood and currently affects more than 170,000 youth in the United States. Alarmingly, diagnosis rates are increasing by 3-5% per year [3]. There is accumulating evidence that diabetes may lead to dementia in both animal models and humans with both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) [4]. T1DM patients are reported to have varying degrees of cognitive dysfunction in different cognitive domains, ranging from general intellectual testing to specific deficits with visuospatial abilities, motor speed, writing, attention, reading, and psychomotor efficiency [5]. The most common cognitive deficits identified in patients with type 1 diabetes are slowing information processing speed and worsening psychomotor efficiency [6]. Three possible causes of the effects of type 1 diabetes on the brain are described in the literature: (i) hypoglycemic episodes; (ii) hyperglycemia; and (iii) deficiencies in C-peptide and/or insulin. Because the brain cannot synthesize or store glucose, it requires a continuous supply. Therefore, it is likely that the

CONTACT Fouzieh Salimi salimif605@gmail.com Student Research Committee, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran

disruption of the glucose supply as a result of hypo- or hyperglycemia will cause disturbances to cognitive function [7]. It is estimated that 40 million people are living with dementia worldwide and this number is expected to double every 20 years to over 110 million in 2050 [4].

Herbal medicines are the oldest remedies known to mankind [8]. Phytotherapies and their combinations demonstrate multiple beneficial anti-diabetic mechanisms, including modulation of carbohydrate metabolism, restoration of beta-cell integrity and function, insulin-releasing activity, improvements in glucose uptake/utilization, antioxidant properties, and a reduction in the risk of diabetic complications [9].

Citrullus colocynthis commonly known as "bitter apple", "colocynth", "vine-of-sodom", and "Tumba" is an annual tropical plant [1] common in countries including Europe, Asia, and Africa [10]. The whole plant has many medicinal properties such as anti-inflammatory, anti-candidal, bacterial, and anti-oxidant, and the fruit is scientifically proved as antidiabetic [8]. Olatunya et al. stated that *C. colocynthis* seeds contain PUFA and the consumption of large amounts of PUFA is linked to a lower incidence of depression, a lower risk of Alzheimer's disease, and decreased prevalence of age-related memory loss [10]. PUFAs might have a positive influence on controlling and developing diabetes. This positive influence includes protecting the pancreatic beta cells from damage, which results from the production of free radicals in diabetes [11]. Furthermore, *C. colocynthis* fruit extract has a pain mitigatory effect in patients with painful diabetic polyneuropathy [12].

In the present study, first, the neurocognitive functions of T1DM rats and healthy rats are compared. Next, in the T1DM rats, the association between cognitive function and *C. colocynthis* intake is examined.

Method and material

Preparation of crude extract

Fresh or dry ripe C. colocynthis fruits were collected from the Saleh Abad region (Ilam province, western Iran) in summer, sliced in half, and their seeds were removed by hand and allowed to dry for 72 h. These seeds have fatty acids such as myristicol, stearic, oleic, linoleic, and linolenic, making them the best source of these acids. Nuts contain 54% fat, 28.4% protein, 2.7% fiber, 3.6% ash, and 8.2% carbohydrate. Furthermore, the seeds are rich in essential amino acids such as arginine, tryptophan, methionine, vitamin B2, B1, niacin, and some kinds of minerals like Ca, Mg, Mn, K, P, Fe, and Zn [13, 14]. About 100 g of seeds were ground with a mixer and added to 1 L of distilled water. Then, it was heated in a water bath to 80 °C for 2 h. The supernatant was recovered and then filtered through Whatman No. 1 filter paper. This operation was repeated several times after each filtration with the renewal of the solvent. At the end of extraction, the fractions obtained were collected in a balloon and lyophilized, yielding the lyophilized aqueous extract.

Animals

All experimental protocols and treatments were approved by the Ethics Committee of the Kerman University of Medical Sciences. We attempted to minimize the discomfort for the animals at all stages of the study (Ethics code: IR.KMU.REC.1398.127). Male Wistar rats (3-4 months old and weighing 200-250 g) were tested in the current study. Animals ($n = 7$ in each group) were caged in groups of four with ad libitum access to food and water. They were housed under controlled temperature (23 ± 1 °C) and 12-h light-dark cycle (lights on 07:00-19:00). A total of 42 intact rats were randomly allocated into the following subgroups: control (healthy rats), Diabetes (The rats received STZ), and Diabetes + Drug. Here, the diabetic rats received Citrullus colocynthis extraction orally. The rats were split into separate groups for open field, elevated plus maze (EPM), novel object recognition (NOR), and Morris Water Maze (MWM) tests. It is noteworthy that these rat groups were different from the ones that were used for the passive avoidance test. The behavioral tests were performed during three consecutive days. On day 1, the open field, EPM, and NOR tests were carried out, in the order of their appearance. On day 2, the learning phase of the MWM test was performed. Finally, on day 3, the probe test of MWM was performed. Additionally, on day 1, a passive avoidance test was done in distinct groups.

Induction of diabetes

The model of experimental diabetes was created after overnight fasting (8 h). The severe diabetes was induced by a single dose of STZ (50 mg/kg body weight), and this group named the "Diabetes" group. STZ was prepared in sodium citrate buffer at pH 4.5 and injected with insulin syringe intraperitoneally [15].

Four days after injection, symptoms of severe diabetes such as frequent urination were observed, but

to ensure if it is diabetes, it is necessary to wait 15 days and then measure fasting blood glucose. The treatment started on day 15 after the induction of diabetes and lasted for 40 days. The blood glucose level more than 200 mg/dl considered as a sign for diabetes induction in rats.

Drug administration

On day 15 after the STZ injection, the treatment was started with C. colocynthis. The aqueous extract of C. colocynthis was taken orally every day for 40 days at a dose of 200 mg/kg [8]. The rats were given glucose (2 g/kg body weight) together with two different doses of Citrullus-colocynthis aqueous extract (CCAE), namely 100 or 200 mg/kg of their body weight. The average blood glucose was 80-126 mg/kg. The blood glucose was measured every 30, 60, 90, and 120 min using a drop of blood taken from their tail by a glucometer. However, the dosage of 200 mg/kg was more suitable for correcting the 2-h postprandial blood sugar levels compared to 100 mg/kg. Consequently, it was chosen for the daily oral treatment of diabetic rats in all the treated groups.

Morris water maze (MWM)

In 1981, the neuroscientist Richard G. Morris designed the Morris Water Maze (MWM) to assess hippocampal-dependent learning through acquiring the spatial learning and long-term spatial memory [16, 17]. The MWM consists of a black circular pool (160 cm diameter and 80 cm height) filled with water maintained at room temperature to a depth of 40 cm. The pool is divided into four quadrants of equal size, with starting points designated at each quadrant as N, S, E, and W. A 10-cm diameter square platform was hidden right below (1.5 cm) the surface of the water in the center of the northeast quadrant. The experiments were carried out in a dimly lit room with various and fixed extra maze geometric images (e.g. circles, squares, or triangles) attached to different points on the walls around the maze. Performances were recorded by a smart video tracing system (Noldus Ethovision® system, version 7, Netherland) and animals could be traced on the screen of a computer. Moreover, the water temperature was fixed at 25 ± 2 °C [18]. To make the rats accustomed to the experimental technique and to decrease their stress, they were exposed to a swimming pool without the platform a day before for 2 min.

In a single training protocol, each rat performed three blocks each separated by a 30-min resting period. Each block consists of four successive trials with 60 s duration and about 60 s inter-trial intervals. All the experimental groups were tested under the natural lights between 9:30 am and 12:30 am. On each trial, the rats were randomly released into the water from one of the four quadrants of the maze facing the wall of the quadrant where it was released. Each rat had 4 different releasing points. During the acquisition step, the location of the platform remained constant and the rats were allowed to swim to the hidden escape platform. After the animal found the platform, it was allowed to remain there for 20-30 s and then was placed in an animal cage to wait for 20-0 s before the start of the next trial. If a rat failed to find the platform in the 60 s, the experimenter guided it toward the platform. After 20-30 s of staying on the platform, the rat was located in an animal cage to wait for 20-30 s before the start of the next trial. The time and distance to find the hidden platform was collected and analyzed later. A single probe trial was performed 24 h after the last training trial to test the spatial memory in the water maze. In this trial, the platform was removed and the rat was allowed to swim for 60 s. The time and distance spent in the target quadrant (the quadrant 4) were analyzed as a measure of spatial memory retention [19].

Open field test

The Open Field test was initially developed in 1934 as a test to measure emotionality in rodents [20, 21]. This test provides some information about locomotor activity and anxiety in rodents. Total distance and velocity are the indices of locomotor activity. Also, Thigmotaxis or outer zone duration (the time that the subject remains adjacent to the outer wall of the maze) indicates anxiety-like behavior. The number of fecal pellets (boli) left in the quadrant is a negatively-related measure of emotionality in rodents and can be used to indicate levels of anxiety. Also, the number of rearing (number of times that a rat reared up on its hindlimbs) is inversely related to anxiety, whereas the number of grooming (number of times that an animal preened its fur or tail with its mouth or forepaws) is directly related to anxiety [21, 22]. Open fields test was performed to study the effects of Diabetes and Citrullus colocynthis on locomotion and anxiety-like behavior. The open field was an opaque Plexiglas arena with dimensions of 90 cm × 90 cm × 45 cm [H]. The arena was segmented into 16 small squares and the time spent in the central or peripheral squares was evaluated using the tracking system. Rat's behavior was recorded and analyzed using an automated video tracking system (Noldus EthovisionV R system, version 7, Netherland) in a 5-min interval. Duration spent in

the center and periphery of the box, velocity, total distance moved, and the number of grooming, rearing, and fecal pellets were recorded and analyzed for each rat [23, 24].

Novel object recognition test

The object recognition test (ORT), also known as the novel object recognition test (NOR), is a relatively fast and efficient means originally described by Ennaceur and Delacour in 1988 for testing different phases of learning and memory in rodents [25, 26]. Because rodents have an innate preference for novelty, a rodent that remembers the familiar object will spend more time exploring the novel object [27]. The NOR evaluates non-spatial learning and objects identity memory, relying on multiple brain regions except the hippocampus [28]. All animals were habituated to their environment with a 10-min habituation session in a plastic cage (60 cm × 60 cm × 40 cm) , where no object was presented and the cage was equally illuminated. Thirty minutes after the habituation period, the animals were allowed to explore two identical objects. The animals were always presented at the same position inside the box, and for a total of 5 min (training session). Memory retention ability was tested during the test session 45 min after the training session. During test sessions with a duration of 3 min, the animals were exposed to familiar and novel objects at the same position that the objects had been presented during the training phase. However, during the test phase, the location of the new object was pseudo-randomly changed to avoid the natural preference of animals for one location or another. All objects (available in duplicate) presented similar material and size, but distinct shape. Between each change of animals, box and objects were cleaned using 70% alcohol and then air-dried. The definition for the exploration time was the duration of sniffing or touching the object with the nose quantified by the camera. Finally, the discrimination ratio (as a recognition index) is the ratio of time spent for exploring one of the objects (in the training session) and the novel object (in the test session) divided by the total time spent for exploring both objects multiplied by 100 [29].

Passive avoidance test

One of the most well-known tests for assessment of learning and memory is fear conditioning or passive avoidance test. This test is performed using a shuttle box, where animals learn to form associations between different stimuli [30]. It has been demonstrated that two neural circuits are involved in fear conditioning; the hippocampus that deals with the learning and memory and the amygdala that deals with the emotional aspect. Nevertheless, it has been found that the amygdala also undergoes some learning during fear conditioning [31]. The passive avoidance task is a fear-aggravated test used to evaluate associative learning and memory in rodents. The animal learns to avoid an environment in which a prior aversive stimulus has been delivered. Here, passive avoidance learning was assessed using an inhibitory passive avoidance paradigm as described hereafter. Briefly, a shuttle box device with dimensions of 100 cm (L) × 25 cm (W) × 25 cm (H), consisting of two compartments (light and dark) separated by a door, was used. In the learning phase of the test, each animal was first habituated to the test equipment by placing in the light chamber (door closed) for 5 min before returning to the home cage. The next day, the animal was returned to the light compartment, the door was opened, and the animal was allowed to move to the dark chamber. After that, the door was closed and upon entering the Dark compartment (TDC), they were given an electric shock (0.5 A for 2 ms) *via* wires embedded in the dark chamber floor. This final part of the process was repeated up to five times at 1-h intervals until the animal learned to avoid TDC (remains in the light compartment for at least 120 s) and the number of shocks required for learning was recorded. The assessment phase of the test was performed 2 h after the learning phase. The animal was placed in the light chamber (door closed) and, after 30 s, the door was opened and the time, until the animal entered the dark chamber, was recorded as the step-through latency (STL). The total time spent in TDC during the 5-min period after door opening was also recorded [19].

Elevated plus-maze

The elevated plus-maze test is one of the most well-known experiments of all currently available animal models of anxiety [32, 33]. The open and closed arms are considered to evoke the same exploratory drive; therefore, the open arms' avoidance is considered to be a result of the induction of higher levels of fear [34]. The number of head dips (downward movement of rodents' head toward the floor from the open arms) and time spent in the open arm is inversely related to anxiety and time spent in the closed arm is directly related to anxiety in rats [35]. The EPM was used to assay the anxiety-like behavior of the animals. The maze is composed of wood and has two open arms and two closed arms (50 cm × 50 cm × 50 cm). Animals were placed in

the middle of the maze and time spent in each arm and the number of head dips was recorded by a video camera mounted above the EPM for 5 min [36].

Measurement of bodyweight and fasting blood glucose (FBS)

The body weight and FBS were measured before the STZ injection, 14 days after STZ injection, 20 days after starting treatment, and 40 days after starting treatment. For FBS evaluation, the blood samples were collected from the tail vein and FBS was determined by a glucometer.

Data analysis

The time and distance spent to find the hidden platform in the MWM training in the acquisition phase were analyzed using a two-way analysis of variance (ANOVA) and a repeated-measures ANOVA to determine the differences in the learning rates of the groups (group and block as the factors). All the data collected from the MWM probe trials swim speed, novel objective, open field, passive avoidance, and EPM tests were analyzed by one-way ANOVA. When statistical significance was found between the groups, Tukey's post hoc multiple comparison test was performed to determine points of significant difference. The data of body weight and FBS measured before and after diabetes induction were analyzed by paired t-test and the data of body weight and FBS measured after treatment were analyzed by repeated measurement test. The data were expressed as means ± SEM and $p < 0.05$ was considered statistically significant. The normality of data was checked by the Kolmogorov Smirnov test [19, 37]

Results

The effects of C. colocynthis administration on spatial learning in diabetic rats

Two-way ANOVA with repeated measures test indicated that the distance moved and escape latency of the Diabetes group significantly increased in block 2 (Figure 1A; $p < 0.01$ and Figure 1B; $p < 0.05$) and block 3 (Figure 1A; $p < 0.01$ and Figure 1B; $p < 0.01$) compared to the control group. The ability of the diabetic rats to find the hidden platform was significantly improved through C. colocynthis administration. This was inferred by the significant reduction in their swimming distance in block 2 (Figure 1A; $p < 0.05$) and block 3 (Figure 1A; $p < 0.05$) and a significant reduction in the escape latency in block 2 (Figure 1B; $p < 0.05$) and block 3 (Figure 1B; $p < 0.05$) compared to the Diabetes group in the MWM test. There was no significant difference in swimming speed among all the groups (Figure 1C).

The effects of C. colocynthis administration on spatial memory in diabetic rats

The probe test was performed 24 h after the acquisition phase to examine long-term spatial memory retention. The obtained results included the mean percentage (%) for a time as well as distance and number of crossing in the target quadrant. The results of the probe test demonstrated that the diabetic rats significantly spent shorter time and distance for crossing in the target quadrant compared to the control group, suggesting long-term memory impairment (Figure 1D; $p < 0.01$, Figure 1E; $p < 0.001$, and Figure 1F; $p < 0.01$). However, this impairment was significantly

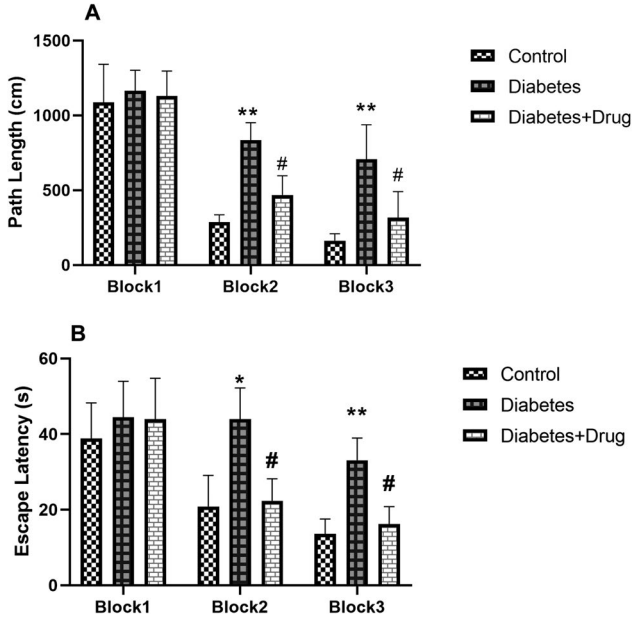

Figure 1. The effects of Diabetes and Citrullus colocynthis administration on spatial learning & Memory in MWM task. Diabetes resulted in significant increase of the traveled distance (A) and Escape latency (B) to find the hidden platform compared to control group. Administration of Citrullus colocynthis in Diabetes group significantly decreased the distance and time to find the hidden platform in blocks compared to Diabetes group. Also, there were no significant difference between all groups in velocity (C). Diabetes resulted in significant decreases in the percentage of time spent (E) and distance traveled (D) in target quadrant and the number of crossing from the platform region (F) compared to control group. Animals of Diabetes + Drug group significantly showed increase in these parameters compared to Diabetes group. Two-way ANOVA and repeated measure used for analysis of learning data and One-way ANOVA used for analysis of memory data. Mean ± SEM, (*) $p < 0.05$ vs control. (**) $p < 0.01$ vs control. (***) $p < 0.001$ vs control. (#) $p < 0.05$ vs Diabetes. (##) $p < 0.01$ vs Diabetes. (###) $p < 0.001$ vs Diabetes.

prevented by 40 days administration of C. colocynthis in the Diabetes + Drug group since they spent more time and distance and crossing in the target quadrant than the diabetic rats (Figure 1D; $p < 0.01$, Figure 1E; $p < 0.001$, and Figure 1F; $p < 0.01$) (one-way ANOVA followed by Tukey's test).

The effects of C. colocynthis administration on anxiety behaviors in open field test in diabetic rats

Rats of the diabetes group had no significant difference in total distance and velocity compared to those in the other groups (Figures 2A and 2B). There was a significant decrease in the time spent in the center of the box or inner zone duration in diabetic rats compared to the control group (Figure 2C; $p < 0.05$). Moreover, the administration of C. colocynthis could increase inner zone duration compared to the Diabetes group significantly (Figure 2C; $p < 0.05$). The time spent in the periphery of the box or outer zone duration significantly increased in diabetic rats compared to control rats (Figure 2D; $p < 0.05$). Besides, the administration of C. colocynthis could decrease this index compared to the Diabetes group (Figure 2D; $p < 0.05$). Rearing numbers significantly decreased in the Diabetes group compared to the control group (Figure 2E; $p < 0.01$). However, grooming frequency significantly increased in the Diabetes group compared to the control group (Figure 2F; $p < 0.05$). No significant difference was observed in the rearing numbers between Diabetes and Diabetes + Drug groups (Figure 2E). C. colocynthis administration significantly decreased the grooming frequency in the Diabetes + Drug group compared to the Diabetes group (Figure 2F; $p < 0.01$). Fecal Pellet number and amount of defecation were assessed between groups and found that the number of fecal pellets in the Diabetes group was significantly more than that in the control group (Figure 2G; $p < 0.05$). Furthermore, C. colocynthis administration decreased this parameter compared to the Diabetes group (Figure 2G; $p < 0.05$) (one-way ANOVA followed by Tukey's test).

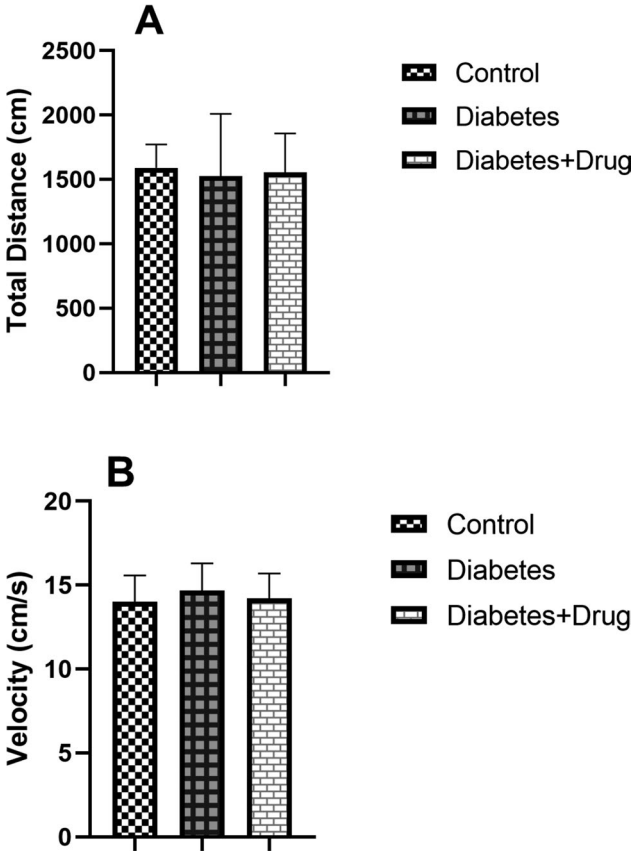

Figure 2. The effects of Diabetes and Citrullus colocynthis administration on the anxiety-like behavior and exploratory activity in the open field test. Total distance and velocity had no significant difference among all of groups (Figure 2A, B). Diabetes increased outerzone duration and decreased innerzone duration compared to control group and conversely Citrullus colocynthis administration could increase innerzone duration and decreased outerzone duration compared to Diabetes group (Figure 2C, D). Rearing number (Figure 2E) decreased, Grooming frequency (Figure 2F) increased and Fecal pellets (Figure 2G) increased in Diabetes group compared to other groups. One-way ANOVA used for analysis these results. Mean ± SEM, (*) $p < 0.05$ vs control. (**) $p < 0.01$ vs. control. (#) $p < 0.05$ vs Diabetes. (##) $p < 0.01$ vs Diabetes. (###) $p < 0.001$ vs. Diabetes.

The effects of C. colocynthis administration on recognition memory in diabetic rats

In the first trial with two novel objects, all the groups spent a similar amount of time exploring the objects

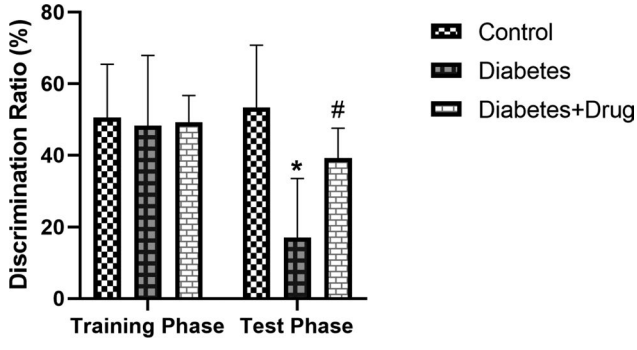

Figure 3. The effects of Diabetes and Citrullus colocynthis administration on novel object recognition test. There was no significant difference in exploration time in training session between all of groups. There was a significant decrease in Exploration time in test session in Diabetes group compared to control group and administration of Citrullus colocynthis in Diabetes + Drug group increased exploration time compared to Diabetes group. One-way ANOVA used for statistical analysis. Mean ± SEM, (*) $p < 0.05$ vs control. (#) $p < 0.05$ vs Diabetes.

and there was no statistically significant difference between them (Figure 3, Training Phase). In the second trial, object recognition memory was assessed by replacing the objects of the first trial with a copy of the original object, and a novel object recognition memory was reflected in a preference for exploring the novel object. The new object recognition memory was significantly disrupted in diabetic rats. The diabetic rats failed to spend a greater amount of time investigating the novel object than the familiar object and they were not significantly biased toward the novel object. In these animals, the discrimination ratio was significantly lower than that in the control group (Figure 3; $p < 0.05$). C. colocynthis administration ameliorated the diabetes-induced impairment of the novel objective recognition test (Figure 3; $p < 0.05$) (one-way ANOVA followed by Tukey's test).

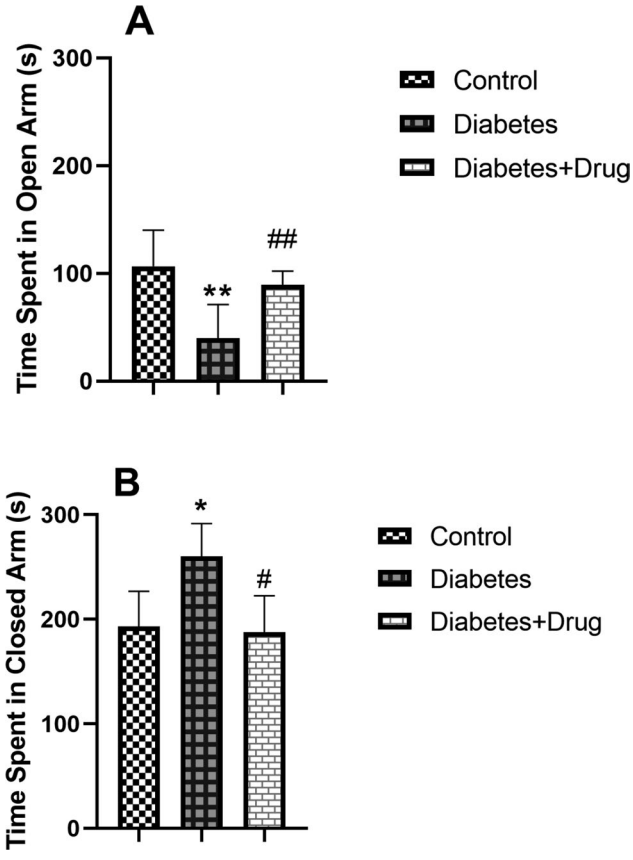

Figure 4. The effects of Citrullus colocynthis administration on anxiety like behaviors in Elevated plus maze task in STZ induced Diabetes rats. STZ increased time spent in closed arm (B) and decreased time spent in open arm (A) and number of head dips (C) significantly that these changes demonstrated increased anxiety due to Diabetes. Citrullus colocynthis in Diabetes + Drug group significantly reversed the effects of Diabetes and improved anxiety. One-way ANOVA used for analysis of these data. Mean ± SEM, (**) $p < 0.01$ vs. control. (*) $p < 0.01$ vs control. (***) $p < 0.001$ vs control. (#) $p < 0.05$ Vs Diabetes. (##) $p < 0.01$ vs Diabetes. (###) $p < 0.001$ vs. Diabetes.

The effects of C. colocynthis administration on anxiety-like behaviors in the elevated plus-maze in diabetic rats

According to EPM data, the rats that received STZ in the Diabetes group showed a significant decrease in time spent in open arms (Figure 4A; $p < 0.01$) and an increase in time spent in closed arms (Fig 4B; $p < 0.05$) compared to the control group. Conversely, C. colocynthis in the Diabetes + drug group displayed a significant increase in time spent in the open arm (Figure 4A; $p < 0.01$) and a decrease in time spent in the closed arm (Figure 4B; $p < 0.05$). Also, studying the number of head dips (downward movement of rodents' head toward the floor from the open arms) showed that the number of head dips significantly decreased in the Diabetes group compared to the control group (Figure 4C; $p < 0.001$) and C. colocynthis administration significantly increased head dips in the treatment

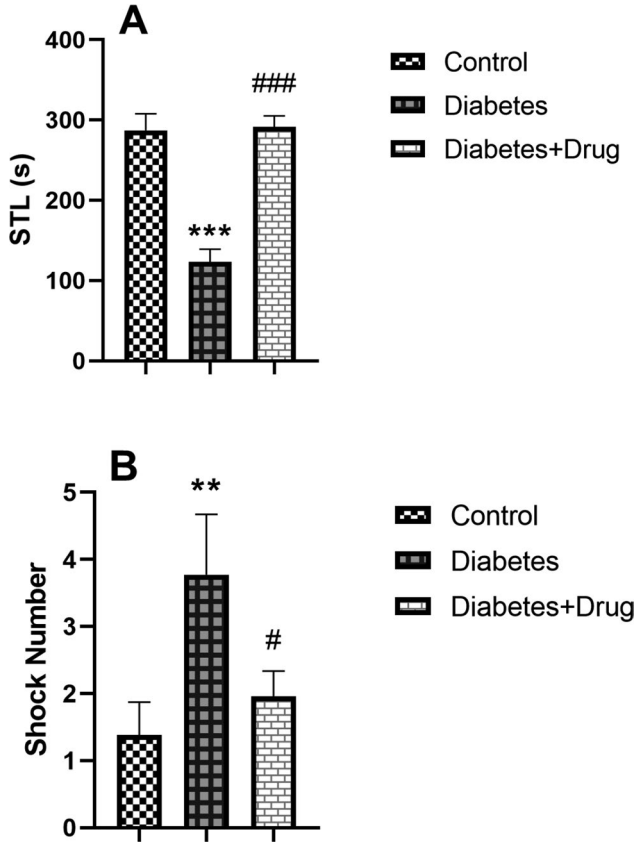

Figure 5. The effects of Diabetes and Citrullus colocynthis administration on learning trial (shock number) (B) and retention test including time spent in dark compartment (C) and STL (step through latency) (A)·Diabetes increased shock number and time spent in dark compartment while decreased STL compared to control group while Citrullus colocynthis reversed these changes. One-way ANOVA used for statistical analysis. Mean ± SEM, (***) $p < 0.001$ vs control. (**) $p < 0.01$ vs control. (###) $p < 0.001$ vs Diabetes. (#) $p < 0.05$ vs Diabetes. in shuttle box test.

group (Figure 4C; $p < 0.001$) (one-way ANOVA followed by Tukey's test).

The effects of C. colocynthis administration on passive avoidance learning and memory in diabetic rats

The influence of diabetes and C. colocynthis administration on passive avoidance learning is shown in Figure 5B. The one-way ANOVA test followed by Tukey's test showed that shock numbers significantly increased in the Diabetes group compared to the control group (Figure 5B; $p < 0.01$). Moreover, C. colocynthis administration significantly decreased shock number in the Diabetes + Drug group compared to the Diabetes group (Figure 5B; $p < 0.05$). Figures 5A and C show the effect of diabetes and C. colocynthis administration on passive avoidance memory. Diabetes significantly increased the time spent in the dark compartment (Figure 5C; $p < 0.001$) but significantly decreased the STL (Figure 5A; $p < 0.001$) compared to the control group. However, C. colocynthis administration significantly decreased time spent in the dark compartment (Figure 5C; $p < 0.001$) but significantly increased the STL (Figure 5A; $p < 0.001$) compared to the Diabetes group.

The effects of C. colocynthis administration on body weight in diabetic rats

According to the results of the paired t-test, the body-weight of rats significantly decreased 14 days after STZ injection compared to before STZ injection in diabetic rats in Diabetes and Diabetes + Drug (before starting treatment) groups (Figure 6A; $p < 0.01$, $n = 14$). Also, repeated measures analysis of body weight after starting treatment showed that 20 and 40 days of C. colocynthis administration significantly increased body weight compared to before starting treatment in Diabetes + Drug group (Figure 6B; $p < 0.01$ for 20- and 40-days treatment, $n = 7$). There was no statistically significant difference between treated and control groups (Figure 6B).

The effects of C. colocynthis administration on fasting blood glucose (FBS) in diabetic rats

The paired t-test results showed that the FBS of rats significantly increased 14 days after STZ injection

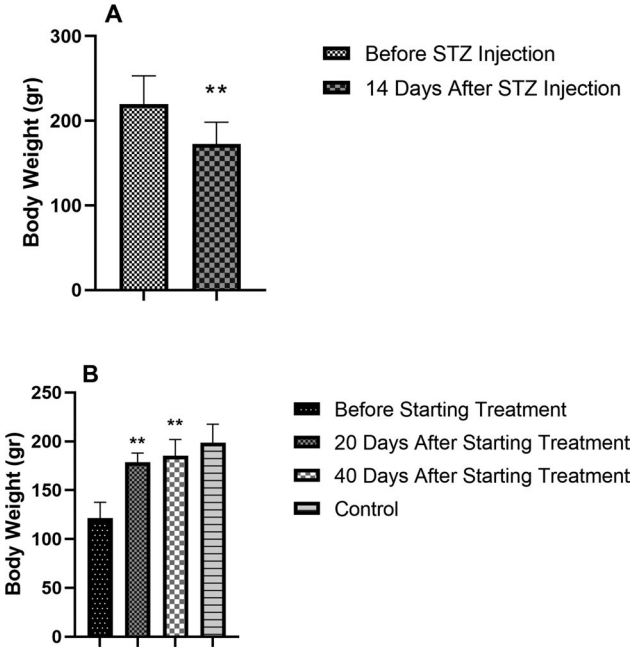

Figure 6. The effects of Diabetes and Citrullus colocynthis administration on Body Weight before and 14 days after STZ injection (A). (Paired T-test used for statistical analysis). Figure 6B shows the effects of Citrullus colocynthis administration on Body Weight 20 & 40 days after Diabetes injection (Repeated measurement used for statistical analysis). Mean ± SEM, (**) $p < 0.01$ vs Before STZ injection in Figure 6A. (**) $p < 0.01$ vs Before starting treatment in Figure 6B.

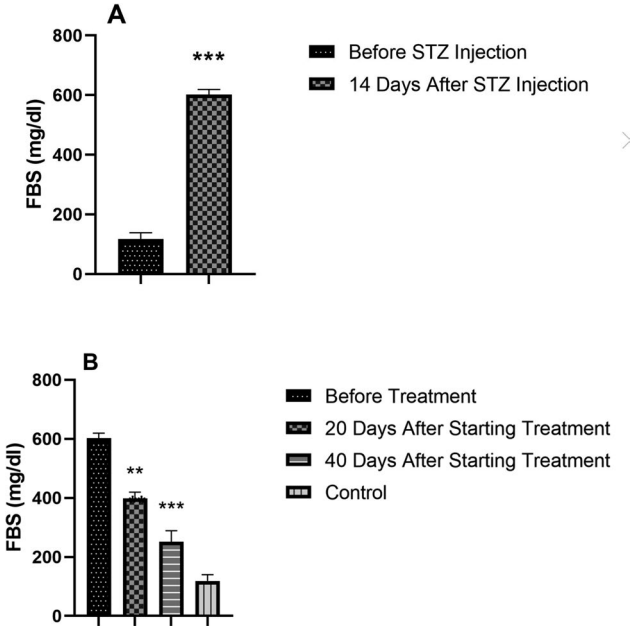

Figure 7. The effects of Diabetes and Citrullus colocynthis administration on FBS before and 14 days after STZ injection (A). (Paired T-test used for statistical analysis). Figure 7B shows the effects of Citrullus colocynthis administration on FBS 20 & 40 days after Diabetes injection (Repeated measurement used for statistical analysis). Mean ± SEM, (***) $p < 0.001$ vs Before STZ injection in Figure 7A. (**) $p < 0.01$ & (***) $p < 0.001$ vs Before starting treatment in Figure 7B.

compared to before STZ injection in diabetic rats in Diabetes and Diabetes + Drug (before starting treatment) groups (Figure 7A; $p < 0.001$, $n = 14$). Also, analysis of FBS after starting treatment showed that 20 and 40 days of C. colocynthis administration significantly decreased FBS compared to before starting treatment in Diabetes + Drug group (Figure 7B; $p < 0.01$ for 20 days treatment and $p < 0.001$ for 40 days treatment, $n = 7$). The results of repeated measures analysis showed no significant difference between treated groups with each other or with the control group.

Discussion

This is the first report on the importance of Citrullus colocynthis as a pharmacological interference on learning and memory impairments and anxiety-like behaviors in diabetic rats. Our findings revealed that diabetes impaired the spatial learning and passive avoidance learning of the male rats. Diabetes also disrupted the long-term spatial memory, passive avoidance memory, and novel object recognition memory of the male rats. It was revealed that diabetes could change anxiety-like behaviors in male rats by increasing the anxiety level. In this study, it was found that Citrullus colocynthis administration could improve learning and memory impairment following diabetes.

Many studies showed that diabetes causes weight loss and increases FBS [38–40]. Consistent with these findings, we witnessed a weight loss and FBS increase after the induction of diabetes by STZ. Also, these results showed that Citrullus colocynthis intensified weight loss and increased FBS. Ghauri et al. showed that Citrullus colocynthis hydro-ethanolic pulpy flesh with seeds extract exerted a substantial anti-hyperglycemic activity in a diabetic rat model by lowering blood glucose, cholesterol, and triglyceride levels [41]. Also, Oryan et al. demonstrated that Citrullus colocynthis can decrease blood glucose in alloxan-induced diabetes rats [42]. Probably, a decrease in the serum concentration of glucose in the treated animals could be related either to the partial regeneration or preservation of the pancreatic β-cell mass by Citrullus colocynthis in rats. The histopathologic sections of the pancreas of the diabetic rats treated by Citrullus colocynthis showed an increase in the size of the islets, with hyper-chromic nucleus and regeneration of the β-cells [42].

It has been evidenced that diabetes leads to cognitive decline and thus dementia in both animal models and humans with both T1DM and T2DM [4, 43]. Adults with T1DM present a subtle decline in cognitive performance compared to age-matched controls, particularly affecting the cognitive domains of intelligence, psychomotor efficiency, and cognitive flexibility [44, 45]. The changes in cognition and hippocampal synaptic plasticity in diabetic rats have been reviewed in other investigations [46, 47]. The performance of these animals on relatively simple behavioral tasks, such as passive avoidance paradigms, is preserved or even improved [47]. In comparison, performance is disturbed on more complex tasks, such as a water maze or a spatial-object learning task [48, 49]. The development of performance deficits on these tasks depends on diabetes duration and the severity of hyperglycemia. Moreover, the deficits can be prevented, but not completely reversed, with intensive insulin treatment [47]. The negative effects of diabetes on learning and memory are attributed to the deleterious changes in LTP [47, 50], glutamatergic neurotransmission [51], neuronal calcium homeostasis [52] and receptors, including N-Methyl D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [53, 54]. Other underlying mechanisms of post-diabetes cognitive impairments may be related to increased oxidative stress and inflammation in hippocampus [55].

The phytosterolin (ipurand), 2 phytosterols, 2 hydrocarbons, a saponin, an alkaloid, a polysaccharide, or glycoside, and tannin can be found in these seeds [56]. In a study, the intensity of hyperglycemia found after the overnight starvation after a 7-day STZ administration was minimized by the injection of the crude aqueous extract of Citrullus colocynthis seeds [57].

In another review, the fatty liver syndrome and the hyperglycemic state related to hyperinsulinemia (type 2 diabetes) caused by exposure to a high-fat diet were extensively corrected *via* the ethanolic extract of Citrullus colocynthis fruit seeds with its rich content of pharmacologically active ingredients including tannin, saponin, and flavanoids. However, this function was dose-dependent [58].

It has been reported that saponins have a hypoglycemic function. Also, because of having antioxidant activity, they can prevent diabetic complications and adjust the blood glucose level. These compounds have an extensive range of biological functions such as the secretion of insulin, which stimulates beta-cell islets to revive enzymes responsible for using glucose [59].

Few studies have been conducted on the effects of Citrullus colocynthis on cognition, behavioral, and brain status. Although we did not find many papers about this issue, the protective effects of Citrullus colocynthis on diabetes have been well approved. However, some studies have demonstrated the protective effects

of Citrullus colocynthis on the central nervous system under different conditions *via* different mechanisms such as anticonvulsant activity by modulating the GABA and benzodiazepine receptors [60], analgesic activity by modulating opioid receptors [61], and antidepressant and sedative activity by modulating of GABA receptors [62]. Studies have shown that the Citrullus colocynthis can have beneficial effects on improving Alzheimer's disease through mechanisms such as antioxidant and anti-inflammatory activity and cholinesterase inhibition [63–65]. In rodents, Citrullus colocynthis investigations showed that diabetes can impaired cognition in behavioral level in some behavioral tasks such as the Morris Water Maze (MWM) [66, 67], Novel object recognition (NOR) [68], passive avoidance [68, 69], Barnes maze [70], T maze [70], and Y maze [69]. Also, at the synaptic level, diabetes disrupts long-term potentiation (LTP) and synaptic plasticity [48, 71] and at the molecular level, diabetes declined BDNF level [72, 73].

The hippocampus is involved in spatial/relational memory [74, 75]. In this regard, water maze specifically tests spatial memory [76]. Our observations revealed that STZ-induced diabetes impaired spatial learning and memory in the MWM task. Other studies confirm our data [77, 78]. Moreover, according to the results of this study, Citrullus colocynthis administration could improve this impairment by reducing distance moved and time spent on finding the hidden platform.

In agreement with some other studies, our results in NOR showed that STZ-induced diabetes impaired the ability of the rat in distinguishing novel objects [79, 80]. Based on our results, this impairment could be improved by the administration of Citrullus colocynthis as it increases the animal's inclination toward a new object.

Our data revealed that STZ-induced diabetes disrupted passive avoidance learning and memory. Other studies are consistent with these findings [81, 82]. Administration of Citrullus colocynthis reversed these disturbances. Citrullus colocynthis decreased shock number, indicating the improved learning process. Besides, it increased STL and decreased time spent in a dark compartment, suggesting the improvement in the memory process.

Our data demonstrated that diabetes increased anxiety-like behaviors in the open field test and elevated plus-maze. Several studies have analyzed the relationship between diabetes and anxiety in animal models and showed that diabetes increases anxiety-like behaviors in an open field and plus maze [22, 83] tasks. Our findings demonstrated that the administration of Citrullus colocynthis could decrease anxiety behaviors.

Citrullus colocynthis decreased grooming, defecation, and time spent in the periphery of the box as an index for high anxiety. Also, it increased rearing and time spent in the center of the box as an index for low anxiety in the open field test. Moreover, Citrullus colocynthis increased the time spent in open arms and head dips as an index for low anxiety and decreased the time spent in closed arms as an index for high anxiety in plus-maze.

In this study, we evaluated the effects of Citrullus colocynthis on cognition in behavioral levels in diabetic rats and found that Citrullus colocynthis could ameliorate the negative effects of diabetes on behavior in rats. It is noteworthy that the behavioral tests alone were not enough to clarify the effects of Citrullus colocynthis on diabetic-associated cognitive impairments. The possible mechanisms of these effects may be related to molecular and synaptic levels such as changes in the BDNF level and its receptor expression or LTP. Therefore, more electrophysiological and molecular investigations are needed to find possible mechanisms for these positive behavioral effects in the future.

Conclusion

In general, our data showed that STZ-induced diabetes could impair spatial and non-spatial or hippocampal-dependent and non-hippocampal dependent learning and memory, as well as increasing anxiety-like behaviors. However, Citrullus colocynthis administration could ameliorate these cognitive impairments, as well as antidiabetic effects such as increasing body weight and decreasing FBS.

Dsiclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Fouzieh Salim http://orcid.org/0000-0002-6460-3666

References

[1] Khalil M, Mohamed G, Dallak M, et al. The effect of Citrullus colocynthis pulp extract on the liver of diabetic rats a light and scanning electron microscopic study. Am J Biochem Biotechnol. 2010;6(3):155–163.

[2] Esteghamati A, Gouya MM, Abbasi M, et al. Prevalence of diabetes and impaired fasting glucose in the adult population of Iran: National Survey of Risk Factors for Non-Communicable Diseases of Iran. Diab Care. 2008;31(1):96–98.

[3] Cato A, Hershey T. Cognition and type 1 diabetes in children and adolescents. Diab Spectr. 2016;29(4):197–202.
[4] Zilliox LA, Chadrasekaran K, Kwan JY, et al. Diabetes and cognitive impairment. Curr Diab Rep. 2016;16(9):87.
[5] Ding X, Fang C, Li X, et al. Type 1 diabetes-associated cognitive impairment and diabetic peripheral neuropathy in Chinese adults: results from a prospective cross-sectional study. BMC Endocr Disord. 2019;19(1):34.
[6] Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. Endocr Rev. 2008;29(4):494–511.
[7] Tonoli C, Heyman E, Roelands B, et al. Type 1 diabetes-associated cognitive decline: A meta-analysis and update of the current literature 1 型糖尿病相关的认知能力下降:一项对最新文献的 meta 分析. J Diab. 2014;6(6):499–513.
[8] Kalva S, Fatima N, Nerella R, et al. Insulinomimetic effect of Citrullus Colocynthis roots in STZ challenged rat model. Iran J Pharm Sci. 2018;14(3):49–66.
[9] Ostovan F, Gol A, Javadi A. Investigating the effects of Citrullus colocynthis pulp on oxidative stress in testes and epididymis in streptozotocin-induced diabetic male rats. Int J Reprod Biomed. 2017;15(1):41.
[10] Olatunya AM, Omojola A, Akinpelu K, et al. Vitamin E, phospholipid, and phytosterol contents of Parkia biglobosa and Citrullus colocynthis seeds and their potential applications to human health. Prev Nutr Food Sci. 2019;24(3):338–343.
[11] Guadarrama-López AL, Valdés-Ramos R, Martínez-Carrillo BE. Type 2 diabetes, PUFAs, and vitamin D: their relation to inflammation. J Immunol Res. 2014;2014:1–13.
[12] Heydari M, Homayouni K, Hashempur MH, et al. Topical C Citrullus colocynthis (bitter apple) extract oil in painful diabetic neuropathy: A double-blind randomized placebo-controlled clinical trial: 外用西瓜属药西瓜瓤（苦苹果） 提取油治疗痛性糖尿病神经病变: 一项双盲随机安慰剂对照的临床试验. J Diab. 2016;8(2):246–252.
[13] Gurudeeban S, Satyavani K, Ramanathan T. Bitter apple (Citrullus colocynthis): An overview of chemical composition and biomedical potentials. Asian J Plant Sci. 2010;9(7):394–401.
[14] Rahimi R, Amin G, Ardekani MRS. A review on Citrullus colocynthis Schrad.: from traditional Iranian medicine to modern phytotherapy. J Altern Complement Med. 2012;18(6):551–554.
[15] Kumarappan C, Mandal SC. Polyphenolic extract of Ichnocarpus frutescens attenuates diabetic complications in streptozotocin-treated diabetic rats. Ren Fail. 2008;30(3):307–322.
[16] Bromley-Brits K, Deng Y, Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. J Vis Exp. 2011;53:e2920.
[17] Morris RG. Spatial localization does not require the presence of local cues. Learn Motiv. 1981;12(2):239–260.
[18] Hajali V, Sheibani V, Esmaeili-Mahani S, et al. Female rats are more susceptible to the deleterious effects of paradoxical sleep deprivation on cognitive performance. Beh Brain Res. 2012;228(2):311–318.
[19] Rajizadeh MA, Esmaeilpour K, Masoumi-Ardakani Y, et al. Voluntary exercise impact on cognitive impairments in sleep-deprived intact female rats. Physiol Behav. 2018;188:58–66.
[20] Hall CS. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol. 1934;18(3):385–403.
[21] Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp. 2015;96:e52434.
[22] Damián JP, Acosta V, Da Cuña M, et al. Effect of resveratrol on behavioral Performanceof Streptozotocin-induced diabetic mice inAnxiety tests. Exp Anim. 2014;63(3):277–287.
[23] Amirazodi F, Mehrabi A, Amirazodi M, et al. The combination effects of resveratrol and swimming HIIT exercise on novel object recognition and open-field tasks in aged rats. Exp Aging Res. 2020;46:1–23.
[24] Rajizadeh MA, Sheibani V, Bejeshk MA, et al. The effects of high-intensity exercise on learning and memory impairments followed by a combination of sleep deprivation and demyelination induced by ethidium bromide. Int J Neurosci. 2019;129(12):1166–1178.
[25] Ennaceur A, Meliani K. A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. Behav Brain Res. 1992;51(1):83–92.
[26] Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. J Vis Exp. 2017;126:e55718.
[27] Ennaceur A. One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res. 2010;215(2):244–254.
[28] Denninger JK, Smith BM, Kirby ED. Novel object recognition and object location behavioral testing in mice on a budget. J Vis Exp. 2018;141:e58593.
[29] Rajizadeh MA, Sheibani V, Esmaeilpour K, et al. Cognitive impairments of sleep deprived ovariectomized (OVX) female rats can be overcome by voluntary exercise. Basic Clin Neurosci. 2020;11(5):573.
[30] Savage S, Ma D. III. Animal behavior testing: memory. Oxford: Oxford University Press; 2014.
[31] Rogan MT, Stäubli UV, LeDoux JE. Fear conditioning induces associative long-term potentiation in the amygdala. Nature. 1997;390(6660):604–607.
[32] Crawley JN. What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice. Hoboken: John Wiley & Sons; 2007.
[33] Komada M, Takao K, Miyakawa T. Elevated plus-maze for mice. J Vis Exp. 2008;22:e1088.
[34] Rodgers R, Dalvi A. Anxiety, defence, and the elevated plus-maze. Neurosci Biobehav Rev. 1997;21(6):801–810.
[35] Walf AA, Frye CA. The use of the elevated plus-maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007;2(2):322–328.
[36] Rajizadeh MA, Afarinesh MR, Zarif M, et al. Does caffeine therapy improve cognitive impairments in the valproic acid rat model of autism?Toxin Reviews. 2019;38:1–11.
[37] Rajizadeh MA, Esmaeilpour K, Haghparast E, et al. Voluntary exercise modulates learning & memory and synaptic plasticity impairments in sleep-deprived female rats. Brain Res. 2020;1729:146598.

[38] Al-Attar AM, Alsalmi FA. Effect of Olea europaea leaves extract on streptozotocin-induced diabetes in male albino rats. Saudi J Biol Sci. 2019;26(1):118–128.
[39] Muzumbukilwa WT, Nlooto M, Owira PMO. Hepatoprotective effects of Moringa oleifera Lam (Moringaceae) leaf extracts in streptozotocin-induced diabetes in rats. J Funct Foods. 2019;57:75–82.
[40] Wang-Fischer Y, Garyantes T. Improving the reliability and utility of streptozotocin-induced rat diabetic model. J Diab Res. 2018;2018:1–14.
[41] Ghauri AO, Ahmad S, Rehman T. In vitro and in vivo anti-diabetic activity of Citrullus colocynthis pulpy flesh with seeds hydro-ethanolic extract. J Complement Integr Med. 2020;17(2):70–73.
[42] Oryan A, Hashemnia M, Hamidi A-R, et al. Effects of hydro-ethanol extract of Citrullus colocynthis on blood glucose levels and pathology of organs in alloxan-induced diabetic rats. Asian Pac J Trop Dis. 2014;4(2):125–130.
[43] Wong RHX, Scholey A, Howe PRC. Assessing premorbid cognitive ability in adults with type 2 diabetes mellitus—a review with implications for future intervention studies. Curr Diab Rep. 2014;14(11):547.
[44] Biessels GJ, Despa F. Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. Nat Rev Endocrinol. 2018;14(10):591–604.
[45] Brands AM, Biessels GJ, De Haan EH, et al. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diab Care. 2005;28(3):726–735.
[46] Biessels GJ, Gispen WH. The impact of diabetes on cognition: what can be learned from rodent models?Neurobiol Aging. 2005;26(1):36–41.
[47] Gispen WH, Biessels G-J. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci. 2000;23(11):542–549.
[48] Biessels G-J, Kamal A, Ramakers GM, et al. Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. Diabetes. 1996;45(9):1259–1266.
[49] Lupien SB, Bluhm EJ, Ishii DN. Systemic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. J Neurosci Res. 2003;74(4):512–523.
[50] Chabot C, Massicotte G, Milot M, et al. Impaired modulation of AMPA receptors by calcium-dependent processes in streptozotocin-induced diabetic rats. Brain Res. 1997;768(1–2):249–256.
[51] Trudeau F, Gagnon S, Massicotte G. Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. Eur J Pharmacol. 2004;490(1–3):177–186.
[52] Kamal A, Artola A, Biessels G, et al. Increased spike broadening and slow afterhyperpolarization in CA1 pyramidal cells of streptozotocin-induced diabetic rats. Neuroscience. 2003;118(2):577–583.
[53] Di Luca M, Ruts L, Gardoni F, et al. NMDA receptor subunits are modified transcriptionally and post-translationally in the brain of streptozotocin-diabetic rats. Diabetologia. 1999;42(6):693–701.
[54] Joël G, Milot M, Gélinas S, et al. Binding properties of glutamate receptors in streptozotocin-induced diabetes in rats. Diabetes. 1997;46(5):841–846.
[55] Mahmoudi N, Kiasalari Z, Rahmani T, et al. Diosgenin attenuates cognitive impairment in streptozotocin-induced diabetic rats: underlying mechanisms. Neuropsychobiology. 2020;80:1–11.
[56] Gharehmatrossian S, Popov Y, Ghorbanli M. Seed germination, dormancy breaking techniques of Citrullus colocynthis (L.) Schrab plant. Iran J Plant Physiol. 2014;4(4):1167–1171.
[57] Benariba N, Bellakdhar W, Djaziri R, et al. Protective action of Citrullus colocynthis seed extracts against the deleterious effect of streptozotocin on both in vitro glucose-stimulated insulin release from rat pancreatic islets and in vivo glucose homeostasis. Biomed Rep. 2013;1(1):119–121.
[58] Ezzat-Ali Esmail O. A Possible protective effect of Citrullus colocynthis melon against diabetes mellitus type-2 is associated with non-alcoholic fatty liver syndrome in rats. J Am Sci. 2012;8:1054–1061.
[59] El Barky AR, Hussein SA, Alm-Eldeen A-E. Saponins and their potential role in diabetes mellitus. Diab Manag. 2017;7(1):148.
[60] Mehrzadi S, Shojaii A, Pur SA, et al. Anticonvulsant activity of hydroalcoholic extract of Citrullus colocynthis fruit: involvement of benzodiazepine and opioid receptors. J Evid Based Complement Altern Med. 2016;21(4):NP31–NP35.
[61] Pashmforosh M, Rajabi Vardanjani H, Rajabi Vardanjani H, et al. Topical anti-inflammatory and analgesic activities of Citrullus colocynthis extract cream in rats. Medicina. 2018;54(4):51.
[62] Nafisi S, Rezazadeh L, Hosseini E, et al. Citrullus colocynthis fruit extract as an anti-depressant in mice. J Basic Res Med Sci 2016;3(4):49–55.
[63] Hosseinkhani A, Sahragard A, Namdari A, et al. Botanical sources for Alzheimer's: A review on reports from traditional Persian medicine. Am J Alzheimers Dis Other Demen. 2017;32(7):429–437.
[64] Kumar S, Kumar D, Saroha K, et al. Antioxidant and free radical scavenging potential of Citrullus colocynthis (L.) Schrad. methanolic fruit extract. Acta Pharm. 2008;58(2):215–220.
[65] Marzouk B, Marzouk Z, Haloui E, et al. Screening of analgesic and anti-inflammatory activities of Citrullus colocynthis from southern Tunisia. J Ethnopharmacol. 2010;128(1):15–19.
[66] Haider S, Ahmed S, Tabassum S, et al. Streptozotocin-induced insulin deficiency leads to the development of behavioral deficits in rats. Acta Neurol Belg. 2013;113(1):35–41.
[67] Shingo AS, Kanabayashi T, Kito S, et al. Intracerebroventricular administration of an insulin analog recovers STZ-induced cognitive decline in rats. Beh Brain Res. 2013;241:105–111.
[68] Jabbarpour Z, Shahidi S, Saidijam M, et al. Effect of tempol on the passive avoidance and novel object recognition task in diabetic rats. Brain Res Bull. 2014;101:51–56.
[69] Roghani M, Joghataie MT, Jalali MR, et al. Time course of changes in passive avoidance and Y-maze performance in male diabetic rats. Iran Biomed J. 2006;10(2):99–104.
[70] Nagayach A, Patro N, Patro I. Astrocytic and microglial response in experimentally induced diabetic rat brain. Metab Brain Dis. 2014;29(3):747–761.

[71] Biessels G-J, Kamal A, Urban IJ, et al. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain Res. 1998;800(1):125–135.
[72] Bathina S, Srinivas N, Das UN. Streptozotocin produces oxidative stress, inflammation, and decreases BDNF concentrations to induce apoptosis of RIN5F cells and type 2 diabetes mellitus in Wistar rats. Biochem Biophys Res Commun. 2017;486(2):406–413.
[73] Utkan T, Yazir Y, Karson A, et al. Etanercept improves cognitive performance and increases eNOS and BDNF expression during experimental vascular dementia in streptozotocin-induced diabetes. CNR. 2015;12(2):135–146.
[74] Kesner R, Evans R, Hunt M. Further evidence in support of the neurobiological bases of an attribute model of memory: role of the hippocampus. Int J Neurol. 1987;21:184–196.
[75] Nunez J. Morris water maze experiment. J Vis Exp. 2008; 19:e897.
[76] D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Rev. 2001;36(1):60–90.
[77] Bhutada P, Mundhada Y, Bansod K, et al. Protection of cholinergic and antioxidant systems contributes to the effect of berberine ameliorating memory dysfunction in a rat model of streptozotocin-induced diabetes. Behav Brain Res. 2011;220(1):30–41.
[78] Palleria C, Leo A, Andreozzi F, et al. Liraglutide prevents cognitive decline in a rat model of streptozotocin-induced diabetes independently from its peripheral metabolic effects. Behav Brain Res. 2017;321:157–169.
[79] Farajpour R, Sadigh-Eteghad S, Ahmadian N, et al. Chronic administration of Rosa canina hydro-alcoholic extract attenuates depressive-like behavior and recognition memory impairment in diabetic mice: a possible role of oxidative stress. Med Princ Pract. 2017;26(3):245–250.
[80] Patel SS, Gupta S, Udayabanu M. Urtica dioica modulates hippocampal insulin signaling and recognition memory deficit in streptozotocin-induced diabetic mice. Metab Brain Dis. 2016;31(3):601–611.
[81] Hasanein P, Shahidi S. Effects of combined treatment with vitamins C and E on passive avoidance learning and memory in diabetic rats. Neurobiol Learn Mem. 2010;93(4):472–478.
[82] Moradkhani S, Salehi I, Abdolmaleki S, et al. Effect of Calendula officinalis hydroalcoholic extract on passive avoidance learning and memory in streptozotocin-induced diabetic rats. Anc Sci Life. 2015;34(3):156.
[83] Yuan P, Zhang J, Li L, et al. Fluoxetine attenuated anxiety-like behaviors in streptozotocin-induced diabetic mice by mitigating the inflammation. Mediators Inflam. 2019;2019:1–8.