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# Effects of endurance exercise and *Urtica dioica* on the functional, histological and molecular aspects of the hippocampus in STZ-Induced diabetic rats

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ABSTRACT

*Ethnopharmacological relevance:* Many body systems and organs, including the hippocampus, are affected by diabetes, and undergo changes that may increase the risk of cognitive decline. *Urtica dioica* (UD) has long been recognized as a medicinal plant with beneficial effects on blood glucose control in diabetes.

*Aim of the study:* The present study aimed to investigate the effect of endurance exercise (Ex), along with *Urtica dioica* (UD) hydro-alcoholic extract on some functional, histological, and molecular aspects of the hippocampus in streptozotocin (STZ)-induced diabetic rats.

*Materials and methods:* 60 male Wistar rats were divided into five groups (N = 12): healthy control (H-C), diabetes control (D-C), diabetes exercise (D-Ex), diabetes *Urtica dioica* (D-UD), and diabetes exercise *Urtica dioica* (D-Ex-UD). Diabetes was induced intraperitoneally by STZ (45 mg/kg) injection. Two weeks after the injection by STZ, Ex (moderate intensity/5day/week) and gavage of UD extract (50mg/kg/day) was performed for six weeks. Cognitive functions were evaluated by the Morris Water Maze test, routine histological examination, and molecular studies were done via Hematoxylin & Eosin stain, and Western blot.

*Results:* Diabetic rats showed spatial learning and memory deficits, as well as negatively affects to the tissue and structure of the hippocampus in the dentate gyrus (DG) and cornu ammonis (CA) areas. Ex + UD treatment caused a decrease of neural disorganization, an increase of neural-microglial density, and thickness of the pyramidal-molecular layer in the hippocampus. In addition, Ex + UD caused a rise of GAP-43 protein levels, a reduction of CAP-1 protein levels, improved hippocampal structure, and improved learning and memory function.

*Conclusions:* These results show that Ex, along with the UD extract, may decrease levels of the central neural complications of diabetes. Given the importance of recognizing non-pharmacological complementary therapies in this field, future studies are warranted.

## 1. Introduction

Diabetes mellitus is a metabolic disease caused by chronic hyperglycemia due to impaired insulin secretion and increased insulin resistance. The long-term complications of diabetes can have disastrous effects on body systems and organs, and it has been proven that diabetes leads to chronic neurological disorders, by causing physiological and biochemical changes in neural cells (Patel et al., 2016). Prolonged hyperglycemia is associated with increased vulnerability to stress and cognitive dysfunction. Therefore, diabetic patients are more sensitive to cognitive impairment than non-diabetic healthy subjects (Umegaki et al., 2017). The hippocampus is a crucial brain area, responsible for storing and retrieving short and long-term memories, and characterized by dental gyrus (DG) and cornu ammonis (CA). The DG includes fascia, and halo indentation, and the CA is anatomically, and functionally subdivided into separate subsets, namely CA1, CA2, CA3, and CA4 (Chiang et al., 2017). Spatial learning and memory are sensitive to changes in the normal glucose range (Bélanger et al., 2004). Long-term diabetes has been linked to abnormalities in the hippocampal neural cells as well as nerve fibers (Zhao et al., 2016), altered hippocampal synaptic plasticity (Biessels et al., 2002), hippocampal atrophy (Wang et al., 2014), cognitive deficits (Noor and Zahid, 2017), and impairments in spatial learning, memory, and problem-solving (Treviño et al., 2017). Growth associated protein 43 (GAP43) is a plastic-related protein that influences axonal growth, and neural network formation caused by skeletal elements and regulated by protein kinase C.

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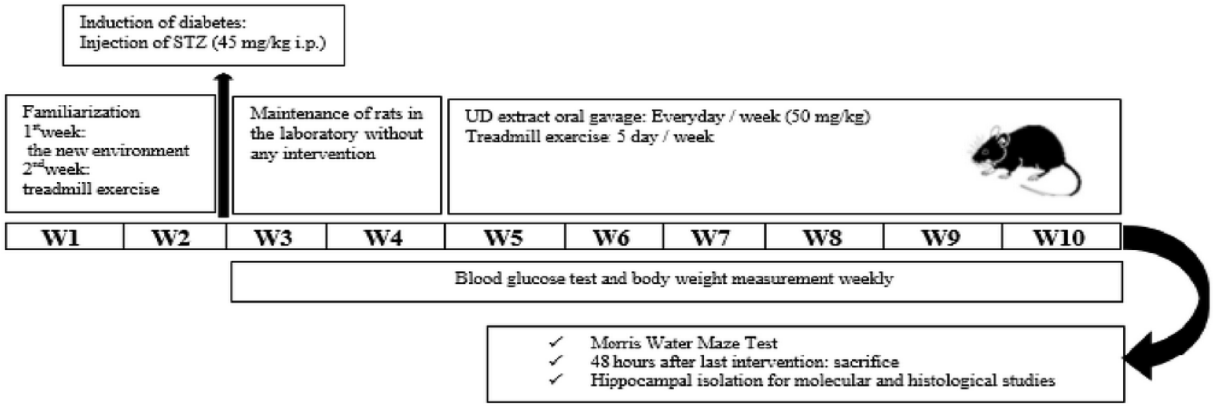


**Fig. 1.** Experimental design: Diabetes was induced by injection of STZ (45 mg/kg, i. p.), and after two weeks, treatment with Ex and UD began for six weeks. After that, during the sixth week, animals were subjected to behavioral studies, and then 48 h after the last intervention, they were killed for histological and molecular studies.

Increased expression of GAP-43 can cause the creation of new synapses, neuronal growth, and post-injury synaptogenesis. Hippocampal GAP-43 protein expression regulates synaptic transmission, and flexibility, such as long-term potentiation, alterations in memory events, and control of plastic-related processes (Holahan, 2015). Adenylyl Cyclase-associated protein 1 (CAP1) is one of the growth cone regulatory proteins in the rat hippocampus, important in postnatal brain growth, and widely expressed in the hippocampus, cerebellum, cortex, and thalamus. CAP1 protein plays an important role in neuronal growth, and is very helpful in learning and memory (Rahmati and Kazemi, 2019).

Now, various studies have investigated the cognitive diseases associated with diabetes. However, no specific diabetes treatment has been identified to prevent or alleviate cognitive impairment (Noor and Zahid, 2017; Seto et al., 2015; Umegaki et al., 2017; Wang et al., 2014). Nevertheless, various studies have shown the remedial effect of exercise training on cognitive deterioration, but the main molecular mechanism of this pathway is still unknown (Voss et al., 2013; Zielinski et al., 2013). Many facets of brain function, and overall brain health are affected by exercise. Exercise can affect learning, and memory with protection against neurodegeneration and reduction of cognitive impairments. Also, exercise can lead to improved synaptic flexibility by directly affecting the structure of synapses, increasing synaptic strength, and strengthening the cellular system, including angiogenesis and neurogenesis. These exercise-induced structural and functional changes have been documented in various brain areas, including the hippocampus. In contrast, diabetes-associated hyperglycemia reduces neurogenesis, and increases oxidative stress and neuronal death (Cotman et al., 2007; de Senna et al., 2011). From another perspective, extracts and natural ingredients are well-known supplements for the prevention and treatment of different diseases (Oz et al., 2017). The consumption of plant materials as neuroprotective factors has been reported by some researchers (Kim et al., 2001; Lim et al., 2002). An extract of these herbal materials can maintain hippocampal neurogenesis and provide amelioration of cognitive disorders in diabetic animals (Lim et al., 2002). In addition to conventional herbal remedies or natural products such as mulberry and tea used to treat diabetes (Ng et al., 2018; Wei et al., 2018), Urtica dioica (UD) has long been recognized as a medicinal plant with beneficial effects on blood glucose control in diabetes. Urtica L., the stinging nettle, is a member of the Urticaceae family, from the Urtica species. UD is one of the famous traditional folk medicines frequently used in Asia for the treatment of diabetes (Farzami et al., 2003; Kavalalı et al., 2003; Zaman, 1989). Its blood glucose (BG) lowering effect has been reported in old manuscripts written by Avicenna (Farzami et al., 2003). UD has a great variety of antioxidant compounds, including polyphenols, flavonoids, lectins, and sterols, which have shown to reduce BG levels and inflammation. In addition, since diabetes is known as a disease caused by oxidative stress, using antioxidants is a therapeutic strategy in the prevention and treatment of diabetes (El Haouari and Rosado, 2019).

However, the neuroprotective mechanisms of endurance exercise (Ex) and the consumption of hydro-alcoholic extract of UD on the histology of the hippocampus are not yet fully understood (Patel et al., 2016; Zielinski et al., 2013). The purpose of this study was to evaluate the effect of Ex with UD hydro alcoholic extract consumption on the functional, histological and molecular parameters of the hippocampus, by observing changes to the thickness of the pyramidal & molecular layers, neural & microglial density, neural disorganization and quantitative evaluation of GAP-43 and CAP-1 proteins in hippocampal tissue of STZ-induced diabetic rats after six weeks.

## 2. Materials and methods

### 2.1. Animals

The experiment was performed on six-weeks-old male Wistar rats, weighing 200–220 g, obtained from a Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences. The animals were maintained in standard conditions, with cycles of light/dark 12:12 h, in transparent polycarbonate cages, 25 ± 1 °C room temperature, and a fed pellet diet, and water *ad libitum*. Before starting the experiment, all animals were maintained at the new environmental condition (climate-controlled room) for a period of one week, and familiarized with treadmill exercise in the second week (10–15 min, 5–10 m/s, five days/week). Randomly, sixty animals were divided into five equal groups of 12 each: healthy-control (H-C), diabetes-control (D-C), diabetes-exercise (D-Ex), diabetes-*Urtica dioica* (D-UD), diabetes-exercise-*Urtica dioica* (D-Ex-UD). All experiments were performed under the supervision of the Lorestan University Animal Ethics Committee (under the code LU. ECRA. 2018.16) and according to the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996). Fig. 1 illustrates a schematic of the experimental design process.

### 2.2. Induction of diabetes

Following an overnight fast, diabetes was induced by a single intraperitoneal injection of STZ (Sigma, St. Louis, MO) dissolved in 0.5 mol/L fresh citrate buffer, pH 4.0 (45 mg/kg of corporal weight), and control animals received an equivalent volume of citrate buffered solution (Rahmati et al., 2015). After the injection of STZ, there were no signs of mistreatment, such as abdominal swelling or digestive problems in animals. Forty-eight hours after STZ injection, blood samples were collected from the tail vein and analyzed using a glucometer

(Emperor, South Korea Isotech). BG levels above 300 mg/dl were considered diabetic, and diabetes was confirmed in all groups (Rahmati et al., 2015). For two weeks after the injection of STZ, without any intervention, the rats were kept in the laboratory. Both body weight (BW) and BG levels of all animals in each group were monitored weekly. BW measurements were obtained one week after the previous BG assessment and just before the next BG evaluation to decrease the impact of food deprivation and stress of blood sampling on BW.

### 2.3. Plant gathering and preparation of extract

Plant material was collected from the mountains of Lorestan province and was authenticated by the agriculture organization of Lorestan (specimen number: 13,776). The UD leaves were dried in the shade and then powdered. The dried powder (500 g) was incubated in 70% ethanol and 30% water for 18 h. The obtained material was passed through a filter paper. To reduce solvent volume and evaporation of ethanol, the UD extract was placed in a centrifuge at 60 °C. After that, the extract was placed in an incubator for 24 h at 60 °C until all ethanol was evaporated, and a dry extract of the plant was obtained (Ahmadi et al., 2014). After preparing the UD extract, the GC/MS method was used to determine the amount of active compounds in the extract. For this purpose, 0.3 g of dried extract was dissolved in 1 ml of water and was centrifuged at 16000g, 4 °C, for 3 min. Then, 2 µl were injected in split mode. GC-MS was performed with an Agilent Technologies 7890A GC system coupled to an Agilent Technologies 7000C triple quadruple mass spectrometer. The extract was analyzed on an HP5MS (30 m × I.D. 0.25 mm × film thickness 0.25 µm). Gas chromatographic analysis of UD is shown in supplementary material S1.

### 2.4. Plant treatment

Two weeks after the induction of diabetes, UD gavage was started. UD was given to rats in D-UD and D-Ex-UD groups, at a dosage of 50 mg/kg BW (Patel et al., 2016) (dried extract of the plant dissolved in 1 ml distilled water) daily oral gavage for six weeks, and 1 ml of distilled water was gavaged to rats in the control group.

### 2.5. Treadmill exercise

Rats in the D-Ex and D-Ex-UD groups were trained on a treadmill, at moderate-intensity exercise, 5 days a week for six weeks, and every training session started with 3 min of warm-up and was completed with 3 min of cool-down. The speed and duration of the treadmill exercise were gradually increased from 10 m/min for 10 min in the first week to 10 m/min for 20 min in the second week, 14–15 m/min for 20 min in the third week, 14–15 m/min for 30 min in the fourth week, and 17–18 m/min for 30 min for the fifth and sixth weeks. To achieve adaptation in training, the intensity (speed and time) of treadmill exercise was kept constant during the sixth week (Rahmati et al., 2015). During the treadmill exercise, a soft brush, without electric shock were used to stimulate the animals, and achieve maximum effort during the experiment. In addition, the front part of the treadmill lines was covered with thick paper to allow the rats to move forward (Rahmati and Kazemi, 2019). During the exercise period, no signs of severe fatigue or movement disorders were observed in the D-Ex and D-Ex-UD groups.

### 2.6. Morris Water Maze test

In the sixth week of Ex and UD interventions, a spatial learning and memory test was performed with a Morris water maze. The Morris water maze consisted of a circular pool (painted black, 200-cm diameter, walls 76 cm depth), filled with water (22 °C ± 2 °C) situated in a room with visual cues on the walls. A platform (10-cm diameter, 35-cm height) was submerged 2 cm under the water surface in one of four quadrants in the pool, and four points were designed as starting positions (N, S, W, or E). A video-recorder of the tracking device hung from the ceiling. The animals were subjected to four trials per session for four consecutive days. For each experiment, the rat was placed in the water, facing the wall of the tank, in one of four start locations. The rat was allowed to search for the platform for 60 s. After finding the platform, the rat was allowed to sit on the platform for 10 s and then to leave the pool. If the rat did not find the platform within 60-s, it was guided by hand, and allowed to remain on the platform for 10 s, and its escape latency was accepted as 60 s. When the rats found the platform, its time was recorded. The animals were tested in this way for four days. Twenty-four hours after the last day of the learning test, a probe trial was conducted (test time 60-s). During this test, the escape platform was removed, and the time spent in the platform quadrant was recorded (Taati et al., 2014).

### 2.7. Tissue preparation

For microscopic studies of hippocampal tissue, rats were anesthetized with chloroform. To accomplish the perfusion process, 100 ml of saline along with 250 ml of 4% paraformaldehyde in 0.1 mg phosphate buffer (pH 7.4) were used. The brain was removed and the hippocampus dissected out, stored in fixative and embedded in paraffin (Sharifi et al., 2002). In each group, five rats were used for Western blot analysis, and five rats for histological studies. The average value was used for statistical analysis. During the investigation, four rats from diabetic groups died after STZ injection.

### 2.8. Histological analysis of hippocampus area

The hippocampus was removed from the brain, four coronal sections were prepared (2 mm each), and formerly immersion fixed in formalin for 24 h before being transferred to 70% ethanol solution, a new process embedded in paraffin, and cut to create 5-µm-thick coronal sections onto slides. The slides were stained by Hematoxylin and Eosin. The histopathological parameters was estimated via the histopathological injury score. Neuropathology was used to assess the level of hippocampus damage, using a light microscope (Olympus, x400). The number, density and disorganization of neurons were measured, and the thickness of the pyramidal and molecular layer were calculated. Neuronal damage in the DG, CA4, CA3, CA2 and CA1 areas of the hippocampus was expressed as the sum of damaged nervous tissue in the total 400X microscope-field cell population (Amin et al., 2013; Oliveira-da-Silva et al., 2009).

### 2.9. Western blot analysis

Western blot analysis was performed based on our previous study (Rahmati and Kazemi, 2019). Polyclonal antibodies for GAP-43 (1:100,000; ab75810, Abcam, Cambridge, MA), CAP-1 (1:1000; ab96354, Abcam, Cambridge, MA) and GAPDH (1:1000; Santa Cruz Biotechnology Inc., Dallas, TX, USA) were used. Data was normalized to GAPDH in the same membrane and expressed as a percentage of control values.

### 2.10. Statistical analysis

Data for all variables was expressed as mean ± SD. The normality of data was analyzed using the Shapiro-Wilk test. Repeated measures ANOVA were used to compare the BG and BW variables. In addition, a two-way ANOVA test was used to investigate the interaction of the day group in spatial learning ability and the interactive effect of independent variables Ex and UD on memory, Kruskal-Wallis analysis was used to compare groups in histological examination of hippocampus area, and one-way ANOVA followed by Tukey's post hoc test to compare groups in other variables. Differences were statistically significant when $p < 0.05$. SPSS 22 for Windows (SPSS Inc., Chicago, IL) was used

in all statistical analyses.

## 3. Results

### 3.1. Treatment with Ex + UD lowers BG and controls BW

Before the STZ injection, the mean BG in all groups was 106 ± 2.58 mg/dl (P > 0.05). Then, BG increased significantly after 48 h of STZ injections in rats of D-C, D-Ex, D-UD, and D-Ex-UD groups compared to H-C groups (P = 0.001), and the mean BG in diabetic groups was 498.07 ± 42.06 mg/dl, demonstrating that diabetes induction was successful. Furthermore, throughout the study, BG levels were higher in the D-C group when compared with the D-Ex, D-UD, and D-Ex-UD groups (P < 0.05). UD consumption, and Ex significantly reduced BG in the D-UD (P = 0.034), and D-Ex-UD (P = 0.005) groups in the first week, and D-Ex group (P = 0.039) in the second week, in comparison to the post-STZ injection stage, and this significant reduction in BG continued until the sixth week (P < 0.05). Comparison of the D-Ex, D-UD, and D-Ex-UD groups in the six-week protocol showed that their BG levels were significantly different only in the sixth week. There were significant differences between the D-UD with D-Ex-UD (P = 0.001), and D-Ex (P = 0.011). Moreover, there was no statistically significant difference between D-Ex, and D-Ex-UD groups in the six-week (P = 0.191) (Fig. 2a). In addition, at the beginning of the study, the mean BW in all groups was 232.74 ± 12.42 g (P > 0.05). BW decreased in the diabetic groups two weeks after STZ injection (first week (W1) of UD consumption, and Ex, compared with the H-C group (P < 0.05), and this decline continued until the sixth week (P < 0.001). The mean BW values of the D-C group were lower than the mean BW values of the D-Ex, D-UD, and D-Ex-UD groups throughout the six-week post-STZ period, particularly in weeks 4, 5 and 6 of the study (P < 0.05). In addition, there was no difference between the BW of D-Ex, D-UD, and D-Ex-UD groups throughout the study (P > 0.05). In general, the results of intra-group comparisons showed that there were no significant changes in the BW of diabetic rats at different stages of the study compared to the post STZ phase (Fig. 2b).

### 3.2. Treatment with Ex + UD increases the level of spatial learning ability and memory function in the morris water maze test

Spatial learning ability was measured using the Morris water maze test (Fig. 3a). The escape latency time (in seconds) was recorded for the four days of reference memory testing. A two-way ANOVA revealed significant main effects of groups (F = 100.567, P = 0.001), and days (F = 68.422, P = 0.001), and did not show a significant interaction between groups*days (F = 1.769, P = 0.063) on the escape latencies. A comparison of groups showed that the escape latencies of H-C, D-Ex, D-UD, and D-Ex-UD groups were significantly lower than that of the D-C group on the 1st, 2nd, 3rd, and 4th days (P < 0.001). The escape latency of the D-Ex-UD group was significantly lower than that of D-UD and D-Ex groups on the first day and fourth day (P < 0.01). The escape latency of the D-Ex-UD group was considerably lower than that of the D-UD group on the second day (P < 0.05), but was not substantially lower than that of the D-Ex group on the second day (P > 0.05). There was no significant difference in the escape latency of the D-Ex, D-UD, and D-Ex-UD groups on the third day (P > 0.05). In contrast, the D-UD group did not show a significant difference compared to the D-Ex group (P > 0.05). Fig. 3b shows the data from the spatial memory test. A two-way ANOVA on the mean scape latency data indicated a significant effect of UD (F = 25.074, P = 0.001), Ex (F = 23.602, P = 0.001), but the interaction between UD*Ex was not substantial (F = 0.573, P = 0.456). A comparison of the groups in the spatial memory test showed that the mean time elapsed in the quadrant containing the hidden platform of the D-C group was significantly lower than the H-C, D-Ex, D-UD, and D-Ex-UD groups (P = 0.001 in all groups). The time elapsed in the quadrant containing the hidden platform of the D-Ex-UD group was longer than that of the D-Ex and D-UD group (P = 0.001). In addition, there was no significant difference between the D-Ex and D-UD groups (P > 0.05). In general, Ex, along with the consumption of UD in the D-Ex-UD group, had the best effect on spatial learning and memory in the STZ-induced diabetic rats.

### 3.3. Histological scoring and semi-quantitative analysis

#### 3.3.1. Ex + UD treatment caused a decrease of neural disorganization in the hippocampus

In cornu ammonis in group D-Ex-UD (Fig. 4) main changes concern the zone of pyramidal cells; to a smaller extent they apply to molecular layer and granular cells. The above mentioned changes most clearly are emphasized in direct and close vicinity of blood vessels. Cells of pyramidal layer reveal cytoplasmic vacuolation of perikaryon. In many cells of that layer, nuclei reveal karyopyknosis and beginnings of degenerative changes. Similar changes but of bigger intensity occur in granular cells. Granular cells in histological image present poorly expressed foam structure, which is the result of going on process of degeneration. In molecular layer there are smaller degree degenerative processes.

The granular cells layers of control (H-C) dentate gyri contained densely arranged neurons with rounded pale vesicular nuclei. In diabetic brains, many granular cells with darkly stained pyknotic nuclei

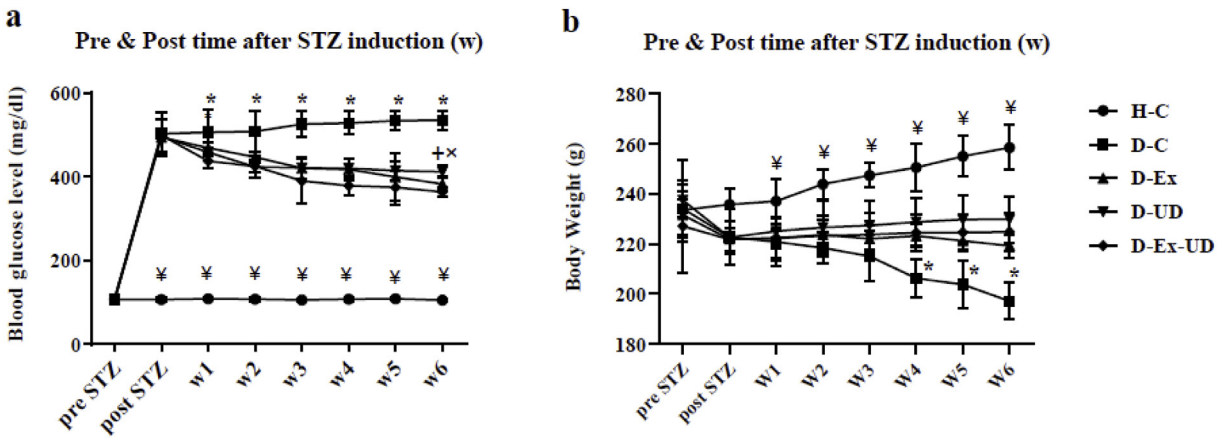


**Fig. 2.** Blood glucose (a), and body weight, (b) values obtained in pre, post, and following experimental weeks after STZ-induction. The groups consisted of healthy-control (H-C), diabetes-control (D-C), diabetes-exercise (D-Ex), diabetes-*Urtica dioica* (D-UD), and diabetes-exercise-*Urtica dioica* (D-Ex-UD). Data is expressed as the mean ± SD. ¥ P < 0.001 for the H-C group *vs*. other groups, *P < 0.001 for the D-C group *vs.* other groups, + P < 0.001 for the D-Ex-UD group *vs.* the D-UD group, and × P < 0.01 for the D-Ex group *vs*. the D-UD group.

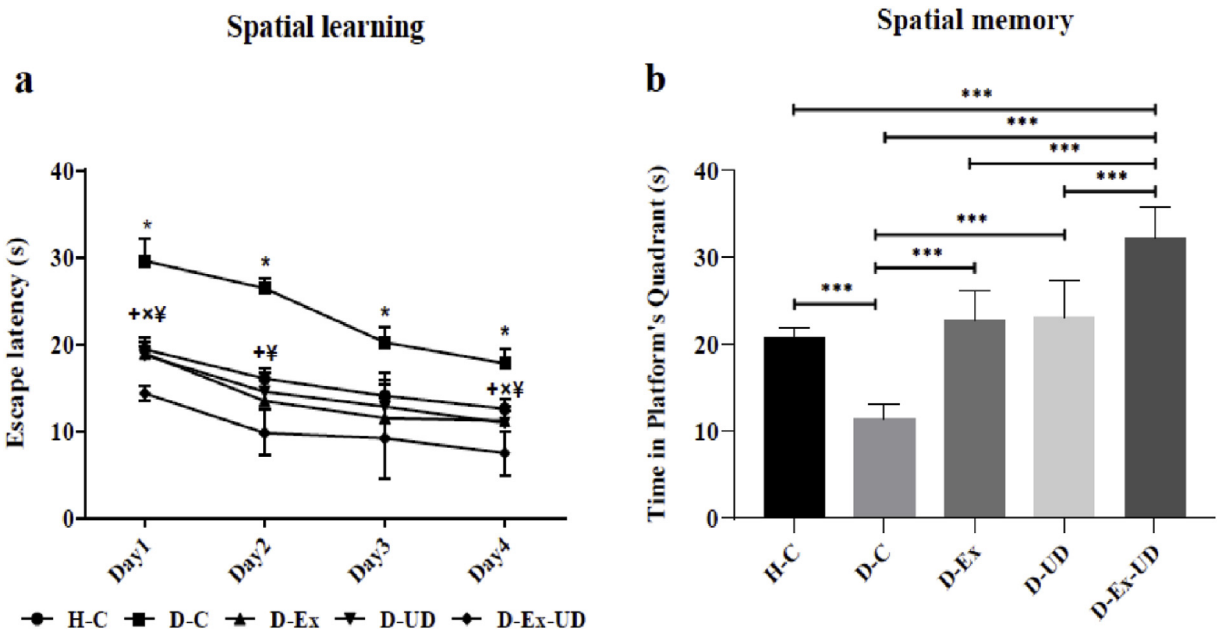


**Fig. 3.** Effect of Ex and UD on spatial learning (a), and memory, (b) in the Morris water maze test. The groups consisted of healthy-control (H-C), diabetes-control (D-C), diabetes-exercise (D-Ex), diabetes-*Urtica dioica* (D-UD), and diabetes-exercise-*Urtica dioica* (D-Ex-UD). Data is expressed as the mean ± SD. * D-C group *vs.* other groups ($P < 0.001$), + D-Ex-UD group *vs.* H-C group ($P < 0.001$), ¥ D-Ex-UD group *vs*. D-UD group ($P < 0.01$), × D-Ex-UD group *vs*. D-Ex group ($P < 0.01$), *** significantly different ($P < 0.001$).

appeared, suggesting apoptosis. Others showed cytoplasmic vacuolizations.

In Urtica dioica extract treated groups, most of the granular neurons appeared normal with pale basophilic vesicular nuclei; however, neuro protective effect of Urtica dioica extract in the brain of streptozotocin-induced diabetic rats. These results indicate that Urtica dioica extract ameliorated the histopathological. Alterations in the dentate gyri of diabetic brains. This result s suggests that Urtica dioica extract significantly prevents apoptosis, and inflammation finally disorganization induced in the dentate gyrus of the diabetic group. In the diabetic brain, almost all neurons appear as small dark cells. Pancreatic nucleus cells, dense cytoplasm and blockade of apoptotic cells were also observed. In contrast, in the diabetic exercise group, less vacuolar and structurally disrupted cells were seen. The D-Ex-UD group had a better performance in reducing neural disorganization in all areas compared to the D-C, D-UD and D-Ex groups (Fig. 4). Histological scoring analysis in the neural disorganization parameter for H&E staining showed in Table 1.

### *3.3.2. Neural and microglial density were elevated in the hippocampus after Ex + UD treatment*

In cornu ammonis in group D-Ex-UD (Fig. 4) main changes concern the zone of granular cells; to a smaller extent they apply to molecular layer and pyramidal cells. Cells of granular layer reveal number of neuron cells. In many cells of that layer in diabetic conditions, neurogenesis suppress and beginnings of degenerative changes. Similar changes but of bigger intensity occur in granular cells. The dentate gyrus also has three layers. The cytoarchitecture differs that of hippocampus in that the pyramidal cell layer is replaced by a granule cell layer of small neurons, which are principle cells of the region.

The granular cell layers of control dentate gyri contained densely arranged neurons. In diabetic brains, Dentate gyri of diabetic brains showed a significant increase in the number of microglial cells (Fig. 4). In the Urtica dioica treated group, this number was dramatically decreased (Fig. 4). These results suggest that Urtica dioica reduces neurogenesis induced in the dentate gyri of diabetic brains. The histopathological injury score analysis of glial was increased in the diabetic dentate gyri compared to control ones (Fig. 4). Treatment protected the dentate gyrus and significantly reduced gliosis in CA2 and CA4 (Fig. 4). Exercise revealed a dramatic decrease in the number of microglial cells among granular cells suggesting suppression in damage to neurogenesis.

In general, this results indicates that Exercise and Urtica dioica enhances the suppressed neurogenesis in the diabetic dentate gyrus. In histopathologic logical and semi-quantitative results in the D-Ex-UD group showed that a combination of Ex with UD consumption had a greater improved mean scores of the hippocampal neurogenesis and microglial density in all areas compared to the D-UD and D-Ex groups (Fig. 4). Histopathologic logical scoring analysis in neural, and microglial density parameters for H&E staining showed in Table 2.

### *3.3.3. The thickness of the pyramidal and the molecular layers increased in response to Ex + UD treatment: cornu ammonis and dentate gyrus*

Cornu ammonis in control group H-C (Fig. 4) is mainly made from pyramidal cells which form a diverse layer. Moreover there are nerve cells which form granular and molecular layer. In the hippocampus, the control group is composed of pyramidal cells as well as multicellular cells forming the molecular layer. In control group with induction of diabetes (group D-C) (Fig. 4), the clearest reaction of hippocampal atrophy, cell loss and reduction of pyramidal layer volume was observed especially in CA1. More over glial proliferation was found. In those places dilutions of neuron weaving are visible and in some nerve cells in the area of perikaryon degeneration changes can be seen. Those changes mainly concern the area of granular nerve cells. To a smaller extent the above mentioned changes affect the layer of pyramidal cells. In Cornu ammonis in group D-C (Fig. 4) there is high degree damage of grey matter of hippocampus; particularly clear degenerative changes can be seen in pyramidal cells and smaller ones in cells of granular and molecular layer. Delaminations between pyramidal cells and adjacent layers are confirmed. In particular pyramidal cells karyopyknosis can be seen as well as translucency of perikaryon cytoplasm. It also concerns the deviation zone of neuron processes. In a near zone (of analyzed neurons) proliferation of glia cells can be noticed. A smaller degree of damage was found in molecular and granular layers. These results suggest that Urtica dioica extract and exercise alone improved or increased the thickness of the molecular layer in all hippocampal areas of diabetic rats.

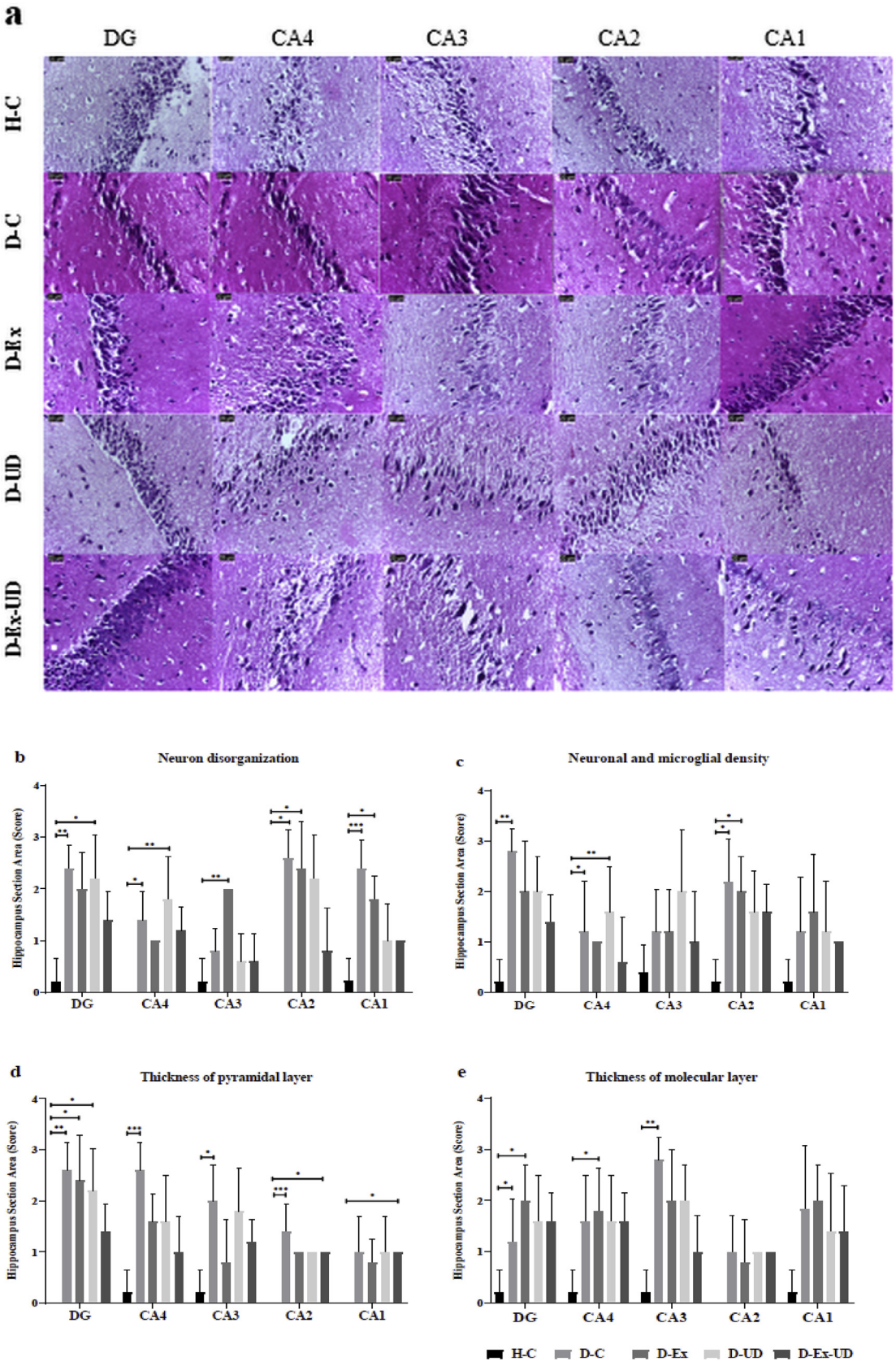


**Fig. 4.** Effect of Ex and UD on histological scoring, and semi-quantitative analysis. H&E staining (a) images of hippocampal areas of rats in healthy-control (H-C), diabetes-control (D-C), diabetes-exercise (D-Ex), diabetes-*Urtica dioica* (D-UD), and diabetes-exercise-*Urtica dioica* (D-Ex-UD) groups shown with the magnification of 400x. In general, the parameters of neural disorganization (b), neural and microglial density (c), thickness of the pyramidal layer (d), and thickness of the molecular layer (e), are shown according to the rating system of 4 points (0 = average, 1 = slightly unusual, 2 = unusual average, and 3 = significantly unusual). *Significantly changed, ($P < 0.05$), **significantly changed, ($P < 0.01$), ***significantly changed, ($P < 0.001$). Data expressed as mean ± SD.

**Table 1**
Histopathological scoring analysis in neural disorganization parameter for H&E staining. This parameter is graded in a semi-quantitative manner according to the rating system of 4 points (0 = average, 1 = slightly unusual, 2 = unusual average, and 3 = significantly unusual).

| Neural disorganization | | | | | |
|---|---|---|---|---|---|
| Hippocampus area ➡ | DG | CA4 | CA3 | CA2 | CA1 |
| Group ⬇ | Mean ± SD ⬇ | | | | |
| H-C | .2±.44 | .0±.0 | .2±.44 | .0±.0 | .22±.43 |
| D-C | 2.40±.54 | 1.40±.54 | .80±.44 | 2.60±.54 | 2.40±.54 |
| D-Ex | 2.00±.70 | 1.00±.00 | 2.00±.00 | 2.40±.89 | 1.80±.44 |
| D-UD | 2.20±.83 | 1.80±.83 | .60±.54 | 2.20±.83 | 1.00±.70 |
| D-Ex-UD | 1.40±.54 | 1.20±.44 | .60±.54 | .80±.83 | 1.00±.00 |
| P value | 0.005* | 0.002* | 0.005* | 0.002* | 0.001* |

In hippocampus in D-Ex-UD group (Fig. 4) a smaller degree of damage of neurons occurs than in others group. The decrease of degenerative changes can be observed. It concerns all hippocampus zones of pyramidal cells as well as cells of granular and molecular layers. There is also a smaller degree of damage with in blood vessels and glial proliferationis less intensified than in histopathological image in group D-Ex-UD.

The molecular layer consists of interacting axons and dendertites. This synaptic layer with continuous with molecular layers of the dentate gyrus and neocortex. The histopathological and semi-quantitative results in D-Ex-UD group showed that exercise with Urtica dioica extract could increase molecular layer thickness in most hippocampal regions (CA1, CA2 and CA3) similar to separate consumption of Urtica dioica and exercise in diabetic animals. In Cornu ammonis in group D-Ex-UD groups (Fig. 4) damage of grey matter can be found to a smaller extent. Fundamental changes occur in pyramidal cells, to a smaller extent in molecular layer and cells of granular layer. However the scope of those changes is smaller than in pyramidal cells(Table 4). Results indicate the Urtica dioica amelotreated the histopathological in Cornu ammonis.

Besides, Histopathological Assessment in Dentate Gyrus. In control group H-C (Fig. 4) stroma for nerve cells is fibrous and protoplasmic astrocytes and oligodendrocytes. The structure of glia stroma is particularly evident in perivascular area. The structure of dentate gyrus presents grey matter arranged in 3 layers: molecular layer, granular layer, and layer of pleomorphic cells. In control group with induction of diabetes (group D-C) (Fig. 4) main changes occurring in the area of dentate gyrus concern granular and pyramidal layer. To a smaller extent they apply to molecular layer. The nature of changes is like in the area of Cornu ammonis but the degree of those changes is bigger particularly in the layer of pyramidal cells. In the layer of granular cells a bigger glial proliferation than in Cornu ammonis can be observed. Moreover, six-weeks of UD extract and the Ex in the D-UD, D-Ex groups increased the thickness of the pyramidal layer and molecular layers in all hippocampal areas compared to the D-C group, but this increase was not statistically significant. Also based on the histopathological score of the D-Ex-UD group showed the least subtractive change in the thickness of the pyramidal and molecular layers. Histopathological scoring analysis in the thickness of the pyramidal layer parameter for H&E staining showed in Table 3 and Table 4 respectively.

### *3.3.4. Ex + UD treatment caused an increase of GAP43 protein expression and a decrease of CAP1 protein expression in the hippocampus*

Molecular results using Western Blot analysis showed that diabetes reduced the expression of GAP43 protein in the hippocampal tissue of the D-C group rats compared to the H-C group ($P = 0.002$). Six-weeks of treatment with Ex and/or UD on diabetic rats caused a significant increase in expression of GAP43 in the hippocampus of the D-UD, D-Ex, and D-Ex-UD groups compared to the D-C group ($P = 0.001$). The mean GAP43 protein expression in the D-UD ($P = 0.014$), and D-Ex-UD ($P = 0.001$) groups was significantly higher than the H-C group, and there was no difference between the D-Ex, and H-C groups ($P > 0.05$). A comparison between D-UD, and D-Ex groups showed that treatment with UD had a more significant increase in GAP43 protein expression compared to therapy with Ex ($P = 0.011$). In contrast, the D-Ex-UD group showed a significantly higher increase in expression of GAP43 protein than the D-Ex and D-UD groups ($P = 0.001$) (Fig. 5a).

Diabetes also increased the expression of CAP1 protein in hippocampal tissue of the D-C group rats compared to the H-C group ($P = 0.001$), and six weeks of treatment with Ex and/or UD on diabetic rats caused a significant decrease in expression of CAP1 in the hippocampus of the D-UD, D-Ex, and D-Ex-UD groups compared to the D-C

**Table 2**
Histopathological scoring analysis in neural and microglial density parameters for H&E staining. These parameters are graded in a semi-quantitative manner according to the rating system of 4 points (0 = average, 1 = slightly unusual, 2 = unusual average, and 3 = significantly unusual).

| Neural and microglial density | | | | | |
|---|---|---|---|---|---|
| Hippocampus area ➡ | DG | CA4 | CA3 | CA2 | CA1 |
| Group ⬇ | Mean ± SD ⬇ | | | | |
| H-C | .2±.44 | .0±.0 | .4±.54 | .2±.44 | .2±.44 |
| D-C | 2.80±.44 | 1.20±.44 | 1.20±.83 | 2.20±.83 | 1.20±1.09 |
| D-Ex | 2.00±1.00 | 1.00±.00 | 1.20±.83 | 2.00±.70 | 1.60±1.14 |
| D-UD | 2.00±.70 | 1.60±.89 | 2.00±1.22 | 1.60±.89 | 1.20±1.09 |
| D-Ex-UD | 1.40±.54 | .60±.89 | 1.00±1.00 | 1.60±.54 | .80±.83 |
| P value | 0.003* | 0.006* | 0.180 | 0.014* | 0.185 |

**Table 3**
Histopathological scoring analysis of the thickness of the pyramidal layer parameter for H&E staining. This parameter is graded in a semi-quantitative manner according to the rating system of 4 points (0 = average, 1 = slightly unusual, 2 = unusual average, and 3 = significantly unusual).

| Thickness of pyramidal layer | | | | | |
|---|---|---|---|---|---|
| Hippocampus area ➡ | DG | CA4 | CA3 | CA2 | CA1 |
| Group ⬇ | | | Mean ± SD ⬇ | | |
| H-C | .0±.0 | .2±.44 | .2±.44 | .0±.0 | .0±.0 |
| D-C | 2.60±.54 | 2.60±.54 | 2.00±.70 | 1.40±.54 | 1.00±.70 |
| D-Ex | 2.40±.89 | 1.60±.54 | .80±.83 | 1.00±.00 | .80±.44 |
| D-UD | 2.20±.83 | 1.60±.54 | 1.80±.83 | 1.00±.00 | 1.00±.70 |
| D-Ex-UD | 1.40±.54 | 1.00±.70 | 1.20±.44 | 1.00±.00 | 1.00±.00 |
| P value | 0.003* | 0.002* | 0.010* | 0.001* | 0.022* |

group (P = 0.001). The mean CAP1 protein expression in the D-Ex (P = 0.001), D-UD (P = 0.004), and D-Ex-UD (P = 0.001) groups were significantly lower than the H-C group. A comparison between D-UD and D-Ex groups showed that treatment with Ex had a more significant decrease in CAP1 protein expression compared to therapy with UD (P = 0.001). The D-Ex-UD group showed a significantly higher reduction in expression of CAP1 protein than the D-Ex, and D-UD groups (P = 0.001) (Fig. 5b).

## 4. Discussion

The present study revealed that BW decreased in the diabetic groups two weeks after STZ injection compared with the H-C group, and this decline continued until the sixth week. The mean BW values of the D-Ex, D-UD, and D-Ex-UD groups were higher than the D-C group, so that the difference in weeks 4, 5, and 6 was significant. Some causes of weight loss in diabetic rats are increased protein catabolism, which releases amino acids for gluconeogenesis, and decreased secretion of anabolic insulin hormone from the beta cells of the pancreas, which produces hyperglycemia (Jain et al., 2014). Further, it was observed that the BG level increased in STZ-induced diabetic animals. Generally, hyperglycemia results from inadequate insulin secretion and/or the inability of tissues to respond to insulin (Association, 2017). Treatment with UD and/or Ex significantly reversed the STZ-induced alteration in BW, and BG of the D-Ex, D-UD, and D-Ex-UD groups compared to the D-C group. In addition, the results clearly showed that diabetes is related to dysfunction in learning performance in the Morris water maze. Eight-weeks after the onset of diabetes, there was a significant increase in time latency to reach the platform. Moreover, it was demonstrated that diabetes decreased hippocampal GAP-43 expression, and UD and/or Ex could change this to normal levels. However, CAP-1 exhibited a reverse response with an increase in expression in the hippocampus of STZ-induced diabetic rats, and UD and/or Ex could modify this to normal levels.

Previous studies have shown that diabetes is associated with several neurological complications of the central nervous system and one of the most important neurological complications of diabetes is learning and memory disorders (Noor and Zahid, 2017; Seto et al., 2015; Umegaki et al., 2017; Wang et al., 2014). Spatial learning and memory process depends on the integrity and function of specific brain areas, such as the hippocampus and striatum (Murray et al., 2014). However, the mechanism of memory impairment has not been elucidated. The evidence shows that STZ-induced diabetes significantly downregulated the mRNA expression of insulin receptor substrate ½ (IRS1/2), insulin receptor (IR), GLUT4, mitogen-activated protein kinase 1 (MAPK1) and peroxisome proliferator-activated receptors (PPARγ) in the hippocampus (Patel et al., 2016). Furthermore, STZ-induced diabetes in rats results in the altered function of N-methyl-D- aspartate (NMDA), and amino hydroxyl propionic acid-type glutamate receptors, which are involved in learning and memory process (Nitta et al., 2002). The results of this study showed chronic treatment with either UD and/or Ex significantly improved the spatial learning and memory dysfunction associated with long-standing diabetes. The D-Ex-UD group, which received both UD and the Ex interventions, exhibited better memory and learning than the D-Ex and D-UD groups. According to the present study and in agreement to Patel et all. study (2016), the constituents of UD are homovanillyl alcohol, p-hydroxybenzaldehyde, β-sitosterol, quercetin, stigmasterol, campesterol, daucosterol, ferulic acid, syringic acid, scopoletin, acetylcholine, choline acetyltransferase, 5-hydroxy tryptamine (5-HT), gallic acid, carvacrol, β-carotene, lutein, kaempferol, and myricetin. The presence of quercetin, scopoletin, esculetin, rutin, and gentisic acid in the extract of UD leaves is known to prevent

**Table 4**
Histopathological scoring analysis in the thickness of the molecular layer parameter for H&E staining. This parameter is graded in a semi-quantitative manner according to the rating system of 4 points (0 = average, 1 = slightly unusual, 2 = unusual average, and 3 = significantly unusual).

| Thickness of molecular layer | | | | | |
|---|---|---|---|---|---|
| Hippocampus area ➡ | DG | CA4 | CA3 | CA2 | CA1 |
| Group ⬇ | | | Mean ± SD ⬇ | | |
| H-C | 0.2±0.44 | 0.2±0.44 | 0.2±0.44 | 0.0±0.0 | 0.2±0.44 |
| D-C | 2.20±.83 | 1.60±.89 | 2.80±.44 | 1.00±.70 | 1.84±1.23 |
| D-Ex | 2.00±.70 | 1.80±.83 | 2.00±1.00 | .80±.83 | 2.00±.70 |
| D-UD | 1.60±.89 | 1.60±.89 | 2.00±.70 | 1.00±.00 | 1.40±1.14 |
| D-Ex-UD | 1.60±.54 | .80±.83 | 1.00±.70 | 0.00±.00 | 1.40±.89 |
| P value | 0.014* | 0.025* | 0.003* | 0.006* | 0.051 |

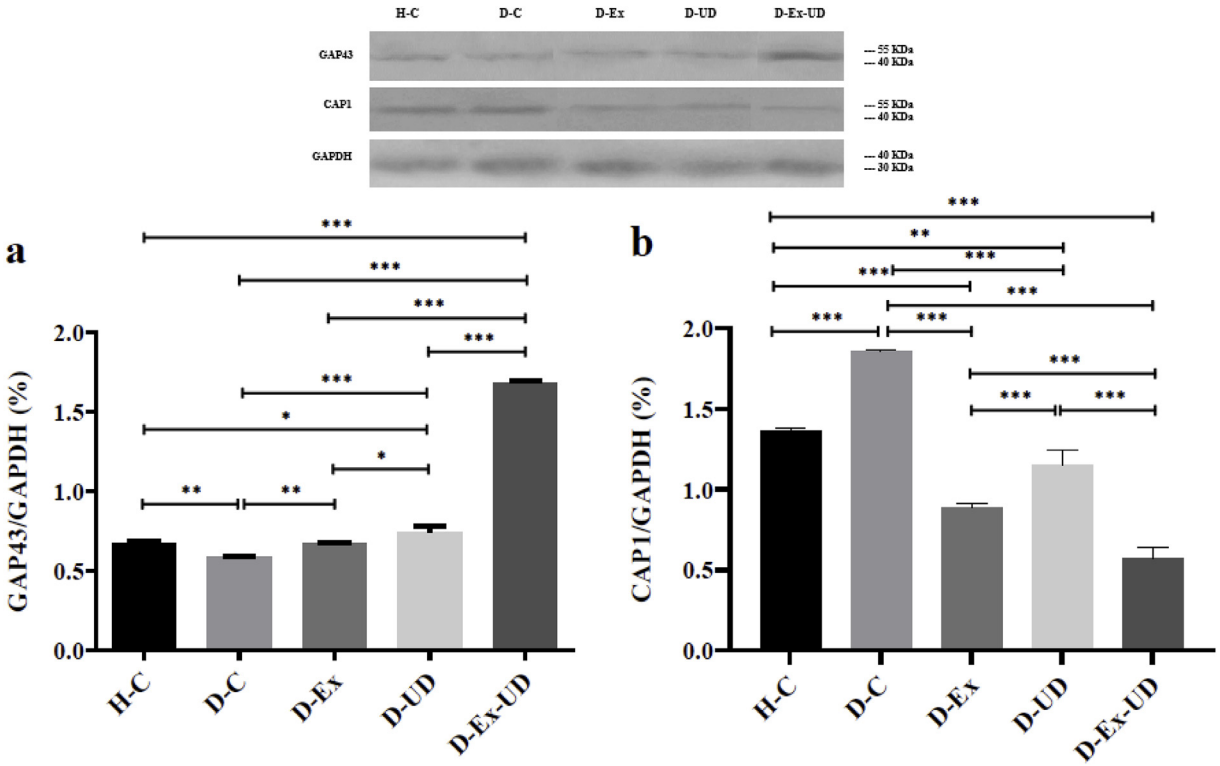


**Fig. 5.** GAP-43 and CAP-1 levels measured in the rat hippocampus. Expression of GAP-43 (a), and CAP-1 (b) further demonstrated by Western blotting normalized to GAPDH. Panels above graph are representative Western blot images from different groups. The groups consisted of healthy-control (H-C), diabetes-control (D-C), diabetes-exercise (D-Ex), diabetes-*Urtica dioica* (D-UD), and diabetes-exercise-*Urtica dioica* (D-Ex-UD). *Significantly changed, ($P < 0.05$), **significantly changed, ($P < 0.01$), ***significantly changed, ($P < 0.001$). Data expressed as mean ± SD.

hyperglycemia. It has reported that quercetin, scopoletin, esculetin, and rutin induce the gene expression of PPARγ, a target receptor for Rosiglitazone (ROSI). ROSI promotes insulin-sensitive action in different cell types and reduces memory deficiency in STZ-induced diabetic rats (Patel et al., 2016). Another ingredient in UD is scopoletin, which by increasing the release of acetylcholine from synaptosomes, enhances hippocampal long-term potentiation and memory. The release of acetylcholine enhances the gene expression of the PPARγ, which promotes angiogenesis and reduces insulin resistance. These factors improve the ability of memory in the hippocampus (Patel et al., 2016). In addition, Ex in diabetic rats showed significant enhancement in spatial learning performance as shown by the Morris water maze test (Lee et al., 2016). It was reported that Ex enhances the neurogenesis level of brain-derived neurotrophic factor (BDNF), and other growth factors, and neurotransmitters, especially in the hippocampus (Honma, 1986). In addition, hippocampal-dependent learning may enhance the survival of cells before spatial learning (Nibuya et al., 1995). It has been shown that physical activity significantly enhances step-through latency, and initial latency through an increase in circulatory BDNF levels, especially when exercise is performed voluntarily (Adlard et al., 2004). Ex also has been shown to facilitate neurogenesis, plasticity, and dendrite proliferation in the hippocampus (Van Praag et al., 1999). The physiological benefits of exercise seen in the hippocampus correlate well with the research findings. showing that increased performance on learning and memory tests results from exercise in rodents (Kiraly and Kiraly, 2005). The histology results of the hippocampus showed diabetes-induced changes in the neural disorganization, neural and microglial density, and thickness of pyramidal and molecular layer parameters in DG, and CA areas. These areas use multiple synaptic paths. Intra and inter hippocampal connections play a specific role in information processing. Overall, the hippocampus seems to play an important role in storing, and retrieving short- and long-term memories. Within this complex process, CA1 is selectively related to autobiographic and long-term memory, the CA2, and CA3 with the encryption process, and the DG with early retrieval and episodic memory formation. Hyperglycemia of individuals with diabetes can affect the cell number, and anatomy of the hippocampus (Murray et al., 2014). Animal models suggest that chronic hyperglycemia can reduce the complexity of neural dendrites and dendritic spines, and damaging these neural processes could lead to a smaller hippocampal volume (Murray et al., 2014). Examination of diabetic hippocampus sections revealed marked effects of diabetes in the form of an increase of neural disorganization, decreased neural, and microglial density, and decreased thickness of pyramidal and molecular layer in the hippocampus of diabetic rats. However, the findings of the present study suggested that neural and microglia cells are sensitive to the neurotoxic effects of diabetes. The results also indicated reductions in neural and microglial cells densities due to diabetes exposures. The negative effects of diabetes on the density of the neural and microglial cells affected all areas of the hippocampus, but this change was more prominent in DG, and CA2 areas. The final point to stress is that the increase of neural and microglial cell densities associated with comparatively normal thicknesses of hippocampal areas suggests that Ex and/or UD treatments result in an increased number of neural and microglial cells in hippocampus areas. This finding constitutes an endorsement of the hypothesis that herbal and exercise interventions affect hippocampus development during diabetes. The increase in neurogenesis may play a role in density results (Benowitz and Routtenberg, 1997). The results of this study showed that six weeks of Ex/UD increased GAP-43 protein expression and decreased CAP-1 expression in hippocampal tissue, which had a positive effect on learning and memory in diabetic rats. According to the present findings, alterations in GAP-43 and CAP-1 levels are essential in regulating memory processes. Results of previous studies have shown that overexpression of GAP-43 protein plays a central role in the learning and memory process (Bolognani et al., 2007). However, the relation between the levels of this protein with an increase of learning

and memory remains unknown. High concentrations of GAP-43 protein have been found in growth cones, which is closely correlated with regenerative, and developmental growth. Terminal remodeling, and neurotransmitter release are dual hypotheses in presynaptic processes, that are likely altered by overexpression of GAP-43 phosphorylation. In addition, a direct relationship between GAP-43 phosphorylation, LTP amplification, and behavioral learning in mnemonic function has been observed. Given the function of neurotransmitters, GAP-43 phosphorylation is associated with increased release of neurotransmitters; overexpression of GAP-43 phosphorylation at the presynaptic terminal is likely to alter cellular function. In addition, a decrease in neurotransmitter release has been observed after lowering GAP-43 levels. Furthermore, increased expression of GAP-43 transgene in hippocampal granule cells could increase signal propagation through the hippocampal circuits, eventually leading to increased memory function in these animals. A second possible mechanism is the enhancement of remodeling of presynaptic neurons, which facilitates the communication between neural circuits, and post-translational modifications of GAP-43 may lead to axonal elongation, since motor growth cones have lower levels of phosphorylated GAP-43 (Holahan et al., 2007). In this study, CAP1 protein levels increased in hippocampal tissue of STZ-induced diabetic rats. Increased CAP1 protein levels are found in some cancers of the brain, CNS, head, neck, pancreas, liver, and kidney. Given the involvement of the pancreas in the induction of STZ-induced diabetes, it is likely that CAP1 protein levels will increase, and since CAP1 is expressed in almost all tissues and cells, diabetes is likely to increase this protein in hippocampal tissue. Overexpression of CAP1 may be a diagnostic marker or a therapeutic target for certain types of diseases and cancers (Xie et al., 2018). In this study, UD/Ex significantly increased the learning and memory of diabetic rats by altering GAP-43, and CAP-1 protein levels. These results support the hypothesis that Ex and/or UD positively affect neurogenesis in the hippocampus. Some of the side effects of diabetes on the brain may be mainly due to the direct consequences of chronic hyperglycemia. Studies on the consumption of UD extracted from hydro-alcoholic leaves have shown a meaningful decrease in glycemic control during diabetes. In animal models, UD has been reported to decrease the amount of BG and glycated hemoglobin during STZ-induced diabetes. Carvacrol in UD leaves has a neuroprotective effect against cerebral injuries, modulates serotonin, and dopamine levels in the hippocampus, and shows the prefrontal cortex (Patel et al., 2016). UD is rich in 5-HT, which is known for its relationship with memory function and insulin secretion (Patel et al., 2016). Generally, persistent chronic hyperglycemia of diabetes is the primary source of increased generation of free radicals through auto-oxidation of glucose that increases the flux of glucose through the polyol pathway. Excessive production of free radicals beyond the scavenging capacities of endogenous antioxidant capacity leads to macro-and microvascular dysfunction, and these changes are also associated with dysfunctions of neural-like alteration of the hippocampal neural density of the central nervous system (Golalipour et al., 2012). UD is one of the plants with strong antioxidant activity due to flavonoids (Patel et al., 2016), which in clinical status are observed to enhance total antioxidant capacity and decrease inflammatory stress (Namazi et al., 2012). It seems that UD affects the control of structural changes in hippocampal neural tissue. In addition, regular exercise is an essential and effective factor in controlling and treating metabolic diseases and diabetes. In this study, Ex significantly reduced BG concentration in the D-Ex group rats. Therefore, these results indicate a chronic adaptation of aerobic exercise on BG reduction in diabetic rats. Several mechanisms act to improve the absorption and elimination of BG after exercise. These include increased blood flow, increased IR turnover, and increased insulin binding to its IR (Gomes et al., 2009). Ex increases the sensitivity of environmental insulin. These results may increase the IR translocation/activation, and the responsiveness of downstream proteins to insulin signaling in the hippocampus. Indeed, increases in the level of insulin have been shown to improve memory in both humans, and experimental animals (Muller et al., 2011). Similarly, Ex positively affects hippocampal plasticity, and memory function (Muller et al., 2011). Regarding the results mentioned above, Ex and UD have positive effects on the molecular and tissue aspects of the hippocampus in diabetic individuals. The results indicated that the Ex and UD might reduce the adverse effects of diabetes on hippocampal tissue, and after improving the molecular aspect, the structure of the hippocampus also becomes normal. Ex and/or UD treatment in all areas significantly indicated an improvement in cell density, and thickness of the hippocampus tissue compared to the DC group. So far, no study has been conducted on the effects of Ex and UD on structural changes in the hippocampus in STZ-induced diabetic rats. According to the results of this study, Ex with UD consumption has a significant effect on the reconstruction of the structure of the hippocampus of diabetic rats.

## 5. Conclusion

In conclusion, the results of this study showed that the EX and/or UD extract led to reduced complications of diabetes on functional, molecular, and tissue changes in the hippocampus. Ex + UD caused a decrease in neural disorganization, an increase in neural, and microglial cell density, an increase in the thickness of pyramidal and molecular layer, an increase of GAP-43 protein expression and a reduction of CAP-1 protein expression in the hippocampus of diabetic rats. Following positive changes in the hippocampal tissue, learning and memory improved because adverse changes in the structure of the hippocampus play an essential role in the impairment of learning, and memory. The combination of Ex with UD extract caused a significant improvement in the hippocampal tissue. Both of these interventions can provide the productive potential for the suppression of changes in the structure of the hippocampus in diabetic rats. Finally, the results demonstrate the advantages of administering UD extract as well as Ex.

## Ethical approval

All experiments were performed under, Lorestan University Animal Ethics Committee (under the code LU. ECRA. 2018.16) and according to the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996).

## Author contributions

Maryam Keshvari and Masoud Rahmati designed this study; Farzaneh Chehelcheraghi performed research and wrote the paper. Maryam Keshvari, Masoud Rahmati, Farzaneh Chehelcheraghi, and Rahim Mirnasouri analyzed the data. Final approval of the version to be published: all authors.

## Declaration of competing interest

There is no conflict of interest, and all authors support the submission to this journal.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112801.

## Abbreviations

| | |
|---|---|
| UD | Urtica dioica |
| Ex | exercise |
| STZ | streptozotocin |
| DG | dentate gyrus |
| CA | cornu ammonis |
| BG | blood glucose |
| BW | body weight |

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