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The effect of Urtica dioica extract on the number of astrocytes in the dentate gyrus of diabetic rats

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Diabetes mellitus is associated with cerebral alterations in both human and animal models of the disease. These alterations include abnormal expression of hypothalamic neuropeptides and hippocampal astrogliosis. Urtica dioica (Nettle) is among several species listed for their use against diabetes in folk medicine. The aim of this study was the evaluation of the astrocyte number in the dentate gyrus of diabetic rats after treatment with nettle.

A total of 21 male albino Wistar rats were used in the present study. The animals were divided into three groups: control, nettle-untreated diabetic, and nettle treated diabetic. Hyperglycaemia was induced by streptozotocin (80 mg/kg) in the animals of the diabetic and treatment groups. One week after injection of the streptozotocin, the animals in the treatment group received a hydroalcoholic extract of Urtica dioica (100 mg/kg/day) for 4 weeks intraperitoneally. After a 5-week survival period, all the rats were sacrificed and coronal sections were taken from the dorsal hippocampal formation of the right cerebral hemispheres. The area densities of the astrocytes were measured and compared between the three groups ($p < 0.05$).

The number of astrocytes increased in the diabetic rats (24.06 ± 9.57) compared with the controls (17.52 ± 6.66). The densities in the treated rats (19.50 ± 6.16) were lower than in the diabetic rats. Furthermore, the control and treated rats showed similar densities.

We concluded that U. dioica extract helped compensate for astrocytes in the treatment rats dentate gyrus in comparison with diabetic rats. (Folia Morphol 2009; 68, 2: 93–97)

Key words: diabetes, astrocyte, dentate gyrus, Urtica dioica

INTRODUCTION

Diabetes mellitus is a common metabolic disorder in humans, which is associated with cerebral changes. More than 1% of the world population suffer from diabetes and their numbers are gradually increasing [12].

A high occurrence of the disease is noted in Iran, especially in urban populations, and is therefore a major health problem [15].

Diabetes mellitus is associated with cerebral changes in both human and animal models of the disease. These changes include abnormal expression

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of hypothalamic neuropeptides, astrogliosis in the hippocampus [18], decreased synaptic plasticity in the hippocampus, neurotoxicity, and changes in glutamate neurotransmission [20].

Increasing evidence has shown that diabetes may be associated with learning and memory deficits in humans [4, 5, 14]. Studies examining the effects of streptozotocin-induced diabetes on memory function in mice and rat models have also shown deficits in memory retention and retrieval, compared to non-diabetic controls [2, 3, 8, 19]. As we showed in our previous studies, astrocytes have the capacity to increase after spatial learning in the hippocampal subfields and dentate gyrus with Morris water maze techniques [10, 11], astrocytes also increased in diabetes [18].

Urtica L., the stinging nettle (Urticacea), is an annual perennial herb that is distinguished by its stinging hairs [13]. Urtica dioica (U. dioica), among the Urtica species, has been known for a long time as a medicinal plant. The decreasing effect on blood sugar of U. dioica as a medicinal herb has been introduced in old writings such as those by Avicenna [6]. In addition, U. dioica is among several species listed for their use against diabetes in folk medicine, in a large pharmacological screen of European species [17].

As the dentate gyrus is an important site for adult neurogenesis in mammals and neuropathological damage occurs in diabetic cases, and also because the number of astrocytes is increased in this disease, and lastly because there is no almost documentation regarding the effects of treatment with nettle on astrocytes in the Dentate gyrus of diabetic rats, the aim of this study was the evaluation of the astrocyte number in the dentate gyrus of diabetic rats treated with nettle.

MATERIAL AND METHODS

Animals

Twenty-one adult male Wistar rats (weighing 250–300 g) were used. The animals were divided into three groups: control, nettle-untreated diabetic, and nettle treated diabetic. All the animals were treated in agreement with the Helsinki Convention on the use of animals in research, approved by the Institutional Review Board. The animals were kept in an air-conditioned animal room (22 ± 2°C) under a 12-hour light/dark cycle; food and water were available.

Induction of diabetes by streptozotocin

The streptozotocin (STZ) used was of analytical grade, obtained from Merck and Sigma. STZ was dissolved in saline for immediate use and injected intraperitoneally. Diabetes was induced with a single IP injection of streptozotocin (STZ) (80 mg/kg) to overnight-fasting rats. Blood samples for glucose measurements were taken from the tail vein. Diabetes was confirmed by measuring the glucose concentration by using the glucometer method. In the experiments, 7 rats were used in each group.

Preparation of the Urtica dioica hydroalcoholic extract

Leaves of U. dioica were collected from a suburb of Gorgan, northern Iran (Golestan, Iran) in 2005 and taxonomically identified by the Department of Pharmacognosy at Mazandaran University of Medical Sciences. A voucher specimen (5-77-1) was deposited in the herbarium of Mazandaran University. Powder of U. dioica leaves was percolated with hydroalcoholic (60°) solvent for 48 hours. The extract was filtered and concentrated in a vacuum at 40°C to make a jelly material.

Study design

This study consisted of three groups: a normal healthy control group that received saline daily for 4 weeks, a diabetic group which received saline daily for 4 weeks after STZ injection, and the treatment group — diabetic rats that were administered 100 mg/kg hydroalcoholic extract of U. dioica [9] daily for 4 weeks.

Intraperitoneal glucose tolerance test (GTT) was performed on 16-hour fasted rats using 2 g glucose/kg body weight. In all groups, blood was collected from the animals by tail snipping at 0, 30, 90, and 120 minutes after glucose load. In addition, glucose tests were performed after IP injection STZ at 1 and 5 weeks.

Tissue processing

After the animals had been sacrificed, the brains were removed and fixed in buffered formaldehyde 10% solution for histological analysis. After processing and embedding, the brains were cut coronally into 7 μm slices. Approximately 10 slices were obtained from each brain. Then they were stained with phosphotungstic acid haematoxylin (PTAH) staining [10, 11]. We used PTAH staining for astrocyte staining because it is one of the special staining methods

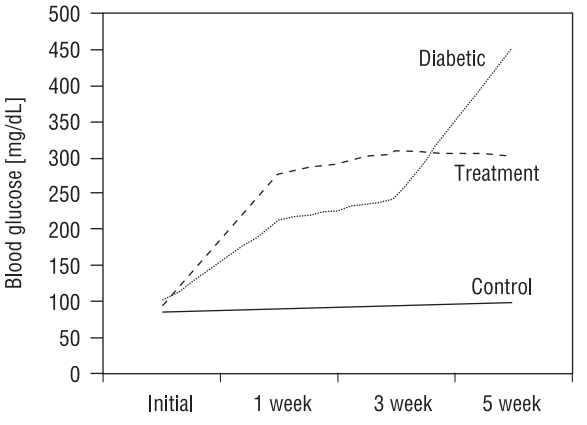

Figure 1. Blood glucose concentration in control, diabetic and treatment groups.

for astrocyte cells and their processes. Using this method the astrocytes appeared blue and the neurons appeared pink [1].

Quantification of the number of astrocytes

A photograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 400X. An area of 20000 μm^2 was selected randomly in the dentate gyrus in all sections. To measure the area density of the astrocytes, the images were transferred onto a computer. Using OLYSIA Autobioreport software, Olympus Co., the appropriate grids were superimposed on the pictures and the cells were counted manually.

Statistical analysis

Experimental results concerning this study were evaluated using SPSS v.11.5 and expressed as mean ± SE. To compare the means of the measured parameters in the three groups by analysis of variance (ANOVA) test, after confirmation of normality, the means were compared by the ANOVA Post Hoc Tukey test; $p < 0.05$ was considered significant.

RESULTS

The GTT of the three groups at the beginning and on the 5th week of the study are shown in Figure 1. As can be seen in Figure 1, the GTT results of all rats were normal at the beginning of the experiment. The GTT results at the end of the study show a marked difference to the previous results in the rats that received STZ, i.e. the rats from the diabetic and treatment groups. The control rats which did not receive STZ showed a normal GTT. In contrast, the diabetic rats which had undergone STZ-induced diabetes on the first day of the study and did not receive the nettle extract showed the most profound impairment in GTT.

Table 1. Mean of astrocytes in dentate gyrus (DG) in control, diabetic, and treatment groups

DG	Mean	Std. error mean	Area [μm^2]
Control	17.52	6.662	20000
Diabetic	24.06	9.576	20000
Treatment	19.50	6.164	20000

Table 2. Mean of astrocytes in dentate gyrus (DG) of all rats in control, diabetic and treatment groups

DG	Control	Diabetes	Treatment
Rat 1	17.2	16.88	23
Rat 2	14.75	26.25	15
Rat 3	12.4	26.36	21.2
Rat 4	25	23.14	20.3
Rat 5	18.8	25.55	18.6
Rat 6	16.9	25.2	20
Rat 7	17.6	25	18.3
Mean	17.52	24.06	19.50

The means of the astrocyte number (per 20000 μm^2) in the control, diabetic, and treatment groups are shown in Table 1, and the mean of the astrocytes in each rat is shown in Table 2.

Morphometric evaluation showed that the number of astrocytes in the diabetic group (24.06 ± 9.57) was higher than in the control group (17.52 ± 6.66), and also that the number of astrocytes in the treatment group (19.50 ± 6.16) was higher than in the control group, but the treatment group had less astrocytes than the diabetic group.

On the other hand, there was a significant difference in astrocyte number between the control and the diabetic groups, but the difference between the treatment group and the other groups was not significant. It seams that nettle can decrease the density of astrocytes that increase in diabetes.

The transitions showed that the anterior-posterior distributions of astrocyte densities were not the same in the three groups (Table 3), but some similarities between the control and treatment groups

Table 3. Mean of astrocytes in dentate gyrus (DG) from anterior to posterior in control, diabetic and treatment groups

DG	Control	Diabetes	Treatment
1	14	34.75	20.5
2	17.5	27	27.5
3	21	27.25	16
4	16.75	20.5	19
5	20.5	22.25	17.5
6	13.25	18.25	16
7	18	20.25	18.5
8	17.75	19.25	18.5
9	16.25	21.75	19
10	18.5	26	19.5

were seen. In addition, no significant regression model was compatible with the transitions drawn up ($p < 0.05$).

DISCUSSION

The present data showed that the astrocytes in the dentate gyrus of diabetic rats, as we thought, increased in comparison with nondiabetic controls, and this difference was significant ($p < 0.05$). Additionally, in the treatment group with hydroalcoholic extract of U. dioica, the number of astrocytes was higher than in the nondiabetic controls, but this difference was not significant ($p < 0.05$). Our data showed that the treatment group had less astrocytes compared with diabetic rats, but this difference was not significant ($p < 0.05$). On the other hand, the number of astrocytes after treatment with extract of U. dioica decreased in the diabetic rats.

An attempt was made to find the anterior-posterior transitions of the astrocyte densities and provide mathematical equations for computational analysis. The transition of the cell density can show the distribution of the density in anterior-posterior transition. The underlying trends of the transitions drawn up were not significantly describable by regression models. This finding is opposite to our previous studies in spatial learning. In our previous studies we found different increases in astrocytes in the anterior and posterior of hippocampal formation [10, 11]. It seems the diabetes, despite of spatial learning examination, does not have a different effect in the anterior-posterior of hippocampal formation.

The increase in cell density seen in the diabetic group was compatible with the results of previous studies. For example, Revsin et al. [20] in 2005 reported that one month after STZ treatment, the number of astrocytes was significantly increased in the STZ-treated group as compared to the vehicle-treated group ($p < 0.001$, $n = 5$); this finding is similar to our results.

Saravia et al. [18] in 2002 showed that the number of GFAP astrocytes increased 3-fold in the hippocampal stratum radiatum of STZ-diabetic female mice compared with age-matched, vehicle-treated nondiabetic controls ($p < 0.005$).

Muranyi et al. [16] showed that the number of GFAP-positive astrocytes increased significantly in the hippocampal formation of diabetic rats. The diameters of astrocyte cell bodies were found to be enlarged, and the number and length of astrocyte foot processes were increased.

What is more, in our previous study in which we worked on the granule cell in the dentate gyrus, we showed that *Urtica dioica* extract can help to restore diabetes-induced granule-cell loss in the rat dentate gyrus [7].

However, by comparison of our results with our previous study it seems that nettle extract administration can be a beneficial treatment for the regulation of cell density in the dentate gyrus of diabetic patients and that this can ameliorate cognitive impairment in the diabetic patient.

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