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## The Preventive and Treatment Effect of *Urtica dioica* on Astrocyte Density in the CA1 and CA3 Subfields of Hippocampus in STZ Induced Diabetic Rats

Efecto Preventivo y del Tratamiento de *Urtica dioica* sobre la Densidad de Astrocitos en los Subcampos CA1 y CA3 del Hipocampo en Ratas con Diabetes Inducida por STZ

Mohammad Jafar Golalipour<sup>\*</sup>; Mehrdad Jahanshahi<sup>\*\*</sup>; Soraya Ghafari<sup>\*\*\*</sup> & Mohammad Afshar<sup>\*\*\*\*</sup>

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**SUMMARY:** Several animal model studies have shown that Diabetes mellitus can affect on the activity of hippocampus astrocytes, but these studies reported controversial findings. This study was done to evaluate the preventive and treatment effect of *Urtica dioica* (*U. dioica*) on astrocytes density in the CA1 and CA3 subfields of hippocampus of streptozotocin (STZ) induced diabetic rats. Twenty-eight male albino Wistar rats were randomly allocated equally into control, diabetic, *U. dioica* treatment and *U. dioica* preventive groups. Hyperglycemia was induced by STZ (80 mg/kg/BW). One week after injection of the streptozotocin, animals in treatment group were received hydroalcoholic extract of *U. dioica* (100 mg/kg/BW/day) for 4 weeks by intraperitoneally. In preventive group, diabetic rats were received 100 mg/kg/BW/ daily hydroalcoholic extract of *U. dioica* for 5 days before STZ injection. Then, animals were sacrificed and coronal sections were taken from the right dorsal hippocampus, stained with PTAH. The area densities of the astrocytes were measured. The number of astrocytes in CA1 of controls, diabetic treatment and preventive groups was  $19.00 \pm 5.5$ ,  $17.14 \pm 6.4$ ,  $21 \pm 8.1$  and  $16.48 \pm 3.2$ , respectively. The densities of astrocytes in CA3 of controls, diabetic, treatment and preventive groups were  $25.45 \pm 7.60$ ,  $21.54 \pm 7.5$ ,  $23.75 \pm 5.6$  and  $19.89 \pm 3.8$ , respectively. The density of astrocytes in diabetic rats reduced in comparison with controls ( $P < 0.05$ ). In CA1 and CA3, in spite of preventive administration, treatment of diabetic rats with *U. dioica* significantly increased the astrocytes. This study showed that treatment with *U. dioica* extract can help compensate for the CA1 and CA3 subfields of hippocampus astrocytes in diabetic rats.

**KEY WORDS:** Diabetes; Astrocyte; *Urtica dioica*; Hippocampus; CA1; CA3; Rat.

### INTRODUCTION

Diabetes mellitus is one of the most common serious metabolic disorders (Gispén & Biessels, 2000). Diabetes mellitus causes a variety of functional and structural alterations including hippocampal astrogliosis (Saravia *et al.*, 2002), decreased hippocampal synaptic plasticity, neurotoxicity and changes in glutamate neurotransmission (Revsin *et al.*, 2005) of the central nervous system.

Astrocytes as glial cells in the central nervous system are effective in Supporting of neurons, scar formation and maintenance of the blood-brain barrier (Afsari *et al.*, 2008), vascular reactivity, regulation of extracellular glutamate

levels, energy metabolism, and protection from reactive oxygen species (Dringen *et al.*, 2000; Tsacopoulos & Magistretti, 1996; Zonta *et al.*, 2003).

Several animal model studies reported that Diabetes mellitus can affect on the activity of hippocampus astrocytes, but these studies reported controversial findings. some researches indicated that diabetes led to increasing of astrocytes (Saravia *et al.*; Baydas *et al.*, 2003a, 2003b; Revsin *et al.*), but other studies have shown that diabetes mellitus reduced the number and activity of astrocytes (Coleman *et al.*, 2004; Coleman *et al.*, 2010).

<sup>\*</sup> Professor, Gorgan Congenital Malformations Research Center, Department of Anatomical Sciences, Golestan University of Medical Sciences, Gorgan, Iran.

<sup>\*\*</sup> Associate Professor, Neuroscience Research Centre, Department of Anatomical sciences, Golestan University of Medical Sciences, Gorgan, Iran.

<sup>\*\*\*</sup> Department of Anatomical Sciences, Golestan University of Medical Sciences, Gorgan, Iran.

<sup>\*\*\*\*</sup> Professor, Department of Anatomical sciences, Birjand University of medical Sciences, Birjand, Iran.

Furthermore, several studies indicated that induction diabetes is associated with a reduction in GFAP-positive astrocytes in the spinal cord and death of hypothalamic astrocytes in poorly controlled diabetic rats (García-Cáceres *et al.*, 2008; Afsari *et al.*).

In recent years, there has been renewed interest in plant medicine for the treatment of different diseases (Kameswara Rao *et al.*, 2003; Ladeji *et al.*, 2003). Isolated studies screened various plants having “folk medicine reputation” by biochemical test for this antidiabetogenic effect (Vats *et al.*, 2002).

*Urtica dioica* L. Stinging nettle (*Urticaceae*) is annual and perennial herb, distinguished with stinging hairs (Kavalali *et al.*, 2003). Among the *Urtica* species, *Urtica dioica* (*U. dioica*) has already been known for a long time as medicinal plants in the world. The blood sugar lowering effect of *U. dioica* as a medicinal herb has been introduced in old script such as those written by Avicenna (Farzami *et al.*, 2003). 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 (Atta-Ur-Rahman & Zaman, 1989).

Since there is controversy about the effect of Diabetes mellitus on astrocytes alterations in the CA1 and CA3 subfields of hippocampus and lack of study regarding the effect of *Urtica dioica* on hippocampal astrocyte density, this study carried out to evaluate the preventive and treatment effect of *U. dioica* on astrocytes density in the CA1 and CA3 subfields of hippocampus of STZ induced diabetic rats.

## MATERIAL AND METHOD

In this experimental study 28 adult male Wistar rats (weighing 250–300 g) were used. All 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 air-conditional animal room ( $22 \pm 2^\circ\text{C}$ ) under a 12 h light/dark cycle.

**Collection of Plant.** Fresh leaves of *U. dioica* were collected from cultivated plant, from suburb of Gorgan, northern Iran (Golestan, Iran) in OCT 2005 and taxonomically identified by Department of Pharmacognosy, Mazandaran University of Medical Sciences. A voucher specimen (5-77-1) was deposited in the herbarium of Mazandaran University.

**Preparation of extract of *Urtica dioica*.** The dried and powdered of *U. dioica* leaves (400 g) were percolated by

Ethanol (45%) solvent. In briefly the dried leaf of *U. dioica* (by using hot air  $35\text{--}40^\circ\text{C}$ ) powdered by mechanical milling. Prelining maceration during 5 hours was done and the product percolated and mixing during 48 hours. The extract was filtrated (0.8 Micron) and spray dried in a lab plant SD4 spray drier (lab plant ltd, England). Spectrophotometric assay was carried out to determine the concentration of phenolic and flavonid content of *U. dioica* leaves extract (Kraus & Spiteller, 1991; Evans, 2002). Also antioxidant activity was measured by DPPH method.

**Chemical.** All chemical such as Streptozotocin (STZ) used were of analytical grade obtained from Merck and Sigma.

**Experiment induction of diabetes.** Streptozotocin was dissolved in saline immediately use and intraperitoneally injected. Diabetes was induced with a single intraperitoneally (IP) injection of Streptozotocin (STZ) (80 mg/kg) to overnight fast rats. Blood samples for glucose measurements were taken from the tail vein. Diabetes was confirmed by measuring the glucose concentration by using Glucometer method.

**Experimental design.** For this study four groups designed: Normal healthy control group (Group I), received Saline daily for 4 weeks. Diabetic group (Group II) received Saline daily for 4 weeks after STZ injection, Treatment group (Group III), diabetic rats that administered 100 mg/kg/BW/ daily hydroalcoholic extract of *U. dioica* (Gülçin *et al.*, 2004; Kavalali *et al.*), for 4 weeks and preventive group, rats were received 100 mg/kg/BW/ daily hydroalcoholic extract of *U. dioica* for five days and then diabetes induced by STZ. In the experiments, seven rats were used in each group.

**Intraperitoneal glucose tolerance test (GTT).** GTT was performed on 16 h fasted rats using 2gram 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. Also glucose test were performed after IP injection STZ in 1 and 5 weeks.

**Histological and Morphometric studies.** After the animals had been scarified, the brains were removed and fixed in buffered formaldehyde 10% solution for histological analysis. Brains were cut coronally into 7  $\mu\text{m}$  slices. Approximately 10 slices obtained from each brain. Then they were processed and were stained with PTAH staining.

Then a photograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 400. An area of 20000  $\mu\text{m}^2$  was selected in the CA1 and CA3 subfields of hippocampus in all sections. To measure the area density of the astrocytes,

the images were transferred to the computer. Using OLYSIA Autobioreport software, Olympus Co, the appropriate grids were superimposed on the pictures and the cells were counted manually.

**Statistical analysis.** Experimental data were evaluated using Spss v.11.5 and one way ANOVA and expressed as Mean±SE P< 0.05 was considered significant.

## RESULTS

Phytochemical analysis of extract showed the presence of phenolic and flavonoids content,  $22.8 \pm 2.7$  and  $41.2 \pm 3.1$  mg/gram dry extract, respectively. Antioxidant activity by DPPH method showed  $25.5 \pm 2.2$  percent of scavenging activity.

The Glucose Tolerance Test (GTT) of the three groups at the beginning and the 5<sup>th</sup> wk of the study are showed in Fig. 1. As shown in Fig. 1, the GTT results of all rats were normal at the beginning of experiment. At the end of study control rats which have not received STZ showed a normal GTT. In contrast, the diabetic rats which have been undergone STZ-induced diabetes at the first day of study showed the most profound impairment GTT. Also result of GTT in treated group was similar to STZ-induced diabetes.

The density of astrocytes (per 20000 mm<sup>2</sup>) in CA1 and CA3 of hippocampus in control, diabetic Treatment and preventive groups are depicted on Fig. 2.

The number of astrocytes in CA1 area of controls, diabetic treatment and preventive groups was  $19.00 \pm 5.5$ ,  $17.14 \pm 6.4$ ,  $21 \pm 8.1$  and  $16.48 \pm 3.2$ , respectively.

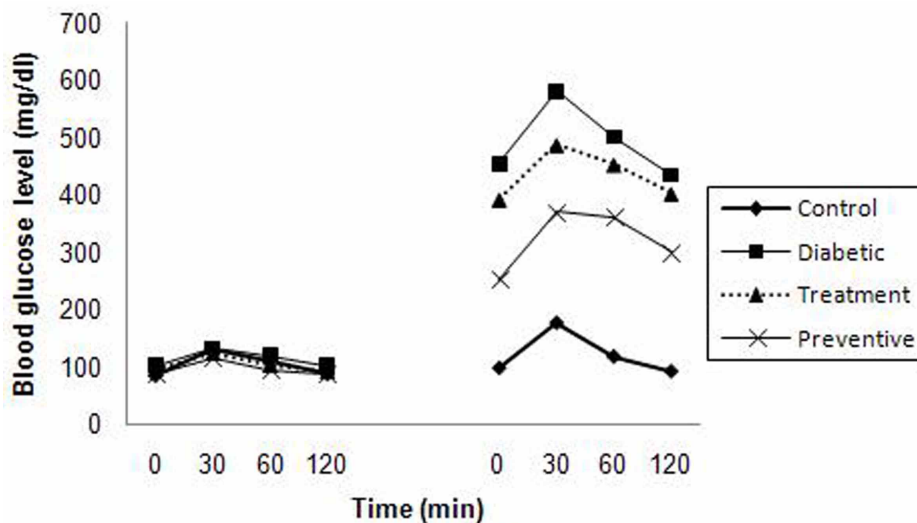


Fig. 1. The Glucose Tolerance Test (GTT) of the four groups at the beginning and the end of the study.

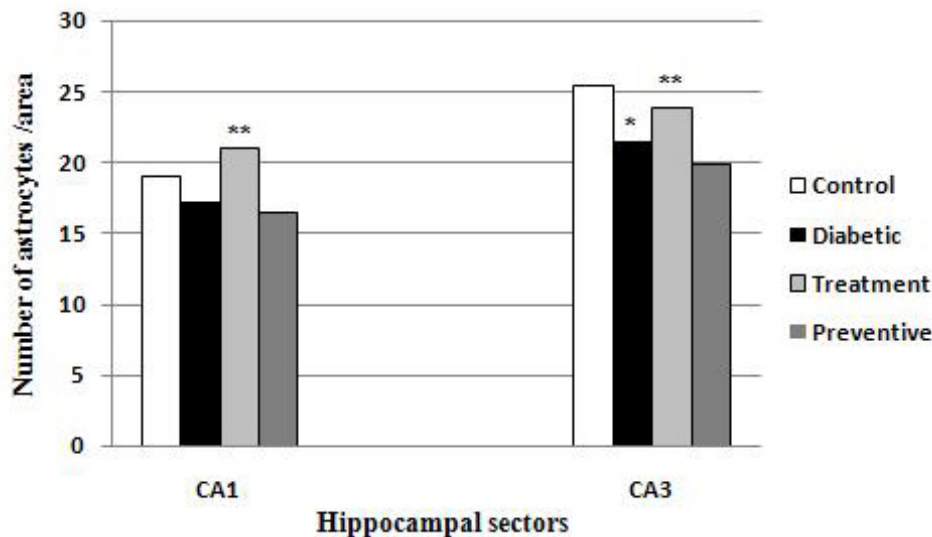


Fig. 2. Astrocytes number in CA1 and CA3 in control, diabetic, treatment and preventive groups (\* compared with control group, \*\* compared with diabetic group, P<0.05)

In CA1, treatment of diabetic rats with *U. dioica* significantly increased the astrocytes as compared to diabetic group, but preventive administration of *U. dioica* does not alter the astrocytes density.

The densities of astrocytes in CA3 area of controls, diabetic, treatment and preventive groups were  $25.45 \pm 7.60$ ,  $21.54 \pm 7.5$ ,  $23.75 \pm 5.6$  and  $19.89 \pm 3.8$ , respectively.

In CA3, the density of astrocytes in diabetic rats reduced as compared with controls ( $P < 0.05$ ). Treatment of diabetic rats with *U. dioica* significantly increased the astrocytes compared to diabetic group, but preventive administration of *U. dioica* had no effect on astrocytes density.

## DISCUSSION

This study showed that astrocytes numbers were non-significantly reduced in CA1 and significantly reduced in CA3 in STZ induced diabetics rats. Our results is similar to previous studies (Coleman *et al.*, 2004, 2010) and is contrast with others (Saravia *et al.*; Baydas *et al.*, 2003a, 2003b; Revsin *et al.*).

Coleman *et al.* (2010) reported that diabetes reduce GFAP content in rats at 4 and 8 weeks of diabetes duration. Also, Lebed *et al.* (2008) indicated that GFAP cell count in CA1 and CA3 areas decrease in 3<sup>rd</sup> day in STZ induced diabetic rats in comparison with controls.

Furthermore, reduction of astrocytes in spinal cord and hypothalamus were reported in didactic animals (García-Cáceres *et al.*; Afsari *et al.*). Reduction of astrocytes in diabetic animals can be due to astrocytes death by apoptosis (García-Cáceres *et al.*).

Apoptotic cell death occurs In diabetes mellitus in numerous tissues (Nishikawa & Araki, 2007; Allen *et al.*, 2005; Arroba *et al.*, 2003, 2005, 2007; Lechuga-Sancho *et al.*, 2006a, 2006b; Klein *et al.*, 2004), with increase of glucose level (Romero *et al.*, 2002; Anitha *et al.*, 2006), decrease insulin or insulin-like growth factor signaling (Ishii, 1995) or an increase in cytokines such as TNF $\alpha$  (Chen & Goeddel, 2002). Because, glial cells control the supply of glucose and its metabolites to neurons (Pellerin *et al.*, 2007), they are the first line of defense against changes in glucose concentrations. Furthermore, when glucose availability is reduced, glycogen stored in astrocytes serves as a fuel source for neurons (Brown & Ransom, 2007). Thus, it is possible that prolonged exposure to elevated glucose levels underlies the increase death of astrocytes.

High glucose levels directly induce death of different glial cell types (Xi *et al.*, 2005; Delaney *et al.*, 2001). However, increased TNF $\alpha$  (Arroba *et al.*, 2005), decreased insulin (Arroba *et al.*, 2007) or insulin growth factor (IGF)-I (Busiguina *et al.*, 1996), could be involved in glial cell death.

The intracellular mechanism of the increase astrocytes death most likely involves nuclear translocation of AIF (apoptosis inducing factor) (García-Cáceres *et al.*, 2008).

Nuclear translocation of AIF is involved in caspase-independent cell death in response to a variety of signals, including oxidative stress, glutamate toxicity, and ischemia. Loss of mitochondrial membrane integrity results in the release of this factor, which can then be translocated to the nucleus where it induces DNA fragmentation and chromatin condensation (Krantic *et al.*, 2007).

In other hand, several studies have reported that increase of number of astrocytes in diabetes mellitus (Saravia *et al.*; Baydas *et al.*, 2003a, 2003b; Revsin *et al.*).

Baydas *et al.*, (2003b) reported an increased GFAP immunostaining in the hippocampus of 6 weeks after induction of diabetes in rats in comparison with control animals.

Also, Saravia *et al.*, study is founded that the number of GFAP cells significantly increase in SFZ-treated mice. Indeed, Saravia *et al.*, reported 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 non diabetic controls.

Furthermore, Muranyi *et al.*, (2006), found that the number of GFAP-positive astrocytes significantly increase in the hippocampus of diabetic rats. Also he reported that the diameters of astrocytes body were found to be enlarged and the number and foot processes length of astrocytes were increased.

Astrocytosis is often related to neurodegenerative diseases and aging, in which neuronal dysfunction or damage can also accompanied (Goss *et al.*, 1991; Magistretti & Pellerin, 1999; Ridet *et al.*, 1997).

The alterations of astrocytes number possibly due to oxidative stress (Baydas *et al.*, 2003b) and free radical formation (Baydas *et al.*, 2003a).

Studies have shown that hippocampus astrocytes of



diabetic rats can changes due to melatonin and vitamin E (Baydas *et al.*, 2003a,b). Vitamin E as an antioxidant agent and melatonin as a direct scavenger and indirect antioxidant can affect on astrocytes in diabetics model (Baydas *et al.*, 2003a). In our study *U. dioica* leaves extract increased the astrocytes number in CA1 and CA3 of diabetic rats. Also, Renno *et al.*, (2008), reported that diabetic rats treated with green tea showed a significant increase in the number GFAP-immunoreactive astrocytes in all the spinal cord gray areas as compared to water-drinking diabetic rats.

In our previous studies, the treatment with *U. dioica* extract after induction of diabetes can help to restore diabetes-induced granule cell and astrocytes loss in the rat dentate gyrus (Fazeli *et al.*, 2008; Jahanshahi *et al.*, 2009)

In the present study, *U. dioica* extract (100 mg/kg/ BW daily for 4 weeks after induction of diabetes) increased the number of astrocyte in CA1 and CA3 of STZ-induced diabetic rats.

Neural protective properties of *U. dioica* extract in the CA1 and CA3 of diabetic rats hippocampus have been attributed to antioxidant and anti-apoptotic properties of *U. dioica* (Fazeli *et al.*, 2010). It is founded that *U. dioica* contains phenolic compounds, particularly flavonoids (Mavi *et al.*, 2004). Flavonoids generally have antioxidant potential (Hall & Cuppett, 1997). Indeed, in our study, the preventive administration of *U. dioica* (100 mg/kg/

BW daily for 5 days before induction of diabetes could not alter the reduction of astrocytes in CA1 and CA3 of STZ-diabetic rats. Also, in our previous study, the preventive administration of *U. dioica* extract can not help to restore diabetes-induced astrocytes loss in the rat dentate gyrus (Golalipour *et al.*, 2011).

Although, neural cell death protective properties of the extract in the CA1 and CA3 of diabetic mice hippocampus have been attributed to antioxidant and anti-apoptotic properties of *U. dioica*, but lack of effect of *U. dioica* in this section of study may be due to low dosage and short duration of this plant before induction of diabetes in animals.

In conclusion, this study showed that astrocytes density of hippocampus reduce in STZ induced diabetic rats and *U. dioica* extract administration after induction of diabetes for 4 weeks can be a beneficial treatment for the regulation of astrocytes density in the in CA1 and CA3 of diabetic rats, but administration of *U. dioica* extract before induction of diabetes can not help compensate for astrocytes in hippocampus.

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GOLALIPUR, M. J.; JAHANSHAH, M.; GHAFARI, S. & AFSHAR, M. Efecto preventivo y del tratamiento de *Urtica dioica* sobre la densidad de astrocitos en los subcampos CA1 y CA3 del hipocampo en ratas con diabetes inducida por STZ. *Int. J. Morphol.*, 31(2):693-699, 2013.

**RESUMEN:** Varios estudios en modelos animales han mostrado que la diabetes mellitus puede afectar la actividad de los astrocitos del hipocampo, pero estos resultados son controvertidos. Este estudio se realizó para evaluar el efecto preventivo y de tratamiento de la *Urtica dioica* (*U. dioica*) en la densidad de los astrocitos en los subcampos CA1 y CA3 del hipocampo en ratas diabéticas inducidas por estreptozotocina (STZ). Veintiocho ratas Wistar albinas macho fueron asignadas al azar por igual en grupos control, diabético, con tratamiento *U. dioica* y preventivo con *U. dioica*. La hiperglucemia se indujo por STZ (80 mg/kg/peso corporal). Una semana después, los animales del grupo tratamiento recibieron el extracto hidroalcohólico de *U. dioica* (100 mg/kg/peso corporal/día) durante 4 semanas vía intraperitoneal. El grupo preventivo, recibió 100 mg/kg/peso corporal/día de extracto hidroalcohólico *U. dioica* durante 5 días antes de la inyección de STZ. Los animales fueron sacrificados, se tomaron secciones coronales del hipocampo dorsal derecho y se tiñeron con PTAH. Fueron medidas las densidades de área de los astrocitos. El número de astrocitos en CA1 de los grupos de ratas control, diabéticas, con tratamiento de *U. dioica* y preventivo con *U. dioica* fue 19,00±5,5, 17,14±6,4, 21±8,1 y 16,48±3,2, respectivamente. Las densidades de los astrocitos en CA3 de los grupos de ratas control, diabéticas, con tratamiento de *U. dioica* y preventivo con *U. dioica* fue 25,45±7,60, 21,54±7,5, 23,75±5,6 y 19,89±3,8, respectivamente. La densidad de los astrocitos en las ratas diabéticas se redujo en comparación con los controles (P <0,05). En CA1 y CA3, a pesar de la administración preventiva, sólo el tratamiento de ratas diabéticas con *U. dioica* aumentó significativamente los astrocitos. Este estudio mostró que el tratamiento con extracto de *U. dioica* puede ayudar a compensar los astrocitos de los subcampos CA1 y CA3 del hipocampo en ratas diabéticas.

**PALABRAS CLAVE:** Diabetes; Astrocitos; *Urtica dioica*; Hipocampo; CA1; CA3; Rata.

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Correspondence to:  
Dr. Mohammad Jafar Golalipour  
Gorgan Congenital Malformations Research center,  
Department of Anatomical sciences,  
Golestan University of medical Sciences,  
P.O. Box: 49175-1141  
Gorgan  
IRAN

Email: mjgolalipour@yahoo.com

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