Proliferation of the β-Cells of Pancreas in Diabetic Rats Treated with *Urtica Dioica*

Proliferación de las Células β del Páncreas en Ratas Diabéticas Tratadas con *Urtica Dioica*

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SUMMARY: This investigation was carried out to evaluate the effect of the extract of Urtica dioica leaves on hyperglycemia and quantitative changes of β-cells in streptozotocin-diabetic rats. Forty male Wistar rats were allocated in groups of normal, diabetic, treatment and protective. Hyperglycemia induced by administrating one dose of 80 mg/kg streptozotocin (STZ) intraperitoneally. Animals in treatment group received *Urtica dioica* (100 mg/kg/day) for 4 weeks intraperitoneally, one week after injection of STZ. In protective group animals received *U. dioica* (100 mg/kg/day) for 5 days before inducing diabetes. After five weeks the animals were sacrificed and whole pancreas removed. Pancreas specimens were used for quantitative morphometric analysis after Chromealum hematoxiline - phloxine staining. The mean ± SE of β-cells in non hyperglycemic animals in protective group was higher than in hyperglycemic animals in the same group (54.33 ± 2.4 versus 1.25 ± 0.5, P<0.05). Hyperglycemia was improved in 6 (60%) of rats in protective group and 1 (10%) rat in treatment group OR=0.07 (CI 95%: 0.0-1.1, p=0.06). The logistic regression analysis showed an association between decrease of blood glucose, increase of number of b-cells and administration of *Urtica* before induction of diabetes. This study showed proliferation of b-cells when of the *U. dioica* leaves extract (100 mg/kg/day) administrated before induction of diabetes in animal model.

KEY WORDS: Streptozotocin; Diabetes; *Urtica dioica*; Rat; Pancreas; β-cells; Blood glucose.

INTRODUCTION

Diabetes is a complex and multifarious group of disorders characterized by hyperglycemia reached epidemic proportions in the present century (Chen *et al.*, 2005).

Plants used in folk medicine to treat diabetes mellitus represent a viable alternative for the control of this disease (Maroo *et al.*, 2002).

Urtica dioica (*U. dioica*), an annual and perennial herb of family Urticaceae is commonly known as medical herb for a long time in the world. This herb is known for its anti- inflammationtory activity (Obertries *et al.,* 1996; Riehemann *et al.*, 1999). There have been also other reports indicating the benefits of using the extract of the leaves or other parts for the use in different conditions, i.e., diabetes (Kavalali *et al.*, 2003; Román Ramos *et al.*, 1992; Petleveski *et al.*, 2003; Farzami *et al.*, 2003) as well as

other disorders like prostatic hyperplasia (Hirano *et al.,* 1994; Lichius & Muth, 1997; Kayser *et al.*, 1995), rheumatoid arthritis, hypertension and allergic rhinitis (Mittman, 1990), stimulation of human lymphocytes (Wagner *et al.*, 1989) and decreasing the lipid peroxidation and liver enzymes (Kanter *et al.*, 2003).

Although, there are some reports regarding the hypoglycemic activity of *U. dioica* in folk medicine (Petleveski *et al.*; Farzami *et al.*) but, in other hand, several investigations have detected hyperglycemic activity of this herb (Neef *et al.,* 1995; Swanston-Flatt *et al.*, 1989).

Therefore, this study was done to evaluate the effects of the hydroalcoholic extract of *U. dioica* leaves on hyperglycemia and Proliferation of β-cells in streptozotocin-diabetic rats.

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MATERIAL AND METHOD

Plant material. *U. dioica* leaves were collected from cultivated plant, from suburb of Gorgan, northern Iran (Golestan, Iran) in 2007 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 the hydroalcoholic extract of Urtica dioica. Powder of *U. dioica* leaves was percolated by hydroalcoholic (60º) solvent for 48 hours. The extract was filtered and concentrated under vacuum at 40ºC to make a jellied material by vacuum spray dryer. In addition to thin layer chromatography and purity tests (foreign matter, total ash, acid insoluble ash and water insoluble ash) for qualificative analysis, monosaccharide-linked spectro-photometric assay were carried out to determine the concentration of polysaccharides in *U. dioica* leaves for standardization of the extract. The results of phytochemical analysis showed presence of high percentage of tannins, steroids and low percentage of flavonoids, carotenoids and saponins in the leaves of *U. dioica.*

Animals. Male adult albino rats (Wistar strain) of 125-175 gr were fed on pellet diet and tap water for full acclimatization. The animals were kept in air-conditional animal room (22± 2˚C) under a 12 h light/dark cycle. The rats were divided into four groups (each group including 10 rats).

Experimental design. Hyperglycemia (blood glucose range of above 200 mg/dl (Rasal *et al.,* 2006) was induced with single IP injection of streptozotocin (STZ) at a dose of 80 mg/kg body weight dissolved in distilled water just before use 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 Accu-check active blood glucose monitor test strip (Jackson-Guilford *et al.*, 2000).

In the experiments, ten rats were used in each group.

Group I: normal control group. Saline daily for 5 weeks.
Group II: diabetic group. Saline daily administered for 5 days and then, hyperglycemia induced by one dose of intraperitoneally injection of 80 mg STZ at day 6[th.]
Group III: treatment Group (STZ-Urtica): hyperglycemia induced by one dose of intraperitoneally injection of 80 mg STZ and then diabetic rats received 100 mg/kg daily hydro-alcoholic extract (Kavalali *et al.*) of *U. dioica,* for 4 weeks.

Group IV: protective group (*Urtica*-STZ): animals received 100 mg/kg daily hydroalcholic extract of *U. dioica*, for 5 days, intraperitoneally and then, hyperglycemia induced by one dose of intraperitoneally injection of 80 mg STZ at day 6th.

Glucose tolerance test. Intraperitoneal glucose tolerance test (GTT) was performed on 16 hrs fasted rats using 2 gram 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, 3 and 5 weeks.

Histopathologic examinations. The animals of three groups are sacrificed by ether anesthesia. Whole pancreas was dissected. The tissue samples were fixed in bouin's fluid and paraffin embedded for microscopic examination in accordance with routine laboratory procedures. Histopathologic examination and grading were carried out on Chromealum hematoxiline – phloxine (Bancroft & Gamble, 1990) stained sections at 5-µm thickness with 30-µm distance were used for and morphometric analyses.

The number of islets and the number of b-cells of each islet were counted by Olympus BX-51T-32E01 research microscope connected to DP 12 Camera with 3.34 million pixel resolution and Olysia Bio software (from: Olympus Optical Co. LTD, Tokyo-Japan).

Statistical analysis. All the grouped data were statistically evaluated using One-Way ANOVA, expressed as the Mean $\pm$ SE from ten rats in each groups. Furthermore, Odds ratios and 95% confidence interval (CI) were calculated using SPSS 11.5. In all of the statistical tests, significance level was set 0.05.

RESULTS

The animals in the end of fifth week according to glucose level (blood glucose range of above 200 mg/dl) and GTT test (Fig. 1) grouped to two groups.

As shown in Table I the mean $\pm$ SE of blood glucose concentrations at the beginning of the study were 85.5±1.7, 102.9± 3.7, 92.2±4.1 and 95.6±3.7 mg/dl in control, diabetic, treatment and protective groups, respectively.

In the end of fifth week the mean $\pm$ SE of the glucose level in control and STZ groups were 99.4 ±5 and 454.7 ± 34.5 respectively. Hyperglycemia was seen in 9 (90%) animals in STZ–*Urtica* and 4 (40%) animals in protective
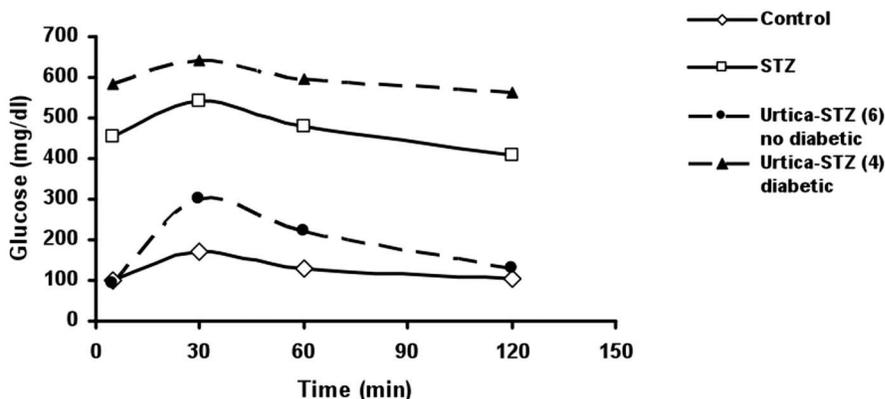
Fig. 1. The curves of Glucose Tolerance Test (GTT) in Control, STZ and protective groups (Day 35).
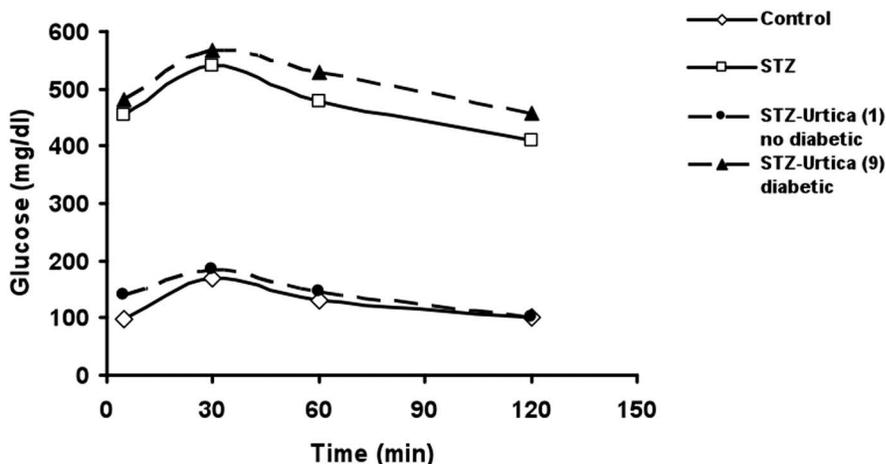

Fig. 2. The curves of Glucose Tolerance Test (GTT) in Control, STZ and treatment groups (Day 35).

Table I. Blood glucose concentration and number of b cells in Control, Diabetic, Treatment and Protective Groups. Results are expressed as Mean±SE of the mean (p-value<0.05). *In the end of fifth week 1 and 6 non diabetic rats in treatment and protective groups respectively.

	Groups	Initial Glucose	In the end of fifth week		
				Glucose	ß cells
I	Control	85.5±1.7	Normal (10)	99.4±5.0	206.5±17.1
			Diabetic (0)	-	-
II	Diabetic	102.9±3.7	Normal (0)	-	-
			Diabetic (10)	454.7±34.5	2.4±0.7
III	Treatment	92.2±4.1	Nondiabetic (1)*	140±0.0	10.0±0.0
			Diabetic (9)	481.55± 28.28	1.8±0.3
IV	Protective	95.6±3.7	Nondiabetic (6)*	93.67±14.09	54.33±2.4
			Diabetic (4)	583.75±10.31	1.25±0.5

401

groups, respectively OR=0.074 (CI 95%: 0.007- 0.835, P value = 0.035).

In the fifth week of the study Mean ± SE of β-cells in control and diabetic group was 206 ± 17 and 2.4 ± 0.7 respectively, also, mean ± SE of beta cells in STZ-*Urtica* and *Urtica*-STZ groups were shown in Table I. The mean ± SE of b-cells in non hyperglycemic animals in protective group (*Urtica*-STZ) was higher than in hyperglycemic animals in the same group (54.33±2.4 versus 1.25±0.5, p<0.05).

According to our results administration of *Urtica dioica* before induction of diabetes, 13.5 times decrease chance of diabetes (Table II) and increasing number of b-cells in streptozotocin diabetic rats (Table III).

Table II. Number and percent of diabetic and non diabetic animals in treatment and protective groups.

Groups	Diabetic (%)	No diabetic	P-value	OR	CI
Treatment	9 (90%)	1 (10%)	0.035	0.074	0.007-0.835
Protective	4 (40%)	6 (60%)			

Table III. A comparison of number of β-cells in treatment and protective groups.

Groups	Less than 10	More than 10	P-value	OR	CI
Treatment	9 (90%)	1 (10%)	0.035	13.50	1.19-152.21
Protective	4 (40%)	6 (60%)			

DISCUSSION

This study showed that the administration of the *U. dioica* leaves before induction of diabetes in animals can increasing proliferation of β-cells and deceasing of blood glucose concentration in 60% of Rats.

The findings of this study are similar to other studies (Bnouham *et al.*, 2003; Petleveski *et al.*; Farzami *et al.*; Kavalali *et al.*).

Acute and chronic hypoglycemic activity of *Urtica* was demonstrated by Farzami *et al.* and Kavalali *et al.* Farzami *et al.* showed that a fraction from *U. dioica* was a potent stimulator of insulin release of β-cells. Also Kavalali et al. reported that lectin fraction of extract of *U. pilulifera* seed has hypoglycemic activity and b-cell regenerative potency.

In the other hand our findings in contrast with two previous studies (Neef *et al*.; Román Ramos *et al.*). These studies have not shown the hypoglycemia activity of aqueous extract of *U. dioica*. In contrast with our study, two studies (Neef *et al*.; Román Ramos *et al*.) have not shown the hypoglycemia activity of aqueous extract of *U. dioica.*

In this study, in treatment group (STZ-*Urtica*) the leaves extract of *U. dioica* can cause hypoglycemia activity and ability to regenerate pancreatic β-cells after four weeks of administrating of *U. dioica* in 10% of streptozotocin-diabetic rats. It may be the animals probably the β cells damage is so extensive that no increase in insulin Secretion is possible. However in protective group *(Urtica*-STZ), we showed proliferation of β-cells diabetic rats in 60 percent of animals.

Therefore, it could be suggested that the contact of the leaves extract might play a protective role in preventing free-radials actions, that may destroy β-cells, those would then cause more secretary ability of β-cells this decrease plasma glucose concentration.

U. dioica probably increased proliferation of β-cells of islets in the pancreas. Our results indicated that decreased blood glucose concentration by *U. dioica* before induction of diabetes might be due to proliferation roles or partial regeneration in the β-cells. However, other extra pancreatic mechanisms such as enhanced glucose transport into the cells, inhibition of the endogenous glucose production can not role out by the results of present study.

Also, some studies reported that inhibition of free radical scavenger enzymes and enhancing production of the superoxide radical are the mechanism of STZ on pancreatic β-cells (Swanston-Flatt *et al.*). Ford *et al.* (1999) showed the role of free radicals in disruption of insulin action and impairing glucose tolerance test. Although STZ produce hyperglycemia in a concentration depended model by selective β-cell cytotoxic effect (Saini *et al.*, 1996).

Our findings suggest that *U. dioica* may have antioxidant or free radical scavenger properties. Antioxidant and free radical scavenging properties of *U. dioica* leaves have been established by several studies (Kanter *et al.*, 2005; Cetinus *et al.*, 2005; Mavi *et al*., 2004).

In conclusion, this study showed that the administration of the *U. dioica* leaves before induction of hyperglycemia in rats can increase proliferation of b-cells in animals and decrease the blood glucose concentration.

Further studies are required for understanding of exact mechanism of *U. dioica* leaves on proliferation of β-cells.

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GOLALIPOUR, M. J.; GHAFARI, S.; KOURI, V. & KESTKAR, A. A. Proliferación de las células β del páncreas en ratas diabéticas tratadas con Urtica dioica. *Int. J. Morphol., 28(2)*:399-404, 2010.

RESUMEN: Este estudio evalúa el efecto del extracto de hojas de *Urtica dioica* sobre la hiperglicemia y de los cambios cuantitativos de células β en ratas diabéticas por estreptozotocina. Cuarenta ratas Wistar macho, fueron distribuidas en grupos normal, diabético, en tratamiento y protector. La hiperglicemia fue inducida, por vía intraperitoneal, a través de la administración de una dosis de 80 mg/kg de estreptozotocina (STZ) . Los animales del grupo en tratamiento recibieron *Urtica dioica* (100 mg/kg/día) durante 4 semanas por vía intraperitoneal, una semana después de la inyección de STZ. En los animales del grupo de protección recibieron *U. dioica* (100 mg/kg/día) durante 5 días antes de inducir la diabetes. Después de cinco semanas, los animales fueron sacrificados y se extirpó el páncreas. Muestras de páncreas se utilizaron para el análisis morfométrico cuantitativo después de la tinción hematoxilina/floxina. La media ± SE de células β en los animales sin hiperglicemia y en el grupo de protección fue mayor que en los animales con hiperglicemia ($54,33\pm2,4$ frente a $1,25\pm0,5$, $p<0,05$). La hiperglicemia mejoró en 6 (60%) de las ratas del grupo de protección y 1 (10%) de ratas en grupo de tratamiento OR=0,07 (IC 95%: 0,0-1,1, p=0,06). El análisis de regresión logística mostró una asociación entre la disminución de la glucosa en sangre, aumento del número de células β y la administración de *Urtica* antes de la inducción de la diabetes. Este estudio mostró una proliferación de las células β cuando el extracto de las hojas de *U. dioica* (100 mg/kg/día) administrado antes de la inducción de la diabetes en modelos animales.

PALABRAS CLAVE: Estreptozotocina; Diabetes; Urtica dioica; Rata; Páncreas; Células b; Glucosa en sangre.

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