The Protective Activity of *Urtica dioica* Leaves on Blood Glucose Concentration and β-cells in Streptozotocin-Diabetic Rats

Mohammad Jafar Golalipour and Vahid Khori

Department of Embryology and Histology, Department of Pharmacology, Gorgan University of Medical Sciences, Gorgan, Iran

Abstract: This study was done to determine the protective activity of the hydroalcoholic extract of *Urtica dioica* leaves on Hyperglycemia and β-cells in hyperglycemic rats. Thirty Wistar rats were allocated in groups of normal, Diabetic and treatment. Hyperglycemia in Rats induced by 80 mg kg⁻¹ streptozotocin. In treatment group, animals received hydroalcoholic extract of *Urtica dioica* 100 mg kg⁻¹ day⁻¹ for five days, intraperitoneally and then hyperglycemia induced by streptozotocin. The blood glucose concentration was measured by using a Glucometer in 1st, 3rd and 5th weeks. In the end of 5th weeks the animals in each group were sacrificed by anesthesia and whole pancreas in three groups extracted and fixed in bouin’s fluid and stained by chromateum hematoxiline-phloxine and beta cells were counted in three groups by Olympus microscope. Mean±SE of blood glucose concentrations in the end of fifth weeks were 99.4±5.0, 454.7±34.5 and 303.6±100.6 in control, diabetic and treatment groups, respectively (p<0.05). The percentages of β-cells in control, diabetic and treatment groups were 73.6, 1.9 and 22.9%, respectively. The percentage of β-cells in treatment group comparing with diabetic group was significant (p<0.05). This study showed that the protective administration of hydroalcoholic extract of *Urtica dioica* has hypoglycemic effect and protective activity of β-cells of langerhans in hyperglycemic rats.

Key words: Hyperglycemia, *Urtica dioica*, β-cells, streptozotocin

INTRODUCTION

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

More than 1% of the entire world populations are victims of diabetes and their numbers are gradually increasing (Kar et al., 2003).

In Iran high occurrence of the disease is noted, especially in urban populations and is therefore a major health problem. 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-inflammatory activity (Obertreis et al., 1996; Riehemann et al., 1999). Furthermore, there have been also other reports indicating the benefits of using the extract of the leaves or other parts of the plant for the use in different conditions, i.e., diabetes (Kavalali et al., 2003; Roman Ramos et al., 1992; Petlevski et al., 2001; Farzami et al., 2003) as well as other disorders like prostatic hyperplasia (Hirono et al., 1994; Lichius and Muth, 1997; Kayser et al., 1995) rheumatoid arthritis, hypertension and allergic rhinitis (Millman, 1990) and stimulation of proliferation of human lymphocytes (Wagner et al., 1989) and decreasing the lipid peroxidation and liver enzymes (Kanter et al., 2003).

Although, there are some reports about the hypoglycemic activity of *U. dioica* in folk medicine (Petlevski et al., 2001; Farzami et al., 2003) but, in other hand, several investigations have detected hyperglycemic activity of this herb (Neef et al., 1995; Swanston-Flatt et al., 1989). Besides in our previous study (Golalipour et al., 2006) chronic administration of the extract of *U. dioica* leaves showed no hypoglycemic effect of it in STZ diabetic rats. With regarding the lack of report about the protective effect of administration of this herb (before making diabetes) this research carried out to evaluate protective effect of the hydroalcoholic extract of *U. dioica* leaves on blood glucose concentration and potential protective roles in β-cells of pancreas in diabetic rats.

Corresponding Author: Mohammad Jafar Golalipour, Department of Histology and Embryology, Gorgan University of Medical Sciences, P.O. Box 49175-553, Gorgan, Iran
Tel/Fax: +98(171)4425165, 4425660
**MATERIALS AND METHODS**

**Plant material:** *U. dioica* leaves were collected from cultivated plant, from suburb of Gorgan, northern Iran (Golestan, Iran) in oct. 2004 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 plant extract:** Powder of *U. dioica* leaves was perculated by hydroalcoholic (60°) solvent for 48 h. The extract was filtered and concentrated under vacuum at 40°C to made a jellies material. In addition to thin layer chromatography and purity tests (foreign matter, total ash, acid insoluble ash and water insoluble ash) for qualification analysis, monosaccharide-linked another reagent assay (spectrophotometry) have been carried out to determine the concentration of polysaccharides in *U. dioica* leaves for standardization of the extract.

The results of phytochemical analysis showed the existence of high percentage of Tannins, Steroids and low levels of flavonoids, carotenoids and saponins in leaves of *U. dioica*.

**Animals:** Male adult albino rats (Wistar strain) of 125-175 g 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 three groups (each including 10 rats).

**Experimental design:** Hyperglycemia was induced with a single intraperitoneally (IP) injection of Streptozotocin (STZ) (80 mg kg⁻¹) to overnight fast rats. Streptozotocin (STZ) purchased from Sigma was dissolved in saline immediately use and intraperitoneally injected (STZ) (80 mg kg⁻¹). Blood samples for glucose measurements were taken from the tail vein. Diabetes was confirmed by measuring the glucose concentration by using Glucometer method.

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

Group I: Normal control group. Saline daily for 5 days.

Group II: Diabetic group. Saline daily for 5 days before STZ injection.

Group III: Treatment Group: animals received 100 mg kg⁻¹ daily hydroalcoholic extract of *U. dioica*, for 5 days, intraperitoneally and then, hyperglycemia induced by intraperitoneally injection of 80 mg STZ.

**Glucose tolerance test:** Intraperitoneal Glucose Tolerance Test (GTT) was performed on 16 h 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 min 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 bouins fluid and paraffin embedded for microscopic examination in accordance with routine laboratory procedures. Histopathologic examination and grading were carried out on Chromalum hematoxiline-phloxine (Gomori, 1941) stained. The number of islets and the number of β-cells of each islet were counted by OLYMPUS microscope and we used OLYSIA software. The islet diameters were measured using calibrated micrometer by taking of fixed number of islet from the two experimental groups and one normal group.

**Statistical analysis:** All the grouped data were statistically evaluated using Student's t-test, expressed as the Mean±SE from ten rats in each groups and λ² test.

**RESULTS**

The mean±SE of blood glucose concentrations before of STZ injection and before administration of *U. dioica* (in treatment group) were 85.5±1.7, 102.9±3.7 and 95.6±3.7 mg dL⁻¹ in control, diabetic and treatment groups, respectively. One week after injection of STZ, the mean±SE of blood glucose concentration were 214.1±23.8 and 277±45.7 mg dL⁻¹ in diabetic and treatment groups, respectively (Table 1).

The effect of STZ in diabetic group showed a gradually increasing of blood glucose level which it was (214.1±23.8) in 1 week and it reached to (454.7±34.5) mg dL⁻¹ in fifth week after injection of STZ.

The mean±SE of Blood Glucose concentration level in treatment group decreased from 309.1±37.4 to 303±100 mg dL⁻¹ in the fifth week.

In control group the mean±SE of Blood Glucose concentration had not any changes. Statistically analysis showed that there was a significant difference between treatment and control groups (p<0.05). Also this difference between diabetic and treatment group was significant (p<0.05).

<table>
<thead>
<tr>
<th>Blood glucose (mg dL⁻¹)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.5±1.7</td>
<td>87.5±1.9</td>
<td>90.2±2.7</td>
<td>99.4±5.0</td>
</tr>
<tr>
<td>Diabetic</td>
<td>102.9±3.7</td>
<td>214.1±23.8</td>
<td>243.5±34.8</td>
<td>454.7±34.5*</td>
</tr>
<tr>
<td>Urtica-Diabetic</td>
<td>95.6±3.7</td>
<td>277.1±45.7</td>
<td>309.1±37.4</td>
<td>303±100 mg dL⁻¹</td>
</tr>
</tbody>
</table>

*The significant difference between group II and group III relative to control in regard to glucose concentration. 1 = Groups, 2 = Treatments
Fig. 1: The result of Glucose Tolerance Test (GTT) that the *Urtica dioica* can cause shift down of GTT curve related to STZ group. [Control: Group (I), STZ: Group (II) and Urtica-STZ: Group (III)]

Table 2: The number of islets, total cells, the number and percents of β cells and islets area (µ) in pancreatic tissues in control, diabetic and treatment groups

<table>
<thead>
<tr>
<th>Islets</th>
<th>Total cells</th>
<th>β-cells</th>
<th>Islets area</th>
<th>P-cells</th>
<th></th>
<th>%</th>
<th>(µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.7±2.2</td>
<td>277.3±17.7</td>
<td>204±15</td>
<td>73.6</td>
<td>589.3±28.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5.8±1.1*</td>
<td>140.9±18.6*</td>
<td>1.7±0.7*</td>
<td>1.9</td>
<td>352.7±36*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>13.0±2.6*</td>
<td>128.3±18.6*</td>
<td>29.4±11*</td>
<td>22.9</td>
<td>399.7±37.4*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In regard to β-cells, differences were shown between group II and III. *(Mean±SE, p<0.001), 1 = Groups, 2 = Treatments*

The same finding was appeared glucose tolerance test such as we had shift-down (recovery) of GTT curve after 4 weeks injection of STZ (Fig. 1).

The histopathologic results depicted in Table 2 and Fig. 2-4.

In this study the number of islets and number of β-cells in pancreas tissue were 14.7±2.2 and 204±15 in control group, respectively. These were 5.8±1.1 and 2.7±0.7 in diabetic groups and 13±2.6 and 29.4±11 in treatment group, respectively.

The percentage of β-cell in control, diabetic and treatment groups were 73.6, 1.9 and 22.9%, respectively. The percentage of β-cells in treatment group with comparing to diabetic group was significant (p<0.05) (Table 2).

**DISCUSSION**

This study showed that the administration of *U. dioica* leaves before induction of hyperglycemia in rats can prevent of severe increasing blood glucose concentration and disruption of β-cells in comparing with no treatment hyperglycemic rats.

The screenings of medical herbs in two studies have not shown the hypoglycemia activity of aqueous extract of *U. dioica* (Neef *et al.*, 1995; Roman Ramos *et al.*, 1992).
Also hypoglycemic activity of *U. dioica* in normal rats has been shown by other researches (Bnouham *et al.*, 2003; Petleveski *et al.*, 2001). Bnouham concluded that anti-hyperglycemic activity of this herb is due to the decrease of transport from small intestine (Bnouham *et al.*, 2003).

Acute and chronic hypoglycemic activity of *Urtica* was demonstrated by Farzami in Iran (Farzami *et al.*, 2003) and Kavalalai in Turkey (Kavalalai *et al.*, 2003). Farzami showed that a fraction from *U. dioica* was a potent stimulator of insulin release of β-cells. Kavalalai tested the effect of lectin fraction of extract of *U. pilulifera* seed for their hypoglycemic activity and found hypoglycemic activity and β-cell regenerative potency with seed of this herb.

In our previous study the leaves extract of *U. dioica* had no hypoglycemic activity and ability to regenerate pancreatic β-cells after four weeks of treatment in streptozotocin-diabetic rats (Golalipour *et al.*, 2006).

In our previous study, the animals probably the β-cells damage is so extensive that no increase in insulin secretion is possible. However, in the present study we show a partial amelioration of β-cell in diabetic rats. If STZ-induced diabetes is reversible, then it could be suggested that the active content of the leaves extract might play a protective role in preventing free-radicals actions, that may destroy β-cells, those would then cause more secretory ability of β-cells this, decrease plasma glucose concentration.

*U. dioica* probably prevented the disruption of β-cells of islets in the pancreas. In recent study, our results indicated that decreased blood glucose concentration by *U. dioica* pretreatment might be due to protective 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 rule out by the results of present study.

STZ produce hyperglycemia in a concentration dependent model by selective β-cell cytotoxic effect (Saini *et al.*, 1999). The mechanism of action of STZ on rodent β-cell is related to uptake of STZ into β-cells and DNA strand breaks which then causes a lethal depletion of NAD, in the β-cells (Swanson-Flatt *et al.*, 1989; Saini *et al.*, 1996).

It has been shown that inhibition of free radical scavenger enzymes and enhancing production of the superoxide radical are the mechanism of STZ on pancreatic β-cells (Swanson-Flatt *et al.*, 1989).

The role of free radicals in disruption of insulin action and impairing glucose tolerance test was shown by Ford (Ford *et al.*, 1996).

Our findings suggest that *U. dioica* may have antioxidant or free radical scavenger properties. Thus, *U. dioica* may have a role in preventing of diabetes or slowing the progress of diabetic disease.

Antioxidant and free radical scavenging properties of *U. dioica* leaves has been established by several studies (Kanter *et al.*, 2005; Cetinus *et al.*, 2005; Mavi *et al.*, 2004). Preliminary phytochemical of the ethanol extract of *U. dioica* was achieved according to the Trease and Evans method (Trease and Evan, 1983). Previous studies showed that antihyperglycemic activity of the *U. dioica* related to active component of *U. dioica* leaves such as flavonoid, peptides and coxmarins (Ellnain *et al.*, 1986; Adamski and Biegonska 1984; Rossikayaya *et al.*, 1985). It has been suggested that the active natural compounds might act by antioxidant and free radical scavenging effect to cause the hypoglycemic activity.

**CONCLUSIONS**

This study showed that the extracts of *Urtica dioica* leaves can prevent from severe increasing of blood glucose concentration and also it protect of β-cells, if it is used before induction of hyperglycemia. Therefore, the usage of this plant can prevent from severity of diabetes in people with background of diabetes.

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