Iranian Journal of Pediatric Society
Volume 1, Number 2, 2007: 25-30

Original Article

Metabolic Effects of Glucose Lowering Substance of Urtica Dioica on Normal and Diabetic Mice, and Patients with Type I Diabetes Mellitus

Gholam Hossein Riazi [1],Maryam Razzaghy-Azar[2], Mohammad Taghikhani[3], Mitra Karimai[3], Masoud Shaabani[1], Seyed Hasan Hasani Komeleh[1], Masoud Yousefi[4]
[1]IBB, Tehran University, [2]Institute of Endocrinology & Metabolism, Iran University of Medical Sciences, [3]Tarbiat Modarres University, [4]Shahid Beheshti University of Medical Sciences, Tehran, Iran.

ABSTRACT

Background: *Urtica dioica is a plant that is used as an edible herb with capability of lowering blood glucose. The aim of this study was to determine the effects of extracted and purified blood glucose lowering substance (BGLS) of Urtica Dioica on mice and then in patients with type I diabetes mellitus.*

Materials and Methods: *The effective substance of the extract was prepared and identified by thin layer chromatography method and then by gel filtration, Fourier transform infra red, mass spectrometry gas chromatography and paper chromatography after acid hydrolysis. Different case control studies were done by this substance on mice. Eight hours before and during the tests all of the mice were fasted. At the first stage 4 separate studies, each in 3 groups of normal mice were done, and blood glucose (BG), liver glycogen, serum insulin and muscle lactic acid were measured after intraperitoneal (ip) injection of BGLS in the first group, distilled water in second group and no injection in the third group. At second stage 20 mice became diabetic by administration of streptozotocin and then divided into two even case and control groups and blood glucose (BG) was measured before and after ip injection of BGLS. At the third stage, BG was measured before and after oral administration of BGLS to normal mice. At fourth stage LD50 of substance was identified in mice and at the 5th stage, in a short 2 day study on 8 patients, the effect of oral administration of one dose of BGLS at 00:07 h on the second day was evaluated.*

Results: *In the first stage, BG of the first group with the mean of 76 ± 5 mg/dL was significantly lower than the second and third groups (108±8 mg/d and 105±9 mg/dL, respectively; p<0.001). Serum insulin level was not significantly different among the 3 groups. Liver glycogen of the 1st group (10.74 ± 0.23 mg/g) was significantly more than the 2nd group (10.08 ± 0.21 mg/g, p = 0.03) and the 3rd group (9.95 ± 0.24, p = 0.016). Lactic acid level of muscles in the 1st group (1.4 ± 0.07 mg/dL) was higher than the 2nd & 3rd group (1.07 ± 0.07 mg/dL and 1.02 ± 0.06 mg/dL respectively) (P < 0.001). In the second stage, BG level was 158 ± 8 mg/dL after 4 hr of ip injection of BGLS in the case group vs 319 ± 14 mg/dL in control group (P < 0.001 . In the third stage). BG decreased from 137 ± 7.1 to 77 ± 17 mg/dL in normal mice after oral administration of BGLS, that was significantly more than decrement in control group (from 146 ± 11 to 120 ± 19 mg/dL) (P < 0.01). In the fourth stage, The amount of LD50 of BGLS was 8.9 mg/ 30 gram mouse. Finally study on patients with type 1 DM resulted in decrement of blood glucose at 2200 h that was significantly lower than the previous day (P=0.001).*

Conclusion: *There is a glucose lowering substance in Urtica dioica that does not increase insulin secretion and can be absorbed through intestinal lumen.*

Key words: *Urtica dioica, Diabetes mellitus, Mice.*

Corresponding author**:** *Maryam Razzaghy Azar, M.D***.**
Address: *Institute of Endocrinology and Metabolism, Behafarin Street, Karimkhan Zand Ave. Firoozgar Hospital*
Tel*: + 98 21 66942903;* ***Fax****: + 98 21 66421054*
Email:*mrazar@iums.ac.ir*

INTRODUCTION

Herbal drugs have been used for treatment of patients with different diseases in many centuries (1, 2). Many drugs in modern medicine have herbal origin. Type 1 diabetes mellitus is one of the most common metabolic diseases in children and its treatment by insulin injection is bothersome for children and their parents. The aim of the present study was to determine the effect of blood glucose lowering substance (BGLS) of Urtica dioica on mice.

MATERIALS AND METHODS

Urtica dioica crude extraction

Fifty grams of dried ground plants was poured in a simple distillation balloon with 1 liter capacity and 200 ml double distilled water was added. After half an hour of soaking, it was heated to evaporation point. Prelim condensed vapor was collected and used in subsequent stages.

BGLS separation and purity determination

At first we used thin layer chromatography (TLC) with plate of silica gel (Merk Co.) and isopropanol and water 5/1 as solvent. After separation of the bands we dissolved them in 1 ml distilled water and microfuged with 10,000 rounds for 5 minutes. Then substances were injected intrapertoneally to mice and we found that one band was effective in decreasing blood sugar of mice. For assessing the purification rate of the band, we used high performance liquid chromatography (HPLC model of Phillips) with reverse phase and C18 = spheriod s50DSL with dimensions 25 X 4.6 mmid column. The amount of injected substance was 20 microliters. The eluent was methanol and water with the ratio of 1/3. The flow rate was 1 ml/min and the temperature was 25°C. The band was not pure, so we used gel filtration chromatography for its purification. It consisted of Sephadex gel (G10) and the eluent was water. Hundred vials were collected and their effectiveness assessed by injections to streptozotocin made diabetic mice (STZ-mice). We found the vials that contained blood glucose lowering substance (BGLS). Then they were examined by spectrophotometer ultraviolet and visible (Uv + Vis) and were injected to 100 STZ-mice. One of them was detected to be effective in lowering blood glucose of STZ-mice. For purification evaluation of this substance HPLC was used again and showed that the substance was pure. For molecular identification we made a special device and dissolved the substance in ether by evaporation technique. White crystalline substance was produced by its dryness. This material studied by fourier transform infrared (FTIR), gas chromatography and mass spectrometry and its molecular weight was determined. Acid hydrolysis and paper chromatography were done (the solvent was acetonitril and ammonium acetate).

Biological studies with BGLS

Streptozocin at high dose (200 mg/kg) was dissolved in monohydrated citric acid and normal saline and was injected to mice intraperitoneally twice with 3 day intervals. After 6 days mice became diabetic (STZ-mice).

Evaluation of the effect of BGLS on blood glucose, serum insulin, liver glycogen and muscle lactic acid in normal mice

Four separate studies were done. In each study 24 normal mice divided into 3 even groups. Their weight and gender were matched. 0.5 ml of the BGLS was injected intraperitoneally (ip) to the first group and 0.5 ml of distilled water to the second group. The third group did not receive anything. Blood glucose level was measured by glucose oxidase kit (Zist Shimi, Iran) before injection (0) and at times 1, 2, and 4 hours after injection. Serum insulin level was measured by RIA (Diagnostic system laboratories DSL, Texas) before and 4 h after injection. Liver glycogen and muscle lactic acid were measured 4 h after injection.

The method of liver glycogen measurement

Liver tissues were put in the tubes contained 2 ml Potas solution (300 g/L) and were heated and shaken for 20 minutes. They were cooled, and then 0.2 ml of saturated sodium sulfate and 5 ml of 95% ethanol were added, respectively. They were centrifuged after 5 minutes cooling. The precipitant was dissolved in 5 ml water and warmed gradually. The volume achieved to 10 ml by adding distilled water. HCl was added to 1 ml of this solution and warmed for 2 hours. Then one drop of red phenol and one drop of NaOH were added until they became yellow. They were diluted with 5 ml of distilled water and glucose was measured by glucose oxidase.

The method of lactic acid measurement

The muscles of sacrificed mice were delivered to aqueous air and 10 ml of 10% trichloroacetic acid (TCA) was added to every one gram of tissue; it was crushed and filtered. By adding TCA to the tubes of standard lactic acid and distilled water, the same volume was made and centrifuged; 0.5 ml of CuSo4 20% and 3.5 ml water were added; then 0.5 g Ca (OH) 2 was poured into the tubes, and were shaken severely. They were put at the room temperature for 30 minutes, and were shaken and centrifuged subsequently. Concentrated sulfuric acid and 4% CuSo4 were added to the tubes. The tubes warmed for 5 minutes then cooled to 20° C and 0.1ml paraphenylphenol was added too. After shaking and maintaining tubes at 30°C for 30 minutes and then in boiling water for 90 seconds and finally at room temperature, specimen's absorption was in 570 nm by spectrophotometer and calculation was performed by this formula:

Lactic acid (mg/dL) = specimen absorption/ standard absorption x 100.

Evaluation of the effect of BGLS on blood glucose of diabetic mice

Twenty STZ-mice divided into 2 even groups. The 1st group received 0.5 ml of BGLS intrapewritoneally(IP) and the 2nd group received 0.5 ml of distilled water IP. Their blood sugar was measured before and 4 hrs after injection.

Evaluation of the effect of orally administered BGLS on blood glucose of normal mice

Twenty mice were divided into 2 groups. After 12 hours of fasting, 1 mg of BGLS was poured directly to the mouth of 1st group and distilled water into the 2nd group. Their blood glucose was measured after 4 hrs and 6 hrs.

Toxicological Evaluation of BGLS

Fifty mice matched for sex, weight and environmental conditions enrolled into the study. They were divided into 5 groups. After 24 hours of food deprivation (exclusively water) fasting, BG was measured. Different doses of BGLS were injected ip (4.99, 6.24, 7.8, 9.75 and 12.18 mg). Every dose was 0.25 more than previous dose. No death occurred 4 hours after the first dose, but mortality rate was 100% after the last dose. According to the method of Reed and Muench LD50 was calculated (3).

Methods of study on patients with type 1 diabetes mellitus

An open label study was performed on 8 patients (4 males, 4 females) with the age range from 7 to 24.6 years (mean ± SD, 13.6 ± 5.3 yrs), and mean duration of diabetes 5.5 ± 4.4 yr (ranging 2-15.5 yr). the exclusion criteria was diabetes of less than 2 years. They were hospitalized for two days. They had three meals and three snacks with the same type and amount, simultaneously in these 2 days. They were put on the regimen of NPH and regular insulin combination half an hour before breakfast and dinner except 2 patients who only received NPH insulin. According to blood glucose monitoring on the first day, the dosage of insulin decreased in some patients on the second day to prevent probable hypoglycemia

due to administration of BGLS (Fig 1). Blood glucose monitoring was done 9 times a day by Glucotrend 2 device (Rosh, Germany) before each meal and 2 hours after finishing the meals plus 15:00, 24:00 and 03:00 hrs. In the morning of the second day, at 07:00 hrs (half an hour before breakfast), 30 ml/M^2 of condensed vapor of urtica dioica without any chemical changes from tubes that was known to have BGLS was administered orally to the patients.

Statistical analysis

Statistical analysis was performed by SPSS software ver. 11. P-value less than 0.05 was considered significant. Two tailed t-student test for animal studies and paired T-test for human study were used for analysis.

RESULTS

Analysis of the extract of Urtica dioica in many stages and study on hundred mice in every stage revealed that there was a blood glucose lowering substance (BGLS) in Urtica dioica. Blood glucose significantly decreased after 4 hours of ip injection of BGLS to normal mice compared to other groups who did not receive this substance ($p<0.001$) (Table 1), but the level of insulin was not significantly different in 3 groups . Increase of liver glycogen in the 1st group was more than 2nd group (P= 0.03) and 3rd group (P=0.016), but lactic acid in muscles of the first group significantly increased comparing to the second group and third groups ($p < 0.001$) (Table 2).

Table 1. Effect of intraperitoneal injection of BGLS on blood glucose in the normal mice

BG* (mg/dL)	1st group	2nd group	3rd group
basal	112 ± 6	105 ± 5	107 ± 7
after 1hr	106 ± 6	107 ± 9	110 ± 12
after 2hr	92 ± 3	104 ± 10	106 ± 13
after 4hr	76 ± 5	108 ± 8	105 ± 9

Table 2. Metabolic effects of BGLS injection on the normal mice

	Insulin (μIU/ml)	liver glycogen (mg/g)	muscle lactic acid (mg/g)
1st group	8.1 ± 2	10.74 ± 0.23	1.4 ± 0.07
2nd group	8.3 ± 3	10.08 ± 0.21	1.07 ± 0.07
3rd group	7.8 ± 1	9.95 ± 0.24	1.02 ± 0.06

* BG: blood glucose. Notice: 1st group, had injection of BGLS, 2nd group, was injected by distilled water and 3rd group, had no injection

Figures 1, 2 and 3 illustrate the differences between the 1st and 2nd group. In STZ-mice, after 4 h of ip injection of BGLS to the cases, blood glucose significantly decreased compared to controls ($P<0.001$), while their basal BG did not show any significant difference (Table 3, Fig. 4).

Table 3. The effect of BGLS injection on blood glucose in the diabetic mice.

BG* (mg/dL)	case	Control
basal	318 ± 10	324 ± 12
after 4hr	158 ± 8	319 ± 14

*BG: blood glucose

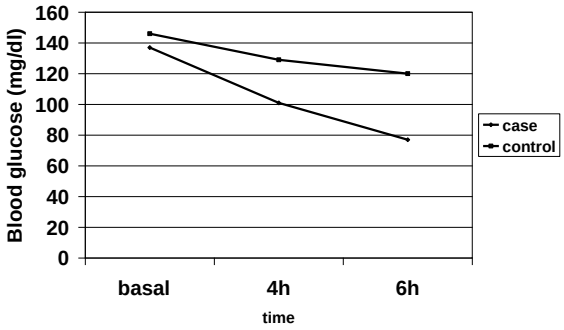

Figure 1. The effect of BGLS on blood glucose in the normal mice

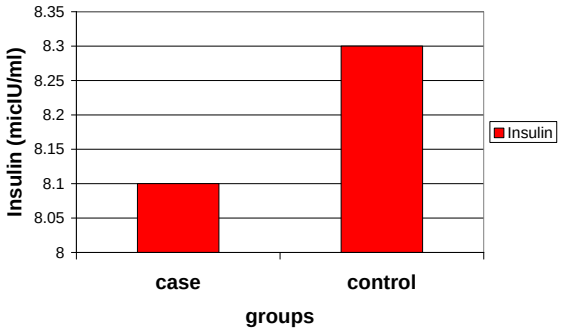

Figure 2. Serum insulin level in the study groups.

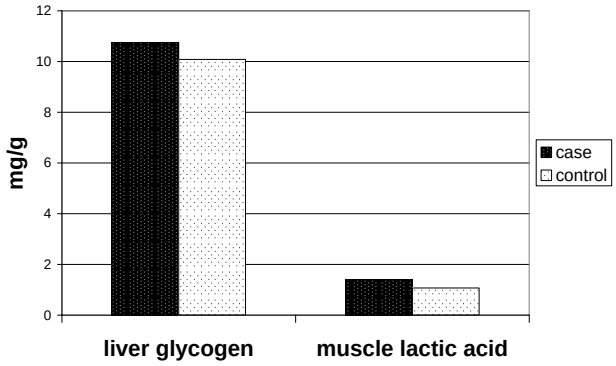

Figure 3. Liver glycogen and muscle lactic acid in the study groups.

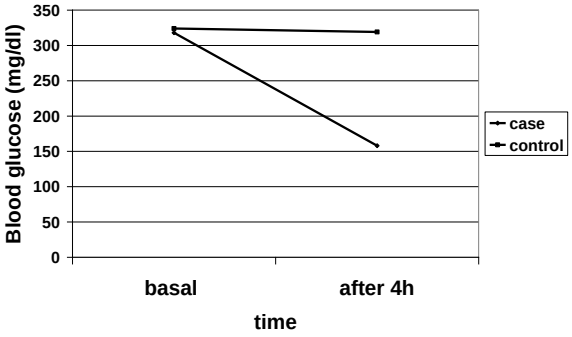

Figure 4. The effect of BGLS on blood glucose of the STZ-mice

In case group, oral administration of BGLS decreased blood glucose after 6 hours, which was significantly lower than blood glucose of control group ($p<0.01$), but basal glucose was not significantly different in two groups (Table 4, Figure 5).

Table 4. The effect of oral administration of BGLS on blood glucose in the normal mice

BG * (mg/dl)	Cases	Controls
Basal	137 ± 7.1	146 ± 11
after 4hr	101 ± 12	129 ± 23
after 6hr	77 ± 17	120 ± 19

* BG: blood glucose

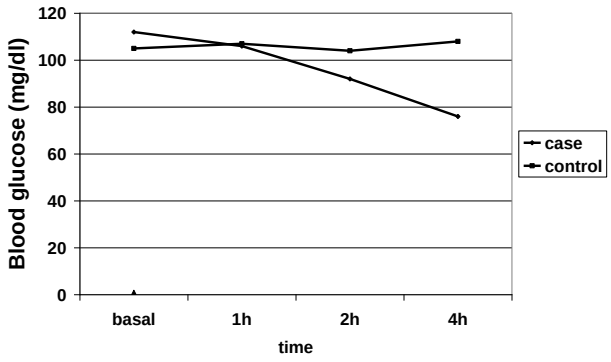

Figure 5. The effect of oral administration of BGLS on blood glucose in the normal mice

LD50 of BGLS

The results of the study about LD50 of BGLS showed that it was 8.9 mg for a mouse weighted 30 gram.

Results of the human study

In the patients with type 1 diabetes, blood glucose decreased to 183.5 ± 82 mg/dl at 22:00 o'clock on the 2nd day that was significantly lower than the blood glucose at the same time on the 1st day (291.3 ± 70 mg/dl) (P=0.001) (Figure 6). The blood glucose was not significantly different at other times during these 2 days.

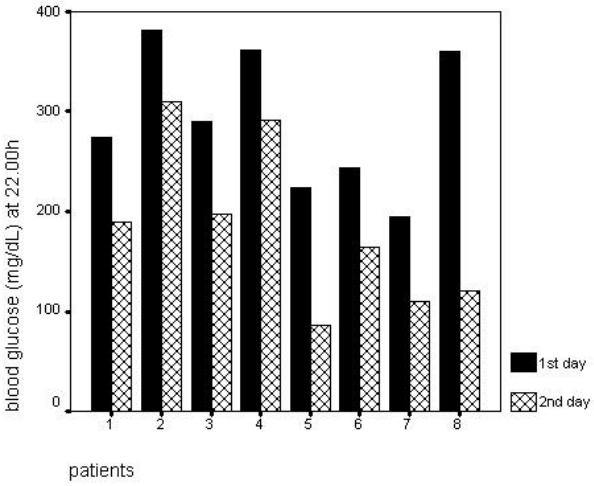

Figure 6. Blood glucose level of every patient at 2200h on 1st day, and 2nd day after oral administration of BGLS.

DISCUSSION

One of the plants that have been traditionally used for diabetes mellitus is Urtica dioica (1). It is named nettle in English and belongs to the family of urticaceae. This plant has different species included large nettle, small nettle and Urtica pilulifera. Large nettle has stinging hairs and its leaves are opposite to each other and are deeply indented and sharp. Urtica dioica has been used for allergy, prostatic hyperplasia and many different disorders. It has been analyzed by different investigators and is considered as a nutrient and is used for different disorders as well as diabetes mellitus(1,2,4-6). In the present study BGLS of Urtica Dioica could lower blood glucose level both by parenteral and oral administration, so its effect is not by delay or prevention of glucose absorption and should have an effect on glucose usage of the cells and it can be absorbed by intestinal canal. Furthermore, it does not increase insulin secretion, so its effect is not induced by this mechanism. In our study, BGLS increased glycogen in the liver, an effect that is similar to insulin and it increased lactic acid in muscles that can be the result of glycolysis. A low LD50 of BGLS in the present study permits its use as a nutrient as reported before (7). In the human study the effect of BGLS appeared after 15 hours, so frequent administration can imitate the role of basal insulin. More investigations should be done to find the mechanism of BGLS function and its exact structure, but this study revealed the glucose lowering effect of a plant that can lead to provide a drug for treatment of type 1 diabetes mellitus.

CONCLUSION

There is a glucose lowering substance in Urtica dioica that can be absorbed from intestinal canal, so can be used orally.

REFERENCES

1. Swanston Flatt SK, Day C, Flatt PR, Gould BJ, Bailey CJ. 1989 Glycemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetes Res. 1989 Feb; 10(2): 69-73.
2. Paul Mittman. Randomized, double - blind study of freeze - dried Urtica dioica in the treatment of allergic rhinitis. ***Planta Medica*** 1989; 56:44-47.
3. Zbinden G, Flury-Roversi M. Significance of the LD50-test for the toxicological evaluation of chemical substances. ***Arch Toxicol*** 1981; 47 (2): 77- 99.
4. Hryb - DJ, Khan MS, Romaso NA, Rosner W. The effect of extracts of the root of the stinging nettle on the interaction of SHBG with its receptor on human prostatic membranes. ***Planta Medica*** 1995; 61:31-2.
5. Kraus R, Spiteller G. Phenolic compounds from roots of urtica dioica. ***Phytochemistry*** 1990; 29: 1653-1659.
6. Matai CK, Talalaj S, Njoroge D, Wamugunda R. Effect of extract of hairs from the herb Urtica massaica, on smooth muscle. ***Toxicon*** 1980; 18: 225-9.
7. Wetherilt H. Evaluation of Urtica dioica species as potential sources of important nutrient. ***DevFood Sci*** 1992; 29:15-25.