Original Article

# *Urtica dioica* (Gazaneh) Distillate Restores Glucose Metabolism in Diabetic Rats

**Fahimeh Zamani-Garmsiri[#], Masoumeh Akmali[#], Ali Gohari, Fatemeh Zal, Atefeh Seghatoleslam**

*Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*[#]Contributed equally*

## Abstract

**Background:** Diabetes has become an important health problem in the world. Natural agents, with antidiabetic property, are potential candidates for improving diabetes. *Urtica Dioica* Distillate (*UDD*) or Araghe Gazaneh is widely used for the treatment of diabetes as per traditional medicine. Despite the tremendous use of *UDD* as an antidiabetic compound in folk medicine, the antidiabetic effects of *UDD* has been neglected by medical scientists. In this study, we aimed to evaluate the effects of *UDD* on the glucose metabolism in diabetic rats. **Methods:** A total of 24 male rats were divided equally into four groups, two treatment and two control groups, each containing normal or Streptozotocin (STZ)–induced diabetic rats. During 4 weeks, control and treatment rats received water or *UDD*, respectively. Fasting blood sugar (FBS), HbA1c, serum creatinine, blood urea nitrogen, and specific activities of hepatic enzymes including glucokinase (GK), hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), and muscle glucose transporter type 4 (GLUT4) and liver phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels were measured. **Results:** FBS and HbA1c increased in diabetic groups. Treatment with *UDD* significantly lowered FBS and prevented weight loss. Decreased FBS level was associated with higher activity levels of GK and HK in *UDD*-treated diabetic rats. G6PD-specific activity decreased in diabetic control rats compared to nondiabetic ones, but *UDD* treatment improved it to the normal levels. A significant decrease in the expression level of GLUT4 was observed in diabetic control rats compared to nondiabetic ones, but *UDD* increased it to the normal levels. **Conclusions:** These findings suggest that *UDD* might exert therapeutic effects against diabetes by improving glucose metabolism and can be used as an alternative or complementary medicine for the treatment of diabetic patients.

**Keywords:** *Diabetes mellitus, diabetic rats, hepatic enzymes, Urtica Dioica distillate*

## Introduction

Traditional Iranian medicine includes a diversity of knowledge and practice used for health maintenance and diagnosis, prevention, and management of disorders.[1] World Health Organization has emphasized that the combination of traditional medicine, as a complementary and/or alternative medicine with conventional drugs, is a suitable, safe, and effective strategy to solve some health issues.[2]

Given the severe long-term complications, diabetes has become a serious problem in modern countries.[3] The increase in the prevalence of diabetes from 2010 to 2030 is predicted to be 69% in developing countries and 20% in developed countries.[4] Treatment of diabetes mellitus using traditional plants as alternative medicines has been reported to have lower side effects.[3,5]

*Urtica dioica (U. dioica)* belongs to the *Urticaceae* family which has been introduced as a hypoglycemic medicinal plant by Avicenna in ancient medicine.[6] The hypoglycemic properties of the plant have been already studied[7] and its antidiabetic effects are also well known in folk medicine.[8,9] There are several methods for using plants in herbal therapy mostly including extraction, purification, fractionation, concentration, fermentation, and distillation.[10] The oldest method is water distillation. It is the most appropriate method to use fresh and dry plant parts which are not damaged by direct heating.[11] Plant distillates (also recognized by other names such as herbal/essential/floral water, hydrolate, and hydrosol) are currently being used in Turkey, Egypt, and Iran widely.[10] The distillate obtained from different medicinal plants (locally named Aragh) is known as a common beverage

**How to cite this article:** Zamani-Garmsiri F, Akmali M, Gohari A, Zal F, Seghatoleslam A. *Urtica dioica* (Gazaneh) distillate restores glucose metabolism in diabetic rats. Int J Prev Med 2023;14:68.

***Address for correspondence:***
*Dr. Atefeh Seghatoleslam, P. O. Box: 71348-45794, Shiraz, Iran.*
*E-mail: seghatolea@sums.ac.ir; seghatoleslama@yahoo.com*

**Access this article online**

**Website:** www.ijpvmjournal.net/www.ijpvm.ir

**DOI:** 10.4103/ijpvm.ijpvm_293_21

**Quick Response Code:**

in different parts of Iran, mostly used to relieve health problems.[12,13]

Despite the antidiabetic effects of *U. dioica* extract,[6-8] a previous study has shown that there are some side effects for the extract of this plant such as nephrotoxicity and hepatotoxicity.[14] On the other hand, folk medicine has targeted the use of herbal distillates in therapeutic interventions; their pharmaceutical and side effects have not yet been studied and tailored to the need by medical researchers. Therefore, the present study aimed to investigate the effect of *UDD* on weight gain, blood glucose, glycosylated hemoglobin (HbA1c), serum creatinine, and blood urea nitrogen (BUN) and the specific activity of the hepatic enzymes involved in glucose metabolism such as glucokinase (GK), hexokinase (HK), and glucose-6-phosphate dehydrogenase (G6PD) in streptozocin (STZ)–induced diabetic rats. The expression levels of the muscle glucose transporter type 4 (GLUT4) and liver phosphoenolpyruvate carboxykinase (PEPCK) genes were also evaluated in the experimental rats.

## Materials and Methods

### Preparation of *UDD*

*U. dioica* plant was collected from the jungles around Sari (Safar Abad region, at 2014 May), Mazandaran province, Iran, and identified by Sari Agricultural Sciences and Natural Resources Office. To obtain 1 liter of *UDD*, we washed 3.75 Kg of the fresh plant, put it into the upper partition of the steam boiler, and added 1.5 liters of water. The procedure was performed as described by Seghatoleslam *et al*.,[13] keeping the temperature at 65°C-70°C. The steam passing through the condenser duct was cooled and *UDD* or Aragh was collected and conserved at 4°C until use.

### Experimental animals

Eight-twelve–week-old male Sprague–Dawley rats (200-250 g) were provided. The animals were kept under standard conditions, 24 ± 2°C, 12 h light/dark cycle.[15] The rats (n = 24) were randomly divided into four groups of six, including nondiabetic control (group I), *UDD*-treated nondiabetic (group II), diabetic control (group III), and *UDD*-treated diabetic (group IV). Table 1 displays a summary of initial weights and initial fasting blood sugar (FBS). For the randomization process, four empty cages were provided and named as the first, second, third, and fourth groups. Then, the 12 nondiabetic rats were randomly but equally divided into the first and second cages, and the 12 diabetic rats were placed in the same way into the third and fourth cages, accordingly. Diabetes was induced in overnight-fasting rats by a single intraperitoneal (IP) injection of STZ (50 mg/kg) (Sigma Chemical Company, St. Louis, MO, USA) dissolved in a vehicle (0.1 M citrate buffer, pH 4.5),[16] whereas the rats in nondiabetic groups only received the vehicle. Fasting blood glucose was determined 72 hours after STZ injection in tail vein blood samples by an Accu-Check Active glucometer (Roche Diagnostics GmbH, Hannheim, Germany).[17] Diabetes was confirmed by fasting blood glucose levels >250 mg/dL. The rats in groups II and IV received 12.5 mL/kg/day (once a day) *UDD* via intragastric gavage and groups I and III were administered 12.5 mL/kg/day distilled water for 27 days.[18] During 4 weeks of the study, all rats received the same diet (standard chow diet). The body weight and FBS of the animals were measured every 9 days (0$^{th}$, 9$^{th}$, 18$^{th}$, and 27$^{th}$). On the 28$^{th}$ day, the nonfasting rats were anesthetized by $CO_2$ and blood samples were collected in EDTA-containing tubes by heart puncture to evaluate HbA1c. The liver and skeletal muscle (soleus muscle) tissues were also isolated and stored at -70°C until use.

**Table 1: Baseline characteristics**

| Parameters<br>Groups | Body weight (gr) | Glucose (mM) |
|---|---|---|
| Non-diabetic control | 229±5.05 | 107.28±5.58 |
| Non-diabetic + *UDD* | 224.83±5.19 | 100.26±4.86 |
| Diabetic control | 218.75±4.08 | 97.92±10.86 |
| Diabetic + *UDD* | 225.00±5.4 | 108.18±11.74 |

Data are represented as mean±SEM (*n*=6)

### Measurement of HbA1c, creatinine, and BUN

The level of HbA1c in the whole blood was determined by a kit-based enzymatic and colorimetric assay (Diazyme Laboratories, USA).[19] Serum creatinine and BUN were determined using Man Company Kit.

### Hematoxylin and eosin (H & E) staining

After fixing in 10% (v/v) buffer formalin and dehydrating in a graded alcohol series, we embedded the liver specimens in paraffin, cut them at 5-μm sections, and stained them with H & E. The slides were, then, examined by a pathologist and the results were analyzed.

### Assessment of enzyme activities

To determine the total HK and GK activities, we minced 1 gram of frozen liver tissue and homogenized it at 4°C in 9 mL of 50 mM HEPES buffer, pH 7.4 (Sigma Chemical Company, St. Louis, MO, USA), using Potter Elvehjem Homogenizer. The supernatant was prepared by centrifugation of the homogenate at 12,000 × g for 1 hour at 4°C for further enzyme assays. The activities of GK and total HK were determined based on the coupled enzyme assay of Davidson and Arion[20] and Ferre *et al*.[21] The formation of NADH was measured by the increase in the absorbance at 340 nm in 25°C to evaluate the enzyme activity.[22]

To investigate the level of G6PD activity, we homogenized the liver samples in 4 volumes of cold 0.154 M KCl. The

homogenate was centrifuged at 27,000 × g for 25 minutes at 4°C and the supernatant was collected for enzyme assay.[22,23] G6PD activity was measured as per the method of Bottomley *et al*.,[24] using glucose 6-P and NADP+ as substrates.[25] Enzyme-specific activities were expressed in mU per mg protein which was determined by the Bradford method.[26]

### Evaluation of gene expression

Liver and muscle tissues were homogenized in BIAZOL total RNA extraction reagent (Bioflux, Japan). Total RNA was extracted as per the manufacturer's instructions and cDNA was synthesized using reverse transcriptase (Thermo Fisher Scientific Company, USA). The levels of GLUT4 and PEPCK mRNA expression were assessed in the skeletal muscle and liver by RT-PCR, using ABI-applied Thermal Cycler (ABI 7500 real-time PCR system, USA) and Eva Green PCR Master Mix (Yekta Tajhiz Azma, Iran), respectively.

All amplifications were performed as duplicates for each sample. Primer sequences were as follows: β-actin: F-5′CCACACCCGCCACCAGTTCG-3', R-5'CTAGGGCGGCCCACGATGGA-3', PEPCK: F-5'CAGGAAGTGAGGAAGTTTGTGGAA-3', R-5'GGAGCCGTCGCAGATGTGAATA-3', GLUT4: F-5'ATGTTGCGGATGCTATGG-3', R-5'TTAGGAAGGTGAAGATGAAGAAG-3'. Expression levels were normalized to β-actin and relative levels were calculated by $2^{-\Delta\Delta Ct}$.

### Statistical analysis

The data were expressed as mean ± SEM as per the statistics consultant's suggestion. Statistical analysis was performed using SPSS 24 and the graphs were designed by GraphPad prism 8. As the values were not normally distributed, the nonparametric Kruskal-Wallis and Mann-Whitney tests were used for a pairwise comparison. $P < .05$ was considered statistically significant.

## Results

### Effect of *UDD* on FBS

To evaluate the possible effects of *UDD* on STZ-induced diabetes, we measured the FBS levels in the experimental rats during the treatment period. As shown in Table 2, the diabetic rats showed a significant increase ($P < .01$) in the FBS levels in a time-dependent manner compared to nondiabetic rats. The treatment of diabetic rats with *UDD* significantly decreased the FBS levels ($P < .01$) up to the day 9 and kept it without significant changes until the day 27. However, the FBS level in nondiabetic rats was not changed by the administration of *UDD*.

### Effect of *UDD* on the level of glycosylated hemoglobin

STZ-induced diabetes resulted in a significant increase in HbA1c levels compared with those in the nondiabetic rats ($P < .01$). As per our data, the treatment of diabetic animals with *UDD* did not exert any significant changes on HbA1c levels compared to the diabetic control rats. The level of HbA1c was not changed significantly in *UDD*-treated nondiabetic rats compared to the nondiabetic control ones [Table 3].

### Effect of *UDD* on the level of serum creatinine and BUN

To investigate the effect of *UDD* administration on renal function, we measured the levels of creatinine and BUN. STZ administration elevated creatinine and BUN levels to 0.65 ± 0.02 and 29.37 ± 1.66 mg/dL, respectively, compared to their levels in nondiabetic control (0.55 ± 0.02 and 18.83 ± 0.98 mg/dL, respectively) [Table 4]. Treatment with *UDD* decreased the levels of BUN in the diabetic group compared to the control diabetic group, but this decrease was not statistically significant. Notably, creatinine and BUN levels of nondiabetic rats after treatment with *UDD* did not significantly differ from the levels in the nondiabetic control group.

### Histopathological findings

Microscopically, the livers of nondiabetic control rats displayed the normal histological structure of hepatic lobules, as shown in Figure 1. There was no abnormal histopathological finding in the diabetic rat liver under the experimental condition, which was similar to the previous study.[27] Notably, nondiabetic and diabetic rats who received the *UDD* showed apparently normal liver architecture with no histopathological changes.

### Effect of *UDD* on the weight gain of the experimental rats

In Figure 2a, the body weight changes are shown after injection of STZ or treatment with *UDD*. The average weight differences between the first day and the 27th day in each

**Table 2: Effects of *UDD* on Blood Glucose levels (mg/dl) in the experimental rats**

| Days<br>Groups | 0 | 9 | 18 | 27 |
|---|---|---|---|---|
| Nondiabetic control | 109.08±5.22 | 88.38±3.42 | 87.12±8.46 | 90.9±3.78 |
| Nondiabetic + *UDD* | 105.84±6.66 | 92.16±0.0 | 99.18±7.92 | 100.08±4.32 |
| Diabetic Control | 328.86±32.58 | 450.36±33.66## | 441.54±17.64## | 537±22.86## |
| Diabetic + *UDD* | 410±17.82 | 249.3±48.06** | 258.12±50.4** | 256.14±41.4** |

Data are represented as mean±SEM ($n$=6). ##$P$<0.01 vs. nondiabetic control rats. **$P$<0.01 vs. diabetic control. *UDD: Urtica Dioica*Distillate

experimental group are shown in Figure 2b. As expected, the body weight was significantly decreased in the diabetic rats compared to the nondiabetic control group after 27 days ($P$ = 0.002). The results showed that treatment with *UDD* significantly reversed these changes ($P$ = 0.004). However, *UDD*-treated nondiabetic rats revealed no significant increase in the body weight compared to the nondiabetic control ones.

### Effects of *UDD* treatment on the specific activities of hexokinase, glucokinase, and G6PD in the liver

To investigate the effectiveness of using *UDD* on glucose metabolism, we measured the specific activities of hepatic HK, GK, and G6PD in the experimental groups. When compared with nondiabetic group, the hepatic specific activities of these enzymes were significantly lowered ($P$ = 0.002 for HK and GK and $P$ = 0.003 for G6PD) in the diabetic group [Figure 3a-c]. After 4 weeks of *UDD* administration, the hepatic HK-specific activity of nondiabetic ($P$ =.01) and diabetic groups ($P$ =.045) was significantly increased compared to the control groups. As illustrated in Figure 3b, *UDD* consumption had no significant effect on the hepatic GK activity in nondiabetic rats, but it significantly increased the GK activity ($P$ =.045) in the diabetic rats. *UDD* administration also significantly elevated the G6PD activity in diabetic rats ($P$ =.005) compared to diabetic control ones. Meanwhile, an increase in the G6PD activity in the *UDD*-treated normoglycemic rats was observed which was not statistically significant under the experimental conditions.

### Effect of *UDD* on GLUT4 gene expression in the skeletal muscle

To further insight into the molecular mechanisms of the antidiabetic effects of *UDD*, we assayed the expression

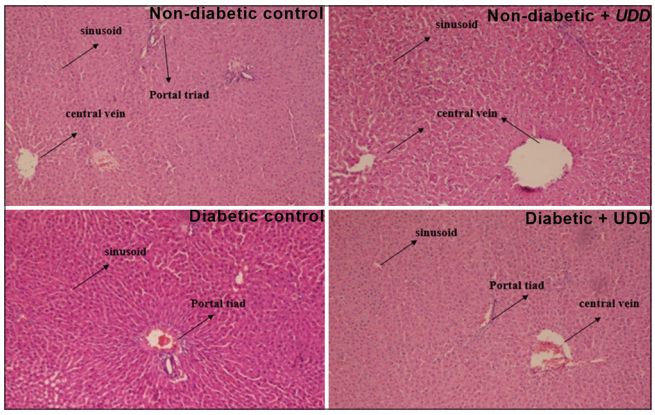


**Figure 1: Effect of *UDD* on the liver tissue. H and E staining for liver sections (X200 magnification). The livers of nondiabetic control rats displayed the normal histological structure of hepatic lobules. Diabetic rats showed apparently normal liver architecture with no histopathological changes. There was no abnormal histopathological finding in the diabetic rat liver who received the *UDD***

**Table 3: Effects of *UDD* on HbA1c levels in four study groups**

| Experimental group | HbA1c (%) |
|---|---|
| Nondiabetic control | 3.78±0.26 |
| Nondiabetic + *UDD* | 3.56±0.09 |
| Diabetic control | 6.41±0.18$^{\#\#}$ |
| Diabetic + *UDD* | 6.66±0.56$^{\#\#}$ |

Data are represented as mean±SEM ($n$=6). $^{\#\#}P$<0.01 vs. nondiabetic control rats

**Table 4: Effects of *UDD* on creatinine and BUN levels in all experimental groups**

| Parameters<br>Groups | Creatinine (mg/dl) | BUN (mg/dl) |
|---|---|---|
| Nondiabetic control | 0.55±0.02 | 18.83±0.98 |
| Nondiabetic + *UDD* | 0.58±0.01 | 18.33±1.22 |
| Diabetic control | 0.65±0.02$^{\#}$ | 29.37±1.66$^{\#\#}$ |
| Diabetic + *UDD* | 0.68±0.01$^{\#}$ | 26.00±1.43$^{\#}$ |

Data are represented as mean±SEM ($n$=6). $^{\#}P$<0.05 vs. nondiabetic control rats , $^{\#\#}P$<0.01 vs. nondiabetic control rats

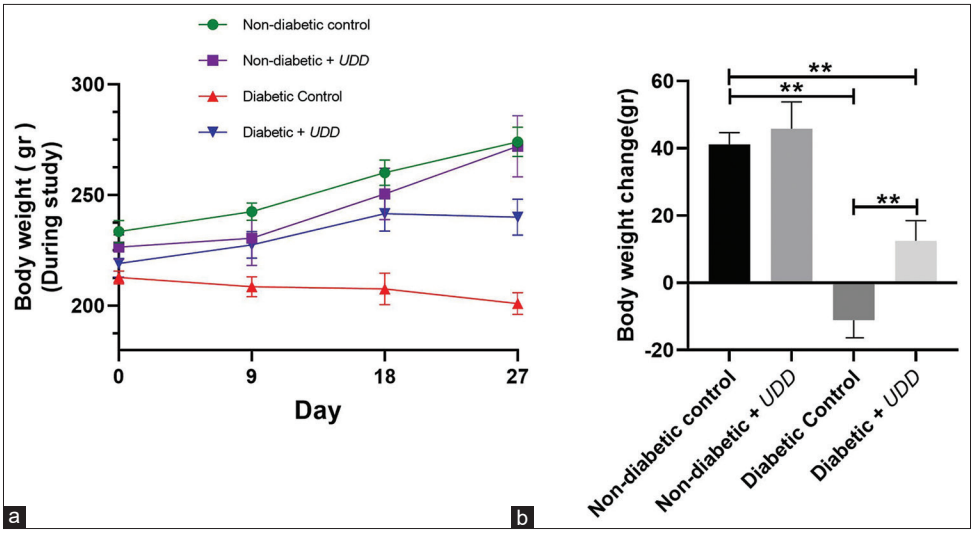


**Figure 2: Effect of *UDD* on the body weight in experimental rats. The changes in the body weight during the study (a) and the average weight differences between the first day and the 27$^{th}$ day (b) are presented. Data are represented as mean ± SEM (n = 6). $^{**}P$ < .01**

levels of muscle GLUT4. As shown in Figure 4a, the expression of GLUT4 was lower in the muscle tissue of the diabetic control group compared to the nondiabetic control group ($P$ =0.04). The administration of *UDD* increased the GLUT4 gene expression of diabetic rats. However, as shown in Figure 4a, this increase was not significant ($P$ = 0.58). No significant change was also observed in the expression of GLUT4 gene in nondiabetic rats treated with *UDD* compared to nondiabetic control ones ($P$ = .88).

### Effect of *UDD* on PEPCK gene expression in the liver

To analyze the efficacy of *UDD* on gluconeogenic-related genes, we measured the mRNA levels of PEPCK. As per our data which are shown in Figure 4b, an increased expression of PEPCK was detected in the muscle of diabetic rats compared to nondiabetic control ones; however, the increase was not statistically significant. Furthermore, administration of *UDD* in diabetic rats did not reveal any significant decrease in the PEPCK gene expression.

## Discussion

In the folk medicine of various countries, *U. dioica* is considered to possess different therapeutic effects. Previous studies have described the hypoglycemic properties of this plant through the improvement of glucose intolerance,[28] and enhancement of insulin secretion and pancreatic function.[7,23,24]

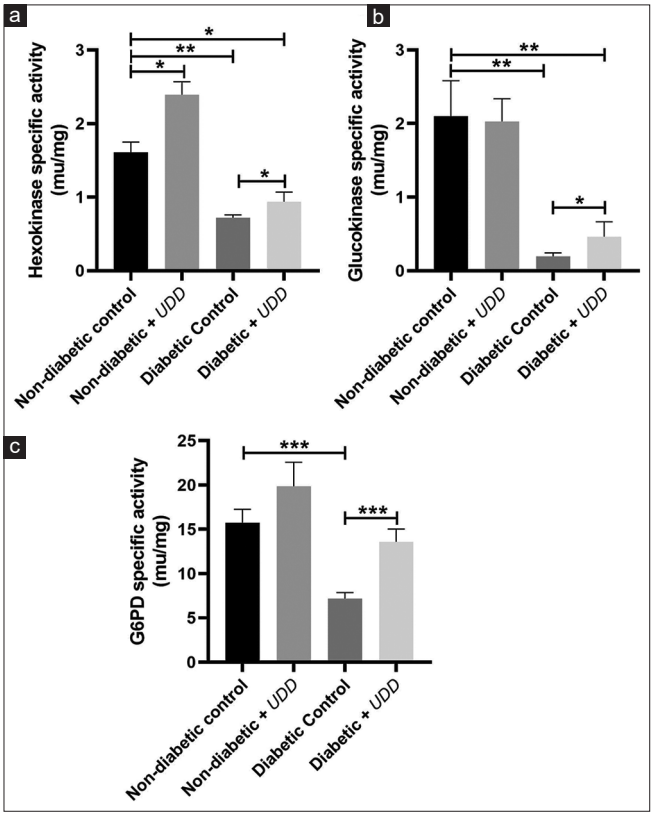


**Figure 3: Effect of *UDD* on the specific activities of hexokinase (a), glucokinase (b), and G6PD is shown in the liver. Data are represented as mean ± SEM (n = 6). ***$P$ <.001, **$P$ <.01, *$P$ <.05**

The possible mechanisms of *U. dioica* antidiabetic activities could be classified into pancreatic and extra-pancreatic mechanisms. Pancreatic mechanisms could function through stimulation of insulin release from β-cells[29] and/or the regenerations of beta cells.[30] Extra-pancreatic mechanisms might work through inhibiting glucose intestinal absorption,[31] alpha-amylase activity,[32] and also forming unique glucose penetrable pores to facilitate the glucose uptake.[33]

However, although antidiabetic effects of *U. dioica* extract are documented by many studies,[6-8] the hypoglycemic properties and the potential toxicity of *UDD* have not been examined yet. The widespread use of herbal distillates in traditional medicine makes it a necessity for further study to improve their clinical application.

There is evidence showing that STZ-induced diabetes leads to pancreatic islets destruction, reduction of insulin secretion, and hyperglycemia.[18] Conversely, *U.dioica,* specially its extract, is known for having antidiabetic properties. In this study, we showed that *UDD* consumption decreased the FBS levels by about 50% without any effects on the levels of HbA1c. Our result is in agreement with that of Qujeq *et al*.'s[7] and Golalipour *et al*.'s[34] studies, reporting that the administration of *U. dioica* extract in diabetic rats increased the secretion of insulin by β-cells. Furthermore, our previous reports showed that the *UDD* could induce β-cell regeneration and increase the serum insulin level in STZ-induced diabetic rats.[18] Therefore, the results of our experiments not only confirmed the previous results but also revealed a mechanism for the hypoglycemic effect of *UDD*. Measurements of HbA1c are used both as an index of mean hyperglycemia and a risk factor for the development of diabetic complications.[35] However, the ameliorative effects need a longer time to be revealed at the level of HbA1c.

Studies have shown that diabetes is associated with a reduction in GK.[21] GK regulates the rate of glucose metabolism in the liver.[36] To further determine the

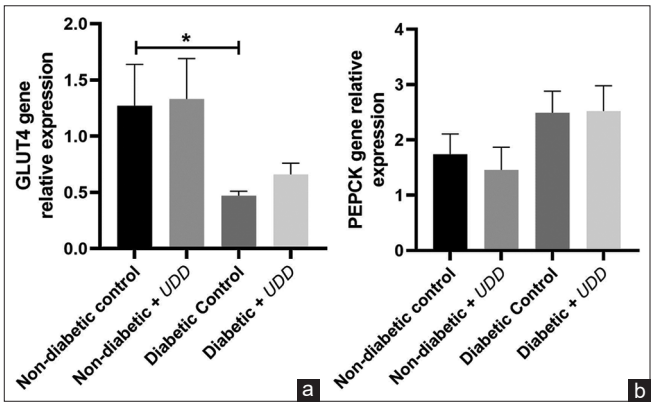


**Figure 4: Effect of *UDD* on GLUT4 gene expression in muscle (a) and PEPCK gene expression (b) in the liver is presented. Values are expressed as mean ± SEM (n = 6). *$P$ <.05**

mechanism of the antidiabetic effects of *UDD*, the specific activity of GK and HK was assessed in the liver tissues. Our data demonstrate that the *UDD* inhibits a diabetes-induced decrease in the specific activity of hepatic enzymes, suggesting that the *UDD* administration exerts the antidiabetic effect by activation of major hepatic enzymes involved in the glucose metabolism.

Regarding to distillate was used in recent study, which ingredients might be responsible for these findings. G6PD, the rate-limiting enzyme in the pentose phosphate pathway, has an important role in the antioxidant defense system by producing NADPH which regulates the cellular redox state against oxidative stress by maintaining reduced glutathione and $H_2O_2$ detoxifying enzyme of glutathione reductase.[25,37-39] Data showed that the levels of G6PD were increased about 1.9 folds after treatment with *UDD* in STZ-induced diabetic rats. In the present study, creatinine and BUN levels were not changed in nondiabetic rats after treatment with *UDD*. Meanwhile, *UDD* administration did not produce alterations in the hepatocytes, sinusoids, and the portal triads. Therefore, under experimental conditions, no nephro/hepato toxicity was observed in the rats who received *UDD*. The activities of HK and G6PD were also increased in *UDD*-treated normal rats, indicating its antioxidant effects. These findings are in agreement with those of Golalipour's study reporting that *U. dioica* extract has antioxidant activities and free radical scavenging properties.[40] However, this outcome is not in accordance with the results of Güneş *et al*.'s[14] study which revealed that *U. dioica* extract not only had no effect on the diabetes but also had toxic effects on the kidneys and liver. The discrepancy between the latter study and ours might be due to the differences in the dose and duration of the treatment and the selection of the different methods of herbal drug preparations (i.e., distillate vs. extract).

Insulin resistance in the muscle is formed by a glucose transport defect. The main insulin-sensitive glucose transporter in the muscle is the insulin-sensitive GLUT4, which removes glucose from the circulation followed by insulin stimulation.[41] In a study by Kadan *et al*.,[42] it was revealed that exposing L6-GLUT4 myc cells to *U. dioica* extract almost doubled the GLUT4 translocation and improved about 1.6 folds in the insulin-stimulated state which was approximately in line with the result of our study in the diabetic group.

PEPCK is a key enzyme of gluconeogenesis, a primary metabolic pathway leading to the production and release of glucose in the liver. At the gene transcription level, insulin downregulates the mRNAs encoding PEPCK.[43] Previous investigations have indicated that oxidative stress impairs the hepatic PEPCK gene expression by an insulin-independent mechanism. In a parallel study, we showed that *UDD* had antioxidant activities (data not published yet). Our data showed that *UDD* administration had no significant effects on PEPCK gene expression in the liver but increased the GLUT4 gene expression in the diabetic rat muscles compared to diabetic rats not receiving *UDD*; however, this increase was not statistically significant. Although there was no evidence of *UDD* impact on the PEPCK gene expression in the literature, perhaps prolonged treatment with *UDD* could be more effective.

This study had potential limitations which include the use of a limited number of male Sprague–Dawley rats as an animal model of diabetes induced by STZ. In addition, there is a possibility that the observed effects of *UDD* in animals differ from how humans would present. In the future investigations, we will aim to identify the most effective and safe dose for human consumption and evaluate the effect of *UDD* on diabetes at the clinical research phase using larger population samples. However, in the category of plant distillate consumption by human, the standardization, dosage, efficacy, and the stability of the distillates require further investigation.

## Conclusion

This article is the first report of hypoglycemic effects of *UDD* or Aragh Gazaneh administration on diabetic rats. The investigation on the actual chemical components of *UDD* is under way; meanwhile, it is known that herbal distillates contain essential oil compounds and organic acids and other water soluble components.[18] Further experiments are also necessary to evaluate the various aspects of the antidiabetic effects of *UDD* and the active constituents involved in the observed effects.

The last but not the least, it can be concluded that the administration of *UDD* in STZ-induced diabetic rats not only lowered the plasma glucose but also improved the liver carbohydrate metabolism through increasing the specific activities of the related key enzymes. Therefore, due to the beneficial effects and lack of toxicity under experimental conditions, it could be recommended as a complementary or alternative medicine to reduce blood sugar in diabetic patients. Moreover, simultaneous administration of *UDD* and antidiabetic drug might be regarded by the physicians to avoid synergic effects.

### Ethics statement

The study protocol was approved by the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978) modified by the Institutional Animal Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC).

### Abbreviations

FBS, Fasting blood sugar; GK, glucokinase; G6PD, Glucose-6-phosphate dehydrogenase; GLUT4, Glucose transporter type 4; HK, hexokinase; PEPCK, Phosphoenolpyruvate carboxykinase; STZ, Streptozocin; UDD, *Urtica Dioica* Distillate.

## Financial support and sponsorship

The present article was extracted from the MSc thesis written by Fahimeh Zamani-Garmsiri and supported by grant number 93-7232 from the Office of the Vice Chancellor for Research, Shiraz University of Medical Sciences, Shiraz, Iran.

## Conflicts of interest

There are no conflicts of interest.

**Received:** 27 Jun 21 **Accepted:** 27 Oct 22
**Published:** 27 May 23

## References

1. Rezaeizadeh H, Alizadeh M, Naseri M, Ardakani M. The traditional Iranian medicine point of view on health and disease. Iran J Public Health 2009;38:169-72.
2. Bodeker G, Ong C-K. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. World Health Organization; 2005.
3. Coman C, Rugina OD, Socaciu C. Plants and natural compounds with antidiabetic action. Not Bot Horti Agrobotanici Cluj-Napoca 2012;40:314-25.
4. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14.
5. Maroo J, Vasu VT, Aalinkeel R, Gupta S. Glucose lowering effect of aqueous extract of Enicostemma littorale Blume in diabetes: A possible mechanism of action. J Ethnopharmacol 2002;81:317-20.
6. Kavalalı G, Tuncel H, Göksel S, Hatemi H. Hypoglycemic activity of Urtica pilulifera in streptozotocin-diabetic rats. J Ethnopharmacol 2003;84:241-5.
7. Qujeq D, Tatar M, Feizi F, Parsian H, Faraji AS, Halalkhor S. Effect of Urtica dioica leaf alcoholic and aqueous extracts on the number and the diameter of the islets in diabetic rats. Int J Mol Cell Med 2013;2:21-6.
8. Burrowes JD, Van Houten G. Herbs and dietary supplement use in patients with stage 5 chronic kidney disease. Nephrol Nurs J 2006;33:85-8.
9. Rashidi AA, Mirhashemi SM, Taghizadeh M, Sarkhail P. Iranian medicinal plants for diabetes mellitus: A systematic review. Pak J Biol Sci 2013;16:401-11.
10. Moore F, Akhbarizadeh R, Keshavarzi B, Tavakoli F. Potential health risk of herbal distillates and decoctions consumption in Shiraz, Iran. Biol Trace Elem Res 2015;167:326-37.
11. Akdağ A, Öztürk E. Distillation methods of essential oils. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi 2018;45:22-31.
12. Fakher S, Seghatoleslam A, Noorafshan A, Karbalay-Doust S, Rahmanifard M, Rashidi M. The impact of echium amoenum distillate, Aragh Gav-Zaban, on naturally boosting fertility: Potential ameliorative role in male mice reproductive parameters. Iran J Med Sci 2019;44:227-35.
13. Seghatoleslam A, Mashkour N, Namavari M, Azarmehr B, Nejabat M. The potential effects of herbal distillates with hot and cold temperament on cell metabolic activity and growth: A preliminary *in vitro* study. J Pharmaceutical Biomedical Sci 2014;4:532-5.
14. Güneş HV, Değirmenci İ, Aydin M, Bozan B, Aral E, Tunalier Z, *et al*. The effects of Rumex patientia L. and Urtica dioica L. on some blood and urine parameters, and liver and kidney histology in diabetic rats. Turk J Med Sci 1999;29:227-32.
15. Council NR. Guide for the Care and Use of Laboratory Animals. National Academies Press; 2010.
16. Fakher S, JALALI M, Tabei S, Zeraati H, Javadi E, Sadeghi M, *et al*. Effect of vitamins A, E, C and omega-3 fatty acids on lipid peroxidation in streptozotocin induced diabetic rats. Iran J Public Health 2007;36:58-63.
17. Khalili A, Nekooeian AA, Khosravi MB, Fakher S. Simultaneous renal hypertension and type 2 diabetes exacerbate vascular endothelial dysfunction in rats. Int J Exp Pathol 2012;93:210-7.
18. Gohari A, Noorafshan A, Akmali M, Zamani-Garmsiri F, Seghatoleslam A. Urtica dioica distillate regenerates pancreatic beta cells in streptozotocin-induced diabetic rats. Iran J Med Sci 2018;43:174-83.
19. Kahn R, Hicks J, Muller M, Panteghini M, John G, Deeb L, *et al*. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care 2007;30:2399-400.
20. Davidson AL, Arion WJ. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: Physiological implications of higher cellular activity. Arch Biochem Biophys 1987;253:156-67.
21. Ferre T, Pujol A, Riu E, Bosch F, Valera A. Correction of diabetic alterations by glucokinase. Proc Natl Acad Sci U S A 1996;93:7225-30.
22. Vesal M, Zal F, Vaseei M. Effects of teucrium polium on oral glucose tolerance test, regeneration of pancreatic islets and activity of hepatic glucokinase in diabetic rats. Arch Iran Med 2003;6:35–9.
23. Akmali M, Ahmadi R, Vessal M. Pre-and post-treatment of streptozocin administered rats with melatonin: Effects on some hepatic enzymes of carbohydrate metabolism. Arch Iran Med (AIM) 2010;13:105-10.
24. Bottomley R, Pitot H, Potter VR, Morris H. Metabolic adaptations in rat hepatomas: V. Reciprocal relationship between threonine dehydrase and glucose-6-phosphate dehydrogenase. Cancer Res 1963;23:400-9.
25. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol 2003;135:357-64.
26. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
27. Chen M, Zheng H, Xu M, Zhao L, Zhang Q, Song J, *et al*. Changes in hepatic metabolic profile during the evolution of STZ-induced diabetic rats via an 1H NMR-based metabonomic investigation. Biosci Rep 2019;39BSR20181379. doi: 10.1042/BSR20181379.
28. Qujeq D, Davary S, Moazzi Z, Mahjoub S. Effect of urtica dioica leaf extract on activities of nucleoside diphosphate kinase and acetyl coenzyme, a carboxylase, in normal and hyperglycemic rats. Afr J Pharm Pharmacol 2011;5:792-6.
29. Bijan F, Ahmadvand D, Vardasbi S, Majin F, Khaghani S. Induction of insulin secretion by a component of Urica dioica leave extract in perfused islets of langerhans and its *in vivo* effects in normal and streptozotocin diabetic rats. Ethnopharmacology 2003;89:47-53.
30. Fazeli S, Gharravi A, Ghafari S, Jahanshahi M, Golalipour M. The granule cell density of the dentate gyrus following administration of Urtica dioica extract to young diabetic rats. Folia Morphol 2008;67:196-204.

31. Bnouham M, Merhfour F-Z, Ziyyat A, Mekhfi H, Aziz M, Legssyer A. Antihyperglycemic activity of the aqueous extract of Urtica dioica. Fitoterapia 2003;74:677-81.
32. Nickavar B, Yousefian N. Evaluation of α-amylase inhibitory activities of selected antidiabetic medicinal plants. J Verbrauch Lebensm 2011;6:191-5.
33. Domola MS, Vu V, Robson-Doucette CA, Sweeney G, Wheeler MB. Insulin mimetics in urtica dioica: Structural and computational analyses of urtica dioica extracts. Phytother Res 2010;24(Suppl 2):S175-82.
34. Golalipour MJ, Ghafari S, Kouri V, Kestkar AA. Proliferation of the β-cells of pancreas in diabetic rats treated with urtica dioica. Int J Morphol 2010;28:399-404.
35. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, *et al*. Tests of glycemia in diabetes. Diabetes Care 2004;27:1761-73.
36. Matschinsky FM, Wilson DF. The central role of glucokinase in glucose homeostasis: A perspective 50 years after demonstrating the presence of the enzyme in islets of Langerhans. Front Physiol 2019;10:148. doi: 10.3389/fphys.2019.00148.
37. Díaz-Flores M, Ibáñez-Hernández MA, Galván RE, Gutiérrez M, Durán-Reyes G, Medina-Navarro R, *et al*. Glucose-6-phosphate dehydrogenase activity and NADPH/NADP+ratio in liver and pancreas are dependent on the severity of hyperglycemia in rat. Life Sci 2006;78:2601-7.
38. Kletzien R, Harris P, Foellmi L. Glucose-6-phosphate dehydrogenase: A" housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. FASEB J 1994;8:174-81.
39. Stumpo DJ, Kletzien RF. Regulation of glucose-6-phosphate dehydrogenase mRNA by insulin and the glucocorticoids in primary cultures of rat hepatocytes. FEBS J 1984;144:497-502.
40. Golalipour MJ, Khori V. The protective activity of Urtica dioica leaves on blood glucose concentration and beta-cells in streptozotocin-diabetic rats. Pak J Biol Sci 2007;10:1200-4.
41. Vargas E, Podder V, Sepulveda MAC. Physiology, Glucose Transporter Type 4 (GLUT4). StatPearls: StatPearls Publishing; 2019.
42. Kadan S, Saad B, Sasson Y, Zaid H. *In vitro* evaluations of cytotoxicity of eight antidiabetic medicinal plants and their effect on GLUT4 translocation. Evid Based Complement Alternat Med 2013;2013:549345. doi: 10.1155/2013/549345.
43. Davies GF, Khandelwal RL, Wu L, Juurlink BH, Roesler WJ. Inhibition of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by troglitazone: A peroxisome proliferator-activated receptor-γ (PPARγ)-independent, antioxidant-related mechanism1. Biochem Pharmacol 2001;62:1071-9.