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The Effect of *Urtica dioica* Hydro-Alcoholic Extract on Glycemic Index and AMP-Activated Protein Kinase Levels in Diabetic Patients: A Randomized Single-Blind Clinical Trial

Bahare Korani,[1] Ali Mirzapour,[2] Ali Akbar Moghadamnia,[3] Soraya Khafri,[4] Nahid Neamati,[1] and Hadi Parsian[5,*]

[1]Student Research Committee, Babol University of Medical Sciences, Babol, Iran
[2]Department of Internal medicine, Babol University of Medical Sciences, Babol, Iran
[3]Department of Pharmacology, Babol University of Medical Sciences, Babol, Iran
[4]Department of Epidemiology, Babol University of Medical Sciences, Babol, Iran
[5]Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

[*]*Corresponding author*: Hadi Parsian, Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran. Tel: +98-1132199936, Fax: +98-1132199936, E-mail: hadiparsian@yahoo.com

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Abstract

Background: Type 2 diabetes (T2DM) is an endocrine disease caused by inadequate secretion or improper utilization of insulin. Studies have shown that AMP-activated protein kinase (AMPK) dysregulation is contributed to the development of T2DM. Urtica dioica (UD) may have anti-hypoglycemic activities in T2DM patients. However, the underlying mechanism is remained unclear. The aim of this study was to assess the UD effect on serum levels of glucose, glycated hemoglobin (HbA1c), insulin concentration, and AMPK levels in diabetic patients.

Objectives: This study aimed to determine the effect of Urtica Dioica hydro-alcoholic extract on glycemic index and AMPK levels in diabetic patients.

Methods: This randomized single-blind clinical trial was conducted in the endocrinology clinic of Rohani hospital (Babol. Iran). Convenience sampling and simple random allocation were used in the study. Sixty diabetic patients were randomly divided into the two drug and control groups. The drug group received 20 mg/kg/d of hydro-alcoholic UD extract three times for 8 weeks and control group received placebo. Fasting blood glucose (FBG), HbA1c, insulin and AMPK were measured and compared at the beginning and end of the study.

Results: FBG levels of the drug group were significantly decreased compared with the placebo group ($P = 0.032$). Quantitative insulin sensitivity check index (QUICKI) increased significantly in drug group compared with the other group ($P < 0.001$). The insulin and AMPK levels in the drug group after taking UD extract increased by 62.5% and 8.0 %, respectively. However, there was no significant changes compared with the placebo group ($P = 0.222$ and $P = 0.542$, respectively).

Conclusions: According to the results, UD is able to decrease glucose level and improve insulin release in T2DM. In addition, as UD is able to induce a small increase in AMPK activity, it is possible that the anti-hyperglycemic effect of UD is mediated by insulin secretion and the possible changes in AMPK levels.

Keywords: *Urtica dioica*, AMP-Activated Protein Kinases, Randomized Controlled Trials, Blood Glucose

1. Background

Diabetes mellitus (DM) is a common metabolic disease worldwide (1). According to the reports of world health organization (WHO), nearly two million people in Iran are involved with the DM and it is predicted that the number of patients will be increased to more than 5 million people in 2025 (2). Type 2 diabetes (T2DM) is resulted from the defects in the insulin production or inappropriate utilization of this hormone (3). Diabetes is associated with disturbances in glucose, protein and lipid metabolism (4). AMP-activated protein kinase (AMPK) is a sensor of energy status that maintains cellular energy homeostasis and plays a critical role to control glucose metabolism (5). This enzyme is activated by hypoxia, ischemia and the low levels of cellular energy (6-8). The evidences indicate that the dysregulation of AMPK activity may be contributed to develop some diseases such as diabetes and metabolic syndrome. In this regard, AMPK can be considered as a therapeutic target to treat diabetes and obesity, as well (9, 10). There have been reports indicating the beneficial effects of U*rtica dioica* (UD) extract in different conditions such as diabetes (11). UD is belonged to the Urticaceae family (12). Some studies have investigated the effects of UD on insulin

secretion (13). As the exact molecular mechanism and mediator(s) effect of the anti-glycemic activity of the UD are unclear and considering the key role of AMPK in glucose hemostasis, we aimed to determine whether this characteristic of UD hydro-alcoholic extract is mediated via AMPK or insulin in T2DM patients. Accordingly, in this clinical trial, we evaluated the effects of 8 weeks prescription of UD hydro-alcoholic extract on T2DM patients by assessment of glucose, glycated hemoglobin (HbA1c), insulin and AMPK before and after drug consumption.

2. Objectives

This study aimed to determine the effect of Urtica Dioica hydro-alcoholic extract on glycemic index and AMP-activated protein kinase levels in diabetic patients.

3. Methods

3.1. Study Design and Participants

This randomized single-blind clinical trial was conducted in the endocrinology clinic of Rohani hospital (Babol. Iran). This research was carried out from January to June 2016. Convenience sampling and simple random allocation were used in the study. The suggested formula for clinical trials (n = sample size, type one error (α) of 0.05 and type two error (β) of 0.20) (power = 80%) was used to calculate the sample size:

$$n = 2\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \times \frac{S^2}{d^2} \quad (1)$$

Sixty diabetic patients were randomly divided into the two groups including drug (n = 30) and control groups (n = 30). The drug group was given 20 mg $kg^{-1}D^{-1}$of hydro-alcoholic Utrica dioica extract three times a day) after breakfast, lunch and dinner(for 8 weeks (safety issues were limiting factor) and the control group received placebo in the same procedure. The flowchart of the study and its design is presented in Figure 1. The informed consent was obtained from the patients before study.

3.2. Inclusion and Exclusion Criteria

Patients with fasting blood glucose (FBG) $\geq$ 126 and HbA1c > 6.5% were included in the study. The diagnosis of diabetes was according to the American Diabetes Association and WHO criteria (4, 14). Patients with cardiovascular, chronic kidney and liver diseases were excluded. Subjects who received any medication affecting the AMPK levels (such as AICAR, GLP-1 mimetic, Thiazolidinediones, metformin, DPP-4 inhibitors, salicylate, α-lipoic acid, resveratrol, and berberine), pregnant and lactating women, , smokers and those who used other medicinal plant supplements were also excluded from the study. The informed consent was obtained from the patients before study. All patients were under supervision of an endocrinologist and they received the same treatment protocol. They were advised to not change their diet and exercise. This study was confirmed by the Iranian registry of clinical trials (IRCT2014100119364N1) and ethics committee of Babol University of Medical Sciences (304930).

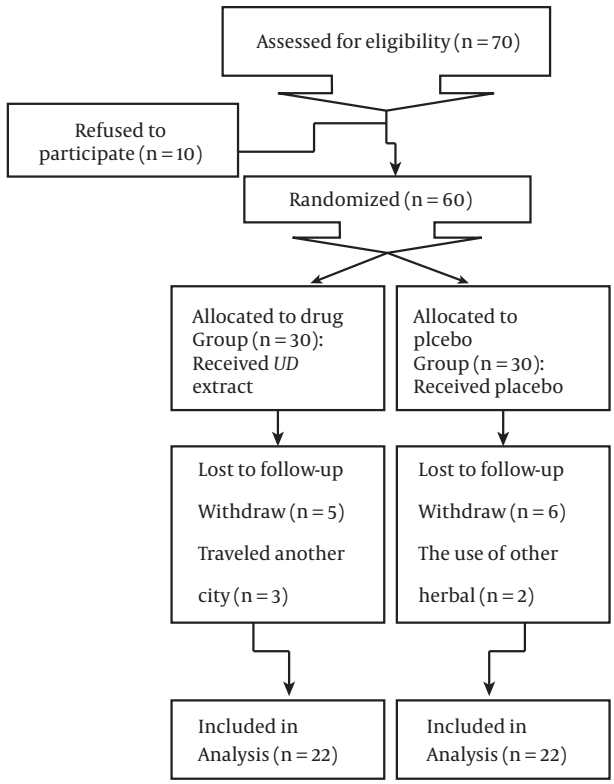


Figure 1. Flowchart of the Study Participants

3.3. Preparation of UD extract

The used UD Hydro-alcoholic extract in this study was a gift from the Giah Essence Pharmaceutical Company (Gorgan, Iran). The final extract solution contained 45% ethanol and 55% water including 2.7 g of dry matter per liter.

3.4. Assessment of Anthropometric and Clinical Measures

Anthropometric measurements were recorded while the patients dressed in light clothing, but without shoes. The weight to the nearest 0.1 kg and the height to the nearest 0.1 cm were measured using a balance-beam laboratory

scale (Seca, GmbH, Germany). BMI was calculated as weight (kg)/ height (m^2).

3.5. Biochemical Analysis

In order to determine the status of biochemical parameters, at the beginning and end of the study after one overnight fasting, 5 mL of blood was taken from each patient. The serum samples were prepared by centrifugation for 10minutes at 3000 g. The obtained serum was stored at -80°C until final analysis. To measure HbA1c, the blood samples were immediately placed in EDTA containing tubes and were analyzed. Blood glucose levels of the studied patients were measured by spectrophotometry method. The assay was based on glucose oxidase enzyme reaction (Ziest Chem kit, Iran) with intra-assay coefficient of variation (CV = 1.6% and inter-assay CV = 4.2%). The levels of AMPK were assessed using ELISA kit (CRYSTAL DAY BIOTECH, China, Cat number: E0746Hu) based on the biotin double antibody sandwich technology. Serum insulin concentration was determined using sandwich immunoassay method (Monobind, USA, product code 5825 - 300). Intra-assay and inter-assay variability of this procedure according to the manufacturer claims was 8.0% and 9.8%, respectively. The assay sensitivity was 0.114 μIU/mL. Ion exchange chromatography column (Biosystemkit, Spain) was used to measure the HbA1c levels. Intra-assay and inter-assay CVs for this assay were < 6.3%, and < 5.9%, respectively. QUICKI was also calculated as 1/log fasting insulin blood +log fasting blood glucose (15).

3.6. Statistical Analysis

To evaluate the normal distribution of variables, Kolmogrov-Smirnov test was applied. The results are reported as Mean ± SD. Mann-Whitney U test and Student's t-test (which was appropriate) were used to analyze the data between the drug and placebo groups. Wilcoxon signed-rank test and paired t-test were used to analyze the data at the beginning and end of the study. P < 0.05 was considered as significant level.

4. Results

4.1. Base Characterizations

Sixty diabetic patients were enrolled in this investigation, whereas 44 patients completed the study. Five male and 17 female patients were in drug group and 3 male and 19 female patients were in the placebo group. The mean age in the drug and placebo group was 48.9 ± 1.9 and 49.3 ± 2.6 years, respectively. At the baseline, no significant differences were observed between the two groups for age, the disease length, weight, height, and BMI (P > 0.05). Demographic characteristics of the studied patients are shown in Table 1.

4.2. Effect of UD on Biochemical Parameters and BMI

FBG levels are summarized in Figure 2. Insulin levels in the drug group after taking UD extract increased by 62.5% and the difference was statistically significant (P = 0.003), but there was no significant change compared with the placebo group. After receiving the UD extract, the QUICKI increased significantly in drug group compared with the other group (P < 0.001). Changes in QUICKI are shown in Figure 3. AMPK levels in the drug group after taking UD extract increased by 8.03%, whereas there were no significant changes compared with the placebo group. Table 2 shows the FBG, HbA1c percent, AMPK levels, insulin, QUICKI and BMI for the drug and placebo groups.

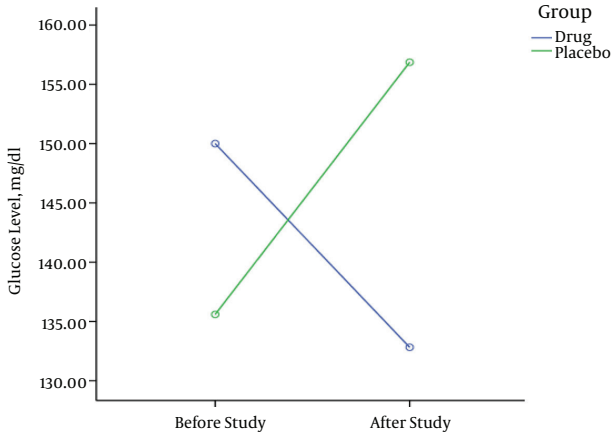


Figure 2. Comparison of Glucose Levels Between Two Groups Before and After the Study

4.3. Safety (Side Effects)

In this study, only one patient (n = 1) suffered from itching in the end of study in the drug group. The difference between the drug and placebo groups regarding the side effects was not significant (P = 0.323).

5. Discussion

Numerous studies have examined the effect of UD on biochemical factors such as blood glucose, HbA1c, and insulin levels. However, there is no report on the effects of UD on AMPK levels in patients with type 2 diabetes, so far. Given the importance of AMPK in the development of diabetes, the aim of this trial was to evaluate the effect of UD

Table 1. Baseline Characteristics[a]

Variable	Drug Group	Placebo Group	P Value
Men, No. (%)	5 (22.7)	3 (13.6)	
Woman, No. (%)	17 (77.3)	19 (86.4)	
Age, y	48.90±9.2	49.31 ± 12.2	(0.851)
Weight, Kg	76.86 ± 11.20	84.27 ± 13.41	(0.710)
Height, m	159.59 ± 8.35	163.45 ± 7.59	(0.100)
Diabetes length, y	2.47 ± 1.57	2.50 ± 1.30	(0.147)

[a]The values are expressed as Mean ± SD.

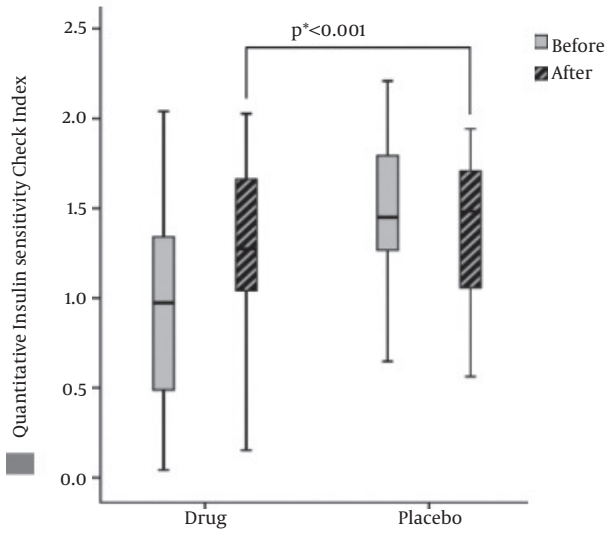
Table 2. Differences in the Studied Parameters in Patients Over 8 Weeks in the Drug and Placebo Groups[a]

Groups	Drug		Placebo		P Value[b]
Parameters	**Before**	**After**	**Before**	**After**	
HbA1C (%)	8.42 ± 1.48	8.73 ± 1.57	9.08 ± 1.32	9.03 ± 1.55	0.577
FBG, mg/dL	150 ± 6.8	132 ± 0.53	135 ± 6.5	156 ± 10.4	0.004
AMPK[c], ng/mL	44.03 ± 22.57	47.61 ± 22.13	46.34 ± 11.24	48.02 ± 11.36	0.542
Insulin[c], μIU/mL	6.4 ± 9.76	10.4 ± 10.72	15.3 ± 13.5	11.6 ± 8.93	0.222
QUICKI[c]	0.85 ± 0.53	2.1 ± 0.67	1.5 ± 0.38	1.4 ± 0.40	< 0.001
BMI	31.3 ± 5.05	30.3 ± 5.03	32.1 ± 4.32	32.4.32	0.213

[a]The values are expressed as Mean ± SD.
[b]P value, comparison between drug and placebo groups after the study.
[c]The values are expressed as Median ± IQR and Mann-Whitney U test and Wilcoxon signed-rank test were used to analyze the data between the drug and placebo groups and to analyze the data at the beginning and end of the study, respectability.

extract on AMPK, glucose, insulin levels, and HbA1c in type 2 diabetes patients. After receiving the UD extract (20 mg kg^{-1} day^{-1}) for 8 weeks, the blood glucose levels decreased significantly in drug group which is consistent with the results of some previous published studies. Namazi et al. (16) found that the consumption of 100 mg kg-1 day-1 of UD extract for 8 weeks can decrease the blood glucose concentrations in diabetic patients. Ahangarpour et al. (17) investigated the hypoglycemic effect of UD extract, at the doses of 50, 100, and 200 mg/kg on male Wistar rats. They observed that the glucose levels in rats receiving the extract at the doses of 100 and 200 mg/kg are significantly decreased compared with the control group. Shahraki et al. (18) reported the similar results in male Wistar rats. Das et al. (19) investigated the hypoglycemic effect of UD extract on type 2 diabetic rats. In that study, 20 rats were divided into three groups including control group receiving water, positive control group treating with glibenclamid and the last group taking 1.25 g/kg aqueous extract of UD for 28 days. They reported that glucose levels in the group taking the UD extract were significantly decreased compared with the control group. Several mechanisms may explain the blood-glucose lowering ability of UD. Several studies have suggested that an increase in glucose uptake by skeletal muscles and also adipose tissues is the major cause of a reduction in blood glucose concentrations after UD consumption (18). It is also suggested that UD has the anti-inflammatory activities that can affect the functional status of the pancreatic β-cells, insulin release, an increase in the uptake of glucose by the cells, and ultimately the blood glucose concentration reduction (20). In addition to the anti-inflammatory activity of the UD, its antioxidant characteristic may be associated with the more intestinal absorption of glucose to cells (16). The mean HbA1c levels in the UD extract receiving group did not statistically change compared with the baseline and also with the placebo group which is inconsistent with the previous studies. Kianbakht et al. (21) reported that UD leaf extract as a capsule (a 500 mg capsule every 8 hours for 3 months) can significantly decrease the level of HbA1c in T2DM. In another study, a significant decrease in HbA1c levels is reported in T2DM who received UD extract (for 8 weeks). Said et al. (22) reported that one tablet of glucolevel (a mixture of the dried leaves extract of *Juglansre-*

Figure 3. Quantitative Insulin Sensitivity Check Index



(QUICKI) was significantly higher in the drug group compared with the placebo group (P* < 0.001).

gia L., *Oleaeuropea* L., *Urticadioica* L,. and *Atriplexhalimus* L.) three doses per day for 4 weeks, significantly reduced the HbA1c levels in 6 out of 16 patients ($P < 0.01$). In our study, although the serum glucose levels of the patients in UD receiving group decreased significantly, the glucose levels were higher than the normal range. Blood HbA1c levels had a significant correlation with the levels of blood glucose. Therefore, the exact control of glucose over the past 2 or 3 months and reaching the blood glucose to the reference interval are necessary to reduce the HbA1c levels (23, 24). Accordingly, it is expectable that the HbA1c levels will not change in these patients. The longer following-up of the patients, probably may result in a significant decrease in the levels of HbA1c. Therefore, it seems that a small decrease in the serum glucose levels is the major reason for no significant decrease in HbA1c levels. Surly, other studies with the larger sample size, longer time of drug prescription, and also the higher doses of UD will be able to highlight this issue, clearly. It seems that the UD extract at the dose of 20 mg kg-1 day-1 was successfully able to improve insulin release and QUICKI in drug group. In a study on diabetic rats induced by alloxan, the insulin levels in diabetic cases which treated with alcoholic extract of UD were significantly increased than the untreated ones (25). Farzami et al. (13) have shown that the aqueous extract of UD may affect the release of insulin from pancreas beta cells in streptozotocin (STZ)-induced diabetic rats. Golalipour et al. (20) reported that pretreatment with the UD hydro-alcoholic extract (100 mg/kg^{-1}/day^{-1}) in the STZ-induced rats might increase proliferation rate of β-cells. As free radicals are involved to impair the pancreatic β-cells, they suggested that the results are probably related to the antioxidant properties of UD extract which can inhibit or scavenge the activity of free radicals. It seems that through the removing free radicals, the probable regeneration of beta cells can lead to the increase of insulin secretion. Inconsistent with these reports, Mobasseri et al. (26) observed that hydro-alcoholic extract of UD is not able to change human insulin sensitivity in muscle cells. In another study, effect of the fresh ginseng extract (HEG) on AMPK activity in HepG2 cells was evaluated (27). The results showed that HEG can stimulate the AMPK activity in this cell line. In this study, it seems that taking the UD extract were able to induce a small increase in the activity of this important enzyme, although the difference wasn't statistically significant. As previously mentioned, dysregulation of AMPK activity can be associated with the development of diabetes. In addition, physiologic or pharmacologic activation of AMPK can improve the quality of diabetic life. Considering the short used treatment period of the present study, a small increase in AMPK activity was observed. Our study had some limitations. According to the evidences, there is no report on the effects of UD on AMPK enzymes in patients with type 2 diabetes. Therefore, further studies with a larger sample size and longer duration of the intervention are needed to achieve more precise results.

5.1. Conclusion

We think that these results can open a new window for further research in traditional medicine and improvement of the life quality of diabetic patients. According to the results, UD is able to decrease glucose level and improve insulin release in T2DM. Furthermore, as UD is able to induce a small increase in AMPK activity, it is possible that the antihyperglycemic effect of UD is mediated by insulin secretion and possible changes in AMPK level.

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Footnotes

Authors' Contribution: Bahare Korani substantially contributed in the conception and design, analysis of the laboratory parameters, drafting the manuscript and analysis and interpretation of the data. Ali Mirzapour was responsible for the designing of the study, revising and drafting the manuscript. Ali Akbar Moghadamnia contributed in designing, interpretation of the results and drafting the manuscript. Soraya Khafri participated in the design of the study and performed the statistical analysis. Nahid Neamati and Hadi Parsian substantially contributed in the conception and design, drafting the manuscript and analysis and interpretation of the data.

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