

Full Length Research Paper

Effect of *Urtica dioica* leaf extract on activities of nucleoside diphosphate kinase and acetyl coenzyme, a carboxylase, in normal and hyperglycemic rats

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Urtica dioica has been shown to have capability of reducing blood glucose through enhancing insulin secretion. The aim of this study was to assess *in vivo* and *in vitro* effects of aqueous and alcoholic extracts of *U. dioica* leaves on activities of Acetyl coenzyme A carboxylase (ACC), Nucleoside diphosphate kinase (NDPK) and insulin level and serum glucose concentration. The experimental animals were randomly distributed among 6 groups of 7 or 8 rats each. The treatment groups received 150 mg/kg of alloxan. Then rats were fed alcoholic and aqueous extracts of *U. dioica* at 50 mg/kg/day doses for a period of two weeks. Subsequently glucose, insulin levels, and ACC, NDPK activity were measured. Our studies showed significantly lower levels of glucose in the group of rats that were treated with ethanol extract of *U. dioica* leaves as compared with the control group (279.6 ± 24.3 vs. 393.6 ± 24.9 mg/dl), ($p < 0.01$). Present results showed significantly elevated levels of insulin in the group of rats treated with ethanol extract of *U. dioica* leaves as compared with the control group (7.6 ± 0.3 vs. 3.6 ± 0.2 , $\mu\text{g/l}$), ($p < 0.01$). Also, results showed significantly elevated activity of ACC and NDPK in the group of rats treated with ethanol and aqueous extract of *U. dioica* leaves as compared with the control group. The results of the present study indicated that alcoholic extract of *U. dioica* leaves was found to reduce glucose level and increase insulin secretion, ACC and NDPK activity in the alloxan diabetic animals.

Key words: *Urtica dioica*, acetyl coenzyme A carboxylase, extract, nucleoside diphosphate kinase

INTRODUCTION

In recent years, medical plants have been the subject of considerable research due to their potential as a control agent of diabetes mellitus, which is considered as a metabolic disorder (Wild et al., 2004; Conget, 2002; Costantine et al., 2006). Previous studies have provided evidence for excessive hepatic glucose production,

peripheral insulin resistance and defective b-cell secretor unction are the main problems caused by diabetes mellitus (Vuksan et al., 2001; Nauck et al., 2004). Based on several studies, *Urtica dioica*, has been traditionally used for treatment of ailments such as diabetes, uterine hemorrhage and coetaneous eruption (Bnouham et al., 2003; Kavalali et al., 2003). Also, there is some experimental evidence to suggest that *U. dioica* extract has hypoglycemic properties, which improves glucose tolerance (Miltman, 1990; Swanston, et al., 1989; Hirono et al., 1994). *U. dioica* decreased the blood glucose of normal and diabetic mice (Krzeski et al., 1993; Schneider et al., 1995; Kayse, et al., 1995).

It is clear that acetyl-CoA carboxylase (ACC; EC 6.4.1.2) catalyzes the biotin dependent conversion of an

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Abbreviations: ACC, Acetyl CoA carboxylase, NDPK, nucleoside diphosphate kinase; mM, milli Molar, kg, kilogram; i.p, intraperitoneal; ml, milliliter.

acetyl CoA, HCO_3^- , and ATP to malonyl CoA (wakil et al., 1958). Glucotoxicity increases the ACC gene expression (Chen et al., 2007). Phosphorylation and dephosphorylation of acetyl-CoA carboxylase cause deactivation and activation of the enzyme, respectively and serve as the enzyme short-term regulatory mechanism (Kim, 1997). Activity of this enzyme can be enhanced in primary cultures of hepatocytes adult rats by insulin as well as by glucose (Norbert et al., 1981) and it is equipped with glucose sensor for insulin secretion. This unique enzyme, which catalyzes the production of malonyl-CoA, shows a significant defect in NDP kinase expression and function under glucolipotoxic conditions (Veluthakal et al., 2009). Nucleoside diphosphate kinase (NDPK, E.C. 2.7.4.6) catalyzes the transfer of the terminal phosphate from nucleoside triphosphate to nucleoside diphosphate (Hammargren et al., 2008) and is closely linked to the energy metabolism of the cell (Park et al., 1973). The purpose of this study was to assess *in vivo* and *in vitro* effects of aqueous and alcoholic extracts of *U. dioica* leaves on activities of ACC, NDPK activities and insulin level, serum glucose concentration.

METHODOLOGY

Collection of plant materials

Leaves of *U. dioica* were collected from Gatab village, located in vicinity of Babol, IRAN in May 2008. It was certified by Center of Agricultural Research and Natural Resources of Mazandaran province.

Preparation of aqueous and alcoholic extracts

Leaves of *U. dioica* were dried at room temperature and then powdered. In order to prepare aqueous concentrate, 100 g of dry leaves of *U. dioica* was boiled in two litres of distilled water for 20 min. After boiling, the mixture was cooled down and filtered through a mesh (0.2 mm) to separate the extract from the solid phase and then, the solution was passed twice through Whatman paper No. 2. The macerate was filtered and the solution was evaporated on a rotator evaporator at a temperature of 60°C to reduce the volume of solution to 1/6 of initial value.

To prepare alcoholic solution, 100 g of dry powder of *U. dioica* was boiled in one litre of ethanol 65 to 70% and then mixed by a shaker for 24 h at room temperature. The same preparation procedure of aqueous concentrate was conducted for producing alcoholic extract. Both aqueous and ethanol solutions were kept 4 days under the hood to let the solvents evaporate, then they were stored at -20°C.

Experimental animal

Rats (totally 45 rats) of 8 to 12 weeks old with approximate weight of 200 to 240 g were used in this study. The rats were provided by animal center of Babol University. Age-matched rats were used as control animals. The rats were placed in suspended bracket cages in an air conditioned room with temperature of 22±5°C and photoperiod of 12 L: 12 D. All animals were carefully maintained under standard animal house conditions with free access to food,

water and *ad libitum*. The rats were fasted 14 h before injection. The approval of the Ethics Committee of Babol University was also obtained (# :P/J/30/1825).

Animals' treatments

The treatments in this study included; (I) non treated animals (control group), which received orally 0.7 ml normal saline, (II) hyperglycemic group that were intraperitoneally injected by 150 mg/kg of fresh solution of alloxan (alloxan monohydrate from Sigma Co., USA) dissolved in distilled water, (III) animals which received aqueous extract, (IV) animals that received ethanolic extract, (V) hyperglycemic animals (received 150 mg/kg alloxan intrape effect of *U. dioica* leaf extract on activities of nucleoside diphosphate kinase and acetyl coenzyme A carboxylase in normal and hyperglycemic rats ritoneal) and treated with aqueous extract, (VI) hyperglycemic animals (received 150 mg/kg alloxan intraperitoneal) and treated with ethanol extract. After 72 h from treatments, blood samples of rats were obtained from conjunctiva and glucose plus insulin of blood serum were measured. At 24 h after blood sampling, rats were treated orally with concentration of 50 mg/kg/d dose of *U. dioica* leaves extract and distilled water (control) respectively for 14 days. Blood samples were taken 24 h after completion of this period to measure glucose and insulin levels. Hyperglycemic was developed gradually as assessed by blood glucose level, which reached approximately 250 mg within 2 days. In each experiment, 0.5 g of rat liver was thawed and suspended in PBS, after stirring for 60 min at 35°C insoluble materials removed by centrifugation at 13000 × g at 4°C. The supernatant was used to measure the enzymes activity.

Protein assay

Protein concentration in the blood samples was determined by dye-binding method of Bradford using BSA as the standard.

NADP kinase activity assay

The activity of NADP kinase was investigated by spectrophotometry using a coupled pyruvate kinase lactate dehydrogenase enzyme system as described previously with modification (Johansson et al., 2008). The assay was carried out in a 1 ml reaction mixture, containing 50 mM Tris-HCl (pH 7.6), 5 mM MgCl_2 , 0.05 mM KCl, 0.1 mM phosphoenolpyruvate, 0.5 mM ATP, 0.1 mM TDP, NADH (0.1 mg/ml), 2 units of pyruvate kinase, and 2.5 units of lactate dehydrogenase. The reaction was initiated by addition of 0.5 to 5 µg of NDP kinase at 25°C adjusted with distilled water to 1 ml. Then 10 µl rat liver homogenized and incubated (for) 10 min at 37°C was added. Monitoring of the decrease in absorbance at 340 nm follows the oxidation of NADH, which reflect ADP formation by NDP kinase. The specific activity of 1 unit of enzyme is defined as the turnover of 1 µmol of substrate in 1 min per milligram of protein.

Acetyl-CoA carboxylase activity assay

ACC activity was determined by spectrophotometry as described previously with modification (Bijleveld et al., 1987). The assay was conducted in a mixture, containing 50 mM Tris -HCl (pH 7.6), 10 mM potassium citrate, 10 mM MgCl_2 , 3.75 mM Glutamate, 0.75 mg bovine serum albumin in 1 ml and 0.125 mM acetyl coa, 20 units of pyruvate kinase and 20 units of lactate dehydrogenase, adjusted with distilled water to 1 ml. Subsequently, 10 µl rat liver homogenized and incubated (for) 10 min at 37°C was added. Monitoring of absorbance at 340 nm follows the oxidation of NADH.

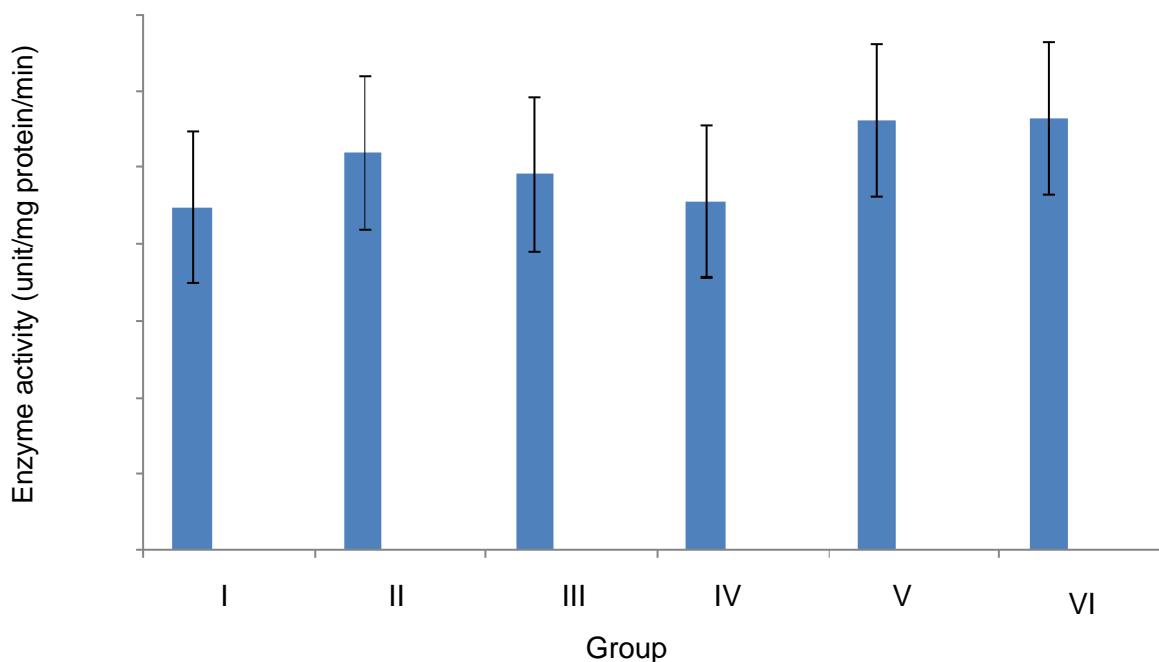


Figure 1. ACC activity in unit/ mg protein/min (Mean \pm S.E.M) after treatment with extract in normal and hyperglycemic rats, P values<0.01.

Table 1. Glucose and insulin (Mean \pm SD.) levels after treatment with extract of *U. dioica* leaves in normal and hyperglycemic rats.

Group	(n)	Glucose (mg/dl)	Insulin(μ g/L)
I	8	86.4 \pm 3.1	17.0 \pm 0.3
II	7	393.6 \pm 24.9	3.6 \pm 0.2
III	8	87.8 \pm 2.9	16.7 \pm 0.4
IV	8	87.5 \pm 3.6	17.1 \pm 0.3
V	7	386.1 \pm 28.4	4.5 \pm 0.4
VI	7	279.6 \pm 24.3	7.6 \pm 0.3

Statistical analysis was done using One-way ANOVA test and P values<0.01 were considered to be statistically significant. (n= number of animals in each group).

The specific activity of 1 unit of enzyme is defined as the turnover of 1 μ mol of substrate in 1 min per milligram of protein.

Determination of glucose and insulin levels

Glucose level was determined by spectrophotometric method (Jenway uv/vis, 6505 model ,Dunmow, UK) using glucose kit of Pars Azmoon Co.,Tehran, Iran. The insulin test was carried out following ELISA assay using insulin kit of rat made by Mercodia Co., Sweden(Stat Fax -2100,Awareness Technology Inc Plam city ,USA,FL 34990).

Statistical analysis

All values were presented as Mean \pm SD Statistical analysis was done using SPSS, version 18.0, One-way ANOVA test and P values <0.01 were considered to be statistically significant.

RESULTS

The alloxan diabetic rats treated with alcoholic and aqueous extracts of *U. dioica* showed higher activity of ACC and NDPK compared to the control group (Figures 1 and 2). Administration of *U. dioica* produced a significant reduction in blood glucose concentration in alloxan hyperglycemic rats, as compared to the controls which received saline only. The effect of extracts on insulin and glucose level are shown in Table 1.

DISCUSSION

The alloxan diabetic rats treated with alcoholic extract of *U. dioica* leaves had a significant rise in serum insulin

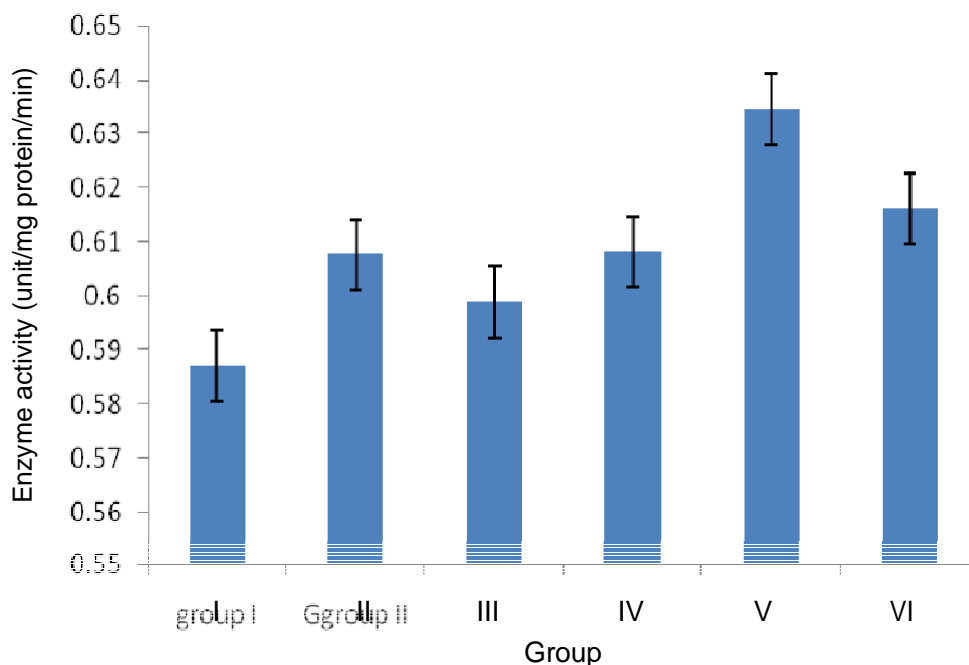


Figure 2. NADPK activity in unit/ mg protein/min (Mean \pm S.E.M) after treatment with extract in normal and hyperglycemic rats, P values $<$ 0.01.

(7.6 ± 0.3 μ g/l) and a decline in glucose level (279.6 ± 24.3 mg/dl) compared to the untreated alloxan-induced rats (3.6 ± 0.2 μ g/l and 393.6 ± 24.9 mg/dl) respectively. These findings are consistent with other studies (Bnouham et al., 2003; Kavalali et al., 2003; Swanston-Flatt et al., 1989). A significantly lower level of blood glucose was recorded in alloxan-induced rats treated with alcoholic extract compared with the control rats ($P < 0.01$), whereas no significant difference was observed in level of blood glucose between alloxan-induced rats treated with aqueous extract and the control rats ($P > 0.01$). Our results were in agreement with those reported previously (Kavalali et al., 2003; Swanston-Flatt et al., 1989). The alcoholic extracts of *U. dioica* leaves reduced glucose level of the rats, indicating the alcoholic extract of this plant has some potential medicinal values. The alloxan diabetic rats treated with alcoholic and aqueous extracts of *U. dioica* (groups V and VI) showed higher activity of ACC and NDPK compared to the control group (groups I and II). Previous investigators have suggested following reasons for reduction of glucose after treatment with plant extract: increase in insulin secretion (Bnouham et al., 2003) increase in environmental metabolism of glucose (Kavalali et al., 2003; Krzeski et al., 1993), increase in the number of pancreas islets (Schneider et al., 1995), and some plants like *U. dioica* is being an antioxidant (Swanston-Flatt et al., 1989; Kayser, 1995). But we hypothesized that the reduction in blood glucose level following the administration of *U. dioica* extract can be attributed to increase activity of ACC as glucose sensor for insulin secretion and NDPK that involves as an energy

metabolism of the cell. In this regard, maybe *U. dioica* extract causes rearrangement of hepatocytes and increase in activity of these enzymes. Anyway, action v mechanism remains unclear, further study is needed to clarify the mechanism.

Conclusion

Our results have shown that *U. dioica* leaves extract given parenterally possesses a hypoglycemic effect in alloxan hyperglycemic rats. Also, for the first time we showed that aqueous and ethanolic extracts of this plant increase the ACC and NDPK activity.

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