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Full Length Research Paper

Effect of extract of *Urtica dioica* on insulin and C-peptide secretion from rats (RIN5F) pancreatic beta cells

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Plants are being used in the treatment of diabetes in traditional system of medicine. *Urtica dioica* (UD) has a variety of uses in traditional medicine. There are few reports about hypoglycemic mechanisms of *U. dioica*. The present study was designed to determine the possible mechanisms of hypoglycemic effects of UD on RIN5F rat pancreatic beta cells *in vitro* models. Beta cells were prepared in multiple flasks containing culture medium. Alcoholic extract of UD at concentrations of 50, 100 and 200 µg/ml was added to flasks. Insulin and C-peptide level were measured at 0, 60, 120 and 80 min. Insulin level in pancreatic cells media before and after addition of UD extract at different concentrations and in different times did not changed significantly (p > 0.2). Also, C-peptide (µg/ml) levels in these media with dose of 50, 100 and 200 µg/ml UD, did not change significantly. The results of the present study demonstrated that alcoholic extract of UD was unable to increase insulin and C-peptide secretion from RIN5F pancreatic beta cells. Hence, the hypoglycemic effects of UD are not based on enhancement of insulin secretion and needs more study.

Key words: *Urtica dioica*, insulin secretion, hypoglycemic activity.

INTRODUCTION

Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat, due to absolute or relative deficiency of insulin secretion or varying degree of insulin resistance (American Diabetes Association, 2011; Farmer and Fox, 2011). The number of adults with diabetes in the world is anticipated to rise from 285 million in 2010 to 439 million in the year 2030 (Shaw et al., 2010).

Patients with diabetes experience significant morbidity and mortality from microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications (heart attack, stroke and peripheral vascular disease). Microvascular disease leads to retinopathy, neuropathy and nephropathy (nephropathy leads to uremia) (Halder et al., 2003; Merlin et al., 2005). Macrovascular disease leads to cardiovascular disease, mainly by accelerating atherosclerosis. These disorders include: coronary artery disease, leading to myocardial infarction (heart attack) or angina, Stroke (mainly ischemic type), peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot (The Advance Collaborative Group, 2008).

The use of herbal remedies has been on the rise worldwide (Naito et al., 2005; Azaizeh et al., 2003; Guarrera, 1999). Plants are being used in the treatment of diabetes in traditional system of medicine (Luke, 2000). *Urtica dioica* (stinging nettle) and *Urtica urens* (dwarf nettle) are members of the Urticaceae family native to Eurasia, and are considered therapeutically

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interchangeable (Blumenthal et al., 1998). We hypothesized that the extracts *U. dioica* can increased insulin and C-peptide secretion from (RIN5F) pancreatic B cell.

METHODS AND MATERIALS

Preparation of extract

Dried UD was purchased from the market and identified by a pharmacognosist. Using an electric mill, plants were crushed into a fine powder. Obtained powder was extracted repeatedly by 70% methanol as solvent (for 5 days) by soak method (maceration). In second stage, this hydro-alcoholic extract were dried completely by rotary evaporator in temperature of 45°C and pressure below 100 mmHg. The dried extract was stored in a refrigerator at temperatures below zero degrees of centigrade for later stages.

Cell culture

RIN-5F (code NCBI: C526) cells type obtained from National Cell bank of Iran (NCBI) affiliated to Pasteur Institute of Iran. Cells were prepared in DMEM [Dulbecco/Vogt modified Eagle's (Harry Eagle) minimal essential medium] and RPMI (Roswell Park Memorial Institute) 1640 medium and were cultured in 5% CO_2 and 10% fetal calf albumin with penicillin G 80 mg and 50 mg streptomycin in sterile conditions. After reaching the required number of specific cells and confluence state, insulin and C-peptide were measured as base line values.

Cell viability

Trypan Blue color was used to assess the live cells proportion in the flask. This color only painted the living cells but could not enter dead cell. In this way, after painting, with NEUBAUER slides and a light microscope determination of percentage of viable and dead cells were become possible. We found that more than 85% of the cells were alive, which technically and according to references were acceptable (Jones and Senft, 1985).

Study protocol

Pancreatic beta cell in Cell Culture Laboratory of Drug Applied Research Center was prepared in DMEMI medium in six flasks. Cells were prepared in the same size case and control flasks. The suspensions of 50, 100 and 200 micrograms of the UD extract in 1ml normal Saline were added to the case flasks. In control flask, beta cells were primed only with 1 ml of normal saline. Insulin and C-peptide levels were measured in before and 60, 120 and 180 min after priming in all case and control samples. Concentration of insulin is determined by Chemiluminescence (CLIA) method according to DiaSorin (LIASONInsulin, 310360, with measuring range of 0.2 to 500 µIU/ml, with less than 10% coefficient of variation). C-Peptide measurement was performed based on Imminoenzymometric method and according to Monobind kite protocol (AccuBind ELISA Microwells, 2725-300, measuring range of 0.01 to 30 ng/ml, with less than 10% coefficient of variation).

Statistical analysis

Statistical analysis was done by means of the statistical package SPSS 16. Values are presented as mean and standard deviation, and 95% confidence interval. Comparison between groups in different time was performed by Repeat Measure Model. A 0.05 level of significant was set.

RESULTS

Table 1 shows concentrations of insulin in medium containing pancreatic beta cells in the control, and case groups before and after adding of UD extract in doses 50, 100, 200 µg/ml and at times before 60, 120 and 180 min. There were no significant changes in insulin levels in all groups with and without UD in different times ($P > 0.05$).

In Table 2, C-peptide concentration in pancreatic beta cells containing media before and after treating of alcoholic extract of UD with concentrations of 50, 100 and 200 µg/ml at times before 60, 120 and 180 min were shown. Between C-peptide concentration at different time and different UD extract concentration in each group, no statistically significant difference was found ($P > 0.05$).

DISCUSSION

Despite all the marvelous advancements in modem medicine, traditional herbal medicine has always been practiced. Alternative therapies have been used by people in our region (as in other regions) who have the faith in spiritual healers. More than 800 plants are reported to have antidiabetic properties (Eddouks and Maghrani, 2004), for example today up to 600 traditional plant medicine has been reported in India for diabetes (Jarald et al., 2008). Ethnopharmacological surveys show that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Kesari et al., 2007). Like all green vegetables, UD leaf densely contains several micronutrients (Wagner et al., 1994). Despite abundance of reports about antidiabetic properties of UD, there is little scientific explanation of these effects. The present study was designed to determine the possible mechanisms of hypoglycemic effects of UD on RIN5F rat pancreatic beta cells.

Insulin discovery in 1921 was the major breakthrough in the treatment of diabetes mellitus (Swanston-Flatt et al., 1991a). Insulin is known as an anabolic hormone that plays an important role in maintenance of body growth and regulation of overall body metabolism (Clark and Wallis, 2003). Before the introduction of insulin, the treatment of diabetes mellitus mainly relied on dietary measures, which included the use of traditional herbal therapies. Many traditional plants have been introduced for treatments of diabetes (Swanston-Flatt et al., 1991a; Gray and Flatt, 1997a). There is data that UD can reduce blood glucose levels, and studies have different results with together (Fawzi and Kamal, 2009). Oral administration of hydroalcoholic extract UD in doses of 100 mg/kg showed a strong glucose lowering effects on streptozocin (STZ) induced diabetes in rats. It has the protective effect on pancreatic cells in animal models

Table 1. Concentration of insulin (µIU/ml) in medium containing beta cells in the control (before) and case (after) groups after addition of UD extract with doses 50 and 100 and 200 µg/ml at different times.

Time (min)	Concentration of insulin in the control group	Concentrations of insulin in media containing 50 µg/ml UD	Concentrations of insulin in media containing 100 µg/ml UD	Concentrations of insulin in media containing 200 µg/ml UD
Before adding	0.16 ± 0.00	1 ± 0.02	0.18 ± 0.00	0.20 ± 0.00
60 min after adding	0.16 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
120 min after adding	0.16 ± 0.00	0.18 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
180 min after adding	0.17 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.20 ± 0.00

Table 2. Concentration of C-peptide (ng/ml) in medium containing beta cells in the control (before) and case (after) groups after addition of UD extract with doses 50 and 100 and 200 µg/ml at different times.

Time (min)	Concentration of C-peptide in the control group	Concentration of C-peptide in media containing 50 µg/ml UD	Concentration of C-peptide in media containing 100 µg/ml UD	Concentration of C-peptide in media containing 200 µg/ml UD	P value¶
Before adding	0.76 ± 0.03	0.31 ± 0.01	0.7 ± 0.02	0.32 ± 0.01	NS
60 min after adding	0.50 ± 0.01	0.33 ± 0.01	0.2 ± 0.02	0.33 ± 0.02	NS
120 min after adding	0.60 ± 0.01	0.86 ± 0.03	0.40 ± 0.01	0.93 ± 0.04	NS
180 min after adding	0.36 ± 0.01	0.80 ± 0.03	0.39 ± 0.01	0.77 ± 0.04	NS
P-value*	NS	NS	NS	NS	

¶ Comparison of different concentration of UD; * Comparison fixed concentration UD in different time; NS: non SIGNIFICANT.

(Kavalal et al., 2003).

In an animal study, diabetic Rats treated with a Methanol extract derived from UD showed a significant decreased in blood glucose level (Fathi Azad et al., 2005). Golalipour and Khori (2007) showed hydroalcoholic extract of UD has hypoglycemic effects in hyperglycemic Rats. According to this study, animals that received hydroalcoholic extract of UD 100 mg/kg for five days had been beneficially affected. On the other hand, in another study by Golalipour et al (2006), chronic administration of UD did not showed hypoglycemic effect or induction of regeneration of beta cells of pancreas in rats. Durdi et al. recently showed that alcoholic extract of *U. dioica* leaves can reduce glucose level and increase insulin secretion, acetyl coenzyme A carboxylase, and nucleoside diphosphate kinase activity in the alloxan diabetic animals (Durdi et al., 2011). Other researchers found more than glycemic effect for UD (Alisi et al., 2008). Bnouham et al. (2003) demonstrated that when UD administered 30 min before glucose loading, a strong glucose lowering effect appeared. However, the aqueous extract of UD (500 mg/kg) did not modify the blood glucose level. They showed that UD has a significant antihyperglycemic effect in oral glucose tolerance test (OGTT) model. They attributed this effect in part to the reduction of intestinal glucose absorption.

In vivo study by Bijan et al. (2003) about the blood glucose lowering effect of the extract of UD showed an enhancement of insulin secretion by Langerhance islets. Results of our study are challenge that study, due to inability of UD extract in enhancing insulin and C-peptide secretion from RIN5F pancreatic beta cells in our study. Based on these results, it seems that hypoglycemic effect of UD, if any, is not the anticipated mechanisms mentioned in the objectives of this study. Probably similar to Bnouham et al.'s study hypoglycemic effects of hydroalcoholic extract of UD may be exerted by reduction of intestinal glucose absorption.

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