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Original Article

Endocrinology Section

Efficacy of the Total Extract of Urtica Dioica on the glucose utilization by the Human Muscle Cells

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ABSTRACT

Objective: Plants are being used in the treatment of diabetes mellitus (DM) in the traditional system of medicine. Urtica Dioica (UD) has a variety of uses in traditional medicine. There are lots of reports about the hypoglycaemic effects of UD, but only few reports about the hypoglycaemic mechanisms of UD can be found in literature. The present study was designed to determine the possible mechanisms of the hypoglycaemic effects of UD on the glucose utilization by the human muscle cells in an in vitro study.

Research Design and Methods: Human muscle cells were grown in multiple flasks which contained the culture medium. An alcoholic extract of UD at concentrations of 50, 100 and 200µ/ml alone and in combination with insulin were added to the muscle cells in the flasks. The glucose levels in the flasks were measured before and 60,120 and 180 minutes after applying the extract or the extract plus insulin.

Results: The mean glucose level in the muscle cell cultures with UD alone and with UD plus insulin at the above mentioned concentrations and time intervals did not change significantly. ($p>0.05$)

Conclusions: The results of the present study demonstrated that the alcoholic extract of UD was unable to enhance the glucose utilization directly or by increasing the insulin sensitivity in the muscle cells and so, for the interpretation of the hypoglycaemic effects of UD (if any), other possibilities must be studied.

Key Words: Urtica dioica, Insulin sensitivity, Hypoglycaemic activity

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder in the metabolism of carbohydrates, proteins, and fat due to an absolute or relative deficiency of insulin secretion or a varying degree of insulin resistance [1, 2]. The increasing epidemy of DM has changed this problem from a medical entity to a social challenge. The number of adults with DM in the world will rise from 285 million in 2010 to 439 million in the year 2030 [3]. The patients with DM experience significant morbidity and mortality from microvascular and macrovascular complications. The microvascular disease leads to retinopathy, neuropathy and nephropathy (nephropathy leads to uraemia) [4, 5]. The 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 ischaemic type) and peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot [6].

The use of herbal remedies has been on the rise worldwide [7-9]. Plants are being used in the treatment of DM in the traditional system of medicine [10]. Urtica Dioica (UD) (stinging nettle) and Urtica urens (dwarf nettle) are members of the Urticaceae family, which are native to Eurasia, and they are considered to be therapeutically interchangeable [11]. There are some studies that showed the hypoglycaemic effects of UD [12, 13]. The mechanism or mechanisms behind the hypoglycaemic action of UD are not clear.

Most of the diabetic patients have varying degrees of insulin resistance, which is defined as a complex nutritional–metabolic state which is characterized by the reduced sensitivity of the target tissues (liver, skeletal muscle and adipose tissue) to the physiological effects of insulin [14]. Some antidiabetic drugs target this resistance. We hypothesized that the extracts of UD could increase the insulin sensitivity in human muscle cells, so that it induced hypoglycaemic effects in diabetic patients. The present study was designed to examine this hypothesis.

METHODS

Preparation of Extract

UD is available in the traditional markets in Tabriz-Iran. Dried DU was purchased from the market and it was identified by a pharmacognosist. By using an electric mill, the plants were crushed into a fine powder. The obtained powder was extracted repeatedly by using 70 percent methanol as a solvent (for 5 days) by the soak method (Maceration). In the second stage, this hydro-alcoholic extract were dried completely in a rotary evaporator at a temperature of 45ºC and at a pressure of below 100 mm Hg. The dried extract was stored in a refrigerator at temperatures below zero degrees of centigrade for further use.

Cell Culture

The HT1080 (code NCBI: C437) cell type was obtained from National Cell bank of Iran (NCBI) which was affiliated to the Pasteur Institute of Iran. The cells were prepared in DMEM [Dulbecco/Vogt modified Eagle's (Harry Eagle) minimal essential medium] and RPMI (Roswell Park Memorial Institute) 1640 medium and they were cultured in 5% C02 and 10% foetal calf albumin with penicillin G 80 mg and 50 mg streptomycin under sterile conditions. After the required number of specific cells was acquired and the confluence state was reached, the insulin and glucose levels were measured as the base line values.

Cell Viability

The Trypan Blue colour was used to assess the proportion of the live cells in the flask. This colour could only paint the living cells and not the dead cells. After painting the cells, by using Neubauer slides and a light microscope, the determination of the percentages of the viable and dead cells was done. We found that more than 85% of the cells were alive, which technically and according to the references was acceptable [15, 16].

Study Protocol

Human muscle cells were prepared in the Cell Culture Laboratory of the Drug Applied Research Center in the DMEMI medium in six flasks. The cells were prepared in the same size as in the case and the control flasks. Before each intervention, the glucose levels in the case and the control flasks were measured. The suspensions, 50, 100 and 200 micrograms of the UD extract in 1ml normal saline were added to the flasks which contained the muscle cells. Thereafter, 1 unit of insulin regular was added to the case flasks. At time zero (before the intervention) and after 60, 120 and 180 minutes (after adding the UD extract and insulin) the level of glucose was measured in all the flasks. The glucose levels were measured by the glucose oxidase method (Pars Azemoon©, Iran). There were no ethical concerns.

STATISTICAL ANALYSIS

The statistical analysis was done by means of the statistical package, SPSS 16. The values were presented as mean and standard deviation, at a 95% confidence interval. Comparison between the groups at different times was performed by using a Repeat Measure Model. The level of significance was set at 0.05.

RESULTS

[Table/Fig-1] shows the concentrations of the glucose levels in the medium which contained the human muscle cells in the control, and the case groups before and after adding the UD extract in doses of 50, 100 and 200 µg/ml and at time periods before, 60, 120 and 180 minutes. There were no significant changes in the glucose levels in all the groups which were with and without UD, at different time periods (P>0.05). Also, the mean glucose level had not changed in the media with different concentrations of the extract.

[Table/Fig-2] shows the glucose concentrations in the media which contained the human muscle cells before and after treating them with the alcoholic extract of UD and insulin at concentrations of 50, 100 and 200 µg/ml at times before, 60, 120 and 180 minutes. There was no significant difference between the glucose concentrations in the different media which contained UD and insulin (p>0.05).

DISCUSSION

Despite all the marvelous advancements in modem medicine, traditional herbal medicine has always been practised. Alternative therapies are being used by people in our region (as in other regions) who have faith in spiritual healers. More than 800 plants have been reported to have antidiabetic properties [17], for example, today, up to 600 traditional herbal medicines have been reported as medicines for DM in India [18]. Ethnopharmacological surveys have shown that more than 1200 plants are being used in the traditional medicine for their alleged hypoglycaemic activities [19]. Like all the green vegetables, the UD leaf densely contains several micronutrients [20]. Despite the abundance of reports about the antidiabetic properties of UD, there is only little scientific explanation which is available on these effects. The present study was designed to determine the possible mechanisms of the hypoglycaemic effects of UD on the human muscle cells and its effects on the insulin sensitivity.

High glucose concentrations cause the development of insulin resistance in the peripheral tissues, including the skeletal muscle, owing to the impairment of both insulin secretion and insulin sensitivity [21]. Many traditional plants have been introduced as the

Time (minute)	Concentrations of glucose in media containing 50µg/ml UD Mean ± SE	Concentrations of glucose in media containing 100µg/ml UD Mean ± SE	Concentrations of glucose in media containing 200µg/ml UD Mean ± SE
Before adding	212±2.35	212±2.11	204±2.35
60 minutes after adding	210±2.11	212±2.35	203±2.11
120 minutes after adding	216±2.35	212±2.35	06±2.11
180 minutes after adding	218±2.94	211±2.11	208±2.94

[Table/Fig-1]: Concentration of glucose (mg/dl) in medium containing human muscle cells in the before and after addition of UD extract with doses 50 and 100 and 200µg/ml in different times.

Time (minute)	Concentrations of glucose in media containing 50µg/ml UD with insulin Mean ± SE	Concentrations of glucose in media containing 100µg/ml UD with insulin Mean ± SE	Concentrations of glucose in media containing 200µg/ml UD with insulin Mean ± SE	P Value¶
Before adding	204±2.11	206±2.11	202±2.11	NS
60 minutes after adding	214±2.94	208±2.35	209±2.35	NS
120 minutes after adding	213±2.94	210±2.35	204±2.11	NS
180 minutes after adding	213±2.35	211±2.94	213±2.35	NS
P Value*	NS	NS	NS	

[Table/Fig-2]: Concentration of glucose (mg/dl) in medium containing human muscle cells in the before and after addition of UD extract with doses 50 and 100 and 200µg/ml with insulin in different times.

Comparison of different concentration of UD
* Comparison fixed concentration UD in different time
NS: Non Significant

treatments for DM [22-24]. There is some data on the mechanisms of UD that can reduce the blood glucose levels and studies have different results with together. The oral administration of the hydroalcoholic extract of UD at a dose of 100 mg/kg showed a strong glucose lowering effect on streptozocin (STZ) induced DM in rats. It showed protective effects on the pancreatic cells in animal models [25].

In an animal study, diabetic rats which were treated with a methanol extract which was derived from UD caused a significant decrease in the blood glucose levels [26, 27]. Golalipour and Khori [28] showed that the hydroalcoholic extract of UD had hypoglycaemic effects on hyperglycaemic rats. According to this study, animals that received the hydroalcoholic extract of UD 100mg/kg for five days had been beneficially affected. On the other hand, in another study which was done by Golalipour et al [29], the chronic administration of UD did not show hypoglycaemic effects or the induction of the regeneration of the beta cells of the pancreas in rats.

Bnouham et al. [12] demonstrated that when UD was administered 30 minutes before the glucose loading, a strong glucose lowering effect was observed. However, the aqueous extract of UD (500 mg/kg) did not modify the blood glucose levels. They showed that UD had a significant antihyperglycaemic effect in an oral glucose tolerance test (OGTT) model. They attributed this effect in part to the reduction of intestinal glucose absorption. The lack of the hypoglycaemic effect of the nettle aqueous extract in alloxan-induced diabetic rats, which was a model of DM with hypo-insulinaemia, demonstrated that this extract could act on glucose homeostasis by the extrapancreatic way.

An in vivo study by Farzami et al [30], on the blood glucose lowering effects of the extract of UD, showed an enhancement of insulin secretion by the islets of Langerhans. The results of our study challenged those of that study based on the inability of the UD extract in increasing the insulin sensitivity and in reducing the glucose levels in vitro. Based on our results, it seems that the hypoglycaemic effects of UD, if any, were not any of the anticipated mechanisms that are mentioned in the objectives of this study. Probably, the hypoglycaemic effects of the hydroalcoholic extract of UD may be exerted by the reduction in the intestinal glucose absorption, as was reported in Bnouham et al's study.

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