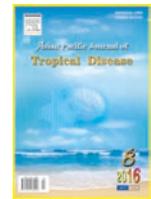




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Effect of hydroalcoholic leaves extract of *Citrullus colocynthis* on induction of insulin secretion from isolated rat islets of Langerhans

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ABSTRACT

Objective: To study the effect of hydroalcoholic leaves extract of *Citrullus colocynthis* (*C. colocynthis*) on insulin secretion in isolated Langerhans islets of rat.

Methods: The islets were isolated from male adult Wistar rats' pancreas by enzymatic digestion method. After isolation, the islets were incubated in different doses of hydroalcoholic leaves extract of *C. colocynthis* (0.01, 0.10 and 1.00 mg/mL Hanks' balanced salt solution for 30 min and then calculated insulin level by ELISA method.

Results: The result showed that *C. colocynthis* has a potential effect on insulin secretion. It was found that dose of 0.1 mg/mL Hanks' balanced salt solution extract has best affect on induction of insulin secretion.

Conclusions: It is suggested that further studies should be done on insulin releasing effect of secondary metabolites (flavones and flavonoids) isolated from *C. colocynthis*.

1. Introduction

Diabetes is a multi-factorial disease that is known as the most common endocrine disorder. Major cause of this disease is the defect of insulin secretion and resistance of peripheral tissue to insulin[1,2]. Diabetes complications such as retinopathy, nephropathy, neuropathy and cardiovascular problems resulted from hyperglycemia during diabetes[3]. Prevalence of diabetes is rising and studies show that about 300 to 328 million individual suffer from diabetes. If this condition continues, its prevalence will be likely to reach to 592 million individual in 2035[4,5].

Insulin is the only hormone that reduces blood sugar. It secretes from the beta cells of Langerhans islets in response to reduction of blood sugar[6,7]. Inappropriate secretion of insulin leads to

disturbances in lipid and carbohydrate metabolism and even energy homeostasis. In diabetic patients glucose is not able to stimulate insulin secretion and subsequently resulted in hyperglycemia[8,9]. Thus, maintaining secretory power in islets can be helpful for preventing or delaying diabetes occurrence[10]. Many compounds have been identified that are stimulators of insulin secretion including glucose, arginine, acetylcholine and also many effective drugs against diabetes act in this way such as sulfonylurea[1,9,11,12]. But overstimulation of sulfonylurea can cause side effects such as hypoglycemia[8].

Recently, more attention has been paid to herbs and their secondary metabolites to treat diabetes and approximately 75% of people around the world used the traditional medicine[8,13]. Extensive researches in the field of medicinal plants suggest that the plants have a potential to treat and control diabetes complications[4,13]. Thus, it has been reported as the common drugs for treatment of diabetes, such as metformin obtained from the plants[14].

Citrullus colocynthis (L.) (*C. colocynthis*) belongs to the Cucurbitaceae family and commonly named wild gourd or bitter apple. It is usually found in desert areas particularly the Middle East and Arabic countries[15]. Studies were confirmed that this plant is effective for the treatment of asthma, jaundice, abortifacient, ulcer and amenorrhea and also has purgative and anti-cancer properties[15,16]. So far, various compounds like cucurbitacins were

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found from *C. colocynthis*[17]. Previous studies have been showed that extracts prepared from seed and fruit of *C. colocynthis* are effective on lowering blood glucose in animal models of diabetes[18-20]. Nevertheless, studies have not been done regarding the effect of its leaves on stimulation of insulin secretion.

The aim of this study was to evaluate effect of hydroalcoholic extract prepared from *C. colocynthis* leaves on insulin secretion in isolated Langerhans islets from male rats. This study was in line with our recent studies on diabetes complications including insulin resistance[21], protective effect on pancreas and heart[22], dyslipidemia[23] protein glycation[24], trace element changes[25], peroxidase/antioxidant balance[26], as well as on the mechanisms of plant ingredients in the treatment of diabetes mellitus[27].

2. Materials and methods

2.1. Chemical reagent

Ethanol, glucose, dimethyl sulfoxide and dithizone were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Ketamine and xylazine were prepared from Alfasan (Woerden, Netherlands). Hanks' balanced salt solution (HBSS), Roswell Park Memorial Institute 1640, 10% fetal bovine serum and collagenase IV were purchased from Gibco (Gibco BrL, New York, USA). Rat insulin kit was also purchased from Mercodia (Uppsala, Sweden).

2.2. Plant collection and authentication

Plant was collected from desert areas of Mehran city located in west of Iran during flowering stage (August to September 2014). Medicinal plants and natural products research center investigated and then authenticated the plant. A voucher specimen of the plant was retained at herbarium of medicinal plants and natural products research center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No. A140740100FP).

2.3. Preparation of hydroalcoholic extract

The preparation method of hydroalcoholic extract was described in our previous study[22]. Briefly, the leaves of *C. colocynthis* were manually isolated and then dried at shade. After drying, the leaves by grinder were fully powdered and soaked at ethanol: water (70:30 v/v) for 48 to 72 h, subsequently were concentrated with rotary machine. Finally they were put in freezer dryer for 24 to 74 h and then obtained hydroalcoholic extract.

2.4. Isolation of rat pancreatic islets

The collagenase digestion method was used for rat islets isolation[28]. The male Wistar rats (220–250 body weight) were anesthetized with ketamine and xylazine. And then the pancreases were isolated and with normal saline fully washed. Subsequently, they were washed by 20 mL cold HBSS for three times (each time 1–2 min). After washing, HBSS was discarded and 1.5–2.0 mg collagenase IV/1 mL HBSS to pancreases were added, then fully pipetted. In next stage, they were placed in shaking incubator for 20 min and vortexed for about 5–6 times. For stop of enzymatic digestion, cold HBSS was added. Finally, digested pancreases were centrifuged (1500 r/min for 60 s) to isolate islet and supernatant was discarded and the islets at the bottom of the tube remained. In order to confirm the isolation, the dithizone-dimethyl

sulfoxide staining was used. After preparation of solution of dissolved dithizone in 5 mL dimethyl sulfoxide which was added to isolated islets and placed in incubator (15 min) and washed with HBSS. The beta cells observed as red color by light microscope due to the high presence of zinc element[29].

2.5. Incubation isolated rat Langerhans islets with extract

Isolated rat islets were placed in the medium (2 mL Roswell Park Memorial Institute 1640, 10% fetal bovine serum) and simultaneously hydroalcoholic extract (0.01, 0.10 and 1.00 mg/mL HBSS) was added overnight at 37 °C. Then 1 mL HBSS with 2.8 mmol glucose were pre-incubated for 30 min. For incubation, liquid pre-incubation was discarded and the wells were divided into two groups including basal group (added 1 mL HBSS with 2.8 mmol glucose) and stimulant group (added 1 mL HBSS with 16.7 mmol glucose) at incubator for 30 min. Then supernatant was collected and stored at –20 °C for measurement of insulin level. Insulin level was determined using ELISA method by rat insulin kit (Mercodia, Uppsala, Sweden).

2.6. Animal ethical committee approval

All experimental procedures involving animals were conducted in accordance to ethical guidelines for working with experimental animals and approved by the Ethics Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

2.7. Statistical analysis

The all results were showed as mean \pm SD. Data analysis was performed to SPSS software by One-way ANOVA followed by Tukey test. $P \leq 0.05$ was considered as significant level.

3. Results

3.1. Effect of leaves extract of *C. colocynthis* on insulin secretion

We treated isolated rat pancreas with different doses of hydroalcoholic leaves extract of *C. colocynthis* (0.01, 0.10 and 1.00 mg/mL HBSS) (Table 1). The results showed that insulin level in control group treated by stimulant dose of glucose (16.7 mmol) was significantly increased compared to group treated by basal dose of glucose (2.8 mmol) approximately 1.64-fold ($P \leq 0.05$). Moreover, the level of insulin in groups treated with 0.01, 0.10 and 1.00 mg/mL HBSS extract along with stimulant dose of glucose (16.7 mmol) was significantly higher than the same groups along with basal dose of glucose (2.8 mmol) about 1.80, 1.98 and 1.85-fold respectively ($P \leq 0.05$).

Table 1

Effect of hydroalcoholic leaves extract of *C. colocynthis* on insulin secretion from isolated rat islets.

Group	Level of insulin (μ IU/mL)	
	Basal glucose (2.8 mmol)	Stimulant glucose (16.7 mmol)
Control group	1.730 \pm 0.105	2.840 \pm 0.651*
T 0.01	4.850 \pm 0.985	8.760 \pm 0.008**
T 0.10	5.330 \pm 0.148	10.600 \pm 0.934#
T 1.00	6.080 \pm 0.658	11.290 \pm 0.015##

Values were presented as mean \pm SD. One-way ANOVA followed by Tukey test. T 0.01: Group of treated with 0.01 mg/mL HBSS hydroalcoholic extract; T 0.10: Group of treated with 0.10 mg/mL HBSS hydroalcoholic extract; T 1.00: Group of treated with 1.00 mg/mL HBSS hydroalcoholic extract. *: significant different with C; **: Significant different with T 0.01; #: Significant different with T 0.10; ##: Significant different with T 1.00. P value ≤ 0.05 .

Although concomitant with increase of dose was showed an increment of insulin level (8.760, 10.600, and 11.290 respectively) but there was not dramatically different between doses of 0.1 to 1.0 mg/mL HBSS extract. In deed, the most effect on insulin secretion was found in treatment by 0.1 mg/mL HBSS extract.

4. Discussion

Insulin is a hormone secreted by the pancreas which is the most important hormone in energy homeostasis. Defect of insulin secretion leads to diabetes. Therefore the discovery of compounds that have feature of stimulation of insulin secretion can be very important in treatment of diabetes[7,30]. This study is the first finding in association with the effect of hydroalcoholic leaves extract of *C. colocynthis* on insulin secretion in isolated islets from male rats. And the finding showed that extract prepared from this plant has property of insulin secretion *in vitro*.

Although glucose is the main stimulant for induction of insulin secretion but along with the progression of the diabetes, this process will be gradually abolished due to the disturbance of glucose entry into beta cells so that glucose-induced insulin secretion reduction is common property in diabetic patients[31]. According to the above sentence, we believed that continuing maintenance of insulin secretion delays many of the side effects of diabetes and focusing on this issue can be successful in diabetes treatment. In previous studies, the promising effects of *C. colocynthis* on improvement of diabetes particularly insulin secretion have been reported so that its seed result in protection of pancreatic beta cell against streptozotocin-induced damage and its seedless pulp leads to normalizing of serum insulin level and increase of insulin in beta cell under diabetes condition in rat[19,32,33]. Two-month treatment of diabetic rats with aqueous extract prepared from leaves of *C. colocynthis* led to increase of liver hexokinase activity and activity reduction of glucose-6-phosphatase and fructose-1, 6-bisphosphatase and ultimately resulted in significant reduction in blood sugar, but in this study the amount of serum insulin did not examined. This study also mentioned that the leaves of *C. colocynthis* contain phenolic compounds[34]. Study has not been conducted on the effect of its leaves on insulin level. Our result indicated that hydroalcoholic leaves extract of *C. colocynthis* resulted in increment of insulin secretion concomitant with dose increase but there was not dramatically change between doses of 0.1 to 1.0 mg/mL HBSS, indeed dose of 0.1 mg/mL HBSS was the best dose for induction of insulin secretion.

The compounds identified in the aerial parts of the plant can be noted to flavones such as isovitexin and isoorientin[35]. In the study, it was found that isovitexin has hypoglycemic effect and is very useful compound for the secretion of insulin[36]. It also seems that this effect is due to the presence of flavonoids in *C. colocynthis*. Because previous studies have been reported that *C. colocynthis* is rich of flavonoids[37-40]. On the other hand, Meena and Patni in 2008 reported that there are flavonoids such as quercetin in different parts of *C. colocynthis* including leaf, stem, fruit and root[41]. Previous studies has been determined that various flavonoids have the ability to secretion of insulin *in vitro*. For example, Cazarolli *et al.* has been reported that apigenin has property of insulin secretion[42]. In addition, it has been showed that quercetin leads to insulin secretion in insulinoma cell line-1 pancreatic beta-cells[10]. In fact, quercetin is one of the candidate's

flavonoids to ameliorate stimulation of insulin secretion[43]. Because it involves with the L-type Ca^{2+} channels resulting in an increase of Ca^{2+} influx and ultimately insulin secretion[44]. In addition, Benariba *et al.* in 2013 suggested that effect of *C. colocynthis* on stimulation of insulin releases in isolated islets from the pancreas of rats associated with the presence of quercetin and myrcetin in this plant[45].

Here, We obtained a novel finding about effect of *C. colocynthis* on insulin releasing from Langerhans islets of rat. Probably flavones and flavonoids can be useful in stimulation of insulin secretion. Therefore, We suggest that to understand the effect mechanism of *C. colocynthis*, further investigations should be done on its secondary metabolites especially isolated flavones and flavonoids.

Conflict of interest statement

We declare that we have no conflict of interest.

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