Study the Effect of *Arabidopsis thaliana* Extract on Reducing Blood Glucose Level in Diabetic White Albino Mice

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Abstract

This study was designed to evaluate the effect of aqueous extract of *Arabidopsis thaliana* seeds on reducing glucose level for white albino mice. Twenty adults mice were used, divided randomly into four groups (five mice per each group). The first group (normal mice) was administrated with 0.1 ml of distilled water as a control, the second group (normal mice) was administrated with 0.1 ml of the plant extract, whereas the third and fourth groups (diabetic mice) were administrated with single dose of alloxan (150 mg/kg of the body weight) to induce diabetes, and the fourth group was administrated with 0.1 ml of the plant extract for 10 days, then blood glucose level was measured for all of the experimental animals (diabetic and non diabetic). Results showed clear increasing in glucose levels in the diabetic mice, while significant reduction was recorded in glucose levels of the normal mice that was treated with the plant extract as compared with the control group. These results indicate that *Arabidopsis thaliana* seeds aqueous extract possesses a hypoglycemic effect.

Key words: Arabidopsis thaliana, glucose level, albino mice

دراسة تأثير مستخلص نبات اذان الفار *Arabidopsis thaliana* في خفض مستوى الكلوكوز في دم الفئران البيضاء المصابة بالسكر

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الخلاصة

صممت هذه الدراسة لتقييم تأثير المستخلص المائي لبذور نبات اذان الفار *Arabidopsis thaliana* على مستوى السكر في دم الفئران البيضاء. استعملت في هذه التجربة 20 فأرة في مرحلة النضوج, قسمت عشوائياً الى اربعة مجاميع (5 فئران لكل مجموعة). جرعت المجموعة الاولى 0,1 مللتر من الماء المقطر , المجموعة الثانية جرعت 0,1 مللتر من مستخلص آذان الفأر (تركيز 200 ملغم/ كغم من وزن الفأر), والمجموعة الثالثة والرابعة حقنت بجرعة مفردة من الالوكسان (150 ملغم / كغم .من وزن الفأر) لأستحداث مرض السكري وقد جرعت المجموعة الرابعة 0,1 مللتر من مستخلص المائي ولمدة 10 ايام. بعدها تم قياس مستوى الكلوكوز بدم الحيوانات المختبرة (المصابة والسليمة). بينت النتائج زيادة واضحة في مستوى الكلوكوز في مجموعة الفئران المعاملة (المصابة), ونقصان واضح في مجموعة الفئران المصابة والتي جرعت بالمستخلص المائي للنبات , وكذلك نقصان في مستوى الكلوكوز للفئران السليمة المجرعة بالمستخلص بالمقارنة مع المجموعة الطبيعية (السيطرة). تشير هذه النتائج الى ان المستخلص المائي لبذور آذان الفار تمتلك تاثير خافض للسكر .

الكلمات المفتاحية:أذان الفار ، مستوى السكر، الفئران الالبينو .

Introduction

Approximately 0.7% of the world's population suffers from insulin-dependent diabetes mellitus [(1)]. It has been estimated that the incidence of diabetes will double to approximately 300 million in the next 25 years. To date, insulin therapy is the only effective treatment for Type 1 diabetes and is also generally required for the treatment of Type 2 diabetes as the disease progresses. Therapyrequires the regular monitoring of blood glucose levels , combined with frequent injection of insulin , in order to avoid the severe debilitating secondary complications associated with chronic hypoglycemia. Meeting this demand will necessitate the development of more cost-effective, higher capacity production in the near future [(2)]. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus.

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So the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus [3]. In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. Marles and Farnsworth estimated that more than 1000 plant species are being used as folk medicine for diabetes [4]. Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels [5, 6, 7]. Several species of herbal drugs have been described in the scientific and popular literature as having antidiabetic activity [8].

Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed [9]. Best reported for the presence of insulin-like substances in plant materials like green tops of onions, lettuce leaves, green bean leaves, barley roots, beet roots and others [10]. The discovery of this hormone in tissues of the higher plants as well as in yeast opened up a new field of research in plant metabolism and afforded another remarkable example of parallelism between certain physiological processes in the plant kingdom with the animal kingdom. Pancreatic insulin's influence on glycogen formation provoked a theoretical concept on the existence of insulin or insulin like protein hormones in organisms rich in glycogen. Collip's efforts on extracts of yeast and onion were successful in altering glucose metabolism. The term glucokinin was proposed by him in order to differentiate insulin of plant origin from that of animals. Following Collip's discovery Charles Best reported on insulin like material in germinating potatoes, rice and even in beetroot [11, 12].*Arabidopsis thaliana*, a small, annual flowering, dicotyledonous plant, was discovered by Johannes Thal (hence, thaliana) in the Harz mountains in the sixteenth century. Arabidopsis is a member of the Brassicaceae family, which includes important crops. It has no agronomic significance, but offers important advantages for basic research in genetics and molecular biology [13].

The aims of this research are to determine the active compounds in Arabidopsis plant seeds, for anti-diabetic treatments of patients with high glucose level in their blood, and to achieve commercial and economic source of insulin.

Materials and Methods

Plant material

Seeds of *Arabidopsis thaliana* were obtained from Dr. Enas Muhgin in Ebn-Albetar Center-Baghdad. The plant was cultivated in north of Iraq and used in genetic engineering experiments in research centers. It was authenticated by Biology Department-College of Science-Baghdad University.

Preparation of the extract

The powdered material of seeds (50 gram) added to 250 ml of distilled water, left over night on stirrer, the extract then dried under reduced pressure and was subjected to various chemical tests to detect the presence of different active phytoconstituents like alkaloids, tannins, flavonoids, saponins, terpens and steroids [14].

Detection of some active compounds-Colorimetric test

This detection was carried out using chemical reagents and depends on appearance of the color to determine the presence of the compound only.

Detection of tannins

(10 gram) of plant powder was mixed with 50 ml distilled water in a magnetic stirrer. The mixture was boiled in a boiling water bath for few minutes, then filtered and the filtrate was treated with few drops of 1% lead acetate solution. The development of greenish-blue precipitate is an indicator for the presence of tannins [15].

Detection of saponins

Saponins were detected by two methods: The first method, aqueous extract of *A. thaliana* seeds powder was shaken vigorously with distilled water in a test tube. The formation of foam standing for a time indicates a positive result. The second method, five milliliters of aqueous extract of the plant was added to 1-3 drops of 3% ferric chloride solution, a white precipitate was developed which indicates a positive result [16].

Detection of terpenes and steroids

(1 ml) of ethanolic extract was participated in a few drops of chloroform, then a drop of acetate anhydride and drop of concentrated sulfuric acid were added, brown precipitate appeared which representing the presence of terpene, and the appearance of dark blue color after few minutes would represent the present of steroids. The color is due to the hydroxyl group (-OH) of the steroids reacting with the reagents and increasing the conjugation of the un-saturation in the adjacent fused ring. Since this

test uses acetic anhydride and sulfuric acid as reagents, caution must be exercised so as not to receive severe burns [17].

Detection of flavonoids

Flavonoids were extracted by well established method of Harborne 1984, and the procedure has also been followed by several others authors. The extraction protocol which has been carried out in this investigation is only for detection of flavonoids in seed extract of *A. thaliana*. Ethanolic extract was partitioned with petroleum ether (1:1v/v), the aqueous layer was mixed with the aluminum solution. The appearance of dark color is an evidence for the presence of flavonoids. Flavonoids react with the reagent and give colour reactions. Spraying reagents 5% fehling solution and 1% AlCl3 solution are exclusively used to detect flavonoids [17].

Detection of alkaloids

(10 gram) of the extract was boiled with 50 ml of distilled water and 4% of hydrochloric acid was added, then the solution was filtered and cooled. 0.5 ml of the supernatant was tested with Mayer solution, appearance of white precipitate indicates the presence of alkaloids [17].

Experimental animals

Healthy 20 adult albino male mice of Swiss albino strain were obtained from the animal house of Biotechnology Research Center, Al-Nahrain University. The age of the mice was 8 weeks, and the weight was 25 gram. The animals were housed in plastic cages, which were cleaned and sterilized weekly with 70% ethanol. Five mice kept in each cage with natural 14 hours light , 10 hours dark , and a controlled temperature at (24-28) C^{o} . The animals were fed chow and water (The protocol was proved by Institutional Animal Ethical Committee JKKMMRF/CP/PhD/ 2008) .

Induction of diabetes

The animals were fasted for 24 hours, then diabetes was induced by a single intraperitoneal (IP) injection of alloxan monohydrated dissolved in distilled water at a dose of 150 mg/kg of mice body weight in volume of 0.1 ml. The diabetic state was confirmed 48 hours after alloxan injection. Blood glucose value was reached 260 mg/dl which indicate hyperglycemia (140 mg/dl as standard before treatment), and there was 5% mortality in animals treated with alloxan. Surviving mice with fasting blood glucose level 250 mg/dl or higher were included in this study [18].

Experimental groups

The animals were divided into four groups (five mice per each group), and the groups were treated as following:

First group, control, normal mice administrated with 0.1 ml distilled water.

Second group, normal mice administrated with 0.1 ml of Arabidopsis seed extract.

Third group, diabetic mice administrated with 0.1 ml of distilled water.

Fourth group, diabetic mice administrated with 0.1 ml of Arabidopsis seed extract.

Blood sample collection

For 10 days after the experiment, blood samples were collected every two days (2, 4, 6, 8, 10) days, from the tail vein of the mice under the experiment, and glucose was assayed immediately using glucometer apparatus.

Results

Results

Chemical detection

The chemical test of the active compounds in A*rabidopsis thaliana* seed extract showed in table (1) indicated that the aqueous extract of this medicinal plant contains tannins, favonoids, alkaloids, saponins, terpenes and steroids.

Table (1): Chemical detection of some active compounds in *Arabidopsis thaliana* seed extract

No.	Compound	Result
1	Tannins	+
2	Flavonoids	+
3	Alkaloids	+
4	Saponins	+
5	Terpenes and Steroids	+
6	Glycoside	-
(+) means positive detection , (-) means negative detection		

Anti-hyperglycemic activity

The effect of treatment with aqueous extract of *Arabidopsis thaliana* on blood glucose levels in normal and diabetic mice are reported in table 2. Blood glucose levels of the treated mice were significantly higher than normal mice (control). Injection of 0.1 ml alloxan induced diabetes in the experimental animals as data showed, higher glucose level reached 249.2 mg/dl (as the average of five investigation) for the diabetic mice, and while it was recorded 166 mg/dl when it was injected with 0.1 ml of Arabidopsis extract

(200 mg/kg of B. Wt). It was reached 163 mg/dl with diabetic mice which were treated with swine insulin. From these results, significant decrease in blood glucose levels was obtained as compared with normal mice (control treatment) which recorded 164.4 mg/dl.

Table 2: Anti-hyperglycemic effects of *Arabidopsis thaliana* seed extract (200 mg/kg of body weight) on induced diabetic mice

Group/Treatment Dose (0.1 ml)	After 2 days (mg/dl)	After 4 days (mg/dl)	After 6 days (mg/dl)	After 8 days (mg/dl)	After 10 days (mg/dl)	AVG (mg/dl)
Normal mice (control)	142	168	162	169	181	164.4
Diabetic mice treated with alloxan only	260	250	242	248	248	249.2
Diabetic mice treatment with insulin	168	164	162	163	164	163
Diabetic mice treated with extract	173	171	161	166	159	166

Discussion

The plant extract studied could be an answer to the people seeking for therapeutic agents from natural sources which is believed to be more efficient with a little or no side effects when compared to the synthetic chemotherapeutic agents. The present experiment indicated that aqueous extract of *Arabidopsis* seeds exhibited a potent blood glucose lowering properties in diabetic white albino mice. Recombinant human insulin in the model plant species of *A. thaliana* seeds was produced by Cory et al. [(2)].

Weili et al., reported that an important therapeutic protein human insulin-like growth factor 1(hIGF-1) called somatomedin C, was expressed in *Arabidopsis thaliana* seeds via oleosin fusion technology. The biological activity of the hIGF-1 as an oleosin-hIGF-1 fusion protein *in vitro* was demonstrated by using human neuroblastoma cells [(19)].

The hypoglycemic activity shown in this study may be related to the presence of flavonoids compounds which had very pronounces effect in the seed extract of this plant. Flavonoids may preserve β-cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes. A prospective study in Finland showed that the intakes of some specific types of flavonoids including quercetin and myricetin were inversely associated with risk of incident type 2 diabetes. In addition, emerging evidence shows that oxidative stress may be involved in the pathogenesis of chronic inflammation underlying insulin resistance, diabetes and cardiovascular disease [(20, 21)].

Many literature reports suggest the existence of proteins with functions similar to proteins that are members of insulin pathways characteristic of vertebrates. Localization of insulin-like protein in plant tissues and it has functions in connection with carbohydrate metabolism [(22)]. Results of this study are in harmony with the results demonstrated by Sheng et al. who found a hypoglycemic activity in *Momordica charantia* and *Canavalia ensiflormis* seed extracts. The results are also in agreement with Panahi et al who found that transgenic plants such as, *Arabidopsis thaliana* producing recombinant human insulin-like growth factor-1 (hIGF-1), the plant-derived hIGF-1 caused differentiation of human neuroblastoma cell line SH-SY5Y, indicating its biological activity [(23, 24, 25)].

It was concluded that it was plant insulin, The presence of insulin-like molecule was recently demonstrated in the seed extract of *Arabidopsis thaliana* , this protein may be responsible for the lowering of blood glucose concentrations when it was injected in diabetic mice. The hypoglycemic activity of the insulin-like protein from seed extract of this plant was similar to that of commercial swine insulin used as control.

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