Effect of the aqueous extract and some proteinous compounds from *Aleo vera* leaves on Blood serum glucose and cholesterol levels in normal mice

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الخلاصة

تم فصل مركبين بروتينيين (II,I) من مستخلص نبات الصبار (*Aloe Barbadenesis Mill*) بتقنية الترشيح الهلامي للراسب البروتيني الناتج من الترسيب بكبريتات الامونيوم وتم تحديد الأوزان الجزيئية التقريبية بالتقنية ذاتها والتي كانت بمدى (63095 و 19952) دالتون على التوالي.

درست فعالية خفض الكلوكوز والكوليسترول للمركبات البروتينية المعزولة فضلاً عن المستخلص الخام في ذكور الفئران البيض السليمة. أشارت النتائج إلى أن التجريع في التجويف البريتوني للمستخلص الخام والمركب البروتيني (II) هما اللذان يمتلكان الفاعلية الأعلى في خفض مستوى سكر مصل الدم وكانت نسبة الانخفاض بحدود (41.77% و 36.71%) على التوالي.

كما أظهرت النتائج أيضاً ان للمركب البروتيني (I) المعطى عن طريق البريتون أدى الى خفض مستوى الكوليسترول في دم الفئران السليمة وبنسبة (19.98%)، بينما اظهر المركب البروتيني (II) بجرعة (40 ملغم/ 10 مل) أعلى نسبة انخفاض في مستوى الكلوكوز عند تجريعه فموياً وبنسبة (15.56%).

Abstract

Two proteinous compounds (I and II) had been isolated by gel filtration chromatography of the precipitate produced by full saturation of the aqueous extract of the *Aloe vera*.

The apparent molecular weights by gel filtration chromatography of the isolated compounds (I and II) were in the range of (63095,19952) Dalton respectively.

The effect of the aqueous extract and the proteinous fractions on glucose and cholesterol levels in normal mice were investigated.

Glucose level was lowered by intraperitoneally administration of the concentrated aqueous extract and the low molecular weight proteinous compound (II) by (41.77% and 36.71%) respectively in normal mice. Furthermore, there was non significant reduction in the cholesterol levels by intraperitoneally administration of high molecular weight proteinous compound (Peak I) by (19.98%) in normal mice. Also there was non significant reduction in the glucose levels by orally administration of (40 mg/10 ml) of low molecular weight proteinous compound (Peak II) by (15.56%).

Introduction

Aloe is a succulent plant, mostly found in East and south Africa that has been used medicinally for centuries. It belongs to the family (Liliaceae)[1] Botanically known as *Aloe barbadenesis*, Mill[2]**.**

The medicinal properties of various parts(Leaf, Aloe gel) which are rich in minerals (magnesium, calicium, chromium, copper, iron, …etc), enzymes, amino acids, vitamins as antioxidant[3]. *Aloe vera* plays an important role in many medical properties as a traditional medicine in many diseases such as Diabetes mellitus, Burns, Ulcerative colitis, Psoriasis, Wound healing[4]. Despite the fact that more than (1200) plants are used around the world in the empirical control of Diabetes mellitus, most of them have not been pharmacologically and chemically investigated[5].

Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels and disturbance in carbohydrates, fats and protein metabolism. Milder hyperglycemia, if present for many years, increase the risk of cardiovascular disease which can manifest as a heart attack, congestive heart failure and kidney failure[6].

The plant contains small chains of polysaccharides, which may regulate blood sugar levels may reduce inflammatory and balance insulin production in the pancrease[7]. In heart disease patients, *Aloe vera* leaf added to the diet, reduced total serum cholesterol, serum triglycerides, fasting and post prandial blood sugar levels[8].

The objective of this investigation was to evaluate the hypoglycemic activity of the aqueous extract and porteinous fractions of *aloe vera* in experimental mice.

Experimental

- Plant Used:

The plant was collected from the nursery of the College of Agriculture and Forests and was classified according to plant taxonomy or plant classification references related to medicinal plants.
Scientific name = (*Aloe vera*) also known (*Aleo Barbadensis*)[9]
Common name = True Aloe or Barbados Aloe.
Family = Liliaceae[10]

-Preparation of aqueous extract:

The cold extract was prepared by grinding (1000 g) of fresh leaves of *Aloe vera* for (10) minutes using a blender, then freezed in a deep freeze. Distilled water (2000 ml) was added and the crude homogenate was stirred for additional one hour then filtered through glass wool. Finally, the mixture was centrifuged for (15) minutes at (4000 xg). The filtrate was concentrated to one third volume by lyophilizer then kept for further investigations.

-Preparation of protein:

Proteinous precipitate was separated by full saturation of ammonium sulphate (75%)[11]. Gradual addition of ammonium sulphate at (4)ºC for (60) minutes accompanied with mixing by electrical mixer. The mixture was left at (0)ºC for (24) hours and then the proteinous precipitate was isolated by centrifugation for (20) minutes at (6000 xg) at (4)ºC. finally, the precipitate was dried by lyophilizer then kept for gel filtration chromatography. Total protein concentration in each step was determined by modified-Lowery method[12].

-Gel Filtration Chromatography:

Total protein was fractionated by gel filtration chromatography using sephadex G-100 in column (2×88 cm). Distilled water was used as a eluent solution. The same technique was used to determine the comparative molecular weights of proteinous fractions[13].

The molecular weight of each proteinous compound (comparative) was obtained from its elution volume under the same conditions of known molecular weights such as (Blue dextran, Bovine serum albumin, Eggs albumin, Trypsin, Insulin, Tryptophan).

-Animal used:

Fifty six male albino mice weighing (20-25)g were obtained from the animal room of College of Education, University of Mosul, were used in experiments. They were housed under standard conditions at temperature (27)ºC and had free access to food and water. The mice were divided randomly into seven groups, each contained four mice. Group

one was kept as control which administered distilled water. Groups (two, three, four, five) were administered orally with gavage needle in increasing concentration (200,300,400,500) mg/10 ml of body weight of crude extract of the plant[14]. Groups (six, seven) were administered orally with dose of (40mg/10ml) of body weight of proteinous peak I,II respectively after fasting for (16) hours[15].

Another set of animals (groups eight, nine, ten, eleven) were administered intraperitoneally in increasing concentrations (200,300,400, 500) mg/10ml of body weight of crude extract. Groups (twelve, thirteen) were administered intraperitoneally with dose of (40 mg/10 ml) of body weight of proteinous peak I,II respectively after fasting for (16) hours.

After (2) hours of administration, blood samples were collected by orbital sinus puncture technique after ether anesthesia[16].

-Parameters measured:

The serum was separated by centrifugation at (4000 xg). Then serum glucose and cholesterol levels were determined using (B10LABO SA. Maizy, France). UV-Visible CECIL CE 1021 single beam spectrophotometer were used to determine these parameters.

-Statistical Analysis:

The statistical methods used to analize the data including mean, standard error while student's-T-test was used to compare between control and experimental mice was at ($P < 0.05$) level[17].

Results and Discussion

-Determination of protein:

Table (1) shows the amount of total protein in the aqueous extract and the proteinous fractions of *Aleo vera*.

Table (1): Amount of total protein in the aqueous extract and the proteinous peaks

Fraction	Proteinous concentration (mg/ml)	Total protein (mg)
Crude homogenate	1.39	3475
Aqueous extract filtrate	1.41	1621.5
Proteinous precipitate solution	2.7	1725.84
Proteinous fraction (Peak I)	0.0915	16.836
Proteinous fraction (Peak II)	0.137	61.65

Fractionation of total protein and determination of comparative molecular weights.

Gel filtration chromatography was used to fractionate the total protein producing two peaks (I,II) as in figure (1).

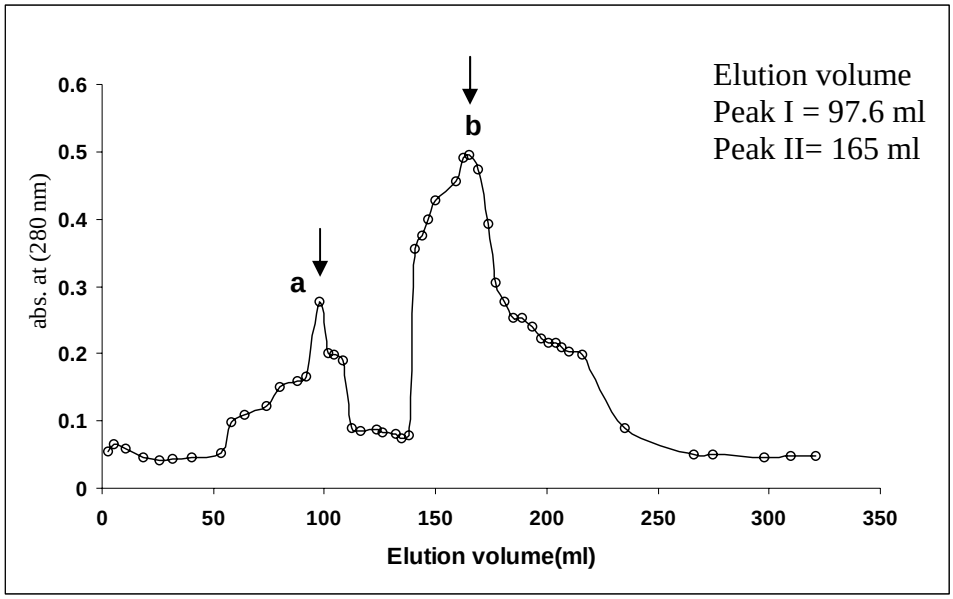

Figure (1): Elution profile of the proteinous materials of *Aleo vera* obtained from Ammonium sulphate precipitation on sephadex G-100.

The arrows (a) and (b) represent the elution volume for the first and second peaks.

By the same technique, the comparative molecular weight of the isolated proteinous compounds were determined on a precalibrated column using known molecular weight. Peak (I) which approximately (63095) Dalton while the peak (II) approximately equal to (19952) Dalton were shown in figure (2).

Table (2): Elution volumes of known molecular weight materials on sephadex G-100

Materials	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	200000	65.8
Bovine serum albumin	67000	101
Eggs albumin	45000	121
Trypsin enzyme	23000	134
Insulin hormone	5734	285
Tryptophan	204	436

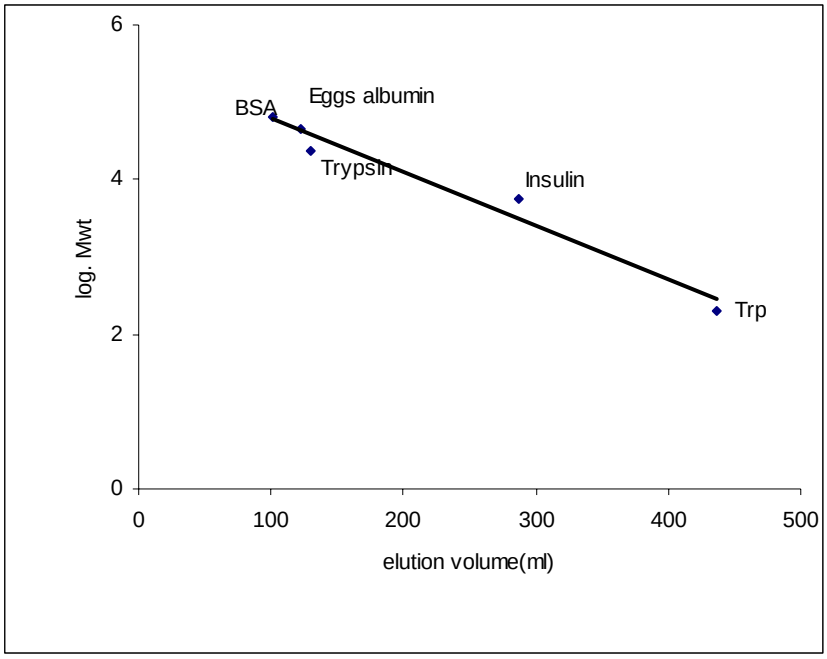

Figure (2): Aplot of the logarithm molecular weight versus elution volumes on a sephadex G-100.

The effect of intraperitoneally administration of crude extract and the proteinous fractions on glucose and cholesterol levels in normal mice

The mean values of blood serum glucose and cholesterol for control and different doses of *Aloe vera* (Intraperitoneally administration) for normal mice were shown in table (3).

The results indicated a significant decrease of serum glucose level in normal mice (with dose of 300,400 mg/10 ml) of crude extract by (-32.97%, -41.77%) respectively. An insulin like structure or action of the proteinous fractions from *Aloe vera* was proposed. These compounds might contain certain sequence of amino acids similar to insulin which binds to specific insulin receptors located on the plasma membrane. Also there was non significant reduction of serum glucose in normal mice with dose of (40 mg/10ml) of proteinous fractions of peak (I,II) by (-30.77 %, -36.71%) respectively and maximum increase being intraperitoneally at adose (500 mg/10 ml) of crude extract by (52.5% and 98.3%) for blood glucose and cholesterol levels respectively. These results might due to the non proteinous compounds of crude extract which increase the biochemical levels.

These results are similar to that results obtained by masterson[(18)]. The decrease might due to that the administration of *Aloe vera* may benefit the patients with type II diabetes by stimulating the synthesis and release of Insulin[(19)] or inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney[(20)].

Also there was non significant reduction in the cholesterol levels by intraperitoneally administration of high molecular weight proteinous (Peak II) by (-19.98%) in normal mice.

The high fiber content of *Aloe vera* had been shown to exert beneficial effects on cardiac disease risk factors by reducing blood levels of cholesterol and glucose[21] therefore, it had a definite role in the prevention and management of atherosclerotic heart disease[18].

Table (3): Mean blood serum (glucose and cholesterol) levels in (mg/dl) ±SE after intraperitoneally administration of different doses of crude extract and proteinous materials of *Aleo vera* in normal fasted mice.

Group No.	Treatment	Serum glucose level mg/dl ± SE	Change %	Serum cholesterol level mg/dl ±SE	Change %
1	Control	70.193± 9.6	----	173.685± 13.3	----
2	Crude extract (200mg/10ml)	76.815 ± 2.4	9.45	222.990 ± 27.9	28.38
3	Crude extract (300mg/10ml)	47.050 ± 4.4 *	-32.97	218.648 ± 8.3	25.89
4	Crude extract (400mg/10ml)	40.870 ± 3.9*	-41.77	262.665 ± 33.3	51.23
5	Crude extract (500mg/10ml)	107.043 ± 17.8	52.5	344.415 ± 35.4	98.3
6	Proteinous Frc.I (40mg/10ml)	48.590 ± 5.1	-30.77	138.975 ± 16.3	-19.98
7	Proteinous Frc.II (40mg/10ml)	44.420 ± 5.2	-36.71	173.405 ± 17.3	-0.16

***** Refers to significance at $p < 0.05$ compared with control group
The values are mean ± SE of 4 mice each group.

The effect of orally administration of crude extract and proteinous fractions on glucose and cholesterol levels in normal mice.

The mean values of blood serum glucose and cholesterol for control and different doses of *Aloe vera* (Oral administration) for normal mice were shown in table (4).

Results indicated not significant decrease of serum glucose level in normal mice (for proteinous fraction. Peak II) at dose (40 mg/10 ml) by (-15.56%) relative to control value. This decrease may be due to an increase peripheral glucose utilization which increase the release of insulin [22].

Table (4): Mean blood serum (glucose and cholesterol) levels in (mg/dl) ±SE after orally adminstration of different doses of crude extract and proteinous materials of *Aleo vera* in normal fasted mice.

Group No.	Treatment	Serum glucose level mg/dl ± SE	Change %	Serum cholesterol level mg/dl ±SE	Change %
1	Control	70.193± 9.6	----	173.685± 13.3	----
2	Crude extract (200mg/10ml)	140.330 ± 8.5	99.93	381.828 ± 53.5	119.83
3	Crude extract (300mg/10ml)	173.563 ± 15.4	147.27	200.230 ± 47.2	15.28
4	Crude extract (400mg/10ml)	72.400 ± 7.7	3.15	309.880 ± 37.2	78.41
5	Crude extract (500mg/10ml)	95.643 ± 9.2	36.26	431.100 ± 35.6	148.2
6	Proteinous Frc.I (40mg/10ml)	90.493 ± 7.1	28.92	199.100 ± 36.7	14.63
7	Proteinous Frc.II (40mg/10ml)	59.270 ± 5.1	-15.56	211.36 ± 58.8	21.69

The values are mean ± SE of 4 mice each group.

Results indicated that the orally administration of crude extract with different doses of the plant and the proteinous Frc (I) caused increase of serum glucose. This might due to the crude extract which contains many materials or constituents such as polysaccharides, proteins, fats, amino acids….The metabolism of these compounds due to an increase of serum glucose, because the glycolysis, glycogenolysis, gluconeogenesis becomes active. Also, the proteinous Frc (I) caused increase of serum glucose because in orally administration, the proteins may be destroyed by the gastric juice or easily inactivated by the proteolytic enzymes. For all of these, the intraperitoneally administration of the extract of the plant has a hypoglycemic effect more than orally administration and it was a preferable route.

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