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Effects of Gel Extract from Aloe Vera Cultivated in Iraq on Blood Glucose Level and Lipid Profile in Induced Diabetic Mice

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Abstract

In Iraq; there is a great demand for handling some epidemic diseases such as hyperglycemia and hyperlipidema through searching some medical plant extracts which the country is rich with and contains an important phytochemicals that may solve the incidence of such cases. Aloe vera that cultivated in Iraq, is known to be rich with biological active constituents. The aim of the present study; the plant gel extract was subjected to treat an induced diabetic lab mice through injecting streptozotocin dose with high fatty food to elevate lipid profile before induction of the gel oral treatment of the plant. In the study results: the oral administration of A.vera gel at concentration of 300mg/kg animal weight gives a reduction in glucose level reached to 122mg/dl at the end of the experiment as well as the anti-diabetic drug Glibenclamide in a dose of 600µg/kg body weight as the glucose level decreased to 123mg/dl glucose in comparison to glucose level 250 mg/dL at zero time. Also the plant gel shows an effect on lipid profile that include cholesterol, triglycerides and High density lipoprotein levels; the A.vera extract after 21 days treatment causes decreasing in all lipid levels; even in normal mice fed with the extract only, except the High density lipoprotein levels had no change after the extract treatment than the negative control.

Keywords: Aloe vera, Blood glucose, lipid profile, Glibenclamide.

تأثير هلام نبات الصبار المزروع في العراق على مستوى السكر في الدم ومستوى الدهون في الفئران المصابة بداء السكري بالحث

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الخلاصة

في العراق؛ هناك طلب كبير على حل بعض الأمراض الوبائية مثل ارتفاع السكر في الدم وارتفاع مستويات الدهون من خلال البحث عن بعض المستخلصات النباتية الطبية التي تزخر بها البلاد وتحتوي على مواد فعالة مهمة قد تكون حلا لمثل هذه الحالات. من المعروف أن الصبار الذي يزرع في العراق غني بالمكونات الحيوية الفعالة . الهدف من هذه الدراسة ؛ هواستخدام مستخلص الهلام لنبات الصبار لعلاج فئران التجارب المصابة بداء السكري بالحث من خلال حقن جرعة من الستربتوزوتوسين مع اعطاء غذاء عالي الدسم لرفع مستوى الدهون قبل المعالجة الفموية للفئران بهلام النبات. اظهرت نتائج الدراسة ان جرعة الهلام بتركيز 300 مغم / كغم من وزن الحيوان سبب انخفاضًا في مستوى السكر إلى 122 ملغم % في نهاية التجربة وكذاك

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عقار جليبنكلاميد المضاد لمرض السكر بجرعة. 600 ميكروغرام / كغم من وزن الجسم حيث انخفض مستوى السكر إلى 123 ملغم % مقارنة بمستوى السكر عند الشروع بالتجربة حيث كان 250 ملغم%. كما يبدو ان لهلام نبات الصبر تأثيرًا على مستويات الدهون الذي تضمن مستويات الكوليسترول والدهون الثلاثية والبروتين الدهني عالي الكثافة بعد 21 يومًا من العلاج فقد سبب انخفاض في جميع مستويات الدهون ؛ حتى في الفئران العادية التي تم تغذيتها بالمستخلص الهلامي فقط ، عدا مستويات البروتين الدهني عالي الكثافة لم يتغير بعد العلاج بالمستخلص مقارنة مع نتائج السيطرة السالبة.

Introduction

Aleo vera plant extracts showed multiple benefits medically and commercially that might gain a considerable clinical importance since ancients [1,2]. Researchers had recorded more than 200 different active components the plant composed which possessed important biological activities [3]. The polysaccharides contained in the gel of *Aloe vera* leaves may primarily attributed to biological effects such as psoriasis [4], burn from sun exposure or radiation [5,6], lichen planus [7], also the plant extract enhanced the healing of damaged skin of different causes [8]. Moreover many industrial products were officinal in markets administer for intestinal problems and reduction of plaque and gingivitis problems [9]. Nowadays the world built a good affords for immune system boosting to face the big health progressed problems that include sugar and lipids high levels through consuming herbal and natural plant rich diets [10], besides; improving human health against different infections and disease such as pathological infection, inflammatory problems, and immune-modulatory for cancer disclosed and decreasing their incidence [11]. This study designed to clarify the applications of *Aloe vera* cultivated in Iraq in reduction of blood sugar levels, and regulating the levels of plasma lipids in induces diabetic mouse with Streptozotocin(STZ), in comparison with traditional anti-diabetic drug (Glibenclamide).

Methodology

1- plant collection and extract preparation

The plant was cultivated at home garden, mature healthy of fresh green *Aleo vera* leaves about 70 cm tall, were cut then washes with tap water. The semi-solid gel in the center of these leaves was taken out of the green leaves with aid of spoon and knife. The straw coloured gel was homogenized by electric blinder, to be filtered and sterile with Millipore filter 0.45 mm, then kept at 4^0C in vacuumed tube until use.

2-Phytochemical Investigation of the Plant Gel [12]

The following chemical test were proceeded to investigate active components in the *Aleo vera* gel extract (7.5g gel in 100 ml distilled water).

a. Detection of Tannins tests

A few drops of the 1% Lead acetate solution were added to the plant extract. A gelatinous or white precipitate was formed that indicated the presence of tannins.

b. Detection of polysaccharides

A liquate of 1 ml of the plant extract was mixed with 2 ml of the Benedict reagent, place the mixture in a boiling bath for 5 minutes and left to cool. The red deposit indicated a presence of polysaccharides.

c. Detection of alkaloids (Dragangroff test)

About 60mg of Bismuth sub-nitrate were dissolved in 0.2ml HCl (solution A). Solution B contains 600mg potassium iodide in 1 ml Distell water. The solution [A + B] were mixed and added to the plant extract, an orange to brown color will indicate the presence of alkaloids.

d. Detection of the Saponins

The detection process will be proceeding by shaking the solution of the plant extract well. Formation of foam at the top of the extract will indicate presence of saponins.

e. Detection of Flavonoids

Alkaline reagent test: by using Sodium hydroxide solution which mixed with few amount plant extract solutions and left, a bright yellow color is obtained in presence of flavonoids.

f. Detection of Polyphenolic Compounds

Few drops of 1% ferric chloride solution were added to the plant extract solution a brown deposition will formed.

2-Induction of Diabetes by streptozotocin

Streptozotocin(STZ), from Sigma Aldrich chemical Co.,U.K. ,was dissolved in ice-cold normal saline immediately before use as in product protocol of the manufacturer. Diabetes was induced in mice by intrapretoneal (i.p) injection in dose of 80mg/kg STZ

3- Experimental Design

Thirty Albino mice(female) (*Mus musculs*), with aged of (8-12) weeks and weight rang (30g) were distributed into five groups, each with 6 mice and kept in a separate plastic cage, All institutional and national guidelines for the care and use of laboratory animals were followed. The laboratory animals groups used in the study included:

Group (1): Normal control mice only with normal feeding---(glucose level =135 mg/dL at zero time)

Group (2): Streptozotocin induced- diabetic control mice --(glucose level=250 mg/dL at zero time)

Group (3): Normal mice treated with 300mg/kg/day *Aloe vera* extract----(glucose level =159 mg/dL at zero time)

Group (4): Streptozotocin induced- diabetic mice treated with 300mg/kg/day *Aloe vera* extract--(glucose level 250 mg/dL at zero time)

Group (5): Streptozotocin induced- diabetic mice treated with 600µg/kg /day Glibenclamide. --(glucose level 250 mg/dL at zero time)

All the drugs were ad ministered orally using an intragastric tube in single dose in the morning for three weeks.

Statistical Analysis

Inorder to investigate the factors and parameters differences effects on all the study results , a statistical analysis system- SAS (2012) program was employed . Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to make significant comparisim between means in the prsent study[13].

Results and Discusion

1-Phytochemical Investigation of the gel Extract

Table 1 ilustated the main active components of the plant extract

Table 1-The phytochemical investigation for main active components of the plant extract

SAMPLE	TEST NAME	REAGENT	RESULT
7.5g.gel in 100ml D.W	Tannins	Lead Acetate !%solution	- **ve**
7.5g.gel in 100ml D.W	polysaccharides	Benedict reagent	**+++ ve**
7.5g.gel in 100ml D.W	alkaloids	Dragangroff reagent	- **ve**
7.5g.gel in 100ml D.W	Saponins	Foam formation	**+++ ve**
7.5g. gel in 100ml D.W	Flavonoids	Alkaline reagent(NaOH)	**+ ve**
7.5g. gel in 100ml D.W	Polyphenolic Compounds	ferric chloride 3%solution	**+ ve**

As shown in Table 1,that the*Aloe vera* gel was rich with many active constituents among them; polysaccharides, polyphenpls, saponins and flavonoids. All these components play important rule in their biological activity.

2- Effect of Aloe vera gel on blood glucose level and lipid profile in induced diabetic mice

As shown in Table 2 and Figure 1, mice glucose level at the beginning of the experiment was elevated from about 135mg/dl up to 250mg/dl after induction diabetic condition through streptozocin (80 mg/kg body weight) intrapretoneal injection to assumed as negative control at zero time. An obvious change was occur following lab animal treatments of plant extract at300mg/kg dose in comparison to the traditional anti diabetic drug Glibenclamide in a dose of, 600µg/kg body weight. After one week from; level of glucose was recorded from 250mg/dl down to 138 mg/dl due to the extract treatment and 133mg/dl with glibenclamide drug. Even in mice fed on Aloe extract without diabetic induction, glucose level was arranged from 159mg/dl down to 133mgldl after week. When the treatments were continued for three weeks later; level of blood glucose was reached to 122mg/dl with *A.vera* gel and 120mg/dl with the commercial drug Glibenclamide, and 110 mg/dl in mice fed *Aloe* extract alone.

Table 2-Average glucose level of different groups of treatment in hyperglycemic mice

Group number	Average Glucose level (zero time)mg/dl	Average Glucose level(after week) mg/dl	Average Glucose level(after 3 weeks) mg/dl
Group(1)	135 ±8.37 b	136 ±6.25 b	135 ±6.04 b
Group(2)	250 ±14.08 a	250 ±14.10 a	256 ±15.73 a
Group(3)	159 ±6.33 b	133 ±7.41 b	110 ±4.68 b
Group(4)	250 ±14.26 a	138 ±6.59 b	122 ±6.26 b
Group(5)	250 ±14.08 a	133 ±6.44 b	120 ±5.03 b
LSD value	58.72 *	47.66 *	51.94 *
Means with the different letters in same column have differed significant value. * (P≤0.05).			

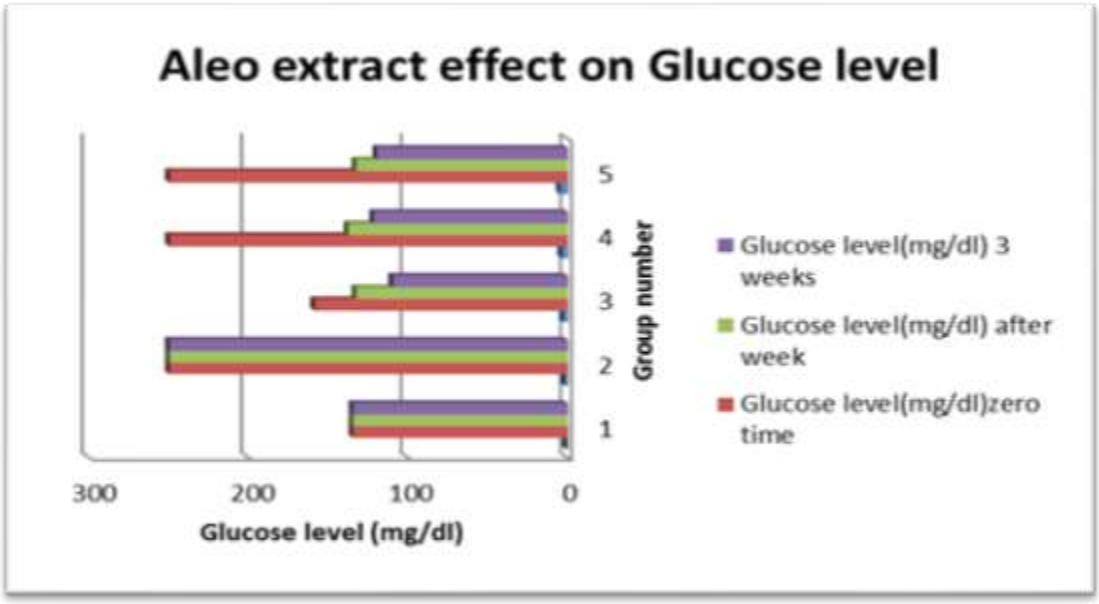

Figure 1-Effect of *Aloe vera* gel extract and Glibenclamide drug on Glucose level in hyperglycemic induced mice

Additionaly; the lipid profile values; an elevation in cholesterol level from normal level (86mg/dl) up to (97mg/dl), and T.A.G levels from normal level (88mg/dl) up to(113mg/dl) in

mice fed on fat rich meals, as in Table 3 and Figure 2. Cholesterol level after three weeks later from the treatment with either Aloe gel and the Glibenclamide, showed a decrease down to (71and 72) mg/dl respectively and in T.A.G level reached to (102 and 90) mg/dl respectively. Moreover; mice that fed with only extract recorded level of cholesterol reached to (79mg/dl). Result showed no change in HDL level for animals taking the extract than the diabetic negative control with some decreasing in animal of Glibenclamide treating.

Table 3-Average cholesterol, triglycerides and HDL levels of different groups of treatment in hyperglycemic mice

Group number	Average Cholesterol level(mg/dl)	Average Triglyceride level(mg/dl)	Average HDL level(mg/dl)
Group(1)	86 ±3.59 a	88 ±3.65 ab	48 b
Group(2)	97 ±4.77 a	113 ±7.02 a	48 b
Group(3)	69 ±2.93 a	79 ±2.38 b	58 a
Group(4)	71 ±2.08 a	102 ±4.92 ab	50 b
Group(5)	72 ±2.37 a	90 ±3.18 ab	45 b
LSD value	28.83 NS	31.66 *	7.42 *
Means having different letters in same column will be with the significant differences. * (P≤0.05).			

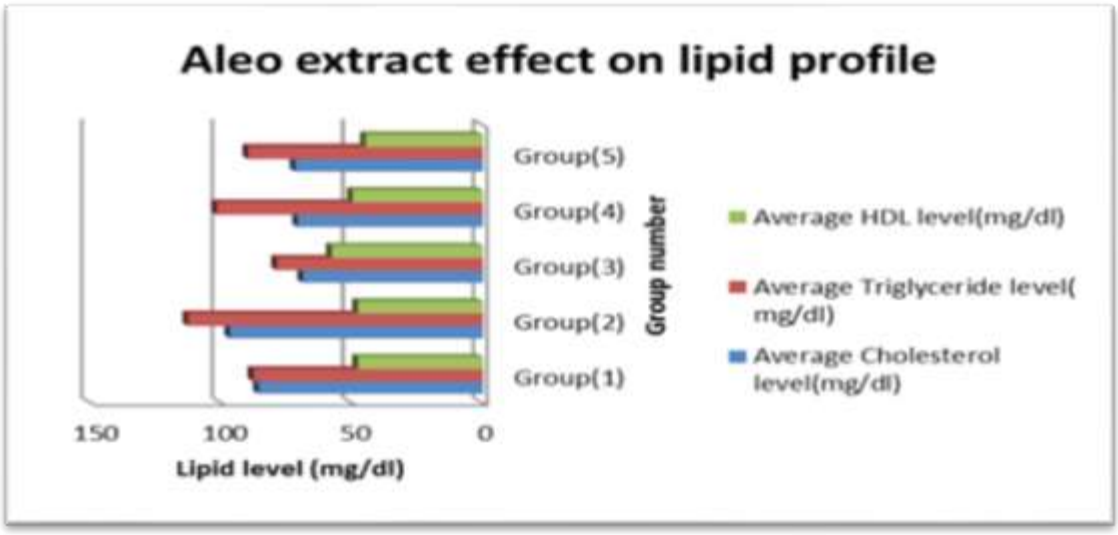

Figure 2- Average cholesterol, triglycerides and HDL levels of different groups of treatment in hyperglycemic mice

The *Aloe vera* species dominates over other 500 types of the genus *"Aloe"* in the biological and medical properties and the worldwide market commercial products [14, 15]. The major components of the leaves were polysaccharides which considered a highly complex molecules found in the *Aloe* gel. Studies showed that the plant rich in polysaccharides could explain the immune boosting and the anti-inflammatory effects [16,17,18]. Data of some studies, about 11 *Aloe* species represented six same polysaccharides components of distinct types, and 90% of monosaccharide composed of (glucose, mannose and xylose) represented in 31 *Aloe* species [19, 20]. The differences in polysaccharides composition affected the biological activity of different species. In one study by *Workineh and co-workers*,2019 which

applied on other *Aleo* species *A. megalacantha* ,concluded that the leaf latex showed a potential effects in treating human glucose which improve the flock use of the plant in such conditions [21].

Results of the present study were coincided with others carried on *Aloe vera* leaf extract grown in different countries, among them a study from Egypt by Enas,2011 who concluded that *A. vera* gel extract administrated orally tend to decrease serum total lipids and glucose significantly, moreover the extract might act to decrease serum Malondialdehyde (MDA) level via increasing nitric oxide level which lead to potentiate the antioxidant capacity as one mechanism of controlling bood glucose level[22]. Two studies by (Subbiah et,al.,.2006 and Manjunath et,al.,.2016)[23,24] who used a traditional anti-diabetic drug "metformin" in dose of 50mg/kg in rats group in comparison to groups treating with *Aloe vera* gel in range (200-400)mg/kg, the elevated blood glucose levels in diabetic induced rats were reduced in all treatment with no significances between all treatments [23,24] .

Beside the polysaccharides bioactivity; *Aleo* gel trace element such as chrome(Cr),manganise (Mn) and zinc (Zn) showed a potentiate anti-diabetic effect for this plant. Also, the gel was very rich with different sources of natural scavenging of free radicals like phenolic and flavonoids secondary metabolites as well as vitamins contents specially vitamins C and E which are responsible for the plant anti-oxidative effect[25].

In case of plant effects on hyper-glycemic conditions related to the degree of β-cell destruction, *A. vera* was supposed to increase plasma insulin, via insulin genic possess in the animal by the plant action [23]. Induction of diabetic condition in lab animal through parenteral dose of streptozotocin(STZ) is accomplished with elevation of total lipid profile due to activation of hormone-sensitive lipase (HSL) which might enhance free fatty acid releasing from adipose tissue [24,26].

The current study concluded that the (STZ) induction of hyperglycemic state in animal was normalized by treating with *A.vera* extract, beside that the extract tends to regulate plasma lipid status, by controlling lipid metabolism.

Conclusion

Aleo gel cultivated in Iraq was rich in phytochimicals besides minerals and many primary metabolites,alltogather might play important rule in regulation blood glucose level and lipid profile even in normal persons.

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