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

Zainab Yaseen Mohammed Hasan1*, Jasim Mohammed Abdullah2
Biotechnology Research Center/AL-Nahrain University; Iraq

Received: 29/9/2020 Accepted: 20/4/2021

Abstract
In Iraq, there is a great demand for handling some epidemic diseases such as hyperglycemia and hyperlipidemia 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.

*Email: zainaby2003@yahoo.com
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 Aloe 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°C 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 liqate 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 musculus), 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 administered 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 present study[13].

Results and Discussion
1-Phytochemical Investigation of the gel Extract
Table 1 illustrated the main active components of the plant extract

| SAMPLE                  | TEST NAME     | REAGENT                        | RESULT  |
|-------------------------|---------------|--------------------------------|---------|
| 7.5g. gel in 100ml D.W | Tannins       | Lead Acetate 1%solution         | -       |
|                         | polycarharides| Benedict reagent               | +++ ve  |
|                         | alkaloids     | Dragangroff reagent            | -       |
|                         | Saponins      | Foam formation                 | +++ ve  |
| 7.5. gel in 100ml D.W  | Flavonoids    | Alkaline reagent(NaOH)         | +       |
| 7.5. 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, polyphenens, 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 133mg/dl 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.

| 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 blood 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), manganese (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 phytochemicals besides minerals and many primary metabolites, all together might play important rule in regulation blood glucose level and lipid profile even in normal persons.

**References**

[1] Hamman JH.. “Composition and applications of Aloe vera leaf gel”. *Molecules*, vol. 13, pp.1599–616, 2008.

[2] Little JW.. “Complementary and alternative medicine: impact on dentistry”. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, vol. 98, pp. 137–45, 2008.

[3] Martyna, Z.D.; Dominika, F.-T.; Monika, D.-J.; and Lucyna, K.S.. “Evaluation of clinical effectiveness of *Aloe vera* – a review”. Journal of Pre-Clinical and Clinical Research, vol. 11, no 1, pp.86-93, 2017.

[4] Paulsen E, Korsholm L, and Brandrup F. “A double-blind, placebo-controlled study of a commercial Aloe vera gel in the treatment of slight to moderate psoriasis vulgaris”. *J Eur Acad Dermatol Venereol*, vol. 19, no. 3, pp. 326–31, 2005.

[5] Ghaderi R, Afsar M, Akhbarie H, and Golalipour MJ. “Comparison of the efficacy of honey and animal oil in accelerating healing of full thickness wound of mice skin”. *Int J Morphol*, vol. 28, no. 1, pp. 193–198, 2010.

[6] Molazem Z, Mohseni F, Younesi M, Keshavarzi S.. “Aloe veragel and cesarean wound healing: randomized controlled clinical trial". *Glob J Health Sci.*, vol. 7, no. 1, pp. 203–9, 2014.

[7] Rajar UD, Majeed R, Parveen N, Sheikh I, and Sushel C. “Efficacy of Aloe veragel in the treatment of vulval lichen planus”. *J Coll Physicians Surg Pak.*, vol. 18, no. 10, pp. 612–4, 2008.
[8] Reuter J, Jocher A, Stump J, Grossjohann B, Franke G, and Schempp CM. “Investigation of the anti-inflammatory potential of Aloe vera gel (97.5%) in the ultraviolet erythema test”. Skin Pharmacol Physiol, vol. 21, no. 2, pp. 106–110, 2008.

[9] Ahlawat KS, and Khatak BS. “Processing, food applications and safety of Aloe vera products: a review”. J Food Sci Technol. 2011; 48: 525–33.

[10] Gupta VK, and Malhotra S. “Pharmacological attribute of Aloe vera: Revalidation through experimental and clinical studies”. Ayu., vol. 33, no. 2, pp. 193–6, 2012.

[11] Boudreau MD, Beland FA. “An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera”. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev., vol. 24, no. 1, pp. 103–54, 2006.

[12] Richard, A.; Thomas, J.; and Barbara, A. Immunology, 4th Edition. W.H. Freeman and Company, USA, 2011.

[13] SAS. 2012. Statistical Analysis System, User’s Guide. Statistical. Version 9.1 th ed. SAS. Inst. Inc. Cary, N.C. USA.

[14] Grace OM, Simmonds MSJ, Smith MF, van Wyk AE. “Documented utility and biocultural value of Aloe L. (Asphodelaceae): A review”. Economic Bot; vol. 63, pp. 167 – 178, 2009.

[15] Grace OM. “Current perspectives on the economic botany of the genus Aloe L. (Xanthorrhoeaceae)”. S Afr J Bot. vol. 77, pp. 980 – 987, 2011.

[16] Paulsen BS, Barsett H. “Bioactive pectic polysaccharides”. Adv Polym Sci., vol. 186, pp. 69 – 101, 2005.

[17] Steenkamp V., Stewart MJ. “Medicinal applications and toxicological activities of Aloe products”. Pharm Biol., vol. 45, pp. 411 – 420, 2007.

[18] Grace OM, Dzajic A, Jäger AK, Nyberg NT, Önder A, Rønsted N. “Monosaccharide analysis of succulent leaf tissue in Aloe”. Phytochemistry. vol. 93, pp. 79 – 87, 2013.

[19] Moller I, Sørensen I, Bernal AJ, Blaukopf C, Lee K, Øbro J, Pettolino F, Roberts A, Mikkelsen JD, Knox JP, Bacic A, Willats WG. “THigh-throughput mapping of cell-wall polymers within and between plants using novel microarrays”. The Plant Journal, vol. 50, pp. 1118 – 1128, 2007.

[20] Fagel JU, Pedersen HL, Melgosa SV, Ahl LI, Salmean AA, Egelund J, Rydahl MG, Clausen MH, Willats WGT. “Carbohydrate microarrays in plant science”. Methods Mol Biol., vol. 9, no. 18, pp. 351 – 362, 2012.

[21] Workineh, W. H.; Yohannes, K. E.; Kefyalew A.,G.; and Wubayehu K. “Antidiabetic and Antihyperlipidemic Activities of the Leaf Latex Extract of Aloe megalacantha Baker (Aloaceae) in Streptozotocin-Induced Diabetic Model”. Hindawi. Evidence-Based Complementary and Alternative Medicine, vol. 9, pp.786, 2019.

[22] Enas, A.K. M. Antidiabetic, “Antihypercholesteremic and Antioxidative Effect of Aloe Vera Gel Extract in Alloxan Induced Diabetic Rats” published in Australian Journal of Basic and Applied Sciences, vol. 5, no. 11, pp.1321-1327, 2011.

[23] Manjunath K.; Bhanu P. G.; Subash, K.R.; Tadvi, N.A.; Manikanta, M.; and Umanaheshwara, R. K. “Effect of Aloe vera leaf extract on blood glucose levels in alloxan induced diabetic rats”. National Journal of Physiology Pharmacy and Pharmacology, vol.6, no.5, 2016.

[24] Subbiah, R.; Kasiappan, R.; Karurun, S. and Sorimuthu S. “Beneficial Effects Of Aloe Vera Leaf Gel Extract On Lipid Profile Status In Rats With Streptozotocin Diabetes”.Clinical and Experimental Pharmacology and Physiology, vol. 33, pp. 232–237, 2006.

[25] Hanaa, S. y.and Ibtisam, S.y. “The effect of Aloe vera extraction on immunity”. Journal of Biotechnology Research Center, vol.8 . no.3, 2014.

[26] Hazhar, M. B. and Ismail S. K. "Indications of Liver and Kidney Functions in Non-Insulin Dependent Diabetic Patients". Iraqi Journal of Science, vol. 62, no. 3, pp: 769-778, 2021.