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Original article

Effect of different extracts of Aloe Vera gel (Aloe Barbadensis) on blood glucose level of alloxan induced hyperglycaemic mice

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Abstract

This study was conducted to evaluate the effect of different extracts of Aloe Vera gel in alloxan induced hyperglycaemic mice. Three different extracts of Aloe Vera gel (dried extract, ethanolic extract and fresh raw extract) were orally administered at 300 mg/kg body weight for 28 days. The fasting blood glucose level was estimated both in normal and alloxan induced hyperglycaemic mice. It was found that, when compared with the control, there was a significant reduction in blood glucose level in all three experimental groups. Ethanolic and fresh raw gel extracts were more effective than the dried extract. The extracts produced similar results when compared with gliclazide. It can be concluded that, the administration of Aloe Vera gel extract significantly decreases blood glucose level in hyperglycaemic mice. Aloe Vera gel can therefore be a natural remedy and a cost effective resource for the management of diabetes.

Keywords: Aloe Vera gel, alloxan induced mice, blood glucose level, diabetes

Introduction

Diabetes Mellitus is a major endocrine disorder and growing health problem in most countries. Epidemiological studies on urbanization and aging influences have shown that the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030.[1] Bangladesh Institute of Research and Rehabilitation In Diabetes And Endocrine Metabolic Disorders (BIRDEM) carried out a recent survey which revealed the prevalence of the disease in the rural population to be about 6.8%.[2] Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries. Population growth, ageing of populations and urbanization with associated lifestyle change is likely to lead to a high prevalence of the disease in Bangladesh. These factors pose a threat to the economic status of the nation. WHO projects those diabetic deaths will double between 2005 and 2030.[1]

Diabetes mellitus is recognized by a triad of classical symptoms of polyuria, polydipsia and polyphagia. These symptoms are the consequences of hyperglycaemia in a diabetic person. Hyperglycaemia results from reduced glycogen storage in the liver and muscle and decreased peripheral utilization of glucose by the body tissues. Uncontrolled blood sugar may occasionally be accompanied by biochemical alterations of glucose and lipid metabolism. Diabetes and its complications have a significant economic impact on individuals, families, health systems and countries.

Many studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus. These effects may delay the development of diabetic complications and correct the metabolic abnormalities. New bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy.[3]

Aloe Vera, a popular houseplant, has a long history as a multipurpose folk remedy in China, Russia, South Africa, United States and India. Useful chemical compounds in the Aloe plant are typically isolated from two materials: latex and gel.[4] Aloe Vera is most commonly used to treat diabetes due to its ability to reduce blood glucose levels.[5] It is a semi tropical perennial succulent plant mainly native to the African continent and Mediterranean.[4]

The word Aloe originated from the Greeks and Vera means true or genuine. Some sources claim that aloe in Arabic means 'bitter'. In Bangladesh, it is well known by the name 'Ghritakumari'. Other global popular names

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include 'lily of the desert', 'miracle plant' and 'silent healer'. At present, it is widely distributed throughout the tropics and subtropics. Spring is the most suitable season for the growth of Aloe Vera. Large scale agricultural production is undertaken in Pakistan, Australia, Bangladesh, Cuba, China, Mexico, India, Jamaica, Kenya and South Africa and USA.

The leaves have an outer protective ring and bitter yellow latex below. The center of each leaf is filled with a viscous gel. This gel contains most of the biologically active ingredients, mainly polysaccharides (glucomannan, acemannan and mannose), antioxidant vitamins, minerals, cholesterol, salicylic acid, prostaglandin precursors and amino acids. The biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance. Ingested Aloe Vera gel is able to reduce blood glucose levels and plasma triglycerides well as many of the secondary symptoms of diabetes that are associated with oxidative stress.

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes.[6] Herbal extracts have been confirmed for its hypoglycaemic effect in humans and animals for type 2 diabetes.[7] Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries.[8]

The present study was carried out to investigate the effect of the different extracts of Aloe Vera gel on blood glucose level of normal and experimentally induced hyperglycaemic mice.

Methods

The study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with the Institute of Nutrition and Food Science (INFS), University of Dhaka.

About 6 kg leaves were cleaned, cut into halves and placed upside down for half an hour to allow drainage of the yellow latex. The leaves were then peeled and the inner clear gel cut into small pieces. They were placed on a large, flat steel pan and covered with a clean gauze cloth, then allowed to dry in air for about 15 days. Dried gel was then ground into fine powder which finally yielded about 35 gms of dried extract.

Similar amount of pieces of gel were obtained as before and then blended with 95% ethanol. It was then placed into a flat pan similar to previous procedure & then allowed to evaporate to dryness. The residue was then ground into powder which was greenish brown in colour.

A small portion of the leaf was peeled and the inner gel was blended. It was weighed accurately and mixed with distilled water to be fed directly. This method was repeated daily to prepare fresh sample.

42 healthy Swiss albino mice were purchased from the animal resource division of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), Mohakhali, Dhaka. They were of 8 to 10 weeks old, of both sex and weighing between 30 to 40 grams. They were housed in clean metallic cages individually in a well ventilated room within room temperature of about 26^0-28^0C in the animal house of the Institute of Nutrition and Food Science, Dhaka University, Dhaka. The animal house was maintained under a constant light and dark cycle alternating every 12 hours. The mice were allowed to feed upon standard food pellets and drink water ad libitum, except for the overnight fast, the day before blood glucose estimation. During fasting, they were allowed free access to water only.

Alloxan monohydrate was purchased from Loba chemicals, India. Gliclazide as Dimerol of Drug International Pvt Ltd was used as positive control. Fresh Aloe Vera leaves were bought from a local market in Old Dhaka.

The animal experiment comprised of experiment I and experiment II. Diabetes was not induced in the mice of experiment I. Alloxan (150mg/kg) was injected to induce diabetes in animals of experiment II.

Experiment – I : This part of experiment was carried out to observe the effect of fresh raw Aloe Vera gel on blood glucose level in normal fasting mice. 12 mice were divided into 2 groups, group A and group B, each comprising 6 mice. All mice were fasted overnight before collection of blood to determine the fasting blood glucose level.

Group-A (Non-diabetic control): Mice were given standard mice feed and water for 28 days. Fasting blood glucose level was estimated on day 1 and day 29 of the experiment.

Group-B (Non-diabetic + Aloe vera control): Mice were given fresh raw Aloe Vera gel (300mg/kg) orally along with mice pellets and water for 28 days. Fasting blood glucose level was estimated on day 1 and day 29 of the experiment.

Experiment – II: This part of study was carried out to see the effect of different extracts of Aloe Vera gel on blood glucose level in alloxan induced hyperglycaemic mice. The glucose lowering effect of the extract was compared with a standard oral antidiabetic drug, Gliclazide. A total of 32 mice were divided into five groups, each comprising 6 mice. All animals were made diabetic by intraperitoneal administration of alloxan at 150 mg/kg body weight.

Group-C (Diabetic control group-negative control): Mice received standard food and water. Fasting blood glucose level was estimated on day 1 (before alloxan injection), on day 4 (after alloxan to confirm diabetes induction) and on day 29 of the experiment.

Group D (Experimental group): This group was divided into group D1, D2 and D3 from day 4 of the experiment (after confirming diabetes). Group D 1 - mice were fed with dried extract of Aloe Vera gel (300mg/kg) orally by means of micropipette along with standard food and water for 28 days. Group D2 - mice were fed with ethanolic extract of Aloe vera gel (300mg/kg) orally by means of micropipette along with standard food and water for 28 days. Group D3 - mice were fed with fresh raw Aloe vera gel (300mg/kg) orally by means of micropipette along with standard food and water for 28 days. Fasting blood glucose level was estimated on day 1 (before alloxan), day 4 (after alloxan) and day 29 of the experiment of all 3 sub groups.

Group E (Anti-diabetic drug group-positive control): Gliclazide (50mg/kg) was given orally along with standard food for 28 days from day 4 onwards. Fasting blood glucose level was estimated on day 1(before alloxan), day 4 (after alloxan) and day 29 of the experiment.

After 28 days of treatment, mice blood sample was collected from the tail vein by tail tipping for the estimation of fasting blood glucose level. All the animals were then sacrificed under light chloroform anesthesia after completion of treatment on day 29. Blood was obtained in eppendorf tubes and centrifuged at 5000 rpm for 15 minutes for the separation of serum. This serum was then used for biochemical analysis.

Determination of serum glucose concentration was done by oxidase and peroxidase (GOD-POD) method using glucose estimation kit (Human, Germany) and microtiter plate reader (Multiskan EX , Labsystems, Finland).

The results are given as mean and standard deviation for the independently performed experiments. Unpaired students't test was used to see the level of significance. A 'p' value of <0.05 was considered as statistically significant. The study protocol was approved by the institutional ethical committee.

Results

In experiment I, the effect of oral administration of fresh raw Aloe Vera gel in non-diabetic mice was observed. Alloxan was not given in this experiment. Group A comprised of 6 non-diabetic control mice & group B comprised of 6 non-diabetic mice treated with Alo Vera. The results showed fresh raw Aloe Vera gel did not produce any significant change (p>0.05) in fasting blood glucose level of Group B compared to control Group A. (Table -I)

Table-I: Effect of fresh raw Aloe Vera gel on fasting blood glucose level in non-diabetic mice

Group (n=6)	Fasting blood glucose level (mmol/L) (mean ± SD)	
Group A	6.07 ± 0.51	p>0.05*
Group B	5.50 ± 0.17 *ns*	

* Cross-table variables and independent sample t test
n = number of mice used

Experiment II showed the effect of different extracts of Aloe Vera gel on fasting blood glucose level of alloxan induced hyperglycaemic mice in Group D1, D2 and D3. Aloe vera gel extracts produced significant change (p<0.001) in blood glucose level in diabetic mice compared to diabetic control (group C). Comparison between the groups receiving different extracts Aloe Vera gel showed significant change (p<0.05) in the groups treated with ethanolic extract and fresh raw gel when compared with the group treated with the dried extract of Aloe vera gel. But there was a non significant change produced between the groups treated with ethanolic extract and fresh raw gel. (Table-II)

The effect of different extracts of Aloe Vera gel (dried extract, ethanolic extract & fresh raw Alo vera gel) in lowering the blood glucose level was compared with the group treated with gliclazide (50mg/kg) for 28 days and the results were not significant (p>0.05). (Table-III)

Table-II: Effect of different extracts of Aloe vera gel on fasting blood glucose level of alloxan induced hyperglycaemic mice

Group (n=8)	Duration of treatment (28 days)	Fasting blood glucose level (mmol/L) (mean ± SD)	Level of significance
Group C	Standard lab diet and water	12.91 ± 0.63	p<0.001* C vs D
Group D1	Dried extract of Aloe vera gel (300mg/kg b.w.)	7.57 ± 0.22	p<0.05
Group D2	Ethanolic extract of Aloe vera gel (300mg/kg b.w.)	6.78 ± 0.63	D1 vs D2 & D3
Group D3	Fresh raw Aloe vera gel (300mg/kg b.w.)	6.67 ± 0.70	p>0.05 D2 vs D3

* Cross-table variables and independent sample t test; n: number of mice used

Table-III: Effect of different extracts of Aloe vera gel and gliclazide on fasting blood glucose level of alloxan induced hyperglycaemic mice

Group (n=8)	Duration of treatment (28 days)	Fasting blood glucose level (mmol/L) (mean ± SD)	Level of significance
Group E	Gliclazide (50mg/kg)	6.76 ± 0.89	p>0.05* E vs D
Group D 1	Dried extract of Aloe Vera gel (300mg/kg)	7.57 ± 0.22	
Group D 2	Ethanolic extract of Aloe Vera gel (300mg/kg b.w.)	6.78 ± 0.63	
Group D 3	Fresh raw Aloe Vera gel (300mg/kg)	6.67 ± 0.70	

* Cross-table variables and independent sample t test; n: number of mice used

Discussion

The results of the present study shows that the different extracts of Aloe Vera gel has blood glucose lowering effect in alloxan induced hyperglycaemic mice, but poses no effect on the blood glucose level of non-diabetic mice. The results in diabetic mice were similar to gliclazide, with the ethanolic extract and fresh raw Aloe Vera gel being more effective.

Absence of blood glucose lowering activity of Aloe Vera gel in non-diabetic mice was also observed by other investigators.[10] But the study of Jafri SA et al[11] do not corroborate with the findings of the present study. It could be due to the absence of aloe latex from the gel used in the present study. They used whole Aloe Vera leaf extract including the laxative component, aloe latex, which might have reduced the blood glucose level in

normal mice significantly compared to that of normal control group.

The antihyperglycaemic activity of Aloe Vera gel was observed by other researchers.[9] In their study, they mentioned a probable mechanism of glucose homeostasis by Aloe Vera gel extract. This may be due to increased activity of hexokinase and decreased activity of glucose-6-phosphatase, lactate dehydrogenase and fructose-1,6-bisphosphatase along with the liver glycogen level returning to near normal range. In 2007, in another study, they represented that Aloe Vera gel has a significant beneficial effect on tissue and plasma glycoprotein content (hexose, hexosamine and sialic acid) in experimentally induced diabetic rats.[12] Consequently, this helps to prevent the development of glycoprotein mediated secondary diabetic complications.

Identification of five phytosterols from Aloe Vera gel was done by Tanaka M et al[13] with an aim to find out the antidiabetic compounds present in Aloe Vera gel. Treatment with 1μg of the five phytosterols, lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol significantly reduced the HbA1c levels in diabetic mice. This work suggested a long term antidiabetic effect of Aloe Vera gel and its phytosterols.

Oral administration of Aloe Vera gel extract have antioxidative effect which is manifested by the significant decrease in serum malonaldialdehyde (MDA) levels with a significant increase in both serum nitric oxide and total antioxidant capacity in diabetic rats. It is confirm that the hypoglycemic effect of Aloe Vera gel extract may be due to the presence of some trace elements such as chromium, zinc and manganese which potentiate insulin action.[10]

Kim et al.[14] demonstrated the activity of Aloe Vera gel against insulin resistance. Aloe formula decreased mRNA expression of PPARγ in liver and adipose tissue of obese mice. It suppressed the ability of PPARγ/LXRα to regulate ATP binding cassette transporter A1 (ABCA1) expression in adipose tissue leading to reduced total fat mass in obese mice. Whether Aloe Vera affected the expression of 11 β-HSD1 was also tested, as 11β-HSD1 inhibition, might counteract the side effect of weight gain and show better anti-diabetic activity. Hepatic β-oxidation enzymes (ACO, CPT1) and PPARα expressions tend to increase in the livers of the lophenol and cycloartanol treated rats compared with those in diabetic fatty control rats.[15] They concluded that orally ingested aloe sterols altered the gene expressions related to glucose and lipid metabolism The findings suggested that aloe sterols could be beneficial in preventing and improving metabolic disorders with obesity and diabetes in rats.

The present results suggest that the extracts of Aloe Vera gel are euglycaemic, which might be a useful blood glucose lowering agent for the treatment of diabetes mellitus. Fresh raw Aloe Vera gel may be a cheap and effective option to manage diabetes mellitus, especially in developing nations. If these experimental data are endorsed in clinical trials in future, Aloe Vera gel extract may emerge as a natural source to offer an alternate or adjuvant remedy for type 2 diabetes.

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