Original Article

# ANTI-HYPERGLYCEMIC EFFECT OF ALOE VERA LEAF GEL EXTRACT IN ALLOXAN INDUCED DIABETIC RABBITS

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**Objective:** To evaluate the anti-diabetic activity of aloe vera ethanolic extract in alloxan induced hyperglycemic rabbits as an alternate mode of treatment for type-2 diabetes.
**Methods:** In the present experiment sixty six healthy male rabbits of local strain weighing 1.0-1.7 kg were divided into 3 groups. They were injected intravenously with alloxan monohydrate according to body weight to induce diabetes. Baseline normal blood sugar level of all the rabbits was recorded. The rabbits turned diabetic within 1-2 weeks after injection of alloxan. A BSR level of = 250 mg/ dl was criterion for diabetes. Group A, served as control and was treated with placebo (5 ml of distilled water daily). Group B was treated with Metformin (135 mg/ kg body weight daily) and Group C was treated with ethanolic extract of aloe vera (300 mg/ kg body weight daily). Blood sugar levels were recorded as 0hrs, 2hrs and 4hrs readings on days 1, 14 and 28 of the treatment. The data was recorded in specially designed proforma and analyzed with the help of computer software SPSS version 16.
**Results:** There was no significant change in the BSL in Group A (the control group, diabetic rabbits treated with 5 ml/ day of distilled water). Overall, in a period of 28 days, there was 44.1% reduction of mean BSL in Metformin group and 25.3% reduction of mean BSL in Aloe Vera group.
**Conclusion:** The ethanolic extract of Aloe Vera leaf gel exhibited anti-diabetic activity in alloxan induced diabetic rabbits. Although antihyperglycaemic effect of Aloe Vera gel extract is lesser than that of Metformin but it has a gradual and sustained pattern.
**Keywords**: Aloe Vera, Diabetes mellitus, Rabbits and Alloxan monohydrate.

## Introduction

Diabetes mellitus is a multi-factorial disease that has a significant impact on health, quality of life and life expectancy of the patients, as well as on health care system. Worldwide, the number of patients is assumed to be doubled in thirteen years from 1997 to 2010.[1] It remains an important risk factor for cardiovascular disease and increasing rate of childhood and adult obesity. Diabetes is likely to become even more prevalent over the coming decade. Diabetes is commonly associated with premature mortality, predominantly due to atherosclerotic vascular disease.[2] The microvascular complications, which affect the small blood vessels in the eye, kidney and nerves, are associated with considerable morbidity. The economic and social costs of diabetes are enormous, both for health care services and through loss of productivity. In developed countries, 10% or more of the total health budget is spent on the management of diabetes and its complications.[3] For type-2 diabetes, treatment begins with a nutritionist designed diet control plan, exercise and weight reduction. Oral hypoglycemic agents are used if these measures fail. Later on, insulin may also be required due to beta cell failure.

The synthetic oral hypoglycemic agents have a number of side effects like gastrointestinal, cutaneous, hepatic and renal. They also have teratogenic effects.[4]. Hence many studies were carried out to investigate the hypoglycemic effect of some plants used traditionally to treat diabetes beside identification of active ingredients, mode of action and safety. Herbal extracts have been confirmed for its hypoglycemic effect in human and animals for type-2 diabetes.[5]

Aloe species are perennial plants, belonging to the family Liliaceae. These are native to North Africa and cultivated in warm climate areas. The plant is the source of two herbal preparations, latex and Aloe gel which is often called Aloe Vera.[6] The gel is composed of mannose-phosphate, acetylated mannan, glucomannans, alprogenglucoprotein and glucosylchromone. Aloe Vera has been used in folk medicine as a remedy for various diseases. However there have been controversial reports on the hypoglycemic activity of Aloe species.[7] The present study was conducted to evaluate the effect of Aloe Vera ethanolic extract on blood glucose level and to compare with that of metformin in alloxan induced diabetic rabbits.

## Material & Methods

The present research was carried out in the department of pharmacology Services Institute of Medical Sciences/ P.G.M.I. Lahore from February to July 2010. A prior approval of the study was obtained from the ethical committee of the PGMI Lahore.

### The Experimental Animals

Healthy male rabbits of local strain weighing 1000-

1700 g were purchased from the veterinary research centre Lahore. The rabbits were kept in the animal house of PGMI Lahore one week prior to the commencement of the study for observation and acclimatization of the animals to the new environment. The animals were fed on green fodder, grains, cereals and plentiful of fresh water. The animals were kept in separate cages.

### Induction of Diabetes in Rabbits

The induction of diabetes in experimental rabbits was done by injecting alloxan monohydrate solution intravenously (in the marginal ear vein).[8] The dose of alloxan monohydrate/kg body weight was calculated according to that mentioned by Puri D et al.[9] Immediately prior to injection of alloxan, 2 grams of glucose/kg body weight dissolved in 10 ml water was administered orally to each rabbit to counteract any expected hypoglycaemia. The required dose was dissolved in 8 ml water and injected in the marginal ear vein of the rabbit using 27 gauge needle and 10 ml syringe.[10]

Eight days after the administration of alloxan, the surviving rabbits (66 in number) of BSR = 250 mg/dl were taken as diabetic and kept for further study. In case of animal found non-diabetic, a low dose repetition was made at an interval of 5-10 days each. For example a rabbit previously given 75 mg/kg was given 55 mg/kg the second time and 30 mg/kg the third time if required. Most of the animals became diabetic after a second dose. The rabbits which died after alloxan injection, due to hypoglycaemia, were excluded from the study.

The rabbits were divided in three groups as under.

### Group-A (Control)

Twenty two alloxan induced diabetic rabbits treated with placebo (5 ml of distilled water/day).

### Group-B (Standard)

Twenty two alloxan induced diabetic rabbits treated with Metformin (135 mg/ kg body weight/day).

### Group-C (Test)

Twenty two alloxan induced diabetic rabbits treated with Aloe Vera extract (300 mg/ kg body weight/day).

### The Plant Material

There are more than 300 species of aloe vera. Aloe Barbadensis Miller is the true aloe which is used in the study **(Fig.-1)**. 100 kg of fresh, mature and healthy leaves of Aloe Vera were purchased from nurseries of Tehsil Pattoki, Distt. Kasoor.

### Preparation of Aloe Vera Ethanolic Extract

The ethanolic extract of Aloe Vera was then prepared with collaboration of the Herbal Heritage Centre, Department of Plant Pathology, University of the Punjab Lahore. Fresh healthy leaves of Aloe Vera, 0.5-0.75 meter in length, were washed with water to remove all the mud and dust particles. The leaves were cut in longitudinal sections carefully to remove the outer hard rind (the skin) and to obtain the colourless transparent gel. The mucilaginous pulp of Aloe Vera leaves was homogenized in a specialized chamber called column. The resultant colourless homogenous fluid was centrifuged at 4000 revolutions /min for a period of 15 minutes and then filtrated to remove the fibres. The colourless filtrate was mixed with 95% ethanol in 1:1 ratio to extract the active ingredients of the gel. The mixture was collected and processed in a rotary evaporator to remove the water content. The resultant solid component was kept in the freeze drier to produce absolute dryness The end product was in the form of greyish white powder which was collected in amber coloured air-tight glass bottles to avoid light and moisture. The powder was stored at room temperature and a known amount of distilled water was added to make suspension in the required dose before administration.

### Determination of dosage

The dose of Aloe Vera gel extract was determined on body weight basis. The required dose in our study was 300 mg/kg body weight/day as a single daily dose before meals. The dose was determined according to Rajeshkaran who used Aloe Vera extract in rats to study effects on serum lipids.[11] Metformin was given

in the dose of 135 mg/kg body weight/day according To Sirtori et al, who used Metformin in rabbits to study its effects on atherosclerosis.[12] Metformin powder is not available in Pakistan. Therefore, tablets Glucophage 250 mg containing 250 mg of metformin BP as active ingredient were purchased and finely crushed with help of mortar and pestle to obtain the powder. The powder obtained from 250 mg tablet was then mixed with 10 ml of distilled water. The resultant suspension contained 25 mg of active ingredient of metformin in each ml. The dose in ml was calculated according to the body weight. For example, a rabbit of 1.2 kg required 6.5 ml of suspension. At the time of administration, the suspension was well shaken. A feeding tube (8 Fr) was passed down into the rabbit stomach. Then the desired drug was dissolved in 10 ml of distilled water and administered with the help of disposable 10 cc syringe via the feeding tube. The drugs were administered as single daily dose.[13]

### Blood Sample collection

The blood sample, 2 ml each time when required, was drawn as described by Akhter et al in 1982[14] according to following technique. The rabbit's ear was dabbed with xylene solution so that the marginal ear vein became prominent. The sample was collected using a 5 ml disposable syringe. The samples were then centrifuged to obtain the sera which then were subjected to further glucose level testing (**Fig.2**).

A baseline BSR of all the rabbits was recorded before induction with alloxan. The weight of each rabbit was also recorded. The drugs (aloe extract, Metformin and distilled water in respective groups) were administered as single daily dose in the morning. BSL was also recorded before administration of drugs (0 hrs reading). The animals were then fed and two more readings recorded according to the schedule (2 hrs and 4 hrs). The data was recorded in specially designed proforma. Blood glucose levels were determined by the glucose oxidase peroxidase method which is specific for glucose, as it responds only to it.

### Statistical analysis

All grouped data were evaluated statistically with SPSS version 16 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Post hoc Tukey test of multiple comparisons.[15] $p < 0.05$ was considered significant. All the numerical values were represented as mean ± SD. Means of blood sugar levels of all the groups were compared on days 1, 14 and 28 of the study independently.

## Results

The data of BSL of control (Group-A) and drugs treated groups (B & C) on Day-1 of the treatment are shown in **Table-1**. The 0 hour readings on Day-1 depict the pre-treatment values of all groups. These values were 291.2 ± 21.8, 283.0 ±15.3 and 288.3 ± 19.4 in groups A, B and C respectively**.** There were no statistically significant differences in these values ($p=0.351$).

The mean BSL readings at 2 hour after administration of the respective drugs were 291.7 ± 21.9 in Group-A, 243.6 ± 22.0 in Group-B and 277.5 ± 19.5 in Group-C respectively. The mean BSL remained almost the same in control Group-A. There was 12.7% reduction of mean BSL in Metformin group and 3.81% reduction of mean BSL in Aloe vera g

**Fig.-2:** Blood sample collection.

The mean BSL readings at 4 hour were 290.409 ± 23.1 in Group-A, 186.6 ± 17.3 in Group-B and 271.3 ± 17.2 in Group-C respectively. Again, there was no change in mean BSL of control Group-A as compared to starting level. The reduction in mean BSL at 4 hours as compared to starting levels in Metformin and Aloe Vera groups were 32.62% and 5.90% respectively. On Day-14 (**Table-2**), the analysis of results within groups showed no significant

Insignificant in Group-B (p=0.105) and Group-C (p=0.391). At 4 hours level, again there were insignificant differences within group analysis of the results in Group-A (p=0.946). This difference was significant in Group-B (p=0.013) and not significant in Group-C (p=0.148).

Upon inter-group analysis of the results, there was highly significant lowering of mean BSL in groups B & C, as compared to Group-A, both at 2 hours and 4 hours level. On comparing Metformin group with Aloe Vera group, the blood sugar lowering effect was more in Metformin group (p< 0.005).

On Day-28 (Table-3), the analysis of results within groups showed no significant change in Group-A at 2 hours level as compared to 0 hours readings (p=0.932). Similarly, the changes were insignificant in Group-B (p=0.528) and Group-C (p=0.931). At 4 hours level, again there were insignificant differences within group analysis of the results of all groups.

Upon inter-group analysis of the results, there were highly significant lowering of mean BSL in groups B & C as compared with Group-A both at 2 hours and 4 hours level (p<0.005). On comparing Metformin group with Aloe Vera group, the blood sugar lowering effect was more in Metformin group (p< 0.005). On 28th day of the experiment, there was 44.16% reduction in the mean BSL of metformin group and 25.34% reduction in the mean BSL of aloe vera group as compared to pre- treatment values on Day-1.

**Fig-3** shows the overall effects of control, metformin and aloe vera on BSL of alloxan induced diabetic rabbits in their respective groups. There was almost no response in the control group and the curve is more or less straight (Blue curve). The response in Metformin group was rapid in onset on the Day-1 and then there was a steady decrease in the mean BSL towards the end (Pink curve). In the Aloe Vera group, the response was gradual. The maximum response was achieved in the second week and then there was a Steady and minimal response in the following days (Green curve).

## Discussion

Due to modernization of lifestyle, non-insulin dependent diabetes mellitus is becoming a major health problem in developing countries.[16] The treatment options have their own drawbacks, ranging

**Table-1:** Mean blood glucose levels in mg/ dl ± SD on day-1 of treatment.

| Time | GP-A | GP-B | GP-C | P-value |
|---|---|---|---|---|
| 0-HRS Mean±SD | 291.2±21.8 | 283.0±15.3 | 288.3±19.4 | 0.456 |
| 2-HRS Mean±SD | 291.7±21.9 | 243.6±22.0 | 277.5±20.6 | 0.009 |
| 4-HRS Mean±SD | 290.4±23.1 | 186.622.017.7 | 271.1±19.5 | 0.006 |
| p-value within group | 0.997 | 0.000 | 0.5 | |

**Table-2:** Mean blood glucose levels in mg/ dl ± SD on day-14 of treatment.

| Time | GP-A | GP-B | GP-C | P-value |
|---|---|---|---|---|
| 0-HRS Mean±SD | 288.1±24.5 | 175.1±11.7 | 235.4±15.3 | 0.00 |
| 2-HRS Mean±SD | 290.1±20.3 | 168.1±11.5 | 230.7±12.9 | 0.00 |
| 4-HRS Mean±SD | 286.4±19.6 | 165.2±10.3 | 228.2±14.5 | 0.00 |
| p-value within group | 0.949 | 0.013 | 0.148 | |

**Table-3:** Mean blood glucose levels in mg/ dl ± SD on day-28 of treatment.

| Time | GP-A | GP-B | GP-C | P-value |
|---|---|---|---|---|
| 0-HRS Mean±SD | 289.4±20.2 | 164.2±9.4 | 221.0±11.8 | 0.00 |
| 2-HRS Mean±SD | 291.8±23.5 | 161.1±8.3 | 218.0±113.0 | 0.00 |
| 4-HRS Mean±SD | 288.9±22.0 | 158.8±10.4 | 215.1±11.0 | 0.00 |
| p-value within group | 0.997 | 0.149 | 0.240 | |

Lack of responsiveness in large segment of patients population[17] As an alternate option, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to control diabetes. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities using variety of mechanisms.

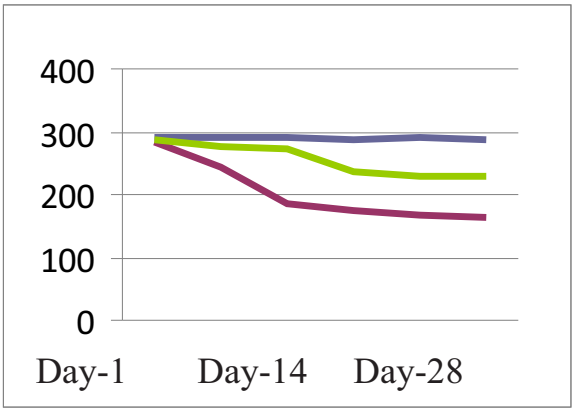


**Fig-3:** Effect of placebo, metformin and aloe vera on BSL of alloxan induced diabetic rabbits in 4 weeks time

During the past few years many phyto-constituents responsible for anti-diabetic effects have been isolated from plants.[18] The various plants tested for their anti-diabetic activity include Aloe Vera, bitter melon, cinnamon, fenugreek, Asian ginseng, American ginseng, gurmar, milk thistle, nopal and chia. In the past 15 years, there have been controversial reports on the hypoglycaemic activity of Aloe Vera species, probably due to differences in the parts of the plant used or to the model of diabetes chosen.[19] So far, the oral hypoglycemic evaluation of Aloe Vera in the form of ethanolic extract has never been done in Pakistan and not substantially even world-wide and that justifies scope of present study. Metformin is a hypoglycemic drug effective in the treatment of Type-2 diabetes. Although metformin increases insulin binding in various cell types, this effect is not universal and does not correlate with stimulation of glucose utilization. In contrast, direct effects of the drug on the glucose-transport system have been demonstrated. Metformin elevates the uptake of non-metabolizable analogues of glucose in both non-diabetic rat adipocytes and diabetic mouse muscle. In the latter, the stimulatory effect of the drug is additive to that of insulin. Thus, it is suggested that the basis for the hypoglycemic effect of this biguanide is probably at the level of skeletal muscle by increasing glucose transport across the cell membrane.[20] Because our results showed that Metformin reduced blood glucose levels in diabetic animals, the state of diabetes in the animals used in the present study was not severe. The β-cell damage was partial because alloxan was carefully administered with relatively lower dose. The hypoglycemic effect of plant extracts is generally dependent upon the degree of β-cell destruction.[21] Aloe Vera gel extract reduced BSR levels in our study. This fact further proves that the β-cell damage was partial.

The administration of 300 mg/kg body weight of Aloe Vera leaf gel in the form of ethanolic extract produced significant BSL lowering effect in alloxan induced diabetic rabbits throughout the 4 week observation period. The reduction in mean BSL on Day-1 was 5.9% and 32.6% in Aloe Vera and Metformin groups respectively. These results show that response on first day of treatment was more abrupt and potent in metformin group as compared to the Aloe Vera group. In the following weeks, there was a sustained anti-hyperglycemic effect in both the Aloe Vera and Metformin groups as compared to the placebo group. On 14$^{th}$ day of treatment, the percentage lowering of mean BSL was 16.05% and 38.16% in the aloe vera and metformin groups respectively. These values show that there was significant response in Aloe Vera group at two weeks of the treatment as far as the blood sugar lowering effect was concerned. On 28$^{th}$ day of treatment, the percentage lowering of mean BSL was 25.3% and 44.1% in the Aloe Vera and metformin groups respectively. These values, though lower than those on Day-14, were not remarkably reduced. These results show that the response with Aloe Vera treatment was achieved over a period of two weeks and after that, it remained sustained in the following weeks. The aforementioned results reveal that both Aloe Vera and metformin have a significant blood sugar lowering effect as compared to placebo in alloxan induced diabetic rabbits. These drugs, however, showed different patterns of hypoglycemic action. The onset of action was slightly delayed in Aloe Vera group as compared to the metformin group. Moreover, the anti-hyperglycemic effect of Aloe Vera leaf gel extract is observed to be lesser than that of metformin in alloxan induced diabetic rabbits. The BSL lowering effect remained 25.7% in the present study. Some of the studies explored the blood sugar lowering effects of various herbs. Kerella (Memordica Charantia) in a 3 months study reduced

Not significant.[22] Cinnamon reduced fasting glucose by 10.3% compared to 3.4% in the placebo group.[23] Feenugreek reduced mean fasting BSL from 151 to 112 mg/dl after 6 months.[24] Gymnema Sylvestre (gurmar) was evaluated in diabetic patients. 22 type 2 diabetic patients on sulfonylurea treatment took 400 mg daily for 18-20 months. Average fasting glucose decreased from a baseline of 174 to 124 mg/dl after 18-20 months.[25] It is evident that all of these plants exhibited a relatively lesser blood glucose lowering effect as compared to Aloe Vera gel used in the present study.

## Conclusion

Aloe Vera leaf gel extract did exhibit some sugar lowering effect but the animals did not turn euglycemic. A combination therapy of aloe vera with oral hypoglycemic agents may reduce the required dose of these agents. Moreover, the preparation of ethanolic extract has not been cost effective. The Aloe Vera leaf gel fresh juice is bitter in taste. Some forms of additives to make it palatable may be used rather than the extract to minimize the cost. Due to its anti-hyperglycemic potential and suspected ability to reduce oxidative stress, Aloe Vera holds promise.

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## Answer Picture Quiz

This man shows dark pigmentation and severe bone marrow suppression resulting in leucopenia and thrombocytopenia. On detailed investigations it was found that he took Isotab tablet (ISDN) which was contaminated with toxic dose of pyrimethamine, an anti folate drug used for malaria prophylaxis. Pyrimethamine can cause skin pigmentation and bone marrow suppression. Treatment consists of early diagnosis, withdrawal of offending agent and folinic acid.