

Original Article

Antidiabetic Effects of Aloe Vera Whole Leaf Extract and Sitagliptin in Streptozotocin-induced Diabetic Rats

Sobia Javaid¹, Akbar Waheed²¹ Post-graduate Trainee, Department of Pharmacology, Riphah University, Islamabad.² Professor and HOD, Department of Pharmacology, Riphah University, Islamabad.**Author's Contribution**¹ Conception of study¹ Experimentation/Study conduction¹ Analysis/Interpretation/Discussion¹ Manuscript Writing² Critical Review¹ Facilitation and Material analysis**Corresponding Author**

Dr. Sobia Javaid,

Post-graduate Trainee,

Department of Pharmacology,

RIPHAH University,

Islamabad

Email: drsobiaqazi@gmail.com

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Abstract

Introduction: *Aloe Vera*, a perennial herb, is being used since ancient times for the cure of various diseases and is recently being used in curing diabetes due to the synergistic activity of its over 75 biologically active ingredients. The objective of the study was to compare the antidiabetic effects of *Aloe Vera* whole leaf extract with standard antidiabetic drug Sitagliptin on streptozotocin-induced diabetic rats.

Material and Methods: The present study was RCT, conducted at the Department of Pharmacology, Islamic International Medical College, Rawalpindi in collaboration with the National Institute of Health, Islamabad, Pakistan, from September 2019 to August 2020. A total of 40, young Sprague Dawley rats weighing between 220-250 grams were taken and randomly divided into Group A (Normal Control) and group B. After induction of Type-2 Diabetes, group B was further subdivided into 3 groups with 10 rats each: Group B1 (Diabetic Control), Group B2 (*Aloe Vera* whole Leaf Extract treated), GROUP B3 (Sitagliptin treated). Terminal Sampling was done to measure FBS and HbA1c on Day 60. Statistical Analysis was done by applying SPSS version 25. A one-way ANOVA test was used for assessing any difference in the mean values. Post-hoc Turkey analysis was conducted to compare any inter-group mean differences. P-value of <0.05 was considered significant.

Results: On completion of the study, at Day 60, mean FBS values for Rats in Group A were 82.40 mg/dl, for Group B1 498.40 mg/dl, for Group B2 89.30 mg/dl, and Group B3 93.00 mg/dl respectively. Mean HbA1c results for rats after terminal sampling in Group A were 3.71%, Group B1 11.84%, Group B2 4.02%, B3 3.73%. Rats in each of Group B2 (*Aloe Vera* whole leaf treated) and Group B3 (Sitagliptin treated) had a significant reduction in fasting blood sugar and HbA1c levels in comparison to Group B1 (diabetic control), with no statistically significant intergroup differences in results of Group A, Group B2 and Group B3.

Conclusion: *Aloe Vera* whole leaf extract significantly decreased fasting blood glucose and HbA1c levels with almost similar efficacy to Sitagliptin in diabetic rats.

Keywords: Diabetes Mellitus, *Aloe Vera*, Sitagliptin, Hypoglycemic Agents, Streptozotocin.

Introduction

Type 2 diabetes mellitus is considered to be due to compromised regulation of carbohydrate, lipid, and protein metabolism, and results from either impaired insulin secretion, insulin action, or a combination of both.¹ Different types of oral hypoglycemic agents are available for the treatment of type 2 diabetes mellitus, including biguanides, sulfonylureas, thiazolidinediones, GLP-1 based agents and DPP-4 inhibitors, etc.² These agents are given either as a sole therapy or in combination.³

Sitagliptin is 1st amongst DPP-4 inhibitors to get approval from the FDA in 2006 for the treatment of Type 2 diabetes mellitus.^{4,5} It is a competitive inhibitor of dipeptidyl peptidase-4 (DPP-4) enzyme and exerts its antidiabetic effects primarily by suppressing glucagon secretion and stimulating insulin release in response to a meal-induced rise in incretin hormones namely, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP).^{6,7} Sitagliptin extends the duration of action of these incretins for several hours by slowing their rate of degradation.⁸ Further, DPP-4 inhibitors are neutral to the body weight.⁹

Oral hypoglycemics used either as monotherapy or in combination form, eventually fail to meet the patient's normoglycemic needs. In addition to economic constraints, reported side effects of these modern drugs encouraged us to search for an economical and safe alternative.¹⁰ *Aloe Barbadosensis* Miller, commonly known as *Aloe Vera*, belonging to the Liliaceae family, is a semitropical to tropical, perennial, succulent xerophyte with more than 200 ingredients of which over 75 are biologically active compounds.^{11,12} It has a great history of use in folk medicine for over 2000 years, and has remained an important part of traditional medicine of diverse cultures, such as China, India, the West Indies, and Japan.¹³ Its leaf exudate and mucilaginous gel hold anti-inflammatory, antioxidant, antibacterial, antifungal, anticancer, cytoprotective, immune-modulatory, and cardiac stimulatory activities.¹⁴ Till recently, no toxicity is reported in manuscripts, of various *Aloe-Vera* extract preparations, used commercially.¹⁵ In the present study, comparative antidiabetic effects of *Aloe Vera* whole leaf extract with new antidiabetic drug Sitagliptin were observed. As far as we know, no comparative studies on antidiabetic effects of *Aloe Vera* whole leaf with sitagliptin are done to date and current work will certainly be a useful tool to bridge

this gap in the struggle of finding a better cure for diabetes.

Materials and Methods

The Randomized control trial (RCT) was carried out at the Pharmacology Department of Islamic International Medical College, Rawalpindi in collaboration with the National Institute of Health (NIH) Islamabad for one year (September 2019-August 2020). A total of 40 adult albino rats, weighing 200-250g were procured from the animal house of NIH. All the animals were housed in standard cages under standard laboratory conditions: temperature $22 \pm 2^\circ\text{C}$, relative humidity $70 \pm 4\%$, and 12 hours light/dark cycle. 10 rats received a normal standard diet and the remaining 30 rats received a high-fat standard diet (protein=20%, carbohydrates=20%, lipids=60%) which was prepared at NIH and administered as standard food pellets manufactured according to the recommendations approved by the universities federation for animal welfare.¹⁶ The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals. After acclimatization for 1 week, the rats were randomly divided into two main groups; 10 rats were allocated to group A and the rest of them (30) were allocated to the experimental group B. Group A was labelled as Normal Control and was given normal saline and normal standard diet whereas the Experimental group was given high-fat standard diet for two weeks. After diabetes induction Group B was further subdivided into three groups with n=10, Group B1 (diabetic control), Group B2 (*Aloe Vera* whole leaf treated), and Group B3 (Sitagliptin treated). Streptozotocin Catalogue number 4191002-1 (714986), CAS No. 18883-66-4, Brand bio world the USA, was purchased from a commercial supplier, as a dry-frozen, pale yellow, sterilized product and kept in a freezer to prevent desiccation. Immediately before injection, STZ was dissolved in the 50mM sodium citrate buffer (pH 4.5). As STZ is liable to degradation within 15 to 20 min after dissolving in the citrate buffer, the STZ solution was prepared just before use and injected within 5 min of dissolution¹⁷. Animals fed on the high-fat diet for 2 weeks before this procedure and kept overnight fast before injecting streptozotocin. A single intraperitoneal injection of streptozotocin at the dose of 35 mg/kg body weight was given. The fasting blood glucose levels were recorded, after 72 hours of streptozotocin administration to confirm Diabetes. Rats with fasting blood glucose equal and above 250 mg/dl were considered diabetic.

Research grade Sitagliptin, Batch No: M-20191010-D05-M06-01 was provided by CCL Pharmaceuticals, Lahore, Pakistan on special request. The drug was administered orally at the dose of 10 mg/kg body weight/day for 40 days.¹⁸ Young 2-3 years old, healthy *Aloe Vera* was bought from a local nursery. Plant material identification was done at Herbarium of Pakistan Quaid-e-Azam University Islamabad, by the Department of Plant Sciences. Accession Number is 132644 and Voucher Specimen Number 125 is preserved at Herbarium of Pakistan (ISL) Quaid-e-Azam University Islamabad Pakistan. For powder formation fresh long *Aloe Vera* leaves were thoroughly washed under tap water, thorny edges were removed, and the yellow material coming out of the cut end was wasted. 1-2 inches from the base and 3-4 inches from the tip was removed. Then thin slices of the whole leaf were made and put in a big tray and dried¹⁹ in the sun. After 5 days these slices were completely dried up and the powder was obtained after grinding them thoroughly, and stored in a tightly closed glass jar, under cool, dry, and dark conditions. Later it was used in the experiment, mixed in feed made for rats (food pellets), at the calculated dose (300 mg/kg body weight/day)²⁰, through the oral route, for 40 days, to treat Diabetes in an experimental rat model.

After completion of the study, at Day 60, blood samples were taken through the cardiac puncture; FBS and HbA1c were measured for all rats in all groups. Microlab 300, a semi-automated, clinical chemistry analyzer was used for estimation of Fasting Blood Sugar, after enzymatic oxidation by glucose oxidase method. For HbA1c estimation, Quo-Lab HbA1c, the semi-automated analyzer was used. Statistical analysis was done by applying the statistical package for Social Sciences version 25 (SPSS-25). Results were documented as mean $\pm$ SEM. Comparisons of quantitative parameters among the four groups were analyzed by using one-way ANOVA (post hoc turkey test). P-value of less than 0.05 was considered as significant.

Results

At the start of the study, levels of Fasting Blood glucose and HbA1c were comparable to each other in all groups. After two weeks of dietary manipulations and then administration of Streptozotocin single I/P injection at a low dose of 35 mg/kg, diabetes was successfully induced in experimental group B. After two weeks of treatment, a significant reduction in Fasting Blood Glucose was observed in both, Group B2

and B3 as compared to Diabetic Control Group B1, P-value <0.001 . No significant difference in results of FBS, in Group B2 and Group B3, was noted, P-value >0.05 . After completion of the study, at day 60, mean FBS values for rats in Group A were 82.40 mg/dl, for Group B1 498.40 mg/dl, for Group B2 89.30 mg/dl, and Group B3 93.00 mg/dl respectively. Mean HbA1c results for rats after terminal sampling in Group A were 3.71%, Group B1 11.84%, Group B2 4.02%, B3 3.73%. Rats in each of Group B2 (*Aloe Vera* whole leaf treated) and Group B3 (Sitagliptin treated) had a significant reduction in fasting blood sugar and HbA1c levels in comparison to the Group B1 (diabetic control), P-value <0.001 , with no statistically significant intergroup differences in results of Group A, Group B2 and Group B3, P-value >0.05 .

Table 1: Mean Value $\pm$ SEM (Standard Error of Mean) of Fasting Blood Glucose (mg/dl) on Day 60 (n = 40)

Groups	Group A Control	Group B1 Diseas e Control	Group B2 Aloe Vera Leaf	Group B3 Sitaglipti n
Mean	82.40	498.40	89.30	93.00
SEM	7.65	21.72	8.91	3.37
P-Value	$<0.001^*$			

Table 2: Mean Value $\pm$ SEM (Standard Error of Mean) of Fasting Blood Glucose (mg/dl) on Day 60 (n = 40)

Groups	Mean Difference	P-Value
A vs B1	416.00 ± 5.57	$<0.001^*$
A vs B2	6.90 ± 5.57	0.607
A vs B3	10.60 ± 5.57	0.245
B1 vs B2	242.70 ± 5.57	$<0.001^*$
B1 vs B3	239.80 ± 5.57	$<0.001^*$
B2 vs B3	2.90 ± 5.57	0.910

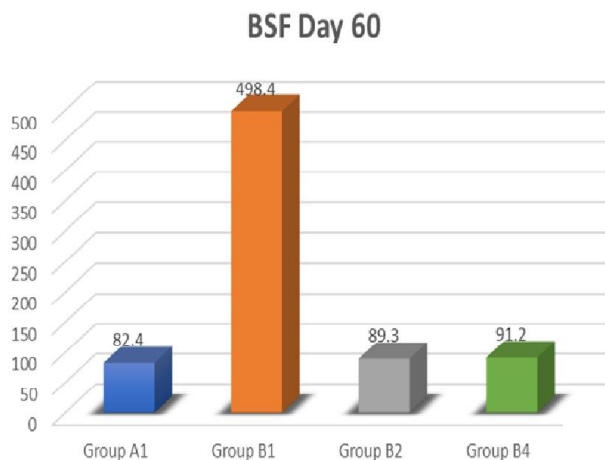


Figure 1: Serum Fasting Blood Glucose (mg/dl) at Day 60

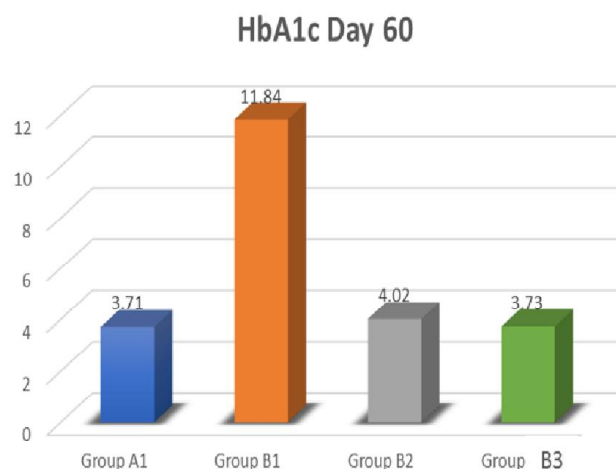


Figure 2: Serum HbA1c (%) at Day 60

Table 3: Mean Value $\pm$ SEM (Standard Error of Mean) of Serum HbA1c (%) on Day 60 (n = 40)

Groups	Group A Contro l	Group B1 Disease Control	Group B2 Aloe Vera Leaf	Group B3 Sitaglipti n
Mean	3.71	11.84	4.02	3.73
SEM	0.11	0.27	0.09	0.34
P-Value	< 0.001*			

Table 4: Comparison of Mean $\pm$ SEM (Standard Error of Mean) of HbA1c (%) on Day 60 (n = 40)

Groups	Mean Difference	P-Value
A vs B1	8.13 $\pm$ 0.22	< 0.001*
A vs B2	0.31 $\pm$ 0.22	0.490
A vs B3	0.02 $\pm$ 0.22	1.000
B1 vs B2	7.82 $\pm$ 0.22	< 0.001*
B1 vs B3	8.11 $\pm$ 0.22	< 0.001*
B2 vs B3	0.29 $\pm$ 0.22	0.546

Discussion

Aloe Vera, an evergreen, cactus-like plant native to South Africa is being used since ancient times for the cure of various diseases. Several studies have been conducted to demonstrate the useful effects of *Aloe Vera* on diabetes cure. The hypoglycemic activity of *Aloe Vera* is postulated to be due to inhibition of hepatic glucose synthesis and an increase in muscle glucose utilization. *Aloe Vera* is richly supplied with minerals, mainly magnesium, calcium, sodium, zinc, and chromium along with proteins and lipids. These minerals are often remembered as hypoglycemic elements as they modulate glucose metabolism. Chromium aids further through increasing insulin-mediated glucose uptake by the cells. Also, the high content of calcium in *Aloe Vera* stimulates beta cells of the pancreas to release insulin and raises hepatic insulin and glycogen concentrations.²¹ *Aloe Vera* safeguards beta cells from destruction and recovers the partly damaged β cells.²² The present study was so designed to bring *Aloe Vera*'s extremely useful metabolic effects in diabetes control under the limelight.

The consequences of the present study are in accordance with the useful work by Meena Gul et al., who conducted RCT to measure the synergistic effects of *Aloe Vera* whole leaf and rosiglitazone on plasma glucose, oxidative stress, and lipid profile in Type-2 Diabetic Sprague-Dawley rats. They induced Type-2 Diabetes in their experimental rats by feeding them on a high-fat diet for two weeks and then a single intraperitoneal injection of streptozotocin at the dose of 35 mg/kg body weight was given. After diabetes induction, rats were randomly divided into two

groups and given treatment for 21 days. The diabetic treatment group received a combination of *Aloe Vera* whole leaf extract 150 mg/kg and rosiglitazone 2.5 mg/kg body weight through the I/P route. After treatment with this combination plasma glucose, serum triglycerides, cholesterol, LDL, and VLDL were significantly reduced in these rats as compared to the diabetic control group.¹⁶ In our study, we similarly found a significant reduction in fasting blood glucose levels of rats of Group B2 who received *Aloe Vera* whole leaf extract, in comparison to Diabetic Control Group B1, P value less than 0.001.

Enas Ali Kamel Mohamed studied Antidiabetic, Antihypercholesteremic and Antioxidative Effect of *Aloe Vera* Extract in Alloxan Induced Diabetic Albino Rats and reported that *Aloe Vera* extracts resulted in a significant reduction in serum glucose, total cholesterol and triacylglycerol levels in those rats and on trace element analysis of *Aloe Vera* extract he found that *Aloe Vera* extracts enclosed substantial amount of Chromium, Manganese and Zinc that may lead to the antidiabetic activity of this medicinal herb.²³

The present study is also supported by the work of Manjunath K et al., on *Aloe Vera* whole leaf extract, determining its hypoglycemic effects in alloxan-induced diabetes in albino rats. They found that after five weeks of treatment with *Aloe Vera* leaf extract, the glycemic control was tremendous, as shown by a significant decrease in blood glucose levels in experimental rats.²⁴

Dr. Joyamma John did his experiment to establish hypoglycemic effects of *Aloe Vera* whole leaf extract on alloxan-induced diabetic albino rats. He reported that aqueous leaf extract of *Aloe Vera* is a beneficial and safe agent in modulating hyperglycemia induced by alloxan in an experimental rat model.¹⁴ This work further supports the findings of the present study where hyperglycemia was significantly reduced by *Aloe Vera* Whole Leaf extract.

Study Limitations

Due to time constraints and availability issues, serum insulin levels, random blood sugar levels, and histopathological studies of the pancreas of experimental rats were not performed.

Conclusion

Aloe Vera whole leaf extract and Sitagliptin significantly lowered fasting blood glucose and HbA1c

levels in HFD-STZ-T2DM rat model, P-value <0.001. *Aloe Vera* whole leaf extract had almost similar efficacy to Sitagliptin with minor statistically insignificant differences (P-value>0.05) in treating diabetes in HFD-STZ-T2DM rat model. Therefore, *Aloe Vera* whole leaf extract and Sitagliptin may be used interchangeably in the treatment of Type 2 diabetes mellitus. This will help patients to attain optimum levels of blood glucose and HbA1c, through safe, cost-effective, easily accessible, eco-friendly means.

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