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Original Article

Aloe Vera Gel for Streptozotocin Induced Diabetes Mellitus in Rats

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ABSTRACT
Objective: To compare anti-diabetic effects of Aloe Vera gel with hypoglycemic agent, sitagliptin on streptozotcin induced diabetic rats.
Materials and Methods:
Study Design: Randomized Control Trial
Place and Duration of Study: The study was done in Department of Pharmacology, Islamic International Medical College, Rawalpindi in collaboration with NIH, Islamabad, Pakistan, from September 2019 to August 2020.
Methodology: 40, young Sprague Dawley rats were taken and randomly divided into Group A and group B. After induction of type 2 diabetes with low dose streptozotocin, group B was subdivided with n=10 each as; GroupB1 (Diabetic Control), Group B2 (*Aloe Vera* gel treated), GROUP B3 (Sitagliptin treated). FBS and HbA1c measured. Statistical Analysis was done by applying SPSS version 25. 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 study, at day 60, Mean FBS of Rats in Group A was 82.40 mg/dl, B1 498.40mg/dl, B2 95.70 mg/dl, B3 93.00mg/dl; Mean HbA1 of Group A was 3.71%, B1 11.84%, B2 4.17% , B3 3.73% respectively. Rats in Group B2 and B3 had significant reduction in FBS and HbA1c levels compared to Group B1, with no statistically significant intergroup difference in Groups A, B2 and B3.
Conclusion:
Aloe Vera gel significantly decreased fasting blood glucose and HbA1c levels with almost similar efficacy to Sitagliptin in diabetic rats.
Key Words: Aloe Vera gel, Hypoglycemic Agents, Sitagliptin, Streptozotocin

Introduction

Type 2 diabetes mellitus, accounting for nearly 90% of overall cases[1] of diabetes is presently a growing pandemic especially for low and middle-income countries where more than three quarters of individuals with type 2 diabetes mellitus are living. [2] It is a major cause of blindness, stroke, heart and kidney failures, lower limb amputation and neuropathies.[3],[4]Various types of hypoglycemic agents including Sulfonylureas, Meglitinides, Biguanides Thiazolidinediones α-Glucosidase and DPP-4 inhibitors etc. are available around the globe for treating such a grave illness[5], but the disease burden is still immense.

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Sitagliptin is an orally available, competitive, beta-amino acid-derived inhibitor of dipeptidyl peptidase - 4 that was approved by the FDA in 2006 as a monotherapy and in combination with other oral hypoglycemics for treating type 2 diabetes mellitus.[6] It was the 1st member of gliptins , oral antidiabetic drugs invented and ever used commercially.[7] It inhibits the degradation of the incretin hormones, namely, glucagon-like peptide-1 (GLP- 1) and glucose-dependent insulinotropic polypeptide (GIP), leading to increased levels of these hormones in the blood which in turn stimulate insulin release from pancreas, with improvement of HbA1c levels in patients with type 2 diabetes.[8] Oral sitagliptin is effectively used for better glycemic control in a broad range of patients with type 2 diabetes , including, overweight , elderly and those with impaired kidney profile as well as, with known cardiovascular problems.[9]
Despite the commercial availability of various anti-diabetic drugs world over, diabetes mellitus with its complications remained a serious challenge for health

system**.** Economic constrains and reported side effects of the antidiabetic drugs, compelled people to look for some cost-effective and nontoxic alternative. *Aloe Barbadensis Miller*, commonly known as *Aloe Vera,* and in local language "*Kuwargandal* "is a perennial, drought resistant, succulent xerophyte with green, dagger- shaped lancet leaves. Its inner mucilaginous gel is reported to contain over 75 different potentially active compounds that are classified into nine categories: anthraquinones, inorganic compounds, enzymes, vitamins, essential amino acids, non-essential amino acids, carbohydrates, fatty acids, and other miscellaneous chemicals[10]. *Aloe Vera*'s cosmetic and medicinal usage dates back to 4th century B.C. UP until now, no toxicity is reported in manuscripts, of various Aloe-*Vera* extract preparations, used globally. In present study, comparative antidiabetic effects of Aloe Vera gel with new oral hypoglycemic agent, Sitagliptin were observed. As far as we know, no comparative studies on antidiabetic effects of *Aloe Vera* gel with sitagliptin are done till date and present work will certainly bridge this gap magnificently.

Materials and Methods

The Randomized control trial (RCT) was carried out in Pharmacology Department of Islamic International Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad for one year (September 2019-August 2020), after approval by the ethical review committee of Riphah University. A total of 40 adult, male albino rats, weighing 200-250 g were procured from animal house of NIH. We excluded female rats as well as rats with body weight below 200 grams. All the animals were housed in standard cages under standard laboratory conditions: temperature 22 ± 2 °C, relative humidity 70 ± 4% and 12 hour light / dark cycle. 10 rats received normal standard diet and the remaining 30 rats received high fat standard diet (protein=20%, carbohydrates=20%, lipids=60%) prepared at NIH and administered as standard food pellets manufactured according to the recommendations approved by the universities federation for animal welfare[11]. The care and handling of rats was in accordance with the internationally accepted standard guidelines for use of animals. After 1 week of acclimatization, 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 given normal saline and normal standard diet whereas the group B 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 gel treated) and Group B3 (Sitagliptin treated).

Streptozotocin Catalogue number 4191002-1(714986), CAS No. 18883-66-4, Brand bio world USA, was purchased from commercial supplier, as a dry-frozen, pale yellow, sterilized product and kept in freezer to prevent desiccation. Immediately prior to 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 dissolution[12]. Animals fed on high fat diet for 2 weeks prior to this procedure and kept overnight fast before injecting streptozotocin. Single intra-peritoneal injection of streptozotocin at the dose of 35 mg/kg body weight was given.[11,13,14] Fasting blood glucose levels were recorded, after 72 hours of streptozotocin administration to confirm Diabetes.[15] Rats with fasting blood glucose equal and above 250mg/dl were considered diabetic.

Research grade Sitagliptin, Batch No: M-20191010-D05-M06-01, was provided by CCL Pharmaceuticals, Lahore, Pakistan. The drug was administered orally at the dose of 10mg/kg body weight/day for 40 days.[16] Young 3 years old, healthy *Aloe Vera* was bought from local nursery. Plant material identification was done at Herbarium of Pakistan- Quaid-e-Azam University Islamabad, by Department of Plant Sciences. Accession Number is 132644 and Voucher Specimen Number 125 is preserved at the Herbarium. Fresh long leaves of *Aloe Vera* were taken, thoroughly washed under tap water. The yellow substance coming out of cut end wasted as it is full of Aloin, the cathartic substance.1-2 inches from base and 3-4 inches from tip end of the leaf were wasted. The thorny edges were removed along with the rind from top and bottom of the leaf and the gel was taken out, washed to remove any impurities and kept in a container. The gel was scooped into an electric blender and then homogenized to obtain the gel extract. The fresh gel extract was daily prepared for each administration over the 40 days of experimental trial period. The fresh gel extract was mixed in rat feed and used in the form of standard food pellets, at the calculated dose (300 mg/kg body weight/day) for treating diabetes in experimental rat model.[17,18] After completion of study, at Day 60, blood samples were taken through 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, 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 +_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 study, levels of Fasting Blood sugar and HbA1c were comparable to each other in all groups. After two weeks of high fat diet 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, significant reduction in Fasting Blood sugar 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 Fasting blood sugar, in Group B2 and Group B3 was noted. P value > 0.05. After completion of study, at day 60, mean FBS values for rats in Group A were 82.40 mg/dl, for Group B1 498.40mg/dl, for Group B2 95.70 mg/dl and for Group B3 93.00mg/dl respectively. Mean HbA1c results for rats after terminal sampling in Group A were 3.71%, Group B1 11.84%, Group B2 4.17%, B3 3.73%. Rats in each of Group B2 (*Aloe Vera* gel treated) and Group B3 (Sitagliptin treated) had 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 + SEM (Standard Error of Mean) of Fasting Blood Glucose (mg/dl) on Day 60 (n = 40)

Groups	Group A Normal Control	Group B1 Disease Control	Group B2 Aloe Vera Gel	Group B3 Sitagliptin
Mean	82.40	498.40	95.70	93.00
SEM	7.65	21.72	4.79	3.37
P Value	< 0.001*			

Table-2: Comparison of Mean ± 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 ± 5.31	< 0.001*
A vs B2	13.30 ± 5.31	0.076
A vs B3	10.60 ± 5.31	0.209
B1 vs B2	402.70 ± 5.31	< 0.001*
B1 vs B3	405.40 ± 5.31	< 0.001*
B2 vs B3	5.31 ± 5.31	0.957

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

Groups	Group A Control	Group B1 Disease Control	Group B2 Aloe Vera Gel	Group B3 Sitagliptin
Mean	3.71	11.84	4.17	3.73
SEM	0.11	0.27	0.08	0.34
P Value	< 0.001*			

Table-4: Comparison of Mean ± SEM (Standard Error of Mean) of HbA1c (%) on Day 60 (n = 40)

Groups	Mean Difference	P Value
A vs B1	8.13 ± 0.21	< 0.001*
A vs B2	0.46 ± 0.21	0.160
A vs B3	0.02 ± 0.21	1.000
B1 vs B2	7.67 ± 0.21	< 0.001*
B1 vs B3	8.11 ± 0.21	< 0.001*
B2 vs B3	0.44 ± 0.21	0.190

Discussion

Aloe Barbadensis Miller has been widely used as a healing plant in the history of mankind due to its excellent biological activities, including anti-inflammatory, anti-diabetic, anti-hyperlipidemic, anti-cancer, anti-fungal and wound healing properties.[19] Hypoglycemic property of *Aloe Vera* is proposed to be due to its excellent effects on insulin synthesis and release by Beta-cells of the Pancreas.[20] Further, *Aloe Vera* extracts might regulate blood sugar levels through enhancing peripheral glucose uptake or decreasing counter regulatory hormones like cortisol, glucagon and growth hormone. [21] Mediha Sefi1 et al,. conducted study to evaluate the hypoglycemic and hypolipidemic activities of *Aloe Vera* mucilage in alloxan- induced diabetic male Wistar rats and found that Aloe Vera mucilage administration to diabetic rats partially and totally corrected glycaemia and liver glycogen content and serum insulin level, respectively. [22] Similar results were seen in present study, where we found significant reduction in fasting blood glucose and HbA1c levels in rats treated with *Aloe Vera* gel extract.

Aloe Vera gel, taken out of the mucilaginous tissue from the middle of the *Aloe Vera* leaves has miscellaneous uses in cosmetics and medicine. It is

richly supplied with many phytochemicals, such as alkaloids, anthraquinones, chromones, coumarin compounds, flavonoids, and phenols etc.[23] Atanu F.O. et al., explored the effects of rigorous glucose regulation with combination of *Aloe Vera* gel extract and metformin in alloxan-induced diabetic male albino rats. They noticed significant improvement in blood glucose measurement and histopathological parameters after, either sole treatment or a combination of 300 mg/kg of *Aloe Vera* gel and 2 mg/kg Metformin in experimental rat model. Their study specifies that *Aloe Vera* gel, or its combination with metformin, induced healing of wounded beta cells of pancreas , leading to enhanced production of insulin, with improvement in glucose uptake by the body cells and cellular integrity , thus, inhibiting risk of cardiovascular diseases and kidney failure associated with diabetes mellitus.[18]

Mubashra Qadeer, Nizwa Itrat, Nida Iftikhar and Uswa Ahmed in 2019 conducted study on *Aloe Vera* gel to evaluate its hypoglycemic effect in diabetic patients. They gave 5mL and 10 mL of *Aloe Vera* gel for four weeks to their experimental subjects and found that all the levels of *Aloe Vera* gel were statistically significant in reducing the blood glucose level of the patients, under study and highest reductions in blood glucose levels were seen with 10 ml of *Aloe Vera* followed by significant reduction in blood glucose levels of patients who had been given 5ml of *Aloe Vera.*[24]

RN Ugbaja et al ., while working on Aloe Vera gel extract for dyslipedemias in streptozotocin induced male albino rats, found that four weeks treatment with Aloe Vera gel extract, caused significant reduction in blood glucose, serum triglycerides, cholesterol and phospholipid levels. He described this improvement in lipid levels to be the result of improved regulation of lipid metabolism and also due to insulinogenic activity of the Aloe Vera gel extract.[17]

These studies strongly support results of current study, where significant decrease in FBS and HbA1c was seen after treatment with Aloe Vera gel extract at 300mg/kg dose in group B2 rats, compared to Diabetic Control Group, B1, P value < 0.001. This may be due to improvement in insulin synthesis and release from pancreas along with decrease insulin resistance, after treatment with *Aloe Vera* gel in these experimental animals.

Study Limitations

Owing to time constrains, cost and availability issues, Post prandial blood glucose, oral glucose tolerance test and serum insulin level estimation were not carried out in the study. Morphological study of pancreas, immune histochemistry and histopathology of pancreas were also not done.

Conclusion

Fresh *Aloe Vera* gel extract and Sitagliptin significantly lowered fasting blood glucose and HbA1c levels in HFD-STZ – T2DM rat model, P value <0.001. *Aloe Vera* gel 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, fresh *Aloe Vera* gel extract and Sitagliptin may be used interchangeably in treatment of diabetes mellitus. This will help patients to attain optimum levels of blood glucose and HbA1c, through nontoxic, economical, easily available, ecofriendly means.

Conflict of Interest: "None to declare."

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C. Interpretation/ Analysis and Discussion