

Effect of Aloe Vera Whole Leaf Extract on Blood Glucose, Hyperinsulinemia and Insulin Resistance in Streptozotocin Induced Type 2 Diabetic Rats

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ABSTRACT

Objective: To determine the effect of Aloe vera whole leaf extract and/or Rosiglitazone on plasma glucose, insulin and insulin resistance in type 2 diabetic Sprague-Dawley rats.

Study Design: Randomized control trail

Place and Duration of Study: This study was conducted at the Department of Physiology Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from April 2009 to Oct 2010.

Materials and Methods: Type 2 DM was induced in 45 healthy Sprague –Dawley rats by feeding high fat diet for 2 weeks and injecting a low dose (35mg/kg) of streptozotocin intra peritoneally. Type 2 diabetic rats were randomly divided into three groups, each group having 15 rats and were labeled as diabetic group, Aloe vera group and rosiglitazone group. The diabetic group was injected normal saline, Aloe vera group was treated with Aloe vera whole leaf extract in dose of 300mg/kg body weight and rosiglitazone group was given 5mg/kg body weight of rosiglitazone I/P for 21 days.

Results: A significant reduction ($p < 0.001$) in plasma glucose (62%), insulin (19%) and TG/HDL ratio (69%) was analyzed in Aloe Vera group as compared to diabetic control group.

Conclusion: The maximum impact in lowering plasma glucose, insulin and TG/HDL ratio was recorded in rosiglitazone group, followed by Aloe vera group. The results of present study provide a scientific basis of using Aloe vera whole leaf extract as antidiabetic in T2DM.

Key Words: T2DM, Aloe vera, Hyperinsulinemia, Insulin Resistance

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which is characterized by hyperglycemia due to the defects in secretion or action of insulin or both.¹ DM is increasing in alarming rate through out the world especially in developing countries. Pakistan ranks sixth in the world's top ten countries with the highest number of diabetics². Managing diabetes is difficult due to the number of side effects associated with drugs used for its treatment. Complementary and alternative medicine (CAM) for the treatment of diabetes mellitus is becoming popular.³ The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated.⁴

Aloe vera comes from a family called Aloaceae and related to the Liliaceae family. Among 360 known species only five have medicinal properties.⁵ The Aloe vera plant has fleshy leaves which consist of gel, latex

and outer green rind. Number of studies has been documented on gel and latex parts but with controversial reports.⁶ A study based on the use of traditional phytotherapy for control and treatment of diabetes by rural inhabitants of district Attock showed that a large majority of people used the extract made from fresh leaves of Aloe vera, according to them this formula is very old and 100% effective.⁷ Increasing incidence of diabetes mellitus in rural population, adverse effects of synthetic medicines and humble financial status of our people necessitates looking for indigenous and inexpensive botanical source of medicines with anti diabetic effects. Therefore present study was designed to analyze the effect of Aloe vera whole leaf extract on plasma glucose, insulin and insulin resistance in type 2 diabetic rats and to compare the results with known antidiabetic drug, rosiglitazone.

MATERIALS AND METHODS

A whole leaf process was employed in making the Aloe juice. Leaves were cut into sections and were pulverized into a soup like structure by placing these in

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a grinding unit. Cellulose was allowed to dissolve in a digestion liquid. Aloe emodin as well as aloin was removed by passage through activated charcoal column.⁸

Forty five healthy Sprague Dawley rats' about 90 days old, weighing between 220±50 grams were taken from National Institute of Health (NIH), Islamabad. Rats with deranged blood glucose levels and that which fail to become diabetic and insulin resistant after two weeks of high fat diet and injection of streptozotocin (35mg/kg) were excluded from the study

For induction of T2DM all animals were fed with high fat diet for 2 weeks after which a single intra-peritoneal injection of streptozotocin (available as 1 gram vial, Bioworld Pharmaceutical) in the dose of 35 mg/kg body weight was given.⁹ For confirmation of T2DM fasting blood glucose levels along with total lipid profile were measured after 72 hours by tail vein sampling. The cut off value for hyperglycemia was of >11.11mmol/l. The development of insulin resistance was measured by using the surrogate marker of TG: HDL ratio. The cut off value of TG: HDL ratio >1.8 was used to establish insulin resistance¹⁰

After induction of T2DM, Sprague Dawley rats were randomly divided into three groups, diabetic control group, Aloe vera and rosiglitazone. Diabetic control group were administered 0.1ml normal saline intraperitoneally (I/P) daily, Aloe vera group were given Aloe vera whole leaf extract in daily dose of 300 mg/kg body weight by gastric tubing and rosiglitazone group treated with 5mg/kg body weight of rosiglitazone I/P for next 21 days. After 21 days of treatment, overnight fasted rats were anesthetized and 5 ml of intra-cardiac blood was collected to analyze plasma glucose, insulin resistance (TG/HDL) and insulin levels.

Analysis of samples was done at Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, Pakistan. Estimation of glucose was done by enzymatic colorimetric (TRINDER'S) method. Triglycerides (TG) and high density lipoprotein (HDL) were estimated simultaneously on automated chemistry analyzer (Vitalab Selectra E) An enzymatic colorimetric method GPO-PAP (Glycerol phosphate oxidase) was used for serum TG estimation. The direct method for quantifying HDL was done and their ratio was taken as marker for insulin resistance.¹¹ Insulin measured by ELISA, based on the direct sandwich technique.

Data was entered into SPSS version 16.0. Mean and standard deviation was employed for all the values. Data within the groups were analyzed by using one-way analysis of variance (ANOVA) followed by Post Hoc (Tukey) test. The "p value" <0.05 was considered statistically significant.

RESULTS

At the end of the study the plasma glucose levels of diabetic control group was 20.15 ± 1.97 mmol/l with reduction in Aloe vera group upto 7.64 ± 0.71 mmol/l

(62%), rosiglitazone group upto 6.54 ± 0.64 mmol/l (68%) which revealed significant reduction (p<0.001) in the two treated groups. However, the reduction in plasma glucose level in rosiglitazone was maximum. The statistical difference among mean plasma glucose levels of the groups was found significant (p<0.001) by one way ANOVA. Post-Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean plasma glucose levels between the groups (table 1). The comparison revealed that mean plasma glucose levels significantly (p<0.001) decreased in Aloe vera and rosiglitazone group as compared to the diabetic group. However a greater reduction in rosiglitazone group was observed.

TG: HDL ratio in diabetic control group was 5.8 ± 1.40, in Aloe vera group; 1.8 ± 0.20, in rosiglitazone group; 1.5 ± 0.30 as shown in table 1.

The statistical difference among mean TG: HDL ratio of the groups was found significant (p<0.001) by one way ANOVA. Post-Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean TG: HDL ratio between the groups (table 1). The comparison revealed that mean TG: HDL ratio significantly (p<0.001) decreased in Aloe vera and rosiglitazone as compared to the diabetic group as shown in table 1.

Table No.1: Comparison of plasma glucose, TG: HDL ratio and insulin levels in different groups by one way ANOVA

Variables	Diabetic control group	Aloevera group	Rosiglitazone group	p Value
Plasma glucose (mmol/l)	20.15 ± 1.97	7.64 ± 0.71	6.54 ± 0.64	<0.001
Triglyceride (mmol/l)	2.16 ± 0.14	1.08 ± 0.10	0.95 ± 0.13	<0.001
HDL (mmol/l)	0.39 ± 0.08	0.58 ± 0.06	0.65 ± 0.07	<0.001
TG:HDL ratio	5.8 ± 1.40	1.8 ± 0.20	1.50 ± 0.30	<0.001
Insulin (µU/ml)	20.63 ± 2.2	16.76 ± 0.95	15.41 ± 1.06	<0.001

All values are presented as mean ±SD for 15 animals in each group

The TG and HDL levels of diabetic control rats were 2.16 ± 0.14 mmol/l and 0.39 ± 0.08 mmol/l respectively however TG decreased in Aloe vera group (1.08 ± 0.10 mmol/l) and rosiglitazone group (0.95 ± 0.13 mmol/l) as compare to the diabetic control group.. The serum HDL levels of diabetic control group was 0.39 ± 0.08 mmol/l, which increased in Aloe vera, and rosiglitazone group upto 0.58 ± 0.06 mmol/l, 0.65 ± 0.07 mmol/l respectively.

Statistical significance of difference between the mean level of lipid parameters were assessed by one way

ANOVA, which revealed significant difference ($p < 0.001$) among the groups. Post-Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean TG levels between the groups (table 1). The comparison revealed that mean plasma TG levels significantly ($p < 0.001$)

decreased while HDL levels increased in Aloe vera and rosiglitazone group as compared to the diabetic group. Plasma insulin levels in diabetic control ($20.63 \pm 2.2 \mu\text{IU/ml}$), which has been found decreased in Aloevera treated group ($16.76 \pm 0.59 \mu\text{U/ml}$) and in rosiglitazone group ($15.41 \pm 1.06 \mu\text{U/ml}$).

Table No.2: Statistical difference of plasma glucose, TG: HDL ratio and insulin levels between different groups using Post-Hoc (Tukey) test

Group comparison	Blood glucose (mmol/l)	Insulin ($\mu\text{U/ml}$)	Triglyceride (mmol/l)	HDL (mmol/l)	TG:HDL
Diabetic Vs Aloevera	<0.001	<0.001	<0.001	< 0.001	<0.001
Diabetic Vs rosiglitazone	<0.001	<0.001	<0.001	<0.001	<0.001
Aloevera Vs rosiglitazone	0.047	0.047	0.039	0.047	0.048

P value <0.005 is statistically significant

Table No.3: Percent reduction in blood glucose, TG:HDL and insulin levels in different treated groups in comparison to the diabetic control

Parameter	Control	Alovera	Rosiglitazone
Blood glucose mmol/l	20.15	62 % ↓	68 % ↓
TG:HDL ratio	5.8	69 % ↓	74 % ↓
Insulin $\mu\text{U/l}$	20.63	19 % ↓	25 % ↓

The comparison of mean plasma insulin levels between all the groups assessed by one way ANOVA, revealed significant difference ($p < 0.001$) amongst the groups. Post-Hoc (Tukey) test was applied to calculate the statistical significance of differences between the mean plasma insulin levels between two groups namely diabetic and Aloevera group, diabetic and rosiglitazone group, (table 1). The comparison revealed that mean plasma insulin level was significantly (<0.001) decreased in Aloevera supplemented group, rosiglitazone group as compared to diabetic control group (Table 2) Post-Hoc (Tukey) test was also applied between interventional groups. The comparison revealed that mean plasma insulin levels were significantly lowered in rosiglitazone group as compared to Aloe vera group.

DISCUSSION

We used the animal model of T2DM developed by Srinivasan, because it closely resembled the natural course and metabolic characteristics of the disease⁹. In previous studies most of the experimental models of T2DM were constructed with alloxan and streptozotocin to destroy only a portion of B cells which resulted in extreme insulin deficiency and overt hyperglycemia in rats and their characteristics were similar to type DM-1 than to DM-2.

Administration of high fat diet for 2 weeks followed by low dose of streptozotocin resulted in frank hyperglycemia, hyperinsulinemia and insulin resistance. These findings were consistent with the published data of different studies¹².

In our study TG/HDL ratio in all groups after inducing T2DM was more than 1.8, manifested the presence of insulin resistance. In a study by Srinivasan et al., (2005), TG/HDL ratio was not measured; however marked hyperinsulinemia ($467.50 \pm 32.43 \text{ pmol/l}$) in high fat fed rats was taken as the indicator of insulin resistance.

Aloe vera supplementation in the present study has resulted in statistically significant ($p < 0.001$) reduction in plasma glucose levels when compared with diabetic control group. A study conducted by Noor et al, resulted in reducing fasting plasma glucose level in streptozotocin induced diabetic rats. They used the same dose and duration of treatment as in our study. However, by the end of the study, fasting blood glucose in their diabetic rats reduced by 41% while in our study blood glucose levels decreased by 62 % of the diabetic control rats¹³. This could be due to the use of whole leaf extract rather only using the gel part and due better extraction of blood sugar-lowering active principles of whole leaf Aloevera extract.

Since it is difficult to quantify insulin resistance in daily practice, there are several methods to estimate it. Most commonly homeostasis model assessment for insulin resistance formula (HOMA- IR) is used¹⁴. However due to financial constraints we could not use HOMA- IR model. We used TG: HDL ratio to quantify it. In present study, there was a marked development of insulin resistance in the diabetic group as revealed by TG: HDL ratio of 5.8. The magnitude of insulin resistance was lowered in Aloevera group by 70%. This could be due to its glucose and lipid lowering property. The insulin sensitizing activity was attributed to the

presence of chromone lephenol and cycloartanol, a phytosterol in Aloe vera extract manifesting the marked insulin sensitizing action of Aloe vera.¹⁵

Kim studied the effect of Aloe vera extract on diet induced obesity (DIO) mice. Aloe vera was given in a dose of 100mg/kg for 8 weeks that significantly lowered insulin resistance¹⁶. However, in their study they used HOMA- IR. The insulin resistance values of DIO group treated with 25, 50, 100 mg/kg Aloe vera extract was 31.4%, 32.1% and 31.1% respectively, of that of the untreated DIO group. However our study results were more significant (70%) than Kim's study. This could be due to higher dose (300mg/kg) of Aloe vera extract used in our study.

At the end of study the plasma insulin level in diabetic control group was $(20.63 \pm 2.2 \mu \text{U/ml})$ consistent with other studies.¹⁷ Treating them with Aloe vera extract resulted in significant decrease ($p < 0.001$) in insulin level by 19%. This may be due to the fact that Aloe vera extract increased the insulin sensitivity by decreasing plasma glucose and lipid levels, thus resulting in reduction in plasma insulin level.

A study conducted by Kim on C57BL/6J mice showed statistically significant ($p < 0.05$) increased level of insulin (71%) after feeding them high fat diet for 12 weeks in comparison to regular diet fed mice.¹⁶ A group of these mice with diet induced obesity (DIO) and hyperglycemia were treated with processed Aloe vera gel (PAG), which resulted in 34% statistically significant decrease in insulin level ($p < 0.05$) in comparison to the diabetic mice. The %age decrease in Kim's study was more profound than our study. This difference could be due to difference in type of model used in study.

Rosiglitazone is a known antidiabetic drug of thiazolidinediones family.¹⁸ It increases insulin sensitivity and improves glycemic control. It also acts as a ligand for the gamma subtype of peroxisome proliferators activated receptor (PPAR- gamma), which is directly involved in the regulation of genes controlling glucose homeostasis and lipid metabolism.¹⁹ In our study the plasma glucose levels are reduced by 68%, insulin 25%, TG 56%, HDL by 66%, TG:HDL ratio 74%. These findings of rosiglitazone group are similar to many clinical trials carried in the past¹⁹.

CONCLUSION

In our study the treatments effects highlighted in percentage terms had recorded maximum impact in lowering blood glucose, insulin and insulin resistance in rosiglitazone treated group followed by Aloe vera. However, with prolonged use, rosiglitazone is associated with weight gain myocardial infarction and heart failure making it an unlikely drug for T2DM.²⁰ This opens the room to explore new strategy of treatment for T2DM, by looking for synergistic action of natural herb with synthetic drug (in half of effective dose). In addition to

minimizing the side effects associated with synthetic drug, may help to lessen the financial burden associated with this disease especially in a country like Pakistan, where socio economic conditions of people are not strong enough to cope with chronic diseases like DM

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; 33(1): 562–569.
2. Shera AS, Fawad F, Maqsood A. Prevalence of diabetes in Pakistan. *Diabetes Res Clin Practice* 2007;76(2):219–222.
3. Rahman A, Zaman K. Medicinal Plants with Hypoglycemic activity. *J Ethnopharmacol* 2004;26: 1–55.
4. Yeh Y, Kaptchuk T, Eisenberg M. Systemic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetic Care* 2003; 26(4):127–94.
5. Michael K, Asim AB, Robert SB. The utility of oral diabetes medication in type 2 diabetes of young. *Curr Diab Rev* 2005;7:83–92.
6. Agarwal M. Whole leaf gel vs standard gel. *Drug. Cosmet Ind* 1997;160(2):22–8.
7. Khan AM, Arshad MA, Zafar M. Ethnophytotherapeutic approaches for the treatment of diabetes by local inhabitant of district attock. *J Ethnobotanical Leaflets* 2004;22(1).
8. Ramachandra, CT, Srinivasa R. Processing of Aloe vera leaf gel: A review. *Am J Agric Biol Sci* 2008; 3(2): 502–51.
9. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005;52:313–20.
10. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 2003;139: 802–9.
11. Hiroshi M, Yasuki I, Shuichi O, Akira F. Method of determining cholesterol content of high-density lipoproteins. *US Patent* 2000; 6(479):249 B2.
12. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research an overview. *Ind J Med Res* 2007;125:451–72.
13. Noor A, Gunasekaran S, Manickam S, Vijayalakshmi MA. Antidiabetic activity of Aloe vera and histology of organs in streptozotocin induced diabetic rats. *Current Sci* 2008;94(8): 1070–76.
14. Mathew DR, Hosker AS, Rudenski BA, Naylor DF, Treacher, Turner RC. Homeostasis model

- assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985;28:412-419.
15. Rajasekaran S, Sivagnanam K, Ravi K, Subramanian S. Hypoglycemic effect of Aloegel on streptozotocin induced diabetes in experimental rats. *J Med Food* Spring 2004;7(1): 61-66.
 16. Kim K, Kim H, Kwon J, Lee S, Kong H, et al. Hypoglycemic and hypolipidemic effects of processed Aloe vera gel in a mouse model of non-insulin-dependent diabetes mellitus. *Phyto-medicine* 2009;16:856-63.
 17. Sood N, Baker W, Coleman C. Effect of glucomannan on plasma lipid and glucose concentrations body weight, and blood pressure: systematic review and meta-analysis. *Am J Clin Nutr* 2008;88:1167-75.
 18. Henry RR. Thiazolidinediones. *Endocrinol Metab Clin N Am* 1997;26:553-573.
 19. Lebovitz H, Dole J, Patwardhan R, Rappaport E, Freed M. Rosiglitazone monotherapy is effective in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2001;86(1):280- 88.
 20. Kaul S, Bolger. A. Thiazolidinedione Drugs and Cardiovascular Risks. American Heart Association and American College of Cardiology Foundation. 2010;121:1868- 77.