EFFECT OF ALOE BARBADENSIS MILLER WHOLE LEAF EXTRACT ON HYPERGLYCEMIA AND INSULIN RESISTANCE IN LOW DOSE STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

BACKGROUND: Managing diabetes is difficult due to the number of side effects associated with drugs used for its treatment. There it is a need of an hour to look for indigenous plants which are safe and cost effective. Present study was planned 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.

DESIGN: Randomized control trail

PLACE AND DURATION OF STUDY: This study was conducted from April 2009 to Oct 2010 at the Department of Physiology Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad.

MATERIAL AND METHOD: Type 2 DM was induced in 60 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 four groups, each group having 15 rats and were labeled as diabetic group, *Aloe vera* group, rosiglitazone group and combined 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, rosiglitazone group was given 5mg/kg body weight of rosiglitazone I/P and combined group diabetic rats were treated with 150mg/kg body weight of *Aloevera* extract and 2.5mg/kg body weight of rosiglitazone (half of their effective dose) for 21 days.

RESULTS: A significant reduction ($p < 0.001$) in plasma glucose (73%), insulin (32%) and TG/HDL ratio (81%) was analyzed in *combined* group as compared to diabetic control group. \

CONCLUSION: The maximum impact in lowering plasma glucose, insulin and TG/HDL ratio was recorded in combined group, followed by rosiglitazone group and then *Aloevera* group.

KEY WORDS: T2DM. *Aloe vera*, insulin resistance

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which is characterized by hyperglycemia due to the defects in secretion or action of insulin or both¹. 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. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated⁴.

Aloe vera comes from a family called Aloaceae and related to the Liliaceae family. Among 360 known species only five have medicinal properties; *Aloe arborescens* Miller, *Aloe perryi* Baker, *Aloe ferox* Miller, *Aloe sepnoria* and *Aloe barbadensis* Miller⁵. 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 reports⁶. A study based on the use of traditional phytotherapy for control and treatment of diabetes by rural inhabitants of district Attock was conducted. It was found 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% effective⁷. 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. In this study we used the animal model of T2DM developed by Srinivasan because it closely resembled the natural course and metabolic characteristics of the disease⁸. In type 2 diabetics post binding defects are primarily responsible for insulin resistance. However return of normoglycemia by weight loss corrects the defect which suggests that defect in receptor is acquired secondary to some combination of hyperglycemia and other metabolic disturbances⁹. In this study a known antidiabetic, rosiglitazone, is used as comparative drug and also in combination with Aloevera to minimize the side effects associated with the drug. Thiazolidinediones consist of rosiglitazone and pioglitazone. These works by binding to peroxisome proliferators activated receptor gamma (PPAR γ), nuclear regulatory protein and regulate glucose and fat metabolisms by improving insulin sensitivity in muscles and liver¹⁰. However, these drugs decrease plasma triglyceride levels but are associated with weight gain an increased in LDL-cholesterol levels, congestive heart failure and left ventricular dysfunction¹¹.

Therefore present study was designed to analyze the effect of *Aloe vera* whole leaf extract alone and in combination with rosiglitazone (in half of their

effective dose) on plasma glucose, insulin and insulin resistance in type 2 diabetic rats and to compare the results with known antidiabetic drug, rosiglitazone.

MATERIAL AND METHOD

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 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 column¹².

The pilot study was conducted on 10 type 2 diabetic rats to find the effective dose of *Aloe vera* whole leaf extract. They were randomly divided into 5 groups, 2 in each group and were given *Aloe vera* whole leaf extract in dose range of 100- 500 mg/kg body weight respectively for 21 days. At the end of study, it was found that *Aloe vera* extract in dose of 300mg/kg body weight was effective in reducing blood glucose in T2DM rats.

Sixty healthy Sprague Dawley rats' about 90 days old, weighing between 220 $\pm$ 50 grams were taken from National Institute of Health (NIH), Islamabad.

For induction of T2DM all animals were fed with high fat diet for 2 weeks after which a single intraperitoneal injection of streptozotocin (available as 1 gram vial, Bioworld Pharmaceutical) in the dose of 35 mg/kg body weight was given¹³. 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 resistance¹⁴.

After induction of T2DM, Sprague Dawley rats were randomly divided into four groups, diabetic control group, *Aloe vera*, rosiglitazone and combined group. 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, rosiglitazone group treated with 5mg/kg body weight of rosiglitazone I/P and combined group were given *Aloevera* extract (150mg/kg body weight) and rosiglitazone (2.5mg/kg body weight) 50% of their effective dose 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.¹⁵ 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.¹⁶ Insulin is 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%) and of combined group 5.41 ± 0.52 mmol/l (73%) which revealed significant reduction ($p < 0.001$) in all the three treated groups. However, the reduction in plasma glucose level in combined group 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 mean plasma glucose levels between the

groups (table 2).

Table 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 (μ U/ml)	Triglyceride (mmol/l)	HDL (mmol/l)	TG:HDL
Diabetic Vs <i>Aloevera</i>	<0.001	<0.001	<0.001	< 0.001	<0.001
Diabetic Vs rosiglitazone	<0.001	<0.001	<0.001	<0.001	<0.001
Diabetic Vs combined	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Aloevera</i> Vs rosiglitazone	0.047	0.047	0.039	0.047	0.048
<i>Aloevera</i> Vs combined	<0.001	<0.001	< 0.001	<0.001	<0.001
Rosiglitazone Vs combined	0.039	0.045	<0.046	0.017	0.036

P value <0.005 is statistically significant

The comparison revealed that mean plasma glucose levels significantly ($p < 0.001$) decreased in *Aloe vera*, rosiglitazone and combined group as compared to the diabetic group. However a greater reduction in combined group was observed as compared to other two groups despite half of their effective doses were used.

TG: HDL ratio in diabetic control group was 5.8 ± 1.40 , in *Aloevera* group; 1.8 ± 0.20 , in rosiglitazone group; 1.5 ± 0.30 and in combined group; 1.1 ± 0.22 as shown in table 1.

Table 1: Comparison of plasma glucose, TG: HDL ratio and insulin levels in different groups by one way ANOVA

Variables	Diabetic control Group	<i>Aloevera</i> Group	Rosiglitazone Group	Combined group	p Value
Plasma glucose (mmol/l)	---	7.64 ± 0.71	6.54 ± 0.64	5.41 ± 0.52	<0.001
Triglyceride (mmol/l)	2.16 ± 0.14	1.08 ± 0.10	0.95 ± 0.13	0.82 ± 0.14	<0.001
HDL (mmol/l)	0.39 ± 0.08	0.58 ± 0.06	0.65 ± 0.07	0.70 ± 0.074	<0.001
TG:HDL Ratio	5.8 ± 1.40	1.8 ± 0.20	1.50 ± 0.30	1.1 ± 0.22	<0.001
Insulin (μ U/ml)	20.63 ± 2.2	16.76 ± 0.95	15.41 ± 1.06	14.05 ± 0.80	<0.001

All values are presented as mean $\pm$ SD for 15 animals in each group.

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 mean TG: HDL ratio between the groups (table 2). The comparison revealed that mean TG: HDL ratio significantly ($p < 0.001$) decreased in *Aloe vera*, rosiglitazone and combined group as

compared to the diabetic group. However a greater reduction in combined group was observed despite half of their effective doses were used as shown in table 1.

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), rosiglitazone group (0.95 ± 0.13 mmol/l) and in the combined group (0.82 ± 0.14 mmol/l) as compared 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*, rosiglitazone group and combined group upto 0.58 ± 0.06 mmol/l, 0.65 ± 0.07 mmol/l and 0.73 ± 0.07 mmol/l respectively.

Statistical significance of difference between the mean level of lipid parameters were assessed by one way ANOV followed by Post-Hoc (Tukey's) test which revealed significant difference ($p < 0.001$) among the groups and between the groups respectively (table 1,2).

Plasma insulin levels in diabetic control (20.63 ± 2.2 μ IU/ml), which has been found decreased in *Aloevera* treated group (16.76 ± 0.59 μ IU/ml), in rosiglitazone group (15.41 ± 1.06 μ IU/ml) and in combined group (14.05 ± 0.80 μ IU/ml) (table 3)

Table 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	Combined
Blood glucose mmol/l	20.15	..	68 % ↓	73 % ↓
TG:HDL ratio	5.8	69 % ↓	74 % ↓	81 % ↓
Insulin μ IU/l	20.63	19 % ↓	25 % ↓	32 % ↓

The comparison of mean plasma insulin levels between all the groups assessed by one way ANOVA followed by Post-Hoc (Tukey) test revealed significant difference ($p < 0.001$) amongst and between the groups (table 1,2). The lowered levels observed in combined group as compared to rosiglitazone group (table 2) despite the fact that half of their effective doses were used.

DISCUSSION

We used the animal model of T2DM developed by

Srinivasan, because it closely resembled the natural course and metabolic characteristics of the disease⁸. In previous studies most of the experimental models of T2DM were constructed with alloxan and streptozotocin. These animal models conceptually deviate from the pattern of T2DM in humans in whom the disease is often preceded by obesity and then subsequently by B cell failure.

Administration of high fat diet for 2 weeks followed by low dose of streptozotocin resulted in frank hyperglycemia, hyperinsulinemia and insulin resistance. High intake of fat diet has been associated with increase in plasma TG. The preferential use of fatty acids for oxidation increases hepatic gluconeogenesis and decreases peripheral (skeletal muscle) glucose utilization. This led to the development of insulin resistance leading to compensatory hyperinsulinemia, hyperglycemia¹⁷. These findings were consistent with the published data of different studies¹⁸.

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 TG/HDL ratio was not measured; however marked hyperinsulinemia (467.50 ± 32.43 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 rats¹⁹. 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 it²⁰. Most commonly homeostasis model assessment for insulin resistance formula (HOMA-IR) is used²¹. 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 *Aloevera* extract manifesting the marked insulin sensitizing action of *Aloevera*²².

Kim studied the effect of *Aloevera* extract on diet induced obesity (DIO) mice. The insulin resistance values of DIO group treated with 25, 50, 100 mg/kg *Aloevera* extract was 31.4%, 32.1% and 31.1% respectively, of that of the untreated DIO group²³. However our study results were more significant (70%) than Kim's study. This could be due to higher dose (300mg/kg) of *Aloevera* 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 studies²⁴. Treating them with *Aloevera* extract resulted in significant decrease ($p < 0.001$) in insulin level by 19%. This may be due to the fact that *Aloevera* 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 with diet induced obesity (DIO) and hyperglycemia were treated with processed *Aloevera* 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. It increases insulin sensitivity and improves glycemic control. 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 past²⁵.

In the present study, we administered half the effective dose of *Aloevera* extract and rosiglitazone in a group of diabetic rats (combined group). This combined administration resulted in reduction in plasma glucose by 73%, insulin by 30%, TG:HDL by 74%. To latest literature survey has revealed that no study has so far been conducted in which *Aloevera* extract and rosiglitazone are used in combination to treat type 2 diabetes mellitus. The data of our study has revealed encouraging results which could help evolve new strategy of treatment for T2DM especially in a country like Pakistan, where socio economic conditions of people are not strong enough to cope with chronic diseases like DM. The use of natural herb with synthetic drug may help to lessen the financial burden associated with this disease. In addition, the side effects associated with prolong use of rosiglitazone such as myocardial infarction and heart failure due to high LDL can be controlled when used with herb in half of their effective dose.

CONCLUSION

Based on the data of the present study, it is concluded that *Aloe vera* whole leaf extract supplementation reduces plasma glucose, insulin and insulin resistance in T2DM rats. However, *Aloevera* and rosiglitazone together (in half of their effective doses) act synergistically and manifest greater antidiabetic actions than each of the chemical (drug) used separately in type 2 diabetic Sprague Dawley rats.

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