Original Article

Aloae Vera on Lipid in Diabetic

Effect of Aloe Vera Whole Leaf Extract on Lipid Profile Status in High Fat Diet and Low Dose Streptozotocin Induced Type 2 Diabetic Rats

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ABSTRACT

Objective To determine the effect of Aloe vera whole leaf extract on lipid profile in type 2 diabetic 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 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 resulted in triglycerides (50%), total cholesterol (49%), low density lipoprotein (57%),, very low density lipoprotein (50%), and increase in high density lipoprotein (50%). The results of present study provide a scientific basis of using Aloe vera whole leaf extract as lipid lowering drug to reduce the complication and mortality associated with DM.
Conclusion: The maximum impact was recorded in rosiglitazone group followed by Aloevera group
Key Words: Lipid Profile, Aloe Vera, Type 2 diabetes

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INTRODUCTION

The core defects in type 2 diabetes mellitus are insulin resistance and beta cell dysfunction which causes chronic hyperglycemia, dyslipidemia and oxidative stress.[1] During the year 2000 globally 2.8% (171 million) people were suffering from diabetes and incidence will increase to 4.4% (366 million) by the year 2030.[2] Diabetes mellitus is more common in developed countries. However, changing lifestyle and fast increasing urbanization have contributed to its increased prevalence in the developing countries.[3] Pakistan ranks sixth in the world's top ten countries with the highest number of diabetics. Diabetes mellitus has affected around 6.9 million people in Pakistan. By a conservative approach, this number may grow up to 13.9 million by the year 2030. [2]
Though oral hypoglycemic drugs are used widely, these treatments have their own drawbacks.[4] Due to chronic nature of disease and associated complication with it DM is causing a huge burden on world's economic resources. There is increasing use of complementary and alternative medicine (CAM) among the general public. With increasing incidence of diabetes mellitus in rural population, its chronic nature and due to adverse effect of synthetic medicine it is a need of an hour to look for indigenous, inexpensive botanical source with antidiabetic and antilipidemic effects. Substantial work has been carried out across the globe regarding hypoglycemic and hypolipidemic effects of plant extracts.[5] Many pharmaceuticals used in conventional medicine today also have natural plant origin. Among them, metformin was derived from flowering Plant, Galega officinalis, which is a common remedy for diabetes. [6]
Aloe vera comes from a family called Aloaceae. The Aloe vera plant has fleshy leaves which consist of gel, latex and outer green rind. Numbers of studies are carried on gel and latex parts but controversial reports are reported. This may be due animal model used, differences in method of extraction and differences in parts used. We used the animal model of T2DM developed by Srinivasan,[7] because it closely resembled the natural course and metabolic characteristics of the

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disease. DM with hyperglycemia and alteration in lipid metabolism leads to the production of reactive oxygen specie (ROS), responsible for development of late complication[1]. So in addition to control blood glucose, normal lipid profile is also necessary to avoid the negative outcomes of DM. Therefore the present study is designed to analyze the effect of Aloe vera on lipid profile in type 2 diabetic rats.

MATERIALS AND METHODS

Preparation of Aloe vera whole leaf extract: A whole leaf process was employed in making the Aloe juice according to previously published procedure with slight modification.[8] Leaves were cut into sections and were pulverized into a soup like structure by placing them in a grinding unit. Cellulose was allowed to dissolved in a digestion liquid containing amine, phosphorous ions and potassium ion under U.V radiation.[9] Aloe emodin as well as aloin was removed by passage through activated charcoal column.[10] The effective dose of 300mg/kg body weight (find through pilot study) was administered once daily by intragastric tubing.

Animals used: Forty five healthy Sprague Dawley rats' about 90 days old were taken from National Institute of Health (NIH), Islamabad. Animal house facility of National Institute of Health (NIH), Islamabad was used. This animal house has a setup according to international standards for breeding and housing of experimental animals. High fat diet (HFD) was specially prepared at NIH according to the standard used elsewhere. [7]

Induction of experimental diabetes: Forty five 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.[7] For confirmation of T2DM fasting blood glucose levels along with total lipid profile were measured after 72 hours. The cut off value for hyperglycemia was of >11.11mmol/l in accordance with the criteria laid down in study.[11] 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[12]

Experimental procedure: After induction of T2DM, Sprague Dawley rats were randomly divided into three groups, diabetic control group, Aloe vera, rosiglitazone group. Diabetic control group were administered 0.1ml normal saline intraperitonealy (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 for next 21 days. After 21 days of treatment, overnight fasted rats were anesthetized with ether for collecting intra-cardiac blood. 2.5ml of collected blood was put in a serum gel separator for the estimation of lipid profile.[12]

Analysis of samples: Analysis of samples was done at Centre for Research in Experimental and Applied Medicine (CREAM) at Army Medical College, Rawalpindi, Pakistan. Triglycerides, Total cholesterol and HDL were estimated simultaneously on automated chemistry analyzer (Vitalab Selectra E). An enzymatic colorimetric method GPO-PAP (Glycerol phosphate oxidase) was used for serum triglycerides estimation.[13] Total Cholesterol (TC) was measured by CHOD-PAP (Cholesterol oxidase phenol ampyrone), an enzymatic quantitative colorimetric method. The direct method for quantifying cholesterol in high-density lipoproteins (HDL) was done.[14] Both LDL and VLDL were calculated by using Friedewald formula. [15]

Data Analysis: Data was entered into SPSS version 17.0. 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 Tukey HSD. The "p value" <0.05 was considered statistically significant.

RESULTS

At the end of study the TG level in diabetic control rats was 2.16 ± 0.14 mmol/l, however it decreased in Aloe vera treated group (1.08 ± 0.10 mmol/l), in rosiglitazone treated group (0.95 ± 0.13 mmol/l) as compared to the diabetic control group significantly when all groups were compared by one way ANOVA.

The serum cholesterol level in diabetic rats was found as 4.83 ± 0.21 mmol/l, while in Aloe vera treated group it is reduced to 2.52 ± 0.13 mmol/l and in rosiglitazone group to 2.33 ± 0.21 mmol/l.

The serum LDL levels of diabetic control group was 4.01 ± 0.23 mmol/l, whereas serum LDL levels in Aloe vera and rosiglitazone the levels reduced to 1.72 ± 0.14 mmol/l and 1.49 ± 0.22 mmol/l respectively as compared to the diabetic control.

Table No.1: Comparison of lipid profile between diabetic control, Aloe vera, rosiglitazone and combined groups by one way ANOVA

Parameter	Diabetic control group (n=15)	Aloe vera group (n=15)	Rosiglotazone group (n=15)	p Value
Triglyceride (mmol/l)	2.16 ± 0.14	1.08 ± 0.10	0.95 ± 0.13	<0.001
Cholesterol (mmol/l)	4.83 ± 0.21	2.52 ± 0.13	2.33 ± 0.21	<0.001
HDL (mmol/l)	0.39 ± 0.08	0.58 ± 0.06	0.65 ± 0.07	<0.001
LDL (mmol/l)	4.01 ±0.23	1.72 ± 0.14	1.49 ±0.22	<0.001
VLDL (mmol/l)	0.98 ±0.07	0.49± 0.05	0.43 ±0.06	<0.001

Values are expressed as mean ± standard deviation for 15 animals in each group P<0.001 compared with diabetic control rats

Table No.2: Statistical difference of serum TG, cholesterol, HDL, LDL and VLDL levels between different groups using Post-Hoc (Tukey) test

Group comparison	Triglyceride (mmol/l)	Cholesterol (mmol/l	LDL (mmol/l)	VLDL (mmol/l)	HDL (mmol/l)
Diabetic Vs Aloe vera	<0.001	<0.001	<0.001	<0.001	< 0.001
Diabetic Vs rosiglitazone	<0.001	<0.001	<0.001	<0.001	<0.001
Aloe vera Vs rosiglitazone	0.039	0.049	0.019	0.039	0.047

The serum VLDL level indicates hepatic insulin resistance and its level in the diabetic control group was found; 0.98 ± 0.07 mmol/l however in Aloe vera, rosiglitazone the VLDL levels decreased to 0.49 ± 0.05 mmol/l and 0.43 ± 0.06 mmol/l respectively as compared to diabetic control.

The serum HDL levels of diabetic control group was 0.39 ± 0.08 mmol/l, while in Aloe vera, rosiglitazone treatment groups revealed serum HDL levels; 0.58 ± 0.06 mmol/l and 0.65 ± 0.07 mmol/l respectively.

Statistical significance of difference between the mean level of all lipid parameters were assessed by one way ANOVA, which revealed significant difference ($p<0.001$) amongst the groups as shown in table 1

All variables of lipid profile with significant p-values were analyzed by Post-Hoc (Tukey) test. The comparison revealed that mean serum TG, cholesterol, LDL and VLDL were significantly decreased ($p<0.001$) in Aloe vera, and rosiglitazone supplemented groups as compared to diabetic control group while HDL levels were significantly (($p<0.001$) raised in Aloe vera, and rosiglitazone group as compared to diabetic control group (table 2).

Post-Hoc (Tukey) test applied between interventional groups revealed that mean serum TG, cholesterol, LDL, VLDL were significantly (<0.05) lowered in rosiglitazone group as compared to Aloe vera group. HDL level were significantly raised in rosiglitazone group as compared to Aloe vera group table 2.

Table No.3: Percent reduction in lipid profile levels in different treated groups in comparison to the diabatic control.

Parameter	Control	Alovera	Rosiglitazone
TG mmol/l	2.16	50 % ↓	56 % ↓
Cholesterol mmol/l	4.83	49 % ↓	51 % ↓
LDL mmol/l	3.98	57 % ↓	62 % ↓
VLDL mmol/l	0.98	50 % ↓	56 % ↓
HDL mmol/l	0.39	50 %↑	66 %↑

DISCUSSION

In our study Sprague Dawley rats were used as experimental animal model. The rodents had been considered the most appropriate and oftenly used models for anti diabetic drug testing due to their easy handling, low price and resemblance with human metabolic characteristics of diabetes mellitus.[16] We used the animal model of T2DM developed by Srinivasan,[7] because it closely resembled the natural course and metabolic characteristics of the disease. Other animal models conceptually deviate from the pattern of T2DM in humans.[17]

In the present study, the plasma lipid profile in diabetic control rats showed severe derangement at the end of three weeks. Hyperlipidemia is one of the established major risk factors of coronary heart disease and cerebrovascular disease. Type 2 DM is associated with increased morbidity and mortality due to cardiovascular disease (CVD) as both share common antecedent; the insulin.[18] Aloe vera and rosiglitazone administration significantly influenced the outcome of high fat diet and advancing age on aforementioned parameters. Aloe vera supplementation in the present study has resulted in statistically significant ($p<0.001$) improvement in plasma TG, cholesterol,

LDL, VLDL and HDL levels when compared with diabetic control group. Aloe vera reduced the levels of TG's by 50%, cholesterol 49%, LDL 57%, VLDL 50% and increased HDL by 50% as compared to diabetic rats at the end study. These findings are supported by the published data of different studies.

A study by Rajesekaran revealed a significant reduction in parameters of lipid profile in streptozotocin induced diabetic rats by giving Aloe vera gel in the dose of 300mg/kg bodyweight extract for 21 days[19]. Our study results were slightly different than Rajesekaran's results. This may be due to the difference in type of experimental animal used and method of induction of diabetes in these experimental animals. A study by Kim revealed that processed Aloe vera gel in a dose 100 mg/kg showed statistically significant result on triglycerides level. They attributed this effect to reduced lipogensis and which was assessed/analyzed by the decrease in adipocyte size. [20]

Sood et al reviewed that effect of glucomannan, one of the major constituents of Aloe vera, on TG, cholesterol, LDL was statistically significant.[21] Glucomannan decreases the ingestion of food, reduces the post prandial rise in plasma glucose, suppresses hepatic cholesterol synthesis and increase fecal elimination of cholesterol containing bile acids. The reason behind glucomannan's ability to preferenntially lower triglycerides may be related to its higher viscosity and

its greater ability to alter the metabolic pathways of hepatic cholesterol and lipoprotein metabolism.[22] Thus antihyperlipidemic effect of Aloe vera extract may be due to number of constituents present in it.

Rosiglitazone is a known antidiabetic drug of thiazolidinediones family. It has been used for the treatment of type 2 DM since 1991. [23] 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.[24] In our study the plasma glucose levels are reduced by 68%, insulin 25%, TG 56%, cholesterol 51%, LDL 62%, VLDL 56% with concomitant increase in HDL by 66%. These findings of rosiglitazone group are similar to many clinical trials carried in the past.

A study was conducted on 18 months old Spargue Dawley rats, in which rosiglitazone was given in a dose of 3mg/kg for 21 days which resulted in significant decrease TG (59%) and insulin (61%). However the changes were associated with increase in body weight.[25] The difference in result may be due to the type of model used and route of administration of rosiglitazone in our study.

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. In our study the treatments effects highlighted in percentage terms had recorded maximum impact in lowering TG, cholesterol, LDL and VLDL in rosiglitazone treated group followed by Aloe vera. However, the side effects associated with prolong use of rosiglitazone such as weight gain, congestive heart failure and left ventricular dysfunction make it an unlikely drug to be used despite its significant results on plasma glucose and TG.[24] Aloe vera in our study has beneficial effect on normalizing the lipid profile in type 2 diabetics. It also leaves a room to explore new combination of treatment by using natural herb with synthetic drug (rosiglitazone half their effective dose) for their synergistic effects and to minimize their side effects associated with synthetic drug.

CONCLUSION

The maximum impact was recorded in rosiglitazone group followed by Aloevera group

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

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