EFFECT OF POMEGRANATE PEEL ALONE AND IN COMBINATION WITH ROSIGLITAZONE ON HYPERGLYCEMIA AND DYSLIPIDEMIA IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate the effect of pomegranate peel extract with or without rosiglitazone on plasma glucose and lipid profile in insulin resistant diabetic rats.

Study Design: Randomized controlled trial.

Place and Duration of Study: Department of Physiology, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (N.I.H), Islamabad from 1st January 2011 to 28th May 2011.

Material and Methods: Type 2 diabetes mellitus was induced in sixty healthy rats. The diabetic rats were divided into four groups, namely diabetic control group which received intraperitoneal injection of normal saline daily, pomegranate group which was treated similar to control group and also received pomegranate peel extract (200mg/kg body weight) orally once daily, rosiglitazone group which received intraperitoneal injection of rosiglitazone (5mg/kg body weight) daily and the combined group received both pomegranate extract (100 mg/kg body weight, orally) and intraperitoneal injection of rosiglitazone (2.5 mg/kg body weight) daily for 28 days. After four weeks of treatment, terminal intracardiac sampling was done to measure plasma glucose and lipid profile.

Results: The plasma glucose and mean serum levels of cholesterol, triglyceride, low density lipoproteins and very low density lipoproteins was significantly reduced (p<0.001) in pomegranate, rosiglitazone and combined groups respectively as compared to the diabetic control. The mean serum levels of high density lipoproteins were significantly (p<0.001) elevated in above mentioned groups as compared to the diabetic control.

Conclusion: Pomegranate peel extract is hypoglycemic and hypolipidemic agent in low doses when used alone or in combination with rosiglitazone in type 2 diabetic rats.

Keywords: Diabetes mellitus, Pomegranate peel extract, Rosiglitazone.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1].

Diabetes is a chronic burdensome disease affecting the large segment of world especially the poor, developing countries in which lack of awareness leads to the complications like cardiovascular disease, diabetic neuropathy, nephropathy, retinopathy and stroke[2].

Pakistan is a poor developing country with a high prevalence of diabetes mellitus especially affecting population of working age group (35-64 years). Globally Pakistan is the 6th leading country affected with diabetes. At present, approximately 7.6 million people are affected with diabetes and this number is expected to increase to 13 million by the year 2025, enhancing the economic burden on the government and people of Pakistan[3].

Despite many advances in the therapeutic management of type-2 diabetes mellitus, still there is a need to explore new treatment modalities with less side effects[4].

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Pomegranates have been prescribed as a remedy for diabetes in the Unani system of medicine since ancient times. Pomegranate contains high levels of a diverse range of phytochemicals including polyphenols, sugars, fatty acids (conjugated and non-conjugated), aromatic compounds, amino acids, tocopherols, sterols, terpenoids, alkaloids which are responsible for its therapeutic benefits as an anti-inflammatory, antioxidant, antineoplastic, hypoglycemic, hypolipidemic and antimicrobial agent[5] Pomegranate is included in the list of "functional foods", which play a vital role in reducing the risk of disease and slowing the progress of chronic diseases, in addition to their basic nutritional advantages[6]. Literature reviews indicated that no studies have so far been undertaken highlighting the antidiabetic potential of pomegranate peel in type 2 diabetic rats. In this backdrop, the present study is aimed to explore the antidiabetic activity of methanolic extract of pomegranate peel in insulin resistant diabetic rats alone and in combination with antidiabetic drug rosiglitazone.

In Pakistan no documented scientific research work has yet been reported to highlight the role of pomegranate in diabetes mellitus.

MATERIAL AND METHODS

This randomized controlled trial (RCT) was conducted at the Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from Jan 2011 to May 2011.

A pilot study was conducted on ten healthy Sprague-Dawley rats to find out the effective and nontoxic dose of pomegranate peel extract in insulin resistant diabetic rats. The minimum effective dose that manifested the hypoglycemic effect was found to be 200mg/kg body weight of the rat.

Actual project was conducted on sixty healthy Sprague-Dawley rats, weighing 250± 50 grams. The rats were obtained from National Institute of Health (NIH) Islamabad, Pakistan and kept under standard conditions (12 hours light and 12 hours dark cycle was maintained).

Fresh Punica granatum (Kandhari anar) were purchased from the local fruit market of Rawalpindi. Voucher specimen number 172, was obtained from Quaid-e-Azam University, Islamabad Pakistan. The whole fruits were thoroughly washed and their peels were removed. The washed peels were air dried for about one month under shade. The dried peels were crushed to powdered form in a mechanical mortar and weighed. 200 grams peel powder was dipped in 1200 ml methanol and then filtered. It was then subjected to mechanical stirring for 24 hours. The solvent was then removed under reduced pressure in a rotary evaporator. The peel extract was transferred to eppendorf tubes and stored at -20^{o} C before use. The extract was prepared at the Department of chemistry, Quaid-e-Azam University, Islamabad.

Diabetes mellitus was induced in all sixty rats. Rats were fed with high fat diet for 2 weeks after which a single intraperitoneal injection of streptozotocin (35mg/ kg body weight) was admininistered[7]. After 72 hrs, fasting blood glucose levels alongwith lipid profile was measured to confirm the development of diabetes and insulin resistance (Triglyceride and high density lipoprotein ratio,TG: HDL > 1.8) [8].

Group I (Control diabetic Rats, n=15)

Diabetic rats were continued on high fat diet ad libitum for 28 days alongwith intra peritoneal injection of normal saline once daily.

Group II (Pomegranate peel group, n=15)

Diabetic rats were administered pomegranate peel extract in the dose 200 mg/kg body weight (calculated by dose response curve after pilot study) orally through gavage needle daily for 28 days[9]. This group was treated similar to group I.

Group III (Rosiglitazone group, n=15)

Diabetic rats were administered injection rosiglitazone intraperitoneally in the dose of 5 mg/ kg body weight daily for 28 days.

Group IV (Pomegranate and rosiglitazone group, n=15)

Diabetic rats were given combined pomegranate peel extract (100 mg/kg body weight) orally and rosiglitazone (2.5mg/kg body weight) intraperitoneally daily for 28 days.

Terminal sample (4-5ml blood) was drawn at the end of fourth week of specific treatment of diabetic rats, by a single intra cardiac injection. Sampling was done in the morning after overnight fast to ensure the fasting sample for serum lipid profile and plasma glucose levels.

Plasma glucose was estimated by enzymatic colorimetric (TRINDER'S) method in which glucose was estimated by glucose oxidase method and a kit supplied by Globe Diagnostics, S.r.l Italy (Cat No.GD057400). Enzymatic colorimetric method was used to estimate TG and total cholesterol (TC) levels by lab kits of Globe Diagnostics S.r.l Italy (Cat No:GA481500 and GA434000) and HDL levels were estimated by lab kit of Human Diagnostics, Germany (Cat No.10084). All assays were done by using automated chemistry analyzer (Vitalab Selectra E), while low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels were calculated by using Friedewald formula.

Table-1: Comparison of plasma glucose, and TG: HDL ratio amongst different groups afterinduction of type-2 diabetes mellitus induction of type-2 diabetes mellitus. expressed as mean ± are standard deviation. There is no statistical difference amongst different groups (p>0.05).

Variables (Normal reference Ranges)	Diabetic rats Group I n=15	Pomegranate peel group II n=15	Rosiglitazone group III n=15	Combined group IV n=15
Plasma glucose (6 - 11mmol/l)	15.8 ± 2.15	16.5 ± 1.41	15. 0 ± 1.95	16.2 ± 2.45
TG: HDL ratio (1.8)	4.7 ± 0.85	4.5 ± 0.22	4.7± 0.19	4.5 ± 0.17

Table-2: Comparison of plasma glucose and total lipid profile by one way ANOVA between different groups. Values are expressed as mean ± standard deviation.

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Plasma glucose (6--11mmol/l)	19.9 ± 2.02	7.34 ± 0.76	6.22 ± 0.43	5.09 ± 0.50	p<0.001
TC (1.86-2.35mmol/l)	4.76 ± 0.22	2.4 9 ± 0.12	2.31 ± 0.17	2.12 ± 0.17	p<0.001
TG (0.38-1.52mmol/l)	2.15 ± 0.12	1.11 ± 0.11	1.00 ± 0.07	0.88 ± 0.11	p<0.001
LDL (0.38-0.62mmol/l)	3.91 ± 0.20	1.58 ± 0.16	1.37 ± 0.19	1.13 ± 0.18	p<0.001
HDL (0.8-1.37(mmol/l)	0.41 ± 0.06	0.68 ± 0.05	0.74 ± 0.07	0.81 ± 0.06	p<0.001
VLDL (0.40-0.46mmol/l)	0.97 ± 0.05	0.50 ± 0.05	0.45 ± 0.03	0.40 ± 0.05	p<0.001

Statistical analysis

Data was entered into SPSS version 16. Mean and standard deviation (S.D) were calculated for all values. Data within the groups were analyzed by using one-way analysis of variance (ANOVA)

followed by Post- Hoc Tukey's test, *p*-value<0.05 was considered statistically significant.

RESULTS

Blood glucose levels and TG/HDL ratio of all the four groups after the induction of diabetes is shown in table-1 which reveals no significant (p>0.05) difference amongst the groups.

After four weeks of specific treatment, plasma glucose, lipid profile, of all four groups were compared by one way ANOVA as presented in table-2. Post-Hock (Tukey's) test was used to calculate the statistical significance of the differences between the mean plasma glucose levels and lipid profile amongst the individual groups (table-3) namely control and pomegranate group, control and rosiglitazone group, control and combined group, pomegranate and rosiglitazone group, pomegranate and combined group and rosiglitazone and combined group.

Table-3: Statistical differences of plasma glucose and various parameters of lipid profile between different groups using Post- Hock (Tukey's) test.

Post-Hock comparison	Plasma glucose	TC	TG	LDL	VLDL	HDL
Pomegranate peel vs. Control	< 0.001	< 0.001	<0.001	<0.001	<0.001	<0.001
Rosiglitazone vs. Control	< 0.001	< 0.001	<0.001	<0.001	<0.001	<0.001
Combined vs. Control	< 0.001	<0.009	<0.001	<0.001	<0.001	<0.001
Pomegranate vs.Rosiglitazone	0.040	0.047	0.043	0.016	0.043	0.048
Pomegranate vs. Combined	0.001	0.001	0.001	0.001	0.001	0.001
Rosiglitazone vs. Combined	0.041	0.020	0.024	0.006	0.024	0.029

This study was conducted to explore the antidiabetic effects of pomegranate peel extract in type-2 diabetes mellitus as revealed by the results especially in combined group in which half doses of rosiglitazone and pomegranate extract when administered revealed the greatest reductions in mean plasma glucose and serum lipid profile parameters as compared to any other groups (table-2).

DISCUSSION

In present study blood glucose levels reduced significantly (p< 0.001) in pomegranate group as compared to the diabetic control group. There was 63% reduction in plasma glucose as compared to the diabetic control group. The extracts of root, rind and flowers of pomegranate have been reported to exert hypoglycemic activity in rodents[10]. Our results were found similar to the work done by Belkacem, et al,[11] who studied the hypoglycemic activity of pomegranate peel extract in STZ-induced diabetic rats for the duration of two weeks. At the end of their study, there was significant reduction (p<0.05) in plasma glucose peel extract treated diabetic rats as compared to diabetic control rats. However they observed 31.8% reduction in plasma glucose as compared to our study in which 63% reduction in plasma glucose was observed after the administration of pomegranate peelsuch a difference was possibly due to difference of durations in both studies[11]. Another difference was in the method of extraction used in our study (that is methanolic) which was considered best for pomegranate extraction as reported by Parmar and Kar[9], while hydroalcholic extraction was used by Belkacem, et al[11]. Our results were similar to the work done by Parmar and Kar[9] who studied the effect of methanolic pomegranate peel extract in a dose of 200 mg/kg body weight in alloxan induced diabetic mice for the duration of four weeks. At the end of four weeks, it was revealed that administration of peel extract caused significant (p<0.001) lowering of blood glucose level. There was 50% reduction in plasma glucose as compared to the diabetic control mice. However reduction in plasma glucose levels in our study was greater, which might be due to the fact that our study was conducted in Sprague-Dawley rats

as compared to mice used in research work done by Parmar and Kar[9] and also the method of induction of diabetes used in present study was differet from the alloxan used by Parmar and Kar[9] for induction of diabetes. In present study there was significant reduction (p<0.001) in blood glucose levels in rosiglitazone treated diabetic rats as compared to the diabetic controls.

In present study diabetic rats treated with combination therapy of rosiglitazone and peel extract revealed significant (p<0.001) glucose lowering effect (5.06 ± 1.08 mmol/ l) as compared to diabetic control group (18.5 ± 6.15 mmol/l). Review of literature did not reveal any study that had evaluated the antidiabetic potential of combined pomegranate peel with insulin sensitizer drug rosiglitazone.

In our study there was significant (p<0.001) reduction in serum TG, TC, LDL and VLDL in diabetic rats treated with pomegranate peel extract as compared to non treated diabetic control rats. A concomitant significant (p<0.001) elevation in HDL levels in pomegranate peel treated group as compared to the diabetic control. Haung et al[14]. studied the effect of oral administration of methanolic pomegranate flower extract (PGF) in a dose of 500 mg/kg body weight in type 2 diabetic rats for the period of 6 weeks. There was significant reduction (p<0.05) in plasma TC (15.5%), and TG (20.83%) levels after four weeks of treatment with PGF extract in the diabetic rats as compared to the diabetic control group. This is different from our study in which more reduction was observed in TC (47.6%) and TG (48.4%) levels; which might be attributed to different strain of rats, Zucker diabetic fatty rats used in that study as compared to Sprague-Dawley rats used in our study.

In present study diabetic rats treated with rosiglitazone revealed significant reduction in serum TG (53%), TC (51%), VLDL (53%), LDL levels (p<0.001) (64%) with significant elevation of HDL (p<0.001) as compared to non- treated diabetic rats.

Hussein, et al[12] studied the effect of oral administration of rosiglitazone in a dose of 5 mg/kg to type 2 diabetic rats on lipid profile for two weeks. At the end of study it was revealed that administration of rosiglitazone decreased triglyceride (14.5%), total cholesterol (22.1%), LDL cholesterol (20%) levels significantly (p<0.003) as compared to the diabetic control rats. Greater reduction had been observed in our study in all above mentioned parameters, which might be due to the longer duration (4 weeks) of administration of rosiglitazone in our study as compared to the shorter duration (2 weeks) of administration of rosiglitazone in their study. In our study, combination treatment of peel extract and rosiglitazone significantly reduced the serum TG (p<0.001) (59%), VLDL (58.7%), LDL levels (71%), TC (54.6%), with significant (p<0.001) elevation of HDL levels as compared to the control diabetic rats.

This study was conducted to explore the antidiabetic effects of pomegranate peel extract in type-2 diabetes mellitus. However it is expected that the side effects of rosiglitazone due to prolong use might be reduced if half of the dose is used in combination

CONCLUSION

Pomegranate peel extract has hypoglycemic and hypolipidemic action in type 2 diabetic rats.

2. Low doses of Pomegranate peel extract exerts potent hypoglycemic and hypolipidemic effects in type 2 diabetic rats when used alone or in combination with antidiabetic drug rosiglitazone.

RECOMMENDATION

Our study suggests that antidiabetic activity of pomegranate peel extract should be evaluated in type 2 diabetic subjects. Owing to its significant hypoglycemic and hypolipidemic effects in type 2 diabetic rats, it can be useful alone or as an adjunct to conventional antidiabetic drugs in humans to control hyperglycemia, and hyperlipidemia in type 2 diabetes mellitus.

CONFLICT OF INTEREST

Abstract and results of this study were accepted and presented in an oral presentationat the International conference on Medical Education, organised by Association for Excellence in Medical Education(AEME) and held on 07th-09th March 2014 at University of Health Sciences(UHS) Lahore, Pakistan. No funding was received from any agency or institution.

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