Original Article

Effect of Pomegranate Peel Alone and in Combination with Rosiglitazone on Oxidative Stress and Insulin Levels in Type 2 Diabetic Rats

Aysha Babar[1], Meena Gul[2], Sadia Moazzam[3] and Mohammad Mazhar Hussain[3]

ABSTRACT

Objectives: To evaluate the effect of pomegranate peel extract with or without rosiglitazone on plasma malondialdehyde (MDA) and insulin levels in insulin resistant diabetic rats.

Study Design: Randomized control trial study

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 (N.I.H), Islamabad from 1st January 2011 to 28th May 2011.

Materials 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.

Results: The plasma MDA levels were significantly ($p< 0.001$) reduced in pomegranate, rosiglitazone and combined groups respectively as compared to the diabetic control. The mean serum levels of insulin ($p< 0.001$) reduced in pomegranate group, in rosiglitazone group and in combined group respectively.

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.

Key Words: Diabetes mellitus, Pomegranate peel extract, Rosiglitazone.

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INTRODUCTION

Diabetes is a chronic burdensome disease affecting the large segment of world especially the poor, developing countries in which lack of awareness have led to the complications like cardiovascular disease, diabetic neuropathy, nephropathy, retinopathy and stroke.[1] (Caro, 2002).

Pakistan is a poor developing country with a high prevalence of diabetes especially affecting population of working age group (35-64 years). Globally Pakistan is 6th leading country affected with diabetes.[2] (Wild et al, 2004). Type 2 diabetes mellitus is characterized by hyperglycemia, due to insufficientsecretion of insulin, insulin resistance in peripheraltissues, and inadequate suppression of glucagon production .[3](Spellman, 2010). In type 2 diabetic patients there is a progressive decline in insulin secreting capability of pancreatic ß-cells attributed to harmful effects of chronic hyperglycemia, elevated free fatty acids (FFAs), increased generation of reactive oxygen species (ROS) and deposition of amyloid in the islets of Langerhans.[4] (Höppener et al., 2000). Diabetes is associated with increased oxidative stress.[5] (West, 2000). ROS generation causes membrane lipid peroxidation.[6] (Sanocka and Kurpisz, 2004). Lipid peroxidation exerts its action on fatty acids and causes alteration in the lipid structure. Enhanced lipid peroxidation produces malondialdehyde (MDA) which is a marker of lipid oxidation; possess deleterious effects on different tissues of the body by altering the function of membrane bound receptors, enzymes, decreasing the fluid state of the cell membrane and causes breakdown of lysine amino acid. The measurement of MDA - thiobarbituric acid (TBA) is most widely used assay for lipid peroxidation due its simple technique. The increase in the level of the MDA correlates with the hyperglycemia in diabetic subjects

[1.] Department of Physiology, Yusra Medical and Dental College, Islamabad.
[2.] Department of Physiology, GKMC, Swabi.
[3.] Department of Physiology, Fazaia Med College, Islamabad

Correspondence: Aysha Babar, Department of Physiology, Yusra Medical and Dental College, Islamabad.
Contact No: 0314-5198024
Email: drmeenagul@hotmail.com

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because of autooxidation of glucose, which causes the generation of free radicals. [7](Acworth, et al., 1997).

Pomegranate (Punicagranatum L.) is a very popular fruit having a growing history of 2000 years and is the predominant member of two species belonging to the punicaceae family. Recent studies have revealed the beneficial constituents in management of diabetes and its complications although their mode of action is still not clear. The peel which is usually discarded is rich in many biologically active compounds such as phenolics, flavonoids, punicalin, pedunculagin, and punicalagin, proanthocyanidin and minerals.[8] (Mirdehghan and Rahemi 2007). The peel contains complex polysaccharides.[9] (Jahfaret al., 2003). Despite of the fact that fruit peels are abundant in many bioactive compounds few studies are available to demonstrate the hypoglycemic activity of peel extracts of different fruits whose many parts like seeds and juice are used worldwide as a remedy of diabetes and its complications.[10] (Parmar and Kar, 2007).

In this backdrop, the present study was aimed to analyze 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 is available to highlight the role of pomegranate in diabetes mellitus.

MATERIALS AND METHODS

This Randomized control trial study was carried at the Department of Physiology, Army Medical College Rawalpindi and National Institute of Health (NIH) Islamabad, Pakistan from 1st January 2011 to 25th May 2011.

In our study, healthy Sprague-Dawley rats of 60-90 days age were purchased from National Institute of Health (NIH), Islamabad. The body weight of each rat ranged between 250±300 grams. These rats were bred in the animal house of NIH and had free access to water and high fat diet.

Rats suffering from any illness as evident from changes in their eating and drinking habits were not included in the study.

Preparation of plant extract: Fresh Punicagranatum (Kandharianar) 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. In the rotary evaporator; the peel extract was passed through a water bath at 45ºC until the solvent was evaporated. The peel extract was transferred toeppendorf tubes and stored at -20º 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 high fat diet for 2 weeks after which a single intraperitoneal injection of streptozotocin (35mg/ kg body weight) was admininistered (Srinivasan, *et al*, 2005).[11] After 72 hrs, fasting blood glucose levelsalong with lipid profile was measured to confirm the development of diabetes and insulin resistance (TG: HDL > 1.8).[12] (McLaugin, *et al.*, 2005).

After induction of type 2 diabetes mellitus in sixty Sprague-Dawley rats, these were divided into four groups as follows:

Group I (n=15)

Control diabetic Rats: Diabetic rats were continued on high fat diet *ad libitum* for 28 days along with intra peritoneal injection of normal saline once daily.

Group II (n=15)

Pomegranate peel group: 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. (Parmar and Kar 2008).

Group III(n=15)

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

Group IV (n=15)

Pomegranate and rosiglitazone group: 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.

For intra cardiac sampling, each rat was placed at its back and after palpation of lower rib cage and sternal margin; syringe needle was inserted into heart taking care not to pierce the posterior wall.

Blood samples from all the groups were transferred to appropriately labeled tubes specific to the group .1.5 ml blood was put in EDTA tubes for plasma MDA and insulin estimation. The samples were transported from NIH to the Centre for Research in Experimental and Applied Medicine (CREAM) at Army Medical College for further processing, storage and assays. After centrifugation the plasma was pippeted out of the sodium fluoride tubes, EDTA tubes and transferred to eppendorf tubes for storage. The plasma for the estimation of insulin levels was stored at -20°C for and at - 70°C for the estimation of plasma MDA levels. Malondialdehyde levels were estimated by thiobarbituric acid reactive substances (TBARS) assay, which is a simple, reproducible and standardized method for assaying lipid peroxidation in plasma, serum, urine, tissue homogenates, and cell lysates.[14,15] (Armstrong and Browne, 1994 and Yagi, 1998). Insulin

(Rat) Elisa kit (DRG. International, U.S.A). Cat no EIA- 2048 was used for estimation of plasma insulin levels.

Statistical Analysis: Data was entered into SPSS version 16. Mean and standard deviation 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 significant.

RESULTS

Rats with fasting blood glucose levels greater than 11 mmol/l (200 mg/dl) were considered diabetic[16](Yassin and Mwafy, 2007) while TG/HDL ratio greater than 1.8 was taken as insulin resistant.[12](McLaugin, *et al*, 2005). After four weeks of specific treatment, plasma insulin and plasma MDA levels of all four groups were compared by One Way ANOVA as presented in table 1 and 2 respectively. Then post Hoc test was applied for comparison between two groups. Post-Hock (Tukey's) test was used to calculate the statistical significance of the differences between the mean plasma insulin levels and mean plasma MDA levels amongst the individual groups. The comparison of plasma insulin and plasma MDA levels also revealed significant difference amongst the treated groups (table 2).

Table No. 1: Comparison of plasma insulin and MDA levels by one way ANOVA between different groups.

Variables	Diabetic control rats n=15	Pomegranate peel group n=15	Rosiglitazone Group n=15	Combined group n=15	P value
Insulin(µU/ml)	20.7 ± 2.12	16.8 ± 0.93	15.2 ± 0.97	14.2 ± 0.65	P<0.001
MDA(µmol/l)	10.0 ± 0.96	5.2 ± 0.60	4.6 ± 0.58	3.36 ± 0.33	P<0.001

MDA (Malondialdehyde levels)

Table No.2: Comparison of insulin and MDA levels between different groups using Post- Hock (Tukey's) test.

Group comparison	Plasma Insulin	Plasma MDA
Control vs. pomegranate peel	< 0.001	< 0.001
Control vs. rosiglitazone	< 0.001	< 0.001
Control vs. combined	< 0.001	< 0.001
Pomegranate vs. rosiglitazone	0.040	0.042
Pomegranate vs. combined	0.001	0.001
Rosiglitazone vs. combined	0.039	0.017

MDA (Malondialdehyde levels

DISCUSSION

In the present study, type 2 diabetes was induced in Sprague-Dawley rats by using the model developed by Srinvasan*et al*, (2005).[11] The rats were given high fat diet for the duration of two weeks, followed by the administration of a single dose of streptozocin in the dose of 35mg/kg body weight. Streptozotocin (STZ)-induced diabetes mellitus is cost effective and rapid technique that can be usedin most strains of rodents.

In our study fasting plasma insulin levels were significantly decreased (P<0.001) (18%) in peel extract treated group as compared to the diabetic control group. McFarlin, *et al.*, in 2009 showed that feeding obese mice with pomegranate seed oil for the duration of 12 weeks caused 13.8% reduction in fasting plasma insulin as compared to the obese control mice.[17] Huang *et al.*, in 2008 found that 5-week treatment with PGF extract improved oral glucose tolerance in ZDF rats, suggested the improvement in insulin receptor sensitivity. Improvement of insulin receptor sensitivity was the predominant mechanism for the antidiabetic efficacy of the PPAR-gamma agonists.[18]

Rosenblat*et al.*, in 2005 studied the effect of administration of 50 ml of pomegranate juice on serum insulin levels in type 2 diabetic subjects for the duration of three months. At the end of three months, there was non-significant decrease in insulin levels (9%) in diabetic subjects as compared to the controls; however there was significant decrease (11.6%) in c- peptide levels in diabetic subjects as compared to the controls.[19]

In our study diabetic rats treated with rosiglitazone showed significant reduction (P<0.001) in plasma insulin levels as compared to diabetic control rats. Hussein, (2001) *et al* studied the effect of oral administration of rosiglitazone in a dose of 5 mg/kg to type 2 diabetic rats on plasma insulin for the duration of two weeks. At the end of study it was revealed that administration of rosiglitazone decreased fasting plasma insulin levels significantly (P < 0.03) by 20.6% as compared to the diabetic control rats. These results were similar to our study. They had induced diabetes in Sprague- Dawley rats by model developed by Srinvisan, (2005) as used in our study.

In our study diabetic rats treated with combination therapy of rosiglitazone and peel revealed significant reduction in fasting plasma insulin levels (P< 0.001) by 31% as compared to the control diabetic rats.

In our study MDA levels were assessed to measure the oxidative stress in type 2 diabetic rats. MDA levels found significantly decreased (P<0.001) by 48% in peel extract treated diabetic rats as compared to the diabetic control. Our results are similar to the work done by Althunibat, *et al.*, in 2010 also investigated the effect of administration of 10 and 20 mg/kg body weight of methanolic pomegranate peel extract in streptozocin

induced diabetic rats on plasma MDA levels.[20]Streptozocin induced diabetic rats showed significant reduction ($P < 0.05$) in MDA levels (27.5%) after peel extract administration.

Manoharan*et al.,* 2009 studied the effect of oral administration of 400mg/kg body weight of ethanolic extract of pomegranate flower extract on plasma MDA levels in streptozocin induced diabetic rats. The extract was administered for the period of 45 days through gavage needle. The study revealed that pomegranate flower extract caused significant reduction in plasma MDA levels (42.4%) in diabetic rats treated with pomegranate flower extract as compared to the diabetic control rats.[21]

Zhang, *et al.,* (2010) studied the antioxidant activity of ethanolic extracts from different parts of pomegranate including its flower, leaf, seed and peel, by adding these into the soybean oil. Estimationof MDA levels and peroxide values revealed that peel extract had highest antioxidant effect among all different parts of pomegranate tested.[22]

Ahmed and Ali, (2010) studied the effect of prophylactic administration of ethanolic extract of pomegranate peel on male albino rats that developed nephrotoxicty after administrating ferric nitrilotriacetic acid (Fe-NTA) acid in a single dose of 9 mg Fe /kg body weight. At the end of the study, it was revealed that rats administered with prophylactic pomegranate peel extract showed enhanced levels of antioxidant enzymes including GR, CAT and GPx as compared to Fe-NTA treated rats.[23]

Ozbek, *et al*., (2010) studied the antioxidant effect of rosiglitazone against gentamicin induced nephrotoxicity in wistar rats. Administration of rosiglitazone in a single dose of 10 mg/kg/day for the duration of two weeks through gavage caused significant reduction in levels of MDA with concomitant elevation in levels of antioxidant enzymes including catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase in comparison to the gentamycin (dose; 100 mg/kg daily) treated rats.

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.

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

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