Original Research Article

Effect of natural polyphenols on diabetes mellitus (type-2) with myocardial infarction: a double-blind placebo controlled trial

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ABSTRACT

Background: Diabetes Mellitus Type-2(T2D); is a leading disease in world wide. T2D is a clinical syndrome characterized by hyperglycaemia. Hyperglycemias are caused by an absolute or relative deficiency of insulin and due to insulin resistance. Diabetic patients are highly prone to Reactive Oxygen Species (ROS) and leads to Cardio vascular complications. Several medicines have been recommended to cure T2D; and still discovery of newer drugs are in process. Now a day, the focus of researches in diabetes includes discovery of newer anti-diabetic agents as well as isolating the active compounds from herbal sources. One such herbal source is pomegranate. Pomegranate is polyphenols and antioxidants rich fruit; which has potency to cure T2D and ROS.
Methods: A Pomegranate Extract of Whole Fruit (PEWF) was prepared as tablet of 300mg to investigate its effects in patients with T2D. Total 40 participants of either gender with nested cases of T2D with Myocardial Infarction (MI) were included in study. All participants were assigned in two groups (20 each). One group was under "Add On" therapy of PEWF and matching placebos of same colour, shape and size were used as comparator agent for second group (300mg BD for 1 month).
Results: Levels of biochemical markers related to T2D were compared to analysed pre and post drug effects by Z test, chi square test and by coefficient of variations. Results highlighted that those participants who were under "add-on" therapy of PEWF showed highly prognostic significance. Thus, PEWF should be consumed in diet as food supplementation.
Conclusions: In conclusion, polyphenols and antioxidants rich fruit supplements should be taken in diet for healthy living.

Keywords: Coronary artery disease, Diabetes mellitus type-2, Fasting plasma glucose, Myocardial infarction, Pomegranate extract of whole fruit, Reactive oxygen species

INTRODUCTION

Diabetes Mellitus Type-2; (T2D) is a pandemic disease. This is becoming challenges to human health.[1] A recent statistics report of International Diabetes Foundation (IDF) reported that there were 366 million people with T2D in worldwide in year 2011 and 371 million people reported in year 2012. Out of these 92.3 million peoples were in china, 63 million peoples were in India and 24.1 million peoples were affected with T2D in USA in year

2012. The incidence of T2D are increasing continuously and it is proposed that approximately 552 million population will be affected with T2D by the year 2030.[2]

T2D is a clinical syndrome characterized by hyperglycaemias. Hyperglycemias are caused by an absolute or relative deficiency of insulin and due to insulin resistance. Diabetic patients are highly prone to Reactive Oxygen Species (ROS) because hyperglycemias deplete natural antioxidants and facilitate the production of free radicals.[3] ROS leads to the oxidation of lipids and a major risk factor for cardiovascular diseases.[4] Several medicines have been recommended to cure T2D; and still discovery of newer drugs are in process. But all these medicines are having certain limitations and side effects.

World Health Organization (WHO) recommends using alternative medicines for treating Diabetes Mellitus (DM).[5] Now a day, the focus of researches in diabetes includes discovery of newer anti-diabetic agents as well as isolating the active compounds from herbal sources.[6] Herbal sources may act on circulatory glucose through different mechanisms; some of them may act as insulin-like substances; some may inhibit insulinase activity; others may cause increase of beta cells in pancreas by activating and regeneration of these cells.[7]

Fruit extracts have been used extensively as these are natural, safe, and readily available herbal sources. One such fruit is pomegranate. Pomegranate studies indicate that parts of pomegranate fractions like peel, flowers and juice have prognostic effects in T2D.[8] Studies shows that the level of polyphenols presents in pomegranate peels (skin and pericarp) are much higher than from seed and pulp. Total polyphenol content from peel was found 85.20±4.87mg gallic acid equivalents per gram of dry weight and total polyphenols content from seeds were 7.94±1.25mg gallic acid equivalents per gram of dry weight. This is well established that pomegranate peel is a richer source of polyphenols.[9]

Literature review suggests that all studies till now have been conducted in human being as well as in experimental animals by administration of pomegranate juice of seed and pulp. However, no study has investigated the effect of pomegranate extract of whole fruit (PEWF) containing the combination of total polyphenols from peels (skin and pericarp), pulps and Seeds. Hence, we prepared pomegranate extract of whole fruit (PEWF) in the form of a tablet of 300mg, which is powerful source of polyphenols and antioxidants to investigate; whether the treatment of T2D with pomegranate extract of whole fruit (PEWF) has any prognostic effect?

As PEWF is rich in natural antioxidants and polyphenols, consumption of this extract may improve disease condition. Null hypothesis (H_0) will be implemented during the trial.[2,3]

METHODS

A randomized, double-blind, placebo controlled, parallel trial was conducted in Base Hospital, Srikot, Pauri-Grahwal, Uttarakhand, India attached to Veer Chandra Singh Garhwali Government Institute of Medical Sciences and Research, Srikot, Pauri Garhwal, Uttarakhand, India (VCSGGIMSR) and Netaji Subash Chander Bose Subharti Medical College (SMC) and C.S. Subharti Hospital, Meerut (U.P.) India, in collaboration with Department of Biochemistry, Pharmacology and Medicine.

A total 100 participants of either gender with MI were enrolled in present study; out of these 40 patients were representing their history of T2D. All participants were screened as per the guidelines of American Diabetic Association (ADA) 2015. The participants; who were satisfying inclusion and exclusion criteria's were enrolled in present study after having a written consent.

Inclusion criteria

- Patients having the classical symptoms of hyperglycemias (polyuria, polydypsia and polyphagia).
- Fasting plasma glucose (FPS) level > 26 mg/dl.
- 2-hours plasma glucose (2HPG) >150 mg/dl.
- HbA1c measurement >6.5%.

Exclusion criteria

- Patients with acute illness, pregnant, lactating, and postoperative patients.
- Patients with CNS disorders, systemic chronic diseases e.g. renal failure and chronic hepatic disease.

Method of randomization

Selected participants were randomized as per criteria given in Table 1 by generating a list of sequential assignments to a treatment group, using the "random seed" function in the Statistical Package for the Social Sciences (SPSS) software program, version 16.0 or its equivalent.

Table 1: Assignment of participants.

	PEWF(Active) 1 BD x 1 month	Placebo 1 BD x 1 month
Diabetes Mellitus Type-2	n=20	n= 20

n: Total number of participants

Assessment of treatment effect

An 4ml venous blood samples (fasting and post prandial) were collected in a sodium fluoride and E.D.T.A. vacutainers. Vacutainers containing sodium fluoride were centrifuged at 8000rpm for 15minutes and plasma was

collected in separate test tube. Samples were processed on the same day for Bio-chemicals markers related to T2D such as Fasting Blood Glucose (FPG), 2 Hour plasma glucose (2HPG) and HbA1c to check pre and post drug effects on fully automated Biochemistry analysers like Cobas 6000.

Trial medicines

Trial medicines were given as "add-on basis" along with other prescribed medicine.

Description of the drug

The active drug has pomegranate extract of whole fruit (PEWF). Matching placebo of same color, shape, size and weight was used. The PEWFs/Placebos were given orally, as tablets of 300 mg twice daily (BD) for one month.

Trial procedure

Duration of treatment

Participants were treated daily with either active medicine or placebo for one month. Regular follow-up of patients were carried by frequents visits and personal communications.

Visit I (Week 0), screening visit (pre dug analysis)

After obtaining an informed consent, nested cases of T2D were included in this study. Venous blood sample was collected for assessment of Biochemical parameters related to T2D. Base line titer has been obtained and recorded in separate sheet.

Visit II (week 1)

The participants were under the "add-on" therapy of PEWF or Placebo (as per table number 1), the doses were issued for 15 days initially and participants were recalled for next visit.

Visit III (week 3)

Follow up information was obtained regarding any adverse effects of treatment. The participants were questioned regarding any missed doses of trial medicine and second dose of medicines were issued for next 15 days.

Visit IV (week 5)-(post drug analysis)

The participants were questioned regarding any missed doses of the trial medicine; biochemical parameter such as FPG, 2HPG was analyzed to check post drug effect.

Visit V-(week 9)-final visit (post drug analysis)

Biochemical parameters related to risk factors for T2Dwere repeated. Biochemical parameter such as HbA1c was analyzed to check post drug effect.

Assessment of compliance

The participants; who had 80% consumption of PEWFs/placebos, will be considered as compliant.

RESULTS

Total 40 participants of either gender belong to age group between 20-60 years were participated in present study (Table 2). A total 07 participants were of age group 40-45 years; 15 participants were of age group belongs to 45-50 years and 18 participants were of age group between 50-55 year. All study participants were categorised in two groups of 20 each. One group of 20 participants (17 men and 03 women) consumed PEWF (active) and second group of 20 participants (18 men and 02 women) consumed placebo medicine. All participants were instructed to continue with prescribed medicines by the clinicians uninterrupted. PEWFs/placebos were given as "add-on" basis for one month.

Table number 03 summarizes the descriptive statistics for PEWF medication in pre and post drug analysis. Serial number 01 highlights that mean level FPG is 220.21 mg/dl in pre drug analysis and 104.33mg/dl in post drug analysis. 2HPG in pre drug analysis is 270.1mg/dl and in post drug analysis is 128.4mg/dl. This highlights the prognostic effect of PEWF in patients with T2D. The level of HbA1c in pre drug analysis is 8.2% and level of HbA1c was examined at 3rd month of post drug effect; it was 6.1%. This is a good sign of prognosis.

Table number 04 summarizes the descriptive statistics for placebo medication in pre and post drug analysis. Serial number 01 highlights that mean FPG is 221.2mg/dl in pre drug analysis and 143.5mg/dl in post drug analysis. 2HPG in pre drug analysis is 280.2mg/dl and in post drug analysis is 128.4mg/dl. The reason behind this is that all participants are on anti-diabetic medications. PEWFs and Placebos are given as "add-on" basis along with other prescribed medicines. The level of HbA1c in pre drug analysis is 7.8% and level of HbA1c was examined at 3rd month of post drug effect; it was 6.8%.

Table number 5 and 6 summarizes the Z statistics PEWF and placebos for post drug in comparison to pre drug analysis. When FPG, 2HPG and HbA1c of pre and post drug effects were compared to each other; statistical results show that $p<0.05$; this indicates the prognostic improvements in patients with T2D. These results indicate the rejection of Null hypothesis (H_0) and acceptance of alternative hypothesis (H1).

Table 7 and 8 summarizes the Chi Square tests of PEWF and Placebos for post drug in comparison to pre drug analysis. In pair 1, 2 and 3 independent variables of FPG,

2HPG and HbA1c are found to be statistically significant (p<0.05) in both active and placebo medications. This suggests prognostic effect found in patients with both active and placebo medication and rejection of Null Hypothesis (H_0). The reason behind this is that all participants are on anti diabetic medications. PEWFs and Placebos are given as "add-on" basis along with other prescribed medicines.

Table 2: Age wise distribution of participants with t2d in groups.

Age (years)	PEWF (active)	Placebo	Total
40-45	03	04	07
45-50	08	07	15
50-55	09	09	18
Total participants	N=20 Men:17 Women:03	N=20 Men:18 Women:02	N=40

n= Total number of participants

Table 3: Descriptive statistics for PEWF (active) medication in pre and post drug analysis.

	Pre dug analysis N=20		Post drug analysis N=20	
Parameters	Mean (X)	Std. Deviation (±SD)	Mean (X)	Std. Deviation (±SD)
Fasting plasma glucose (mg/dl)	220.21	63.1	104.7	15.53
Two hour plasma glucose (mg/dl)	270.1	87.85	128.4	27.86
HbA1c (%)	8.2	1.6	6.1	0.8

n= Total number of participants

Table 4: Descriptive statistics for placebo in pre and post drug analysis.

	Pre dug analysis N=20		Post drug analysis N=20	
Parameters	Mean (X)	Std. Deviation (±SD)	Mean (X)	Std. Deviation (±SD)
Fasting plasma glucose (mg/dl)	221.8	59.89	143.5	24.10
Two hour plasma Glucose (mg/dl)	280.2	69.44	143.5	32.30
Hba1c (%)	7.8	1.6	6.8	1.3

n= Total number of participants

Table 5: Z statistics of PEWF (active) for post drug in comparison to pre drug analysis.

Paired Samples Test				
Pairs	Parameters	Z Test	Degree of Freedom	Sign (2 Tailed)
Pair01	Fasting plasma glucose for pre and post drug analysis	154.15	7254	0.00
Pair02	Two hour plasma glucose for pre and post drug analysis	189.67	7254	0.00
Pair03	HbA1c pre and post drug analysis.	211.34	7254	0.00

Table 6: Z Statistics of placebo for post drug in comparison to pre drug analysis.

Paired Samples Test				
Pairs	Parameters	Z Test	Degree of Freedom	Sign (2 Tailed)
Pair01	Fasting plasma glucose for pre and post drug analysis	67.046	4528	0.00
Pair02	Two hour plasma glucose for pre and post drug analysis	69.266	4528	0.00
Pair03	HbA1c pre and post drug analysis.	125.67	4528	0.00

Table 7: Chi square test of PEWF (active) for post drug in comparison to pre drug analysis.

Paired Samples Test				
Pairs	Parameters	Chi square	Degree of Freedom	Sign (2 Tailed)
Pair01	Fasting plasma glucose for pre and post drug analysis	91.193	18	0.00
Pair02	Two hour plasma glucose for pre and post drug analysis	108.265	33	0.00
Pair03	HbA1c pre and post drug analysis.	49.81	139	0.00

Table 8: Chi square test of placebo for post drug in comparison to pre drug analysis.

Paired Samples Test				
Pairs	Parameters	Chi square	Degree of Freedom	Sign (2 Tailed)
Pair01	Fasting plasma glucose for pre and post drug analysis	99.156	15	0.00
Pair02	Two hour plasma glucose for pre and post drug analysis	126.624	30	0.00
Pair03	HbA1c pre and post drug analysis	72.13	139	0.00

Table 9: Coefficient of variations for PEWF (active) and placebo medicine after post drug analysis.

Descriptive statistics	PEWF (active)			Placebo		
Post drug analysis	Mean (X)	Std. Deviation (±SD)	C.V.	Mean (X)	Std. Deviation (±SD)	C.V.
Fasting plasma glucose	111.99	15.53	0.13	129.01	24.10	0.18
Two hour plasma glucose	132.36	27.86	0.17	151.47	32.31	0.21
Hba1c	6.1	0.8	0.09	6.8	1.3	0.11

Table 9 is highlighting the coefficient of variations (CV) for PEWFs and placebo medicine in post drug analysis. The mean levels of independent variables were compared to each other and statistical analysis states that CV of FPG, 2HPG and HbA1c for PEWF are 0.13, 0.17 and 0.09. CV of FPG, 2HPG and HbA1c for placebo are 0.18, 0.21 and 0.11. This indicates that CV of PEWF is lower than Placebo. This highlights that statistical results of PEWFs are much highly significant than placebos. This indicates that PEWF has higher prognostic significance.

DISCUSSION

Study results and statistical analysis highlighted that natural polyphenols have high prognostic effects in T2D. A number of mechanisms have been proposed for this. A key mechanism by which pomegranate fractions affect T2D is by reducing oxidative stress and lipid per-oxidation. This reduction may occur directly by neutralizing the generated ROS.[10]

Another mechanism by which pomegranate cure T2D is by increasing the level of antioxidative enzymes, inducing metal chelation activity and by modification of certain transcription factors such as Nuclear Factor kB (NF-kB) and peroxisome proliferator activated receptor γ(PPAR- γ).[11]

NF-kB is a redox sensitive transcription factor; which is activated by ROS.[12] After activation; NF-kB activates the endothelial derived tissue factors. Endothelial derived tissue factors activate the coagulation cascade and leads to cardiovascular complications.[13] This is the basis that T2D is major risk factor for CAD. Pomegranate polyphenols consume ROS, thus inhibit the factor NF-kB.[14] This helps in the prognosis of MI.

PPAR- γ increase insulin resistance by repressing glucose receptor GLUT-4.[15] ROS activates the PPAR- γ and leads to T2D. Studies reported that pomegranate polyphenols suppress the activity of PPAR-γ and leads to the prognosis of T2D.[16]

Literature review suggests that natural polyphenols of Pomegranate stimulate beta cells to release insulin hormone and also help in the activation, regeneration and to increase the number of β cells.[17,18] Studies also indicate that polyphenols and antioxidant rich food items also exhibit extra pancreatic effects via peripheral glucose utilization.[19] Thus, pomegranate polyphenols has highly prognostic effects to cure T2D. Consumption of PEWF is easiest, safe and natural way to improve disease conditions. The food items such as PEWF and herbal sources should be included as an integral part of human diet and should be given as food supplements.

CONCLUSION

T2D is effecting worldwide. Patients with T2D are highly prone to Oxidative stress and leads to cardiac complications in future. Medicine which are generally prescribes to treat T2D patients are having certain limitations. Present study results recommend that consumption of natural polyphenols of pomegranate is a safest way to cure T2D without having any side effects. In conclusion, polyphenols and antioxidants rich fruit supplements should be taken in diet for healthy living.

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