RESEARCH ARTICLE

ANTIOXIDATIVE EFFECT OF PUNICA GRANATUM (POMEGRANATE) ON BIOCHEMICAL PARAMETERS IN PATIENTS WITH DIABETES MELLITUS (TYPE 2) AND MYOCARDIAL INFARCTION: A DOUBLE BLIND PLACEBO CONTROLLED TRIAL.

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Abstract

Diabetes Mellitus Type 2(T2D) is one of the leading disease in worldwide. Approximately 177 million peoples in worldwide are effected with T2D. T2D is a heterogeneous metabolic disorder, which is characterized by hyperglycemia and cardiovascular complications. Pathogenesis of T2D is absolute or relative deficiency of insulin and insulin resistance. Patients with T2D are highly prone oxidative stress because hyperglycemia depletes natural antioxidants.

Hypoglycemic drugs; which are usually prescribe to cure T2D are either too expensive or have sides effects on Hematological, Liver and kidney functions. WHO recommends to use alternative medicine to cure T2D because herbal products are natural and don’t have such side effects. One of such herb is pomegranate; usually consumed as fruit. As per Ayurvedic literature, Pomegranate seeds may cure T2D and pulps contain lots of antioxidants. This prompted us to find out; whether the presence strong antioxidants in pomegranate have any prognostic effects in patients with T2D? So we prepared a Whole Fruit Extract of Pomegranate (PEWF) to investigate its effect in patients with T2D.

Total 40 patients of either gender with T2D and MI were included in this study. Demographic information, Clinical and Biochemical investigation related to T2D were processed in 4 ml fasting venous blood. All participants were assigned in two groups of 20 each. One group were under “Add On” therapy of Active Drug which includes Pomegranate extract of whole fruit (PEWF) (300 mg twice daily for One Month), matching Placebo of same color, shape and size was used as comparator agent for second group. The Drug was issued to the Patients initially for 15 days after which they were recalled for clinical valuation, compliance monitoring, adverse effect monitoring if any; and drug refill for next 15 days. At the end of one month, the base line investigations were repeated. Patients were followed for next 3 months; on 4 month level of HbA1C was monitored to check the status of T2D.

Results were analyzed, Z test and chi square indicates that significant difference has been observed after active medication. Coefficient of Variations (C.V.) shows that when mean of active and placebo were compare to each other; patients who were under “add-on” therapy of PEWF for one
month, showed highly significant. This indicates the prognostic effect of PEWF in patients with T2D with MI.

Introduction:-
Diabetes Mellitus Type 2(T2D) is a clinical syndrome characterized by hyperglycaemia caused by an absolute or relative deficiency of insulin and insulin resistance. Diabetic patients are highly prone to oxidative stress because hyperglycaemia depletes natural antioxidants and facilitate the production of free radicals. These free radicals are known as reactive oxygen species (ROS). Several medicines have been recommended to cure T2D; and still discovery of newer drugs are in process.

World Health Organization (WHO) recommended the use of alternative medicines for treating Diabetes Mellitus (DM). Now a days the focus of research in diabetes includes discovery of newer anti-diabetic agents as well as isolating the active compounds from herbal sources. Herbal sources may act on blood glucose through different mechanisms; some of them may act as insulin-like substances, Some may inhibit insulinase activity, others may cause increase beta cells in pancreas by activating and regeneration of these cells. Fruit extracts have been used extensively as these are natural, safe, and readily available. One such example is pomegranate *Punica Granatum Linn* (Family Punicaceae), a fruit native to the Middle East. The therapeutic potential of pomegranate fractions are due to the presence of unique bioactive compounds with antioxidant, anti-inflammatory, anti-infective, anti-atherogenic, anti-carcinogenic, and anti-hyperglycemic effects. A study reported that pomegranate extracts and their active compounds could be effective in the treatment and prevention of T2D. A key mechanism by which pomegranate fractions affect T2D is by reducing oxidative stress and lipid per-oxidation. This reduction may occur by directly neutralizing the generated ROS.

The presence of strong antioxidant principles of Pomegranate thus prompted us to design the present study to investigate; whether the treatment of T2D with Pomegranate Extract of Whole Fruit (PEWF) has any prognostic effect?

Hypothesis:-
As PEWF is rich in natural antioxidants and Polyphenols, the consumption of this extract may improve disease condition. Null Hypothesis ($H_0$) will be implemented during the trial.

Material & methods:-

Trial Design:-
A randomized, double-blind, placebo controlled, parallel trial was conducted in Base Hospital, Srikot, Pauri-Garhwal, Uttarakhand, India attached to Veer Chandra Singh Garwali Government Institute of Medical Sciences & Research, Srikot, Pauri Garhwal, Uttarakhand ,India (VCSSGIMSR) and Netaji Subhash Chandra Bose Subharti Medical College (SMC) associated with Chhatrapati Shivaji Subharti Hospital, Meerut(U.P.) India, in collaboration with Department of Biochemistry, Pharmacology and Medicine.

Inclusion and Exclusion criteria:-
A total 100 confirmed cases of MI were enrolled in study; out of these 40 patients were representing the history of T2D. Patients who were satisfying inclusion and exclusion criteria’s were enrolled in the present study after obtaining a written consent.

Inclusion Criteria:-
Patients with age group 20-55 years and who were representing the history of T2D with MI as per the guidelines of American Diabetic Association (ADA) 2015.
- Patients with classical symptoms of hyperglycemia. (polyuria, polydypsia and polyphagia)
- Fasting Plasma Glucose (FBS) level> 126 mg/dl.
- Plasma postprandial Glucose level> 150 mg/dl
- Random Plasma Glucose (RBS) level>200 mg/dl.
- HbA1C measurement>6.5%
Exclusion Criteria:-
- Patients with acute illness, pregnant, lactating, and postoperative patients.
- Patients with CNS disorders, systemic chronic diseases like renal failure, chronic hepatic disease.

Method of Randomization:-
Selected participants were randomized as per Table 1 given below, 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

| Dose                                      | PEWF(Active) 1 BD x 1 month | Placebo 1 BD x 1 month |
|-------------------------------------------|----------------------------|------------------------|
| Diabetes Mellitus (type 2) with MI.       | n=20                       | n=20                   |

“n” is number of participants.

Assessment of treatment effect:-
Overnight fasting, 4 ml venous blood samples were collected in a plane and EDTA vacutainer. After coagulation, samples were centrifuged at 8000 RPM for 15 minutes and serum was collected in separate test tube. Samples were processed on the same day for Biochemical markers related to T2D and ROS such as Plasma Glucose (Fasting and Postprandial), HbA1C, Total antioxidant Activity (TAA), Glutathion Peroxidase (GPX), Super oxide dismutase (S.O.D.) and Glutathion Reductase (GSH) on fully automated Biochemistry analysers; Cobas 6000 and semi automatic analyser Transasisa Chem 5 Plus.

Trial Medicines:-
Trial medicines were given as “add-on basis” along with other prescribed medicine.

Description of Drug:-
The active drug has pomegranate extract of whole fruit (PEWF). Matching placebo of same color, shape, size and weight was used. The PEWF/Placebo was 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 out by frequent visits and personal communications.

Visit I (Week 0), screening visit (Pre Drug Analysis):-
After obtaining an informed consent, patients with MI were included in present study. 4 ml fasting venous blood samples were collected for Biochemical parameters related to T2D and ROS from study participants. Base line titers were obtained and recorded in separate sheet.

Visit II (Week 1):-
Participants were under the “add-on” therapy of PEWF or Placebo (as per table: 1), the dose was issued for 15 days initially and participants were recalled for next visit.

Visit III (Week 3):-
Follow up information were obtained regarding whatever there were 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, All Biochemical parameters related to T2D and ROS were repeated.
Visit V (week 14), Final Visit:-
Participants were questioned regarding any adverse effects of treatment. Blood samples were collected to evaluate the level of HbA1C.

Assessment of Compliance:-
The participants who had 80% consumption of PEWF / Placebo, will be considered as compliant.

Results:-
A total 40 participants of either men and women of aged between 20-55 year were participated in study. Out of these, 10 participants were of age group belongs to 40-45 years, 16 participant were of age group 45-50 years, 14 participants were of age group between 50-55 years. A total, 20 participants (17 men and 03 women) consumed PEWF (active) and 20 participants (18 men and 02 women) consumed Placebo medicine.

Table number 2, 3 summarizes the Descriptive Statistics for active and placebo medication in both Pre and Post Drug effects. Mean and Standard Deviation of Pre and Post Drug analysis shows the reduction of Bio-chemical markers related to T2D and ROS after active medication.

Table:2: Descriptive statistics for PEWF (active) medication in Pre and Post Drug Analysis.

| Sr.no | Parameters                              | Pre Dug analysis n=20 | Post Drug Analysis n=20 |
|-------|-----------------------------------------|-----------------------|-------------------------|
| 1     | Fasting Blood Glucose ( mg/dl)          | 220.21                | 111.98                  |
| 2     | Postprandial Blood Glucose (mg/dl)      | 333.18                | 132.36                  |
| 3     | Total Antioxidant activity (mmmol/l)    | 0.61                  | 2.92                    |
| 4     | Glutathion Peroxidase (U/l)             | 2929.88               | 9673.40                 |
| 5     | Glutathion Redctase( U/L)               | 24.79                 | 65.30                   |
| 6     | Super oxide dismutase ( U/L)            | 118.63                | 295.95                  |
| 7     | HbA1C                                   | 8.6                   | 5.2                     |

n=number of participants.

Table 3: Descriptive statistics for Placebo medication in Pre and Post Drug Analysis.

| Sr.no | Parameters                              | Pre Dug analysis n=20 | Post Drug Analysis n=20 |
|-------|-----------------------------------------|-----------------------|-------------------------|
| 1     | Fasting Blood Glucose ( mg/dl)          | 191.85                | 129.01                  |
| 2     | Postprandial Blood Glucose (mg/dl)      | 226.23                | 151.47                  |
| 3     | Total Antioxidant activity (mmmol/l)    | 0.75                  | 0.96                    |
| 4     | Glutathion Peroxidase (U/l)             | 2505.26               | 2934.91                 |
| 5     | Glutathion Redctase( U/L)               | 27.50                 | 34.34                   |
| 6     | Super oxide dismutase ( U/L)            | 111.33                | 130.52                  |
| 7     | HbA1C                                   | 7.8                   | 6.9                     |

n=number of participants.

Table number 4, 5 shows the Z test for active and placebo medication of post drug effect in comparison to pre drug analysis. In table 4, 5 pair 1 and 2 shows that p<0.05, this indicates that significance difference has been found of mean for FBS and PP in pre and post drug analysis after active and placebo medications. Pair 3, 4, 5 and 6 shows that p<0.05, this indicates that antioxidant status of TAA, GPX, GSH and SOD has been improved in post drug analysis after active and placebo medications. Pair 7 indicated that mean level of HbA1C is under controlled limit after medication. Statistical significance indicates the rejection of Null Hypothesis (H0), which means that Alternative Hypothesis (H1) will be implemented. Z test analysis shows statistically significance because all the patients were on T2D therapy. Pomegranate extract of whole fruit was given as “ADD-ON” basis.
Table 4: Z test of PEWF (active) for Post Drug in comparison to pre drug analysis.

| Pairs     | Parameters                        | Z Test | Degree of Freedom | Sign(2 Tailed) |
|-----------|-----------------------------------|--------|-------------------|----------------|
| Pair1     | Fasting Blood Glucose Pre and Post Drug Analysis | 154.15 | 7254              | 0.00           |
| Pair2     | Post Prandial Glucose Pre and Post Drug Analysis | 189.67 | 7254              | 0.00           |
| Pair3     | Total Antioxidant activity Pre and Post Drug Analysis | 228.47 | 7254              | 0.00           |
| Pair4     | Glutathion Peroxidase Pre and Post Drug Analysis | 285.95 | 7254              | 0.00           |
| Pair5     | Glutathion Reductase Pre and Post Drug Analysis | 271.14 | 7254              | 0.00           |
| Pair6     | Super oxide dismutase Pre and Post Drug Analysis | 353.25 | 7254              | 0.00           |
| Pair7     | HbA1C Pre and Post Drug Analysis. | 211.34 | 7254              | 0.00           |

Table 5: Z test of Placebo for Post Drug in comparison to pre drug analysis.

| Pairs     | Parameters                        | Z Test | Degree of Freedom | Sign(2 Tailed) |
|-----------|-----------------------------------|--------|-------------------|----------------|
| Pair1     | Fasting Blood Glucose Pre and Post Drug Analysis | 67.04  | 4528              | 0.00           |
| Pair2     | Post Prandial Glucose Pre and Post Drug Analysis | 69.26  | 4528              | 0.00           |
| Pair3     | Total Antioxidant activity Pre and Post Drug Analysis | 158.42 | 4528              | 0.00           |
| Pair4     | Glutathion Peroxidase Pre and Post Drug Analysis | 12.95  | 4528              | 0.00           |
| Pair5     | Glutathion Reductase Pre and Post Drug Analysis | 33.91  | 4528              | 0.00           |
| Pair6     | Super oxide dismutase Pre and Post Drug Analysis | 86.43  | 4528              | 0.00           |
| Pair7     | HbA1C Pre and Post Drug Analysis. | 125.67 | 4528              | 0.00           |

Table number 6, 7 shows, chi square test for active and placebo medications in post drug analysis, independent variables were found to be statistically significant(p<0.05) in both active and placebo medications. Which mean prognostic effect has been seen in patients with both active and placebo medication and rejection of Null Hypothesis (H₀).
Table 6:- Chi square test of PEWF (active) for Post Drug in comparison to pre drug analysis.

| Pairs   | Parameters                                      | chi square | Degree of Freedom | Sign(2 Tailed) |
|---------|-------------------------------------------------|------------|-------------------|----------------|
| Pair1   | Fasting Blood Glucose Pre and Post Drug Analysis| 91.19      | 18                | 0.00           |
| Pair2   | Post Prandial Glucose Pre and Post Drug Analysis| 108.26     | 33                | 0.00           |
| Pair3   | Total Antioxidant activity Pre and Post Drug Analysis| 132.1     | 1                 | 0.00           |
| Pair4   | Glutathion Peroxidase Pre and Post Drug Analysis| 8.51       | 2                 | 0.00           |
| Pair5   | Glutathion Reductase Pre and Post Drug Analysis| 117.01     | 1                 | 0.00           |
| Pair6   | Super oxide dismutase Pre and Post Drug Analysis| 14.79      | 3                 | 0.00           |
| Pair7   | HbA1C Pre and Post Drug Analysis.               | 49.81      | 139               | 0.00           |

Table 7:- Chi square test of Placebo for Post Drug in comparison to pre drug analysis.

| Pairs   | Parameters                                      | chi square | Degree of Freedom | Sign(2 Tailed) |
|---------|-------------------------------------------------|------------|-------------------|----------------|
| Pair1   | Fasting Blood Glucose Pre and Post Drug Analysis| 99.15      | 15                | 0.00           |
| Pair2   | Post Prandial Glucose Pre and Post Drug Analysis| 126.62     | 30                | 0.00           |
| Pair3   | Total Antioxidant activity Pre and Post Drug Analysis| 100.00  | 4                 | 0.00           |
| Pair4   | Glutathion Peroxidase Pre and Post Drug Analysis| 65.69      | 1                 | 0.00           |
| Pair5   | Glutathion Reductase Pre and Post Drug Analysis| 14.74      | 1                 | 0.00           |
| Pair6   | Super oxide dismutase Pre and Post Drug Analysis| 21.87      | 4                 | 0.00           |
| Pair7   | HbA1C Pre and Post Drug Analysis.               | 72.13      | 139               | 0.00           |

Table number 8 shows the difference in coefficient of variations (C.V.) in post drug analysis for active and placebo. Parameters like FBS, PP, TAA, GPX, GSH and SOD shows the C.V. is 0.13, 0.17, 0.22, 0.18, 0.13 and 0.12 for active, This indicates that C.V. of active is less then placebo, which suggest that active medicine has high prognostic effect in T2D. Significant improvements in Antioxidative status indicate the good sign of prognosis.

Table 08:- Coefficient of Variation of PEWF (active) & Placebo medicine.

| Descriptive Statistics | PEWF (ACTIVE)     | Placebo   |
|------------------------|-------------------|-----------|
|                        | Mean | Std. Deviation | C.V. | Mean | Std. Deviation | C.V. |
| Fasting Blood Glucose  | 111.99 | 15.53 | 0.13 | 129.01 | 24.10 | 0.18 |
| Postprandial           | 132.36 | 27.86 | 0.17 | 151.47 | 32.31 | 0.21 |
| Total Antioxidant activity | 2.92 | 0.65 | 0.22 | 0.96 | 0.32 | 0.33 |
| Glutathion Peroxidase | 9673.40 | 1804.06 | 0.18 | 2934.92 | 2567.07 | 0.87 |
| Glutathion Reductase  | 65.30 | 8.05 | 0.13 | 34.34 | 7.40 | 0.21 |
| Super oxide dismutase | 295.95 | 37.11 | 0.12 | 130.53 | 17.55 | 0.16 |
| HbA1C                  | 5.2 | 0.8 | 0.09 | 6.9 | 1.3 | 0.11 |
Discussion:

Literature review suggests that oxidative stress in T2D seems to be caused by both an increased production of free radicals and reduction in antioxidative defense. Increase of oxidative stress depletes the antioxidative defense mechanism of body. Now a day’s lots of natural and artificial supplements are available to improve antioxidative status. Antioxidative rich beverages including 100% fruit juice, iced tea and red wine are common; among these PEWF has most potent antioxidant capacity.

Results were analyzed and statistically presentation shows that in Z test; the mean level of FSB and PP in both active and placebo shows statistically significance (p<0.05). This indicates the rejection of null hypothesis (H₀) and acceptance of alternative hypothesis (H₁). Which mean that Blood Glucose levels significantly reduced in post drug effects in patients with T2D. The level of TAA, GSH, GPX and SOD were increased, which shows that an antioxidants level in blood has been improved; which indicates the prognosis. HbA1C level of active and placebo shows statistically significance (p<0.05) this indicates that prognostic effect has been seen in patients with T2D.

In chi square test; Post drug analysis of independent variables found statistically significant (p<0.05) in both active and placebo medications. This indicates the rejection of Null Hypothesis (H₀). Present study indicates that the administration of PEWF as supplementation to participants with T2D for one month: (a) Improvement in T2D status (b) Improvement in Antioxidative status.

Mechanism is still unclear, available studies shows that Polyphenols and Antioxidative rich plants such as pomegranate; stimulate beta cells to release insulin and may also help in regeneration or increase in the number of beta cells. Plants such as Punica granatum l(Pomegranate) exhibit extra pancreatic effects via peripheral glucose utilization. These herbs are better than other available oral hypoglycemic agents, because no toxicity symptoms has been observed till date, which is an important finding because none of the conventional oral hypoglycemic agents exhibit this action.

In conclusion, the Polyphenols and Antioxidants rich fruit supplements containing pomegranate extract of whole fruit found beneficial in patients with T2D with MI.

Conclusion:

Statistically presentation shows that PEWF supplementation as an “ADD-ON” basis with regular medication for T2D shows the decrease of markers for Diabetes and improvement of antioxidant status in blood. In conclusion, The Polyphenols and Antioxidants rich fruit supplements containing PEWF has found beneficial in patients with MI & CHD.

Ethical consideration:

Ethical clearance has been obtained in written form, Institute Ethics Committee (IEC) of VCSGGIMS&R & SMC. Clearance from Clinical Trial Registry India (CTRI) (A unit of Government of India Undertaking) has been obtained before to initiation of present trial. This trial was conducted according to Good Clinical Practice and the Declaration of Helsinki.

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References:-
1. Martin Gallan P, Carrascosa A, Gussinye M, Dominguez C. Biomarkers of diabetes associated oxidative stress and antioxidant status in young diabetic patient with or without subclinical complications. Free Radical Biology and Medicine. 2003; 34:1563-1574.
2. World Health Organization Expert Committee on diabetes mellitus. Technical report series. Geneva: World Health Organization, 1980
3. Collier E., Watkinson A., Cleland C.F., and Roth j. Partial purification and characterization of an insulin-like material from spinach and lemna gibba. Journal of Biological Chemistry.1987; 262: 6238-6241.
4. Shanmugasundaram E.R., Gopith k.l., Radha S.K., and Rajendran V.M. Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given gymnema sylvestere leaf extract. Journal of Ethnopharmacology.1990; 30: 265-269.
5. Abdel Moneim A., El-Feki M. and Salah E.,Effect of Nigella Sativa, Fish oil and Gliclazide on alloxan diabetic rats. Journal of Biochemical and Histopathological studies.2007; 23: 237-265.
6. Johanniingsmeier SD, Harris GK. Pomegranate as a functional food and nutraceutical source. Annual Review of Food Science and Technology. 2011; 2:181–201.
7. Medjakovic S, Jungbauer A. Pomegranate: a fruit that ameliorates metabolic syndrome. Food Functions. 2013; 4:19–39
8. Katz SR, Newman RA, Lansky EP. Punica granatum: heuristic treatment for diabetes mellitus. Journal of Medicinal Food.2007; 10:213–217.
9. Banihani S, Swedan S, Alguraan Z.Pomegranate and type 2 diabetes. Nutrition Research.2013; 33(5):341-348.
10. Colak E, Majkio Singh, Stankovie Set al, Parameters of Antioxidative defence in type 2 diabetic patients with cardiovascular complications. Annals of Medicine.2005; 37:613-620.
11. Nazirogilu M., Butterworth P. Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. Canadian Journal of Applied Physiology.2005; 30:172–185.
12. Seeram NP, Aviram M, Zhang Y et al. Comparison of antioxidant potency of commonly consumed polyphenol rich beverages in the United States. Journal of Agriculture and Food Chemistry. 2008;56:1415-1422
13. Ahmed I, Adeghate E, Sharma AK, Pallot DJ, Singh J. Effects of Momordica charantia fruit juice on islet morphology in the pancreas of the streptozotocin-diabetic rats. Diabetes Research and Clinical Practice 1998;40:145–151.
14. Chakravarthy BK, Gupta S, Gambhir SS, Gode KD. Pancreatic beta-cell regeneration in rats by epicatechin. Lancet. 1981; 2:759–760.
15. Shanmugasundaram ER, Gopinath KL, Radha Shanmugasundaram K, Rajendra VM. Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given Gymnema sylvestre leaf extracts. Journal of Ethnopharmacology. 1990;30:265–279.
16. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Journal of Pharmacology. 1996; 27:431–434.
17. Sharma MK, Khare AK, Feroz H. Effect of Neem oil on blood sugar levels of normal hyperglycemic and diabetic animals. Indian Medical Gazette 1983;11:380–383.
18. Rahul Goyal, S. Nagtilak, Vijay Thawani, Monika Pathania & Shavetika Jindal. An antioxidative effect of Punika Granatum (Pomegranate) on biochemical parameters in patients with myocardial infarction: a double blind placebo controlled trial. European Journal of Biomedical & Pharmaceutical Science, 3(5), 662-667, 2016.