Effect of Dietary intervention by Dry Pomegranate (Punica Granatum L) Powder on some biochemical factors in Mild Cardio Vascular Disease Patients
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ABSTRACT:
This study aimed to examine the effect of dry pomegranate (Punica Granatum) (DPG) intervention on biochemical parameters in mild cardiovascular disease (CVD) patients. Sixty adult males > 45 years suffering from mild CVD were selected from the outpatient clinic of the cardiopulmonary department in El-Fayoum General Hospital in El-Fayoum city. The patients were divided randomly into two equal groups (A&B 30 patients/ group): Group A (Control group); who had received a placebo for 8 weeks. Group B (Study group); supplemented with 50g of dry pomegranates daily for the same period. Mean daily nutrient intake using 24 Hours recall, anthropometric measurements [body mass index (BMI), waist-hip ratio (WHR), arm circumference (AC)], blood pressure, and heart rate were assessed. Blood analysis for lipid profiles and malondialdehyde (MDA) and some antioxidants “Superoxide dismutase (SOD), Glutathione (GSH), Catalase enzyme (CAT), and ascorbic acid (VIT C)” were determined for both groups before and after treatment. Results showed that in the control group, there were no significant differences in all parameters measurements before and after supplementation. In-group B, there were significant differences in the mean values of systolic blood pressure, diastolic blood pressure, cholesterol, MDA, SOD, CAT, and VIT C compared to pre-post supplementation values. There were no significant differences in the mean values of AC, WHR, triglyceride, low-density lipoprotein cholesterol LDL-C, high-density lipoprotein cholesterol HDL-C, and GSH post-intervention. The study concluded that dry pomegranate could be used for the prevention of lipid peroxidation and to enhance antioxidant status in patients.

Keywords: Dry Pomegranate, CVD, Lipid Peroxidation, Antioxidant, Lipid profile.
INTRODUCTION

More than 17 million people die annually from cardiovascular disease (CVD) worldwide (WHO, 2017), and many could be saved by better controlling high blood pressure (responsible for the bulk of heart disease-related deaths annually), high levels of blood cholesterol, and other condition that raise the risk for heart disease and stroke (WHO, 2017). High blood pressure affects between 16 and 37% of the population globally (Poulter et al., 2015). In 2010, hypertension was believed to have been a factor in 18% representing 9.4 million globally of all deaths (Campbell et al., 2015). The World Health Organization (WHO) reported that cardiovascular diseases are the leading cause of death in Egypt, accounting for 46% of total deaths (WHO, 2017)

Bioactive components, particularly polyphenols, have been studied for their potential beneficial effects on health (Resnick et al., 2000). A natural source of phenolic compounds, pomegranate is loaded with antioxidants such as tannin, polyphenol, flavonoid, and vitamins C. Other antioxidants in pomegranate include tocopherols and anthocyanin were demonstrated to have protective and therapeutic qualities. The bioactivities of this phytochemicals are mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential (El-Falleh et al., 2012).

Clinical research by kumarie et al., (2012) shows that pomegranates, when part of a healthy diet, might help prevent heart disease, heart attacks, and strokes. This is due to the potential effect of pomegranates thin the blood, increase blood flow to the heart, reduce blood pressure, reduce plaque in the arteries, and reduce bad cholesterol, while increasing good cholesterol. Pomegranate extracts have been shown to scavenge free radicals, decrease macrophage oxidative stress, and lipid peroxidation in animals and increase plasma antioxidant capacity in elderly humans.

Hence, this study aims to examine the effect of dry pomegranate powder on lipid profiles, lipid peroxidation biomarkers and some antioxidants status in mild CVD patients.
SUBJECTS, MATERIALS, & METHODS

Subjects: Sixty adult males > 45 years, diagnosed with mild CVD (stage 1) were randomly selected from the outpatient clinic of the cardiopulmonary department in El-Fayoum General Hospital in El-Fayoum city, Egypt, and were participated in this study after their approval to participate in the study. They were selected on the following criteria:

Inclusion criteria: their ages were > 45 years old, diagnosed as a mild CVD patient (stage 1).

Exclusion criteria: Patients with bronchial asthma, diabetes mellitus, mental health problems such as depression or anxiety and Illiterate patients are excluded from the study.

Study Design: Sixty mild CVD patients were subjected to clinical, nutritional; biochemical and anthropometric measurements, then they were randomly divided into two main groups:

(Control group): This group consisted of thirty patients with mild CVD (stage 1). They received red-colored (starch) powder for 8 weeks (placebo).

(Intervention group): consisted of thirty patients with mild CVD (stage 1), and supplemented with 50gm of dry pomegranate (DP) daily (on breakfast as a powder dissolved in one cup of water), for 8 weeks. All patients were subjected to clinical and biochemical measurements before and after study period (8 weeks).

II- Materials:

1- Fruit samples of Pomegranate were obtained from the local market in El-Fayoum city, Egypt.

2- Reagent kits for biochemical measurements were purchased from DiaSys Diagnostic System GmbH, Dokki, Giza.

Methods:

- Fruit drying method: pomegranate (PG) peeled, washed, and mashed. Then the moisture content was reduced by using a water bath and then resulted in the derided at a temperature of 40°C, then ground to obtain the powder.

- Determination of phenolic and flavonoids compounds

Polyphenols:
The phenolic compounds were determined by the HPLC technique according to the method of Goupy et al. (1999). As follows; 5 g of PG samples were mixed with methanol and centrifuged at 10000 rpm for 10 min. The supernatant was filtered through a 0.2μm Millipore membrane filter then 1-3 ml was composed in a vial for injection into the HPLC Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column type was an ODS column with a measurement of 5μm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml/min. Standard phenolic acids from Sigma Company was dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by Hewllet packed software to calculate the concentration of phenolic compounds.

**Flavonoid:**

The flavonoid compounds were determined by the HPLC technique according to the method of Mattila et al., (2000). As follows; 5 g of samples were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2μm Millipore membrane filter. Then 1-3 ml was composed in a vial for injection into the HPLC [Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050)]. The column type was an ODS column with a measurement of 5μm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml/min. Standard Flavonoids from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by HEWLLET packed software to calculate the concentration of flavonoid compounds.

- **Free radical scavenging activity for pomegranate using 2,2-DiPheny1-1- Picry1 Hydrazy1 (DPPH+) method**

By lightening, the purple color of (2.2 Diphenyl -1-picryl hydroxyl) according to Pratap et al., (2013), the DPPH+ free radical scavenging activity of PG samples at varied doses was evaluated. A Spekol 11 (Carl Zeiss -Jena)
spectrophotometer was used to measure the absorbance at 517 nm. Using the current equation, the percentage inhibition was determined.

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\text{Inhibition \(\%\)} = \left( \frac{\text{A Blank} - \text{A Test}}{\text{A Blank}} \right) \times 100.
\]

**Anthropometric measurements:**
The anthropometric measurements used in this study were, Weight, Height, Body Mass Index (BMI), mid upper arm circumference and waist to hip ratio were measured in accord to the guide of the National Health and Nutrition Examination Survey (NHANES) methods (NHANES, 2007).

**Nutritional Assessment:**
A- Mean daily nutrient intake was estimated using 24 Hours recall for 3 consecutive days.

B- Dietary History.

**Clinical examination:** Blood pressure and heart pulse rate were measured for all participants in both groups before and after supplementation.

**Blood Samples collected:** These following parameters were measured pre and post dry pomegranate supplementation in blood of study’s subjects. The blood analysis required a 12-h fasting.

**Blood biochemical analysis:** Serum total cholesterol level was determined colorimetrically according to the method described by Allain et al., (1974). For determination of serum, triglycerides (TG) level the method described by Fassati and Precipè, (1982) was used. Serum high-density lipoprotein cholesterol (HDL-c) level was the estimated spectrophotometrically as claimed by the Lopes-Virella et al., (1977), serum low-density lipoprotein-cholesterol (LDL-c) concentration was evaluated as stated by the method of Wieland and Seidel (1983). Nishikimi et al. (1972) estimated Blood Super Oxide Dismutase (SOD) activity on the report of the method. Where blood Catalase (CAT) activity was evaluated in the opinion of Aebi (1984). Blood glutathione (GSH) level was determined according to the method of Beutler et al. (1963). For the determination of blood lipid peroxidation end product (MDA) the method of Satoh, (1978) was used. Limitation of serum ascorbic acid was determined according to the colorimetric method of Harris and Ray (1935).
Statistical analysis:
Statistical analysis was performed using statistical package software (SPSS, Inc. Chicago, USA). Data were analyzed using one-way analysis of variance (ANOVA) to show differences among variables before and after pomegranate supplementation and the level of significance was set at \( P \leq 0.05 \) (SAS, 2006).

RESULTS AND DISCUSSION

I- Subjects

Anthropometric measurements of patients in both two groups (A&B).

A substantial amount of evidence demonstrates that the distribution of abdominal fat is more directly linked to metabolic risks than BMI (Chan et al., 2002). Waist hip ratio (WHR) was found to be more significantly connected with CVD risk factors (HDL, triglycerides, diabetes, and hypertension) than BMI (Bell et al., 2018). The study done by Romero-Corral et al., (2010) showed that the best indicator of real adiposity is body fat percentage, which has the strongest links to mortality and CVD risk. A study by Ashwell et al., (2012) indicated the superiority of WHR to BMI concerning CVD.

Nutritional Assessment

1- Dietary intake

24 Hours recall:

From Figure (1), The mean value of daily total energy, protein, carbohydrates, fat, riboflavin, thiamine, Vit C, Vit A, copper, zinc, iron, magnesium, phosphors, calcium, potassium and sodium is 114%, 178.5% 194.3%, 209%, 80%, 76%, 44%, 46%, 85%, 112%, 231%, 29.9%, 169%, 82%, 59% and 241.5% of the percent RDA.

As shown in fig. (3) The mean daily intake of total energy, protein, carbohydrates, fat, iron, zinc, phosphorus, and sodium were higher than the RDA, whereas intake of riboflavin, thiamine, vitamin C, vitamin A, copper, magnesium, calcium, and potassium were lower than the RDA. It was recommended that an individual's nutrient intake be within established recommended dietary intake levels in the prevention and treatment of diet-related chronic disease (WHO and FAO, 2003). Fats, protein and carbohydrate consumption should contribute 15–30%, 10–15%, and 55–65% respectively of total
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The results of this study revealed that subjects consumed more fat (209%) above the recommended daily allowance (RDA) of 15–30% for optimal health. A high-fat diet is considered as a risk factor for the cardiovascular system. (Nelms et al., 2011).

Clinical trials show that reducing or modifying dietary fat consumption reduces the risk of combined cardiovascular events by 16 percent (Hooper et al., 2001).

Imbalanced diets and lower physical activity are important variables contributing to the acceleration of CVD epidemics (Reddy, 2002). The participants in this study consumed more carbohydrates than the RDA, and excessive dietary carbohydrate intake, particularly simple sugars, has been associated with atherogenic dyslipidemia (Lairon et al., 2005).

Higher carbohydrate intake was found to be significantly associated with blood triglycerides and adversely associated with serum HDL-cholesterol (Castro et al., 2006). Micronutrients such as sodium excess and potassium deficiency are closely associated with the risk of developing CVD.

The study's findings revealed that the average dietary sodium intake was (5554.958±1142.263) of all the participants were higher than the recommended intake (<2300 mg). Dietary sodium has long been thought to play a role in the development of high blood pressure (Mccarron, 2008).

The study participants had a dietary potassium intake of (2798.374 ±478.76) mg per day, which was lower than the recommended intake of 4700 mg/day. This could be attributed to the participants' inadequate intake of fruits and vegetables.

The dietary pattern of the participants in this study and their nutritional status is of great concern; as they consume simpler carbohydrates especially eat red meat excessively. Rarely eat fish, do not take the minimum amount of fresh or cooked vegetables and fruits, rarely eat olive oil or nuts, while they exceed the limit in consuming hydrogenated fats, so it is recommended to follow the RDA.

Identification of phenolic compounds by HPLC analysis

Figure (2) shows a chromatogram of phenolic compounds in the dry pomegranate.
As indirect antioxidants, catechins regulate protein synthesis and signaling pathways (Fan et al., 2017). In addition, catechins can up-regulate antioxidant enzymes (Rodriguez-Ramiro et al., 2011). Mice given 0.2% catechins in drinking water showed significantly increased activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Khan et al., 1992), which play key roles in scavenging Reactive oxygenated spices (ROS) (Negishi et al., 2004). Catechins appear to be able both to generate and to scavenge free radicals and show their beneficial effects due to a combination of both mechanisms (Oliveira-Marques et al., 2009). The antioxidant efficacy of catechins is exerted through (1) direct mechanisms scavenging ROS, chelating metal ions; and (2) indirect mechanisms inducing antioxidant enzymes, inhibiting pro-oxidant enzymes, and producing phase II detoxification enzymes and antioxidant enzymes) Youn et al., 2006).

Ellagic acid and hydrolyzable ellagitannins, as well as their high antioxidant potential, have been linked to atherogenesis prevention. Punicalagin is the most abundant ellagitannin in pomegranate juice (PJ), it is this molecule that gives strong antioxidant activity (Tzulker et al., 2007). Studies by Artik et al. (1998) and Poyrazoglu et al. (2002), illustrated the presence of gallic acid, quercetin, catechin, chlorogenic acid, and o-coumaric acid in pomegranate juices. Gallic acid 4.55 ± 8.55 mg/L, catechin 3.72 ± 2.29 mg/L, chlorogenic acid 1.24 ± 1.42 mg/L, caffeic acid 0.78 ± 0.79 mg/L, ferulic acid 0.01 ± 0.02 mg/L, phloridzin 0.99 ± 1.47 mg/L, and quercetin 2.50 ± 1.96 mg/L were the amounts of phenolic compounds. The findings showed that ferulic acid, quercetin, and rutin were all detected in the same pomegranate varieties.

**Total polyphenols and total flavonoids content:**

The total phenolic and flavonoids in fresh and dry fruit. Fresh fruit had the highest concentrations of total polyphenols and flavonoids, (124.00 ± 5.4 mg GAE/g) and (11.03 ± 2.99 mg QE/g) respectively. The DPG was followed by (109.23 ± 3.54 mg GAE/g) and (10.74 ± 1.34 mg QE/g) in that order.

Pomegranate juice (PJ) has a greater concentration of total polyphenols
(5 mmol/L) than other fruit juices (orange, grapefruit, grape, cranberry, pear, pineapple, apple, and peach juices), according to Seeram et al., (2008), PJ was the most effective, followed by orange juice, grapefruit juice, and peach juice.

The antioxidant concentration of PJ was found to be higher than that of blueberry, cranberry, and orange juices, as well as red wine (Aviram et al., 2002).

Variations in phenolic and other components in pomegranate could be attributed to different varieties Gadze et al. (2012), level ripeness, different processing type or, a different part of the plant consumed Free radical scavenging capacity of pomegranate fruit using (DPPH\(^+\)) radical method

From table (2), the free radical scavenging activity using DPPH radicals ranged from (0.138 to 0.085\(\mu l\)) at concentrations 5 and 40mg/ml, of fresh fruit, followed by the dry fruits, (0.149 and 0.097\(\mu l\)), at the same concentrate.

DPG has a free scavenging capacity comparable to the fresh fruit values, which resulted from the high polyphenolic content.

The antioxidant activity of pomegranate fruit extracts was 0.109, 0.074, 0.936, and 0.33g/ml, respectively, of ethanolic soxhlet, ethanolic maceration extract, hexane maceration extract, and butylated hydroxyl toluene, as determined by Khan et al. (2017). Fernandes et al. (2017), found that the antioxidant activity using (IC\(_{50}\) DPPH) of juices of nine pomegranates (Mollar de Elche, Valenciana, White, CG8, Cis 127, Katirbasi, Parfianka, Wonderful 1, Wonderful 2), were 9.78, 9.78, 6.37, 7.16, 7.65, 4.97, 16.62, 7.59, and 1.97\(\mu l\) juice/ml, respectively. Al-Sanabani et al. (2016), found that the DPPH radical scavenging activity of fresh pomegranate seeds, frozen pomegranate seeds, and freeze-dried pomegranate seeds, were (79.54, 77.39 and 68.91), respectively. Basiri, (2015), the antioxidant activity using (IC\(_{50}\) DPPH), of pomegranate seeds extracts, were (0.30, 0.15, 0.34, 1.77, 2.01, and 4.23mg/ml), of water, methanol, acetone, ethyl acetate, and hexane extracts, respectively. Zeghad et al. (2019), found that the antioxidant capacity using (IC\(_{50}\) DPPH), was (0.600mg/mL), for Punica granatum fruit.
Clinical examination (Heart rate, systolic blood pressure and diastolic blood pressure)

Table (3) illustrated that, in group A (control group), there were insignificant decreases in the mean value of HR, SBP and DBP measured at post-treatment (94.06±10.84, 134.5±3.79 and 85.16 ±3.59) when compared with its pre-treatment measure (94.06±10.84, 134.5±3.79 and 85±3.47) respectively.

In-group B (supplementation group), there were a significant decrease in the mean value of HR, SBP and DBP measured at post-treatment (93.26±6.94, 125.33±4.53 and 80.66±1.72) when compared with its pre-treatment measure (97.13 ±7.23, 134.66 ±3.92 and 84.16 ±2.96).

Sumner et al., (2005) The PJ-treated group exhibited better arterial flexibility, according to measurements of arterial stiffness of the common carotid arteries in 73 individuals with at least one cardiovascular risk factor who received PJ (240 mL/day for 1 year). Tsang et al. (2012) studied the effects of 500 ml/d Pomegranate juice or placebo consumption in 28 obese subjects who did not have a metabolic syndrome for 30 days. SBP and DBP were found to be significantly lower. A previous study found that consuming 50 ml/d of Pomegranate juice for 14 days in patients with hypertension could result in a 36 % reduction in Angiotensin converting enzyme (ACE) activity and a 5% reduction in SBP (Aviram and Rosenblat, 2012). Pomegranate juice consumption for two weeks reduced blood pressure in hypertensive individuals (Asgary et al., 2014). The results of this study were in line with Gonzalez-Ortiz et al., (2011) found that the blood pressure-lowering action of PJ has been attributed to a number of processes. The first is angiotensin-converting enzyme (ACE) inhibition. Lyn et al., (2012), found a significant reduction in systolic blood pressure (SBP) and (DBP) in healthy individuals after receiving 330 ml/d PJ for 30 days.

Data in the table (4) showed that, in group A, there was an insignificant decrease in the mean value of cholesterol, triglyceride, HDL-C and LDL-C measured at post-treatment (195.27±5.37, 163.7 ±5.02, 38.33±1.22 and 122.97±4.39) when compared with its pre-treatment measure (195.63±5.42,
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164.13±5.16, 38.1±1.29 and 123.67±4.42) respectively.

In a group of patients supplemented with pomegranate powder, there were insignificant differences in the mean value of triglyceride, HDL-C and LDL-C measured at post-treatment when compared with its pre-treatment measures. There was a significant decrease in the mean value of cholesterol measured at post-treatment (178.3±5.23 mg/dl) when compared with its pre-treatment measure (187.3 ±5.29 mg/dl).

Pomegranate powder or extract's powerful antioxidant activity against lipid peroxidation could be the key to the antiatherogenic effects of pomegranate peel powder or extract's on lipoproteins (Labib and Hossin, 2009). Pomegranate Ellagitannin has been found as an active antioxidant ingredient with anticancer properties that protect low-density lipoprotein cholesterol (LDL-C) from oxidation in vivo, which is a critical phase in the pathophysiology of atherosclerosis (Krummel, 2008).

Hyperlipidemia has been related to an increased risk of CVD. Certain chronic illnesses cause abnormality in lipid metabolism, resulting in aberrant blood lipid amounts when compared to normal levels. Increased blood levels of cholesterol (TC), triglycerides (TAGs), and low-density lipoprotein cholesterol (LDL-c) are all important risk factors for cardio metabolic illnesses. Increased levels of high-density lipoprotein cholesterol (HDL-c) are regarded to be helpful because they aid in cholesterol elimination (Karr, 2017).

The results of this study were in line with Gonzalez-Ortiz et al., (2011) found a similar significant decrease in TC and LDL levels in the intervention group, although these changes were not significant when compared to the changes in the control group.

Table (5) showed that, in-group A, there were insignificant differences in the mean value of MDA, SOD, GSH, CAT, and VIT C measured at post-treatment when compared with its pre-treatment measures.

In-group B post supplementation, there was a significant difference in the mean values of MDA, SOD, CAT, and VIT C measured at post-treatment when compared with its pre-treatment measure.
However, the result of GSH revealed that there was an insignificant difference in the mean value measured at post-treatment when compared with its pretreatment measures.

Polyphenols are vascular protective, according to the studies discussed in this article. However, as revealed by Loke et al., (2010) study, different groups of polyphenols may have distinct substantial impacts on cardiovascular disease. Quercetin (flavone) decreased aortic F2-isoprostane, vascular superoxide, vascular leukotriene B4, and plasma P-selectin concentrations in an atherosclerotic rat model while increasing vascular eNOS activity, heme oxygenase-1 protein, and urinary nitrate excretion. Epicatechin (flavanol) and theaflavin (dimeric catechin) had similar but less significant effects.

Morvaridzadeh et al., (2020), reported that CPJ consumption might inhibit the activity of ROS by its antioxidant content and through which induce an antihypertensive effect and a reducing effect of pomegranate consumption on malondialdehyde (MDA), as a biomarker of oxidative stress, in adults. The result of this study agreed with Pirinccioglu et al., (2012), who reported that dietary supplementation with pomegranate decreased the formation of MDA and protein carbonyl levels in the cortex and hippocampus of mice, pomegranate has been shown to have direct antioxidant benefits, and daily administration of pomegranate juice resulted in lower MDA and protein carbonyl levels.

Ajaikumar et al., (2005), indicating the efficacy of pomegranate in avoiding oxidative damage and related changes, reported the reversal of GSH levels in gastric ulceration following pomegranate treatment. Pomegranate has been shown, to directly decrease the generation of superoxide anion, which can activate SOD and CAT. This finding implies that pomegranate's neuroprotective properties are attributable to its antioxidant activity (Sudheesh and Vijayalakshmi, 2005). Subash et al. (2014) reported that a diet supplemented by 4% of pomegranate extract attenuates oxidative damage by a decrease in lipoperoxidation and protein carboxylation and restoration the level of the antioxidant enzyme such as SOD, catalase (CAT), Glutathione peroxidase (GPx) and
Glutathione (GSH) and inhibition of AchE activity. Antioxidant enzymes, such as SOD and CAT, are the first line of defense against ROS in cells, scavenging the harmful intermediate of incomplete oxidation. A decrease in the activity of these antioxidant enzymes can lead to an excess of (O2) and H2O2, which can lead to the creation of OH°, which can contribute to the onset and propagation of lipid peroxidation (LPO). By scavenging (O2°), SOD protects cells against ROS, which destroys membranes and biological structures (Arivazhagam et al., 2000). SOD has the ability to catalyze the dismutation of (O2°) into H2O2, which is then deactivated to H2O by CAT (Murugan and Pari, 2006). As a result, SOD can serve as a primary defense against ROS, preventing the creation of more free radicals. SOD activity was found to be reduced in diabetic people due to H2O2 inactivation or glycation of the enzyme, both of which have been linked to diabetes. CAT is a hemeprotein found nearly universally in mammalian cells that is responsible for reducing H2O2 and protecting cells from extremely hazardous OH radicals (Sozmen et al., 2001). Inactivation of the SOD enzyme by glycation could also cause a decrease in CAT activity. Any combination of antioxidant characteristics may help to reduce oxidative damage to some extent or completely. As a result, eliminating O2° and OH° radicals are probably one of the most efficient disease defenses (Sankaranarayanan and Pari, 2011). The antioxidant concentration of pomegranate juice is higher than that of most other fruit juices. It also contains three times the amount of antioxidants found in green tea. Pomegranate juice's antioxidants can assist in the removal of free radicals, the protection of cells from harm, and the reduction of inflammation (Rosenblat et al., 2006). Anahita et al., (2014), found that pomegranate administration improved SOD and CAT activity in diabetic rats as compared to diabetic control rats. Furthermore, the pomegranate-induced increase in SOD activity may accelerate the dismutation of superoxide to H2O2, which is rapidly eliminated by CAT, protecting diabetic rats' tissues from highly reactive and poisonous OH° and thereby preventing LPO.

CONCLUSION

It was concluded that the intervention with dry pomegranate
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powder could be used as prophylactic and prevention of lipid peroxidation to enhance antioxidant status in patients and in dietary management for mild cardiovascular disease.

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Table (1): Anthropometric measurements of patients in both two groups (A&B).

<table>
<thead>
<tr>
<th>Items</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.23±3.92</td>
<td>50.16±3.29</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>96.7±17.87</td>
<td>96.08±16.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.46±7.84</td>
<td>173.6±6.25</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>31.3±4.69</td>
<td>31.87±5.03</td>
</tr>
<tr>
<td>Arm circumference (cm)</td>
<td>49.46±5.70</td>
<td>49.46±5.70</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.99±0.08</td>
<td>0.99±0.08</td>
</tr>
</tbody>
</table>

Fig. (1): Mean Daily Nutrients Intake of CVD patients and percentage of the RDA
Effect of Dietary intervention by Dry Pomegranate (Punica Granatum L) Powder on some biochemical factors in Mild Cardio Vascular Disease Patients

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Fig. (2): chromatogram of phenolic compounds in pomegranate.

Table (2): Free radical scavenging capacity of pomegranate fruit using (DPPH⁺) radical method

| Concentration mg/ml | Free radical scavenging capacity pomegranate using (DPPH⁺) radical (µl) |
|---------------------|------------------------------------------------|-----------------|
|                     | Fresh pomegranate | Dry pomegranate |
| 5                   | 0.138             | 0.149           |
| 10                  | 0.113             | 0.127           |
| 20                  | 0.099             | 0.108           |
| 40                  | 0.085             | 0.097           |
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Table (3): Mean ±SD of Heart rate, Systolic blood pressure and Diastolic blood pressure pre and posttest at both groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control pre</th>
<th>Control post</th>
<th>Treat. Pre</th>
<th>Treat. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (p/min)</td>
<td>94.06 ±10.84</td>
<td>94.06 ±10.84</td>
<td>97.13 ±7.23</td>
<td>93.26 ±6.94**</td>
</tr>
<tr>
<td>Systolic blood pressure (mm/Hg)</td>
<td>134.5 ±3.79</td>
<td>134.5 ±3.79</td>
<td>134.66 ±3.92</td>
<td>125.33 ±4.53**</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm/Hg)</td>
<td>85 ±3.47</td>
<td>85.16 ±3.59</td>
<td>84.16 ±2.96</td>
<td>80.66 ±1.72**</td>
</tr>
</tbody>
</table>

*Significant level is set at alpha level <0.05 **Significant level is set at alpha level <0.01

Table (4): Mean ±SE of cholesterol, triglyceride, HDL and LDL pre and posttest at both groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cont. pre</th>
<th>Cont. post</th>
<th>Treat. pre</th>
<th>Treat. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride mg/dl</td>
<td>164.13 ±5.16</td>
<td>163.7 ±5.02</td>
<td>158.57 ±5.04</td>
<td>152.53 ±4.95</td>
</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td>195.63 ±5.42</td>
<td>195.27 ±5.37</td>
<td>187.3 ±5.29</td>
<td>178.3 ±5.23**</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>38.1 ±1.29</td>
<td>38.33 ±1.22</td>
<td>38.81 ±2.23</td>
<td>38.93 ±1.47</td>
</tr>
<tr>
<td>LDL-C mg/dl</td>
<td>124.71 ±4.42</td>
<td>124.2 ±4.39</td>
<td>116.78 ±4.70</td>
<td>108.86 ±4.71</td>
</tr>
<tr>
<td>VLDL mg/dl</td>
<td>32.83 ±1.03</td>
<td>32.74 ±1.0</td>
<td>31.71 ±1.01</td>
<td>30.51 ±0.99</td>
</tr>
<tr>
<td>AI (LDL/HDL) mg/dl</td>
<td>3.24 ±0.13</td>
<td>3.24 ±0.12</td>
<td>3.00 ±0.65</td>
<td>2.79 ±0.84</td>
</tr>
</tbody>
</table>
Table (5): Mean ±SE of MDA, SOD, GSH, CAT, and VIT C pre and post-test at both groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cont. pre</th>
<th>Cont. post</th>
<th>Treat. pre</th>
<th>Treat. Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA nmol/ml</td>
<td>7.89±0.13</td>
<td>7.77±0.11</td>
<td>8.04 ±0.16</td>
<td>5.66±0.13a*<strong>b</strong></td>
</tr>
<tr>
<td>SOD u/ml</td>
<td>3.57±0.20</td>
<td>3.38 ±0.98</td>
<td>3.47±0.20</td>
<td>6±0.21a*b**</td>
</tr>
<tr>
<td>CAT U/L</td>
<td>1.87±0.12</td>
<td>1.93 ±0.13</td>
<td>1.93 ±0.12</td>
<td>3.46±0.12a<strong>b</strong></td>
</tr>
<tr>
<td>GSH mmol/L</td>
<td>39.93±1.02</td>
<td>38.69 ±0.89</td>
<td>38.43 ±0.83</td>
<td>40.8±0.88</td>
</tr>
<tr>
<td>VIT C µg/dl</td>
<td>2.57±0.14</td>
<td>2.67±0.13</td>
<td>2.73 ±0.12</td>
<td>4±0.12a<strong>b</strong></td>
</tr>
</tbody>
</table>
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