Cardioprotective Role of Pometone (Pomegranate seed oil) on Electrocardiography in Methionine Overload Rabbits (Part 1)

Likaa Najim Abdullah, Baraa Najim Al-Okialy*
*Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.
Corresponding author:
Baraa Najim Al-Okialy

Abstract

This study was designed to investigate the probable role of pomegranate seed oil as antioxidant in ameliorating the deleterious effects of methionine overload on cardiovascular function of adult female rabbits. Thirty-Two female rabbits were randomly assigned into four equal groups (eight animals each) and treated for 42 days daily as follows: the first group was drenched drinking corn oil, serving as control (group C), the second group (G1) was intubated orally with methionine 100mg/kg. B.W, while the third group (G2) was intubated orally with methionine 100mg/kg. B.W and pomegranate seed oil (PSO)30 mg /Kg. B.W, while the animals in the group(G3) were intubated orally with pomegranate seed oil 30 mg /Kg. B.W. After fasting the animals blood samples were collected at 0, 21and 42 days of the experiment to assess: serum troponin I and calcium concentrations .The electrocardiographs (ECG) were recorded for rabbits in all experimental groups at the same interval of the experiment .The results showed a significant increase in concentrations of serum troponin-I and calcium ion in group G1 after 42 days of the experiment. The analysis of ECG in rabbits treated with methionine (groupG1) showed a significant decrease in p wave, QRS wave and T wave amplitude , also significant increase in QRS and T wave interval as well as significant prolongation in P-Q and Q-T interval. While PSO maintains the normal shape of QRS waves and prevent prolongation of Q-T interval in group G3 and it was less effective in group G2 where the oil fail to restore normal level of P, P-Q, Q-T waves . In conclusion, the deleterious effects of methionine overload on heart function represented by abnormality of ECG component, and documented the cardioprotective role of PSO.

KEYWORDS: Pomengranate seed oil, ECG, Troponin-I, Heart rate, Calcium.

Introduction

Methionine is an essential amino acid found in both animal and plant proteins converted via enzymatic trans] methylation to homocysteine (Hcy). The common natural sources of this amino acid have traditionally been fish meal and meat meal, specially for starter chicks and broilers (Hoeler and Hooge , 2003). Rice and casein (Lewis and Baley, 1995). Methionine is a lipotropic and protective factor against various types of liver damage, but excessive dietary methionine is hepatotoxic (Oz *et al*., 2008). Methionine in the diet is the main source of Hcy in the blood. In addition to methionine overload, hyperhomocysteinemia (HHcy) may result from genetic defects in the enzymes involved in the metabolism of homocysteine or from deficiencies of enzyme cofactors or cosubstrat:folic acid, vitamin B6 (pyridoxine), or vitamin B12 (cyanocobalmine), or some chronic medical conditions and drugs (White *et al* . 2001 and Sahi *et al*. 2006).

New suggestion revealed the role of HHcy in the induction lipid peroxidation and oxidative damage (Delvin *et al* ., 2007) leading to occurrence of different

disease condition (Jamison *et al* ., 2007; Tounyz and Schiffrin , 2008). Several studies have suggested that HHcy is one of the independent risk factor for cardiovascular disease and coronary artery disease including coronary, carotid, aortic vessels, myocardial infarction and deep venous thrombosis (Suematsu *et al*., 2007; Hillenbrand *et al*.,2008 and Agoston-Coldea et al.,2011) , and menopause (Gambaccia and Mannlla,2007).

Almost all parts of a pomegranate (*Punicagranatum* Linn.) have well known as antioxidant activity (Noda *et al*.,2002 and Althunibat *et al*.,2010)and are used for the treatment of numerous diseases. In folk medicine, pomegranate was commonly used as an antithelmintic, antipyretic, antibacterial agent and to cure diarrhea ,hemorrhage ,acidosis and ulcer (Larrosa *et al*.,2010 and Lee *et al*.,2010).Animal studies showed that pre-treatment with pomegranate extracts can protect cardiac cells from death those arises from excessive adrenaline-like compounds and from damage by the potent oxidant-inducing chemotherapy drug doxorubicin (Mohan *et al* .,2010 and HassanpourFard *et al* .,2011) in both investigations, pomegranate extracts improve EKG abnormalities and elevat serum markers of heart muscle injury. The present study was designed to investigate the role of pometon (Pomegranate seed oil) in ameliorating and the effect of methionine over load on induction of cardiac dysfunction.

Materials and Methods

Thirty two adult female local rabbit (1250-2000gm /B.w) were used in this investigation .Their ages ranged between (6-8) months. Animals were housed in cages in conditioned room (22-25°C) in the Animal House of College of Veterinary Medicine -University of Baghdad for the period from November 2012 up to February2013. Animals had free access to water and standard pellet diet along the experimental period. Rabbits were randomly divided into four equal groups (8/ group), and they were treated daily for 42 days as follows:-

Group C: -rabbit in this group were administrated ordinary corn oil orally, serving as control (C) ; group G_1**:**-rabbit in this group were treated with methionine (Segma) (100 mg/kg BW) dissolved in corn oil orally **.**While rabbit in group G_2 were treated with methionine (100mg/kg BW) plus pometon (pomegranate seed oil) 30mg/kg BW (VitanPharma -Germany) and rabbits in G3 were administered orally pometone 30 mg/kg BW only. Fasting blood samples (8 hrs) were collected from animals by cardiac puncture technique (Lucas *et al* .,2004) at 0, 21,42, days of the experiment and serum was isolated and frozen at -18°C until measuring of serum troponin I(Tn-I) concentration by using troponin kit (Biomerieux -France) and serum calcium concentration was determined by a method as described by Connerty and Bigs(1966).

Preparation of Animals for Recording ECG:

The rabbits were placed on a table and then immobilized by ligation the four limbs and they were kept about 5-10 minutes to get calm . Electrode gel was rubbed into the skin in the area where the alligator clips were attached . Electrodes were attached to the skin at the triceps brachial muscle of the right and left limbs and biceps femoral muscle of the right and left hips. ECGs were recorded by using electrocardiogram (NihonKohden,Co., LTD Germany). All ECGs were standardized at 1mV=10mm, with a chart speed of 50mm/sec. Lead II were recorded at 0, 21,24days.

Statistical analysis: All data were performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$) and Least Significant

Differences test (LSD) was using to determine the differences among different groups (Snedecor and Cochran, 1973).

Results and Discussion

A significant ($P<0.05$) increase in Troponin-I (Tn-I) concentration was detected after 21, 42days of the experiment in group G_1 comparing to the control and other treated groups (G_2, G_3) (table-1). Besides there was no significant differences in serum Tn- I concentration in group (G_2) during the experimental period as compared to the control group .While, significant ($P<0.05$) decrease in serum Tn-I was observed during 42 days after treatment of animals with pomegranate seed oil (group G_3) compared to control.

Table (1): Effect of pomegranate seed oil on serum troponin–I (Tn-I) concentration (ngl/L) in methionine overload treated female rabbits.

Groups / Days	C	G1	G2	G3
Pretreatment	0.48±0.02 A a	0.48±0.03 A b	0.46±0.02 A a	0.45±0.02 A a
21 day	0.45±0.01 B a	0.57±0.01 A a	0.46±0.03 B a	0.43±0.01 B a
42 day	0.49±0.02 B a	0.60±0.01 A a	0.52±0.01 B a	0.40±0.01 C a

Value express as mean ± SE .n=8 .c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (pso), G3:Animals received 30mg/kg BW Pometon (PSO). Small letters denote within group difference $p<0.05$. Capital letters denote between groups difference $p<0.05$.

Troponin-I is the 'inhibitory' unit of the troponin complex associated with the thin filament, and inhibits actomyosin interactions at diastolic levels of intracellular Ca^{2+}.Bindingof $Ca^{2+}$to troponin C (TnC) during systole induces conformational changes those relieve the inhibitory influence of cardiac Tn-I (cTnI), thereby promoting actomyosin crossbridge formation and contraction (Solaro,2001).The results showed an alternation in contractile function in group G1 comparing these result from changes in the size or duration of the Ca^{2+}transient and/or changes in myofilament response to Ca^{2+} ,while the pivotal role of Ca^{2+}in regulating contraction and relaxation is well recognized, it has become increasingly apparent that myofilament properties also have a major role in the dynamic modulation of contractile function (Solaro and Rarick ,1998 and Solaro, 2001)

Recent evidence also suggests that altered thin filament function plays an important role in the contractile dysfunction associated with human heart failure (Noguchi *et al* , 2004) and may reflect changes in the balance between kinase and phosphatase activities

(Metzger and Westfall,2004)Significant decrease of serumcTn-I concentration in pomegranate seed oil groups (G_2 and G_3) may be due to the antioxidant properties of PSO.

The mean values of serum calcium concentration were clarified in table (2). Significant ($P<0.05$) increase in serum calcium concentration was recorded after 21days of the experiment in group G_1 compared to the control. Also the results showed a significant ($P<0.05$) increase in this parameter after 42 days of treatment in groups G1 and G2 compared to the control and G3groups. While the effect of pometone caused a significant ($P<0.05$) reduction in serum calcium concentration in groupG_3 comparing to G_1 and G_2.

Table (2): Effect of pomegranate seed oil on serum calcium concentration (mg/dl) in methionine overload treated female rabbits.

Groups / Days	C	G1	G2	G3
Pretreatmet	9.69±0.01 A a	9.73±0.03 A c	9.78±0.1 A b	9.63±0.02 A a
21 days	9.66±0.02 B a	10.35±0.01 A b	10.00± 0.01 AB b	9.66±0.02 B a
42 days	9.65±0.07 C a	13.93± 0.1 A a	12.52±0.1 B a	9.69± 0.02 C a

Value express as mean ± SE .n=8 .c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (pso), G3:Animals received 30mg/kg BW Pometon (PSO). Small letters denote within group difference $p<0.05$. Capital letters denote between groups difference $p<0.05$.

The elevation in Hcy concentration and the case of HHcy is regarded a major risk factors of methionine overload .HHcy is a potent risk factor for cardiovascular disease and is associated with impaired endothelium-dependent vasodilation in both experimental animals (Jiang *et al* , 2005 and Cheng *et al* ., 2009) and human (Chao *et al* , 2000).

Clarke *et al*, (2006) reported that sever HHcy impaired endothelium –dependent relaxation in mouse aorta and EDHF in renal arteries. EDHF activates Kca and voltage dependent Ca^{+2} channel(Kohler and Hoyer, 2007).Voltage-gated calcium ions channels play an important role in regulating calcium influx at physiological level(Henley and Poo, 2004)and in pathophysiological events such as ischemia or hypoxia (Fung,2000). Hcy is one of the predictors of bone minerals density, and HHcy is associated with lower bone mineral density and in elderly women on a vegetarian diet seem to be at higher risk of osteoporosis development than non-vegetarian women(Krivosikova *et al*., 2010).Hcy is an agonist of NMDA-R, known to be present in cardiac tissue, and when activated, the increases intracellular calcium leading to increase cell excitability(Maldonado *et al*., 2010).

The results also showed a significant decrease in serum calcium concentration in group G_2 received methionine plus pomegranate seeds oil compare to group G_1.There were

strong evidence revealed that pomegranate elicits ameliorating health effect in several disease (Jurenka, 2008). Many pomegranates beneficial effects have been widely related to the presence of ellagic acid and ellagitannis, specially puncalsgins, punicalins and gallagic acid (Viladomiu *et al*, 2013) . Pomegranate juice is a rich source of polyphenols, tannins andanthocyanin's, which are important in maintaining calcium concentration and protect neuronal cells in mice from oxidative stress by increase in influx(Vroegrijk*et al*., 2011)such effect may be due to intracellular GSH, directly lowering levels of ROS, and preventing the influx of Ca^{2+} despite high levels of ROS (Ishige *et al.*, 2001). Another study showed that the pomegranate extract is a rich source of polyphenols and play an important role in the prevention of bone loss and is able to enhance bone formation and increase in bone Ca^{+2}content in mouse embryos (Monsefi *et al*., 2012). In addition to their antioxidant properties of flavonoids, the beneficial effects of flavoniods could be based on their ability to bind to Ca^{2+}-ATPase's thus change their activity resulting in maintaining Ca^{2+}level(Horáková,2011).

The Electrocardiogram

1 -P-Wave interval and amplitude:

Statistical differences were absent (P>0.05) in P-wave interval and amplitude values between experimental groups after 21and 42 days of treatment as compared to control group, as well as, when they were compared with each other .With exception a significant (P<0.05) reduction in the mean value of P-wave amplitude in group G_1 at 42 days of the experiment comparing to the control and G_2 groups (table-3).

2-QRS complex:

The data in the table-4 illustrated a significant (p<0.05) decrease in the mean values of QRS complex amplitude (mv) in experimental groups G_1 and G_2, after 42 days of the experiment as compared with G_3 and control groups. Further more ,groups G1 and G2 showed significant(p<0.05) elevation in the mean value of QRS interval and significant(p<0.05) decrease in the mean value of QRS amplitude (mv) as compared to the control group and other treated group (G_3). The results have also clarified that treatment of rabbits with methionine plus pomegranate seed oil did not cause any differences in the mean value of QRS-interval after 42 days of experiment as compared to the control. While a significant (p<0.05) elevation observed in QRS amplitude in G_3 at 42 days as compared to control group.

3-T-Wave :

After 21,42 days from started of the experiment there were significant (p<0.05) increase in T-wave interval and significant (p<0.05) decrease in T-wave amplitude were observed in group G_1 when compared to control ,G_2 and G_3 groups. Besides significant (p<0.05) decrease after21 and 42 days in the mean value of this parameter was observed in group G_3, compared with the same time of the experiment. Also there were no significant differences in the mean values of T-wave amplitude for G_3 group after 21 and 42 days of the experimental period when compared to control and T_2 group as well as to each other (table-5).

4-P-Q and Q-T interval:

A significant(p<0.05) increase in P-Q interval was observed after 21 and 42 days of the experiment in group G1 comparing to the control and group G_3,while Q-T interval showed a significant increase (p<0.05) in G1 group as compared to control, G_2 and G_3 groups at 42 days of experiment (table-6). The results have also revealed a significant (p<0.05) decrease in the mean value of both P-Q and Q-T interval in methionine plus pomegranate seed oil (G_2) after 42 days of the experimental as compared to group G_1 .Depending on statistical results, each of pomegranate seed oil (group G_3) and control

did not show any significant differences in P-Q and Q-T intervals at 21 and 42 days of treatment when they compared with each other.

Table (3): Effect of pomegranate seed oil on P wave interval (sec) and amplitude(mv) in electrocardiogram of methionine overload treated female rabbits.

Groups Days	C		G1		G2		G3	
	P int	P m.v	P int	P m.v	P int	P m.v	P int	P m.v
0	0.041± 0.001 A a	0.12± 0.01 A a	0.042± 0.006 A a	0.16± 0.02 A a	0.041± 0.001 A a	0.15± 0.03 A a	0.043± 0.003 A a	0.12± 0.01 A a
21 days	0.041± 0.001 A a	0.15± 0.02 A a	0.041± 0.008 A a	0.16± 0.02 A a	0.041± 0.001 A a	0.16± 0.01 A a	0.041± 0.001 A a	0.12± 0.01 A a
42 days	0.040± 0.001 A a	0.16± 0.02 A a	0.038± 0.001 A a	0.11± 0.01 B b	0.041± 0.001 A a	0.18± 0.01 A a	0.040± 0.001 A a	0.14± 0.01 AB a

Value express as mean ± SE,- n=8 c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (PSO), G3:Animals received 30mg/kg BW Pometon (PSO) Small letters denote within group difference $p<0.05$.Capital letters denote between groups difference $p<0.05$.

Table (4):Effect of pomegranate seed oil on QRS complex wave interval(sec) and amplitude(m.v) in electrocardiogram of methionine overload treated female rabbits

Groups Days	C		G1		G2		G3	
	QRS int	QRS m.v	QRS int	QRS m.v	QRS int	QRS m.v	QRS int	QRS m.v
0	0.020± 0.001 A a	0. 45± 0.002 A a	0.018± 0.001 A b	0. 45± 0.001 A a	0.018± 0.001 A b	0. 46± 0.002 A a	0.018± 0.001 A a	0. 46± 0.002 A b
21 days	0.018± 0.01 A a	0.44± 0.08 A a	0.018± 0.001 A b	0.31± 0.01 C b	0.018± 0.01 A b	0.40± 0.02 B b	0.18± 0.001 A a	0.46± 0.02 A b
42 days	0.018± 0.001 C a	0.44± 0.02 B	0.038± 0.001 A a	0.28± 0.02 D c	0.028± 0.001 B a	0.35± 0.02 C c	0.018± 0.001 C a	0.63± 0.02 A a

Value express as mean ± SE,- n=8 c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (PSO), G3:Animals received 30mg/kg BW Pometon (PSO) Small letters denote within group difference $p<0.05$.Capital letters denote between groups difference $p<0.05$

Table(5): Effect of pomegranate seed oil on T wave interval(sec) and amplitude(mv) in electrocardiogram of methioninoverload treated female rabbits.

Groups / Days	C		G1		G2		G3	
	T int	T m.v	T int	T m.v	T int	T m.v	T int	T m.v
0	0.080± 0.004 A a	0.24± 0.001 A a	0.081± 0.001 A c	0.25± 0.002 A a	0.081± 0.002 A b	0.26± 0.001 A a	0.081± 0.001 A a	0.26± 0.002 A b
21 days	0.081± 0.001 B a	0.26± 0.02 A a	0.086± 0.003 A b	0.22± 0.02 B b	0.082± 0.004 B b	0.25± 0.02 A a	0.058± 0.001 C b	0.24± 0.01 A b
42 days	0.080± 0.001 C a	0.26± 0.02 A a	0.116± 0.02 A a	0.22± 0.02 B b	0.94± 0.02 B a	0.26± 0.02 A a	0.058± 0.001 D b	0.28± 0.01 A a

Value express as mean ± SE,- n=8 c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (PSO), G3:Animals received 30mg/kg BW Pometon (PSO) Small letters denote within group difference $p<0.05$.Capital letters denote between groups difference $p<0.05$

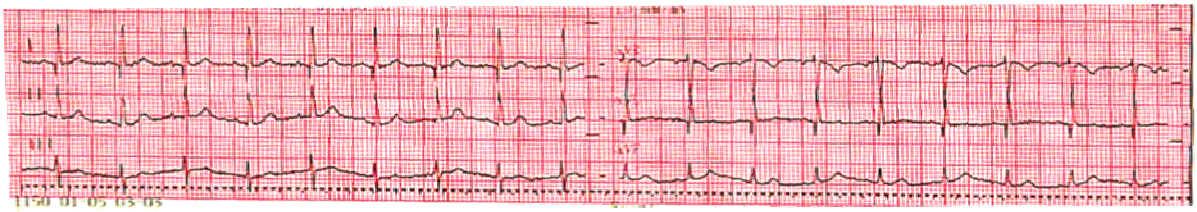
Table (6):Effect of pomegranate seed oil on P-Q and Q-T wave interval(sec) in electrocardiogram of methionine overload treated female rabbits.

Groups / Days	C		G1		G2		G3	
	P- Qint	Q-T int	P- Q int	Q-T int	P- Q int	Q-T int	P- Q int	Q-T int
0	0.076± 0.004 A a	0.130± 0.004 A a	0.078± 0.001 A c	0.130± 0.003 A b	0.080± 0.004 A b	0.130± 0.004 A b	0.078± 0.001 A a	0.130± 0.004 A a
21 days	0.076± 0.004 B a	0.130± 0.004 A a	0.089± 0.003 A b	0.130± 0.004 A b	0.081± 0.003 B b	0.130± 0.004 A b	0.081± 0.003 B a	0.130± 0.003 A a
42 days	0.078± 0.001 C a	0.130± 0.004 C a	0.121± 0.001 A a	0.170± 0.004 A a	0.106± 0.001 B a	0.140± 0.004 B a	0.078± 0.001 C a	0.130± 0.004 C a

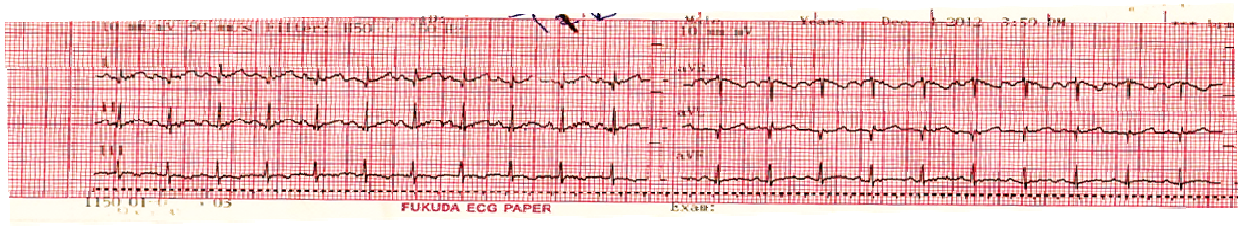
Value express as mean ± SE,- n=8 c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (PSO), G3:Animals received 30mg/kg BW Pometon (PSO) Small letters denote within group difference $p<0.05$.Capital letters denote between groups difference $p<0.05$.

Figure(1) Effect of pomegranate seed oil on electrocardiogram (LII) at pretreated period:

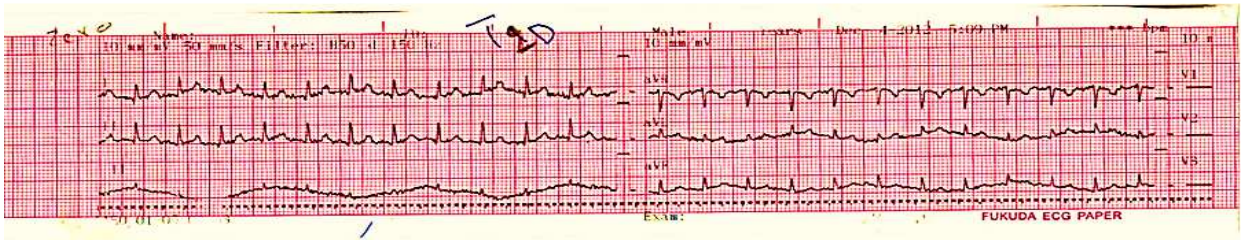
Control



G1



G2



G3

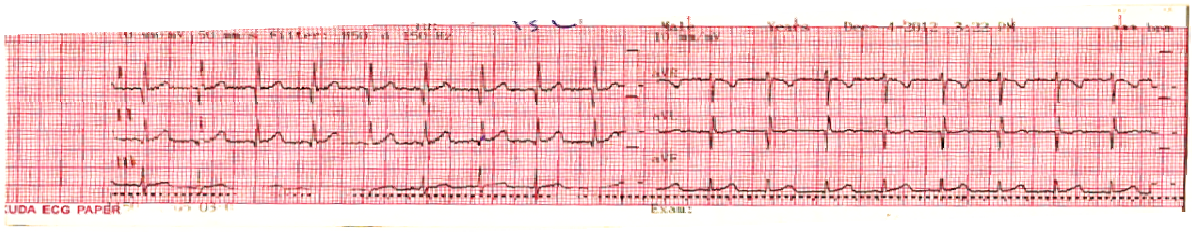
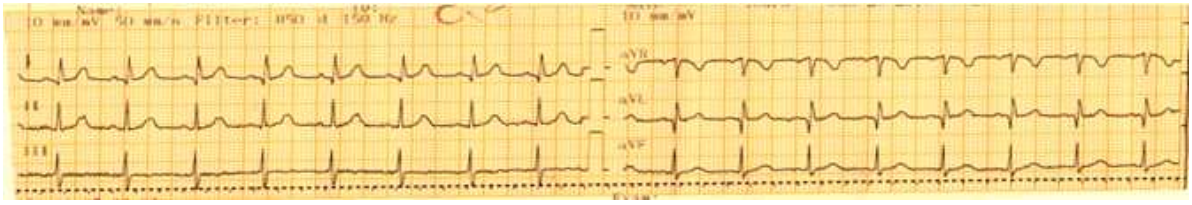
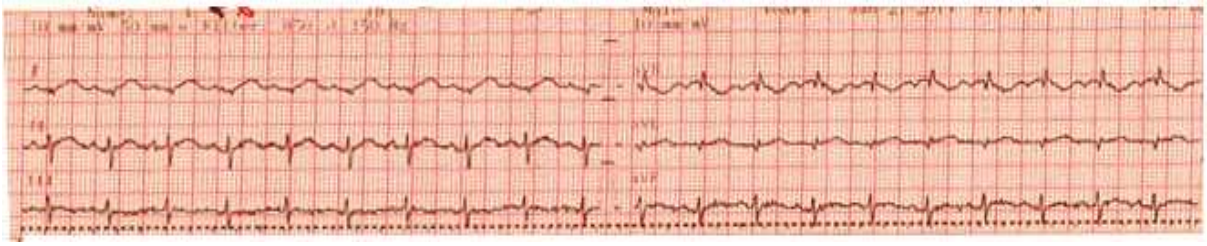


Figure (2) Effect of pomegranate seed oil on electrocardiogram (LII) after 21 days:-

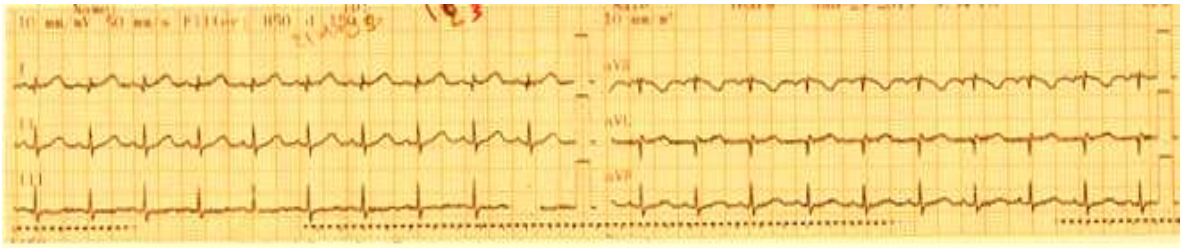
Control



G1



G2



G3

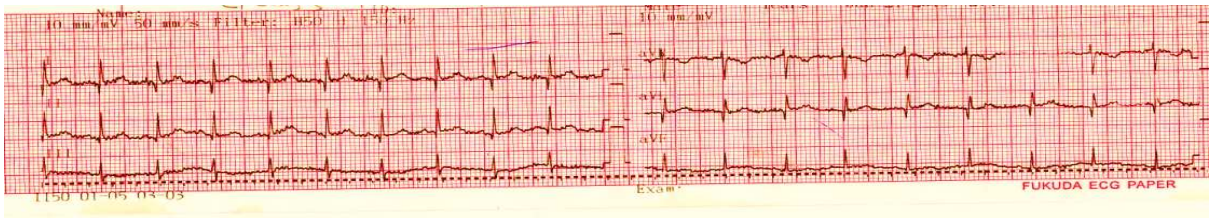
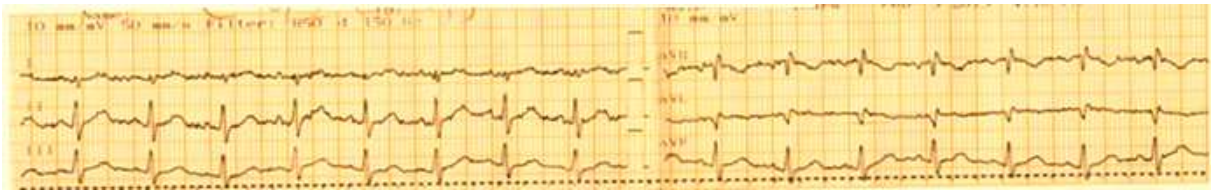
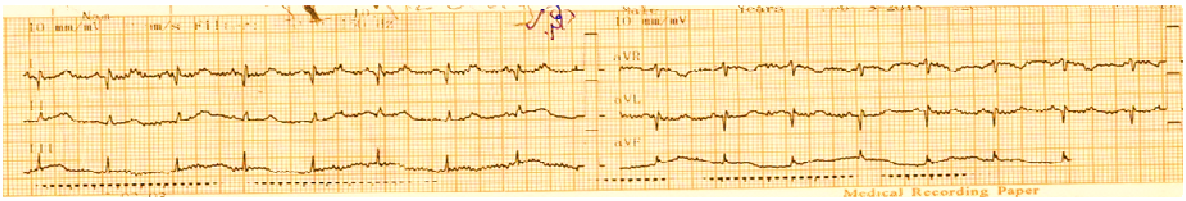


Figure (3) Effect of pomegranate seed oil on electrocardiogram (LII) after 42 days:-

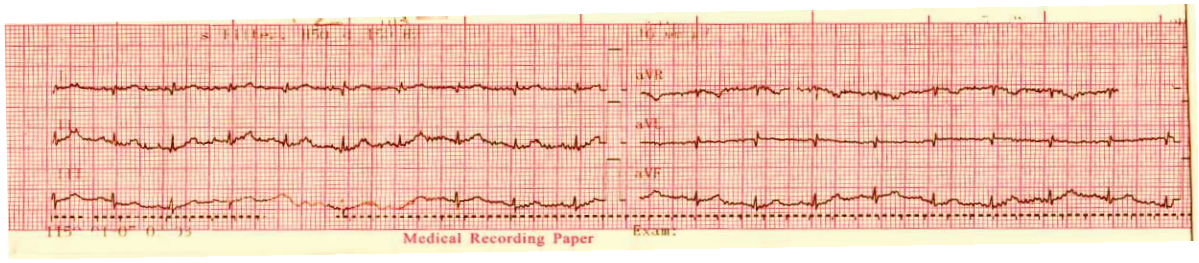
Control



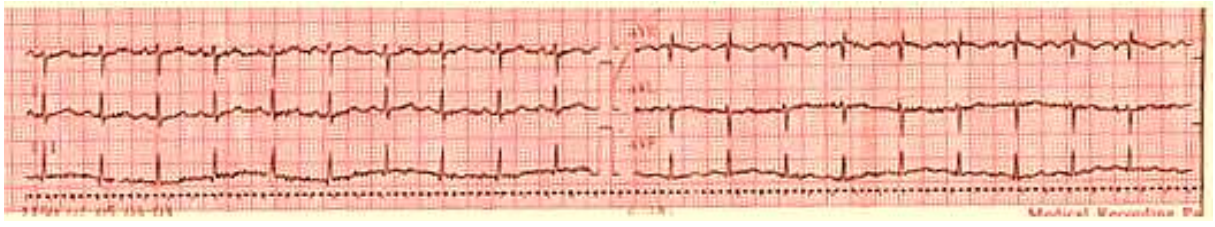
G1



G2



G3



The changes in the ECG indicating changes in the left ventricular regional wall motion abnormalities (RWMA) with change in the conducting system documented as arrythemia (Rosenberger, *et al*.,2006). Although the high level of Hcy (HHcy) are associated with vascular seizure, dementia and arrhythmias, the mechanism of Hcy-mediated cardiac arrhythmias is unclear. Long PR interval reflects slow conduction through the atrioventricular (AV) node and bundle of Hiss , and may indicate a disease of conducting tissue predisposing to bradyarrhythemia through high-grade AV block (Davey, 2010).

Hypercholesterolemia induced due to HHcy might be involved in this issue (Bamash, *et al*.,2013).Previous studies in animals as well as in humans has been reported a prolongation of Q-T in hypercholesterolemic state and is considered to be due to increased oxidative stress and myocardial remodeling (Szabo *et al*., 2005; Chih-Sheng *et al* ., 2007 and Acampa *et al* ., 2011). Oxidation of Hcy can generate free radicals that can damage arterial endothelium (Malinow *et al*., 1989). Furthermore Hcy also promotes the oxidation of LDL-cholesterol, which can lead to heart disease (Eikelboom , 1999). So, it can be suggested that oxidative stress induce pulmonary hypertension and right ventricular hypertrophy (Dachun *et al* ., 2011) leading to increase Q-T interval(AL-kinani *et al*.,2011). Besides, methionine overload may induce Q-T prolongation through change in K^+ conduction in myocardium. Meanwhile Rosenberger et al, (2011) explained that Hcy enriched diet lead to prolongation of Q-T interval.

The increase in Q-T interval in PSO group (G2) was lesser than Q-T interval in rabbits treated with methionine, suggesting the preventive and beneficial role of pomegranate seed oil to attenuate the repolarization characteristics of rabbits exposed to methionine overload . The mechanisms for reduction in Q-T interval in rabbits in group G2 in this experiment is unexplained .It has been shown that pomegranate caused down regulation in the expression of NOIII induced by oxidized LDL in human coronary endothelial cells (De Nigirs *el al*.,2006) in addition to its ability to protect NO against oxidative destruction and enhance biological action of nitric oxide (Ignarro *et al*., 2006). Accordingly, it can be speculated that pomegranate seed oil may increased NO production and in turn alter the activity of ATP dependent K^+ channels hence repolarization. So the reduction in Q-T prolongation in G_2 treated group may lead to reduct in the rate of arrhythmias.

Table (7) showed a significant ($P<0.05$) decrease in the mean values of heart rate in G1 group after 21and 42 days of intubation as compared to G3and control groups, as well as the results clarified absence of significance ($P<0.05$) between G_1 and G_2 when compared with each other at the same periods. Besides there was no significant ($P<0.05$) differences in this parameters at different intervals of the experiment in group G3 as compared to the control group.

Hhcy has been considered as an independent risk factor for various cardiovascular disease like endothelial dysfunction, vascular inflammation, atherosclerosis, hypertension, cardiac hypertrophy and heart failure (Ankur *et al.,* 2012). Previous reports suggest that Hcy increase peroxidation, and ROS production and oxidative stress (Rosenberger *et al*., 2006 and 2007), causing arrhythmia and sudden cardiac death (SCD).Several studies assert an association between HHcy and atrial fibrillation (Naji *et al*., 2010 and Acampa *et al*., 2011). Reduction in heart rate by HHCY may alter the excitability of sino atrial (SA)and atrioventricular (AV) nodal cells, resulting in abnormal nodal condition (Soni,2012). Besides, elevated level of HCY may cause cardiac arrythemia by an increasing mitochondrial NOs activities , metalloproteinase activity, disrupts connexin-43(gap junction protein of heart conducting

system),accumulation of collagen fiber and interrupted cardiac conductivity (Givvimani *et al* .,2011).

Table (7): Effect of pomegranate seed oil on Heart Rate (b/m) in methionine overload treated female rabbits

Groups / Day	C	T1	T2	T3
Zero time	276 ± 4.3 A a	281± 5.9 A a	281± 5.9 A a	271± 4.1 A a
21 days	276± 4.2 A a	236± 3.3 B b	246± 4.2 B b	276±4.2 A a
42 days	276± 4.3 A a	225 ±1.6 B b	228 ± 1.6 B c	278± 4.4 A a

Value express as mean ± SE,- n=8 .c:- control group , G1: Animals received methionine 100 mg / kg B.W , G2 : Animal received methionine 100mg/ kg BW plus 30 mg /kg BW pometon (PSO), G3:Animals received 30mg/kg BW Pometon (PSO) Small letters denote within group difference $p<0.05$.Capital letters denote between groups difference $p<0.05$.

There are several plausible mechanisms by which elevated Hcy may account for these changes. Hcy activates N-methyle-D-aspartate receptors (NMDA receptors), as well as modulates sodium and potassium channel. Activation of cardiac NMDA receptors, especially those at the SA and AV nodes , allows more calcium influx , resulting in bathmotropic and dromotropic effects. High levels of Hcy increase sodium currents while inhibiting potassium currents in human atrial myocytes (Cai *et al* .2007 and 2009) caused an increase sodium currents enhancement of cell excitability, while inhibition of potassium currents prolongs action potential duration (APD). HHCY was shown to increase intracellular and mitochondrial Ca+2 ion level resulting in oxidative stress (Tyagi *et al*,2010) and it decreases myocyte contractile performance by agonizing the NMDA-R1 receptors (Gao *et al*, 2007 and Vacek et al,2012) .Increased intracellular Ca+2 by agonizing the NMDA-R1 receptors, impairing ability of NCX-I proteins to extrude Ca+2 from cell in exchange for sodium ion, impairs uptake of endoplasmic reticulum Ca+2, this increases Ca+2 mitochondria, disrupting electrone transport leading to impaire membrane potential ,thereby disrupting mitochondrial function (Zhaou *et al*, 2008) ,as well as, reducing the ability of ATP production resulting decline in myocardiac contractility (Moshal *et al*, 2008 c). Recent study suggest that HHCY increased mitochondrial NO level and mitochondrial permeability leading to poor cardiac performance (Moshal *et al*,2009).

The present study showed a significant decrease in heart rate of PSO treated group as compared to control and G3 groups. Pomegranate fruit could be considered as functional food because of its antioxidant activity. Polyphenolic compound of pomegranate are predominant phytochemicals responsible for its functional properties (Viuda-Martos *et al*,2010). The antioxidant activity of pomegranate components has been the subject of many *invitro* and *invivo* studies (Zhang *et al.*, 2008 ; Am, *et al*,2009 and Tezcan *et al*,2009 and Pedriali *et al*, 2010). Polyphenolic compound of pomegranate and PSO like elliagic acid derivatives, punicalagin isomers and anthocyanins are known of their antioxidant activity through free radical scavenger and inhibition of lipid peroxidation

invitro (Noda *et al*,2002 and Boussetta *et al*,2009)). Pomegranate polyphenolic molecules undergo redox reaction because phenolic hydroxyl groups readily donate hydrogen reducing agent (Magrigal-Carballo *et al*,2009) or some can react with certain precursor of peroxide that preventing peroxide formation (Naveena *et al*, 2008). Pomegranate improve cardiovascular health (Davidsone *et al*, 2009 and Fuhrman *et al*, 2010) and exert benifecial effect on the clinical vascular complication and atherogenesis by enhancing nitric oxide synthase III (De Nigirs *et al*, 2006), in addition to its capability in protection NO against oxidative destruction and enhance its biological action (Ignarro *et al*,2006).In Conclusion the effect of antioxidant properties of pomegranate seed oil on heart rate may cannot overpass the effect of methionine and consequently there were no significant changes between two treated groups (T1 and T2) as compared between them in some study parameters.

References

Acampa, M.; Lazzerini, P.E.; Guideri, F.; Rechichi, S.; Capecchi, P.L.; Maccerini, M and Laghi-Pasini, F.2011. Homocysteine and P wave dispersion in patients with heart transplantation.Clin. Transplant.,25:119-125.

Agoston-Coldea,L.;Mocan,T.;Gatfosse,M.;Lupus,S. and Dumitrascu,D.L. 2011. Plasma homocysteine and the severity of heart failure in patient with previous myocardial infarction. Cardiol J.,18:55-62.

AL-Kinani, R. K.M.; Abed allatef.; Saad, H.;Albazii and Wefaq, J.2011.The protective Role of follic asid In stress induced by Methionine over Load On Electrocardiograph on female New Zealand rabbits. J. Scientific Karbala University ; 9: (2).

Althunibat, O.Y.; Al-Mustafa, A.H; Tarawneh, K.; Khleifa, K.M.; Ridzwan, B.H.and Qaralleh, H.N. 2010. Protective role of Punica granatum L. peel extract against oxidative damage in experimental diabetic rats. Process Biochem45(4): 581–5.

Ankur,R.;Pooja,D.;Seema,R.Amarjeet,D. and Ashok,K.2012. Hyperhomocystenemia and Cardiovascular Disease: A Transitory Glance. Int. J. Drug Dev. Res.,4(2);70-75.

Bamashmoos,S.A.; Al-Nuzaily,M.A.K.; Al-Meer,A.M.and Ali,F.H.H.2013. Relationship between total homocysteine ,total cholesterol and creatinine levels in overt hypothyroid patients. Speringer.Plus,2:423.

Boussetta,T.;Raad,H.Letteron,P.;Gougerot-Pocidalo,M.A.;Driss,F. and EL-Benna, J. 2009. Punicic acid, a conjugated linolenic acid, inhibits TNFα-induced neutrophil hyperactivation and protects from experimental colon inflammation in rats .PLoS One 4(7):6458.

Cai, B.Z.; Gong, D.M.; Liu, Y.; Pan, Z.W.;Xu, C.Q.; Bai, Y.L.; Qiao, G.F.; Lu, Y.J and Yang, B.F.2007.Homocysteine inhibits potassium channels in human atrial myocytes. Clin Exp Pharmacol Physiol. 34(9):851-5.

Cai,B.;Shan,L.;Gong,D.;Pan,Z.;Ai,J.;Xu,C.;Lu,Y.and Yang,B.2009.Homocysteine modulates sodium channel currents in human atrial myocytes.Toxicology.265(3):201-206.

C¸ am, M.; Hısıl, Y. and Durmaz, G. 2009. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food Chem,112:721–6.

Chao, C.L.; Kuo,T.L and Lee, Y.T. 2000. Effects of methionine induced hyper homocysteinemia on endothelium dependent vasodilation and oxidative status in healthy adults. Circulation. 101(5):485-490.

Cheng, Z.; Yang, X and Wang, H. 2009. Hyperhomocysteinemia and endothelial dysfunction. Curr. Hypertens Rev.;5(2):158-165.

Chih-Sheng ,C. ; Kun-Tai ,L. and Shuo-Tsan L. 2007. Effects of atorvastatin on ventricular late potentials and repolarization dispersion in patients with hypercholesterolemia. Kaohsiung J. Med. Sci., 23:217–24.

Clarke, Z.L.; Moat, S.J. ;Miller, A.L.; Randall, M.D.; Lewis, M.J and Lang, D. 2006. Differential effects of low and high dose folic acid on endothelial dysfunction in a murine model of mild hyperhomocysteinaemia. Eur J Pharmacol.;551(1-3):92-97.

Connerty, H.V. and Biggs, A.R . 1966. Determination of serum calcium by means of ortho-cresolphthalein complexone . Am.J. Clin. Path.,45:290-6 .

Dachun,Xu.; Guo,H.;Xin, X.; Zhongbing, Lu.; John, F.; Xinli ,Hu.; Yawei ,Xu.; Tang,Q.; Hu,D.; Somani,A.; Aron, M. G.; Ostertag,E.; Robert J. B.; Kenneth, W.E. and Yingjie, C.2011.Exacerbated pulmonary arterial hypertension and right ventricular hypertrophy in animals with loss of function of extracellular superoxide dismutase. J. Hypertension., (58): 303-309.

Davey,P. 2010. Electrocadiograph (ECG). Elesevier Medicine , 38(7):348-356.

Davidson MH, Maki KC, Dicklin MR, Feinstein SB, Witchger MS, Bell M,McGuire DK, Provos JC, Liker H, Aviram M. 2009. Effects of consumption of pomegranate juice on carotid intima-media thickness in men and women at moderate risk for coronary heart disease. Am. J. Cardiol., 104(7):936–42.

Delvin, A.;Singlh, R.;Wads,E.;Innes, S.;Bottiglieri, T and Lentz, S. 2007. Hyper homocysteinemia .J. Bio .Chem. , 282(5):37082-37088.

De Nigris, F.; Williams-Ignarro, S.; Botti, C.; Sica, V.; Ignarro, L.J.and Napoli, C.2006. Pomegranate juice reduces oxidized low-density lipoprotein down regulation of endothelial nitric oxide synthase in human coronary endothelial cells. Nitric Oxide 15(3):259–63.

Eikelboom, J.1999. Homocysteine and cardiovascular disease : Acritical review of the epidemiological evidence .Ann. Intern. Med. ;131: 363- 375.

Fuhrman, B.; Volkova, N.and Aviran, M. 2010. Pomegranate juice polyphenols increase recombinant paraoxonase-1 binding to high-density lipoprotein: studies in vitroand in diabetic patients. Nutr.; 26(4):359–66.

Fung, M.L. 2000 . Role of voltage-gated Na+ channels in hypoxia-induced neuronal injuries, Clin. Exp. Pharmacol. Physiol., 27 569–574.

Gambaccia, M. and Mannella, P. 2007.Homocysteine, menopause and cardiovascular disease. Menopause Int., 13:23-2.

Gao, X.; Xu, X.; Pang, J.; Zhang, C.; Ding, J.M.; Peng, X.; Liu, Y.and Cao, J.M. 2007. NMDA receptor activation induces mitochondrial dysfunction, oxidative stress and apoptosis in cultured neonatal rat cardiomyocytes. Physiol. Res. ;56(5):559–69.

Givvimani, S.; Qipshidze, N.; Tyagi, N.; Mishra, P.K.; Sen, U and Tyagi, S.C. 2011. Synergism between arrhythmia and hyperhomo-cysteinemia in structural heart disease Int. J. Physiol. Pathophysiol. Pharmacol., 30; 3(2): 107–119.

HassanpourFard ,M.; Ghule, A.E.; Bodhankar, S.L. and Dikshit, M. 2011. Cardioprotective effect of whole fruit extract of pomegranate on doxorubicin-induced toxicity in rat. *Pharm .Biol.*, Apr; 49(4):377-82.

Henley, J and Poo, M.M. 2004. Guiding neuronal growth cones using Ca2+ signals. Trends Cell Biol. 14 320–330.

Hillenbrand ,R. ; Hillenbrand ,A. ;Liewald ,F. and Zimmermann ,J. 2008.Hyperhomocysteinemia and currant carotid stenosis. Cardiovascular Disorders., 8:1.

Hoeler, D. and Hooge,D.M. 2003. Relative effectiveness of methionine sources in turkeys-scientific and new commercial data. Inter. J. Poul .Sci., 2(5):361-366.

Horáková, L.2011.Flavonoids in prevention of diseases with respect to modulation of Ca-pump function.J. Interdiscip. Toxicol., 4(3): 114–124.

Ignarro, L.J.; Byrns, R.E.; Sumi, D.; de Nigris, F.and Napoli, C. 2006. Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide. Nitric. Oxide 15:93–102.

Ishige, K.; Schubert, V and Sagara, Y.2001. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanism. J . Free Radical Bio. Med. 30, (4): 433–446.

Jamison,R.L.;Hartigan,P.;Kaufman,J.S.;Goldfarb,D.S.;Warren.S.R.;Guarino,P.Dand Gaziano, J.M. 2007 . Effect of homocysteine lowering on mortality and vascular disease and end –stage renal disease :a randomized control trial.J.A.M.A.,298(10):1163-1170.

Jiang, X.; Yang, F.; Tan, H and et al.2005. Hyperhomocystinemia impairs endothelial function and eNOS activity via PKC activation. *Arterioscler Thromb* VascBiol.;25(12):2515-2521.

Jurenka, J. 2008. "Therapeutic applications of pomegranate (Punica granatum L.): a review," Alternative Med. Rev., 13(2) :128–144.

Kohler, R and Hoyer, J. 2007.The endothelium-derived hyperpolarizing factor: insights from genetic animal models. Kidney Int.;72(2):145-150.

Krivosíková, Z.; Krajcovicová-Kudlácková, M.; Spustová, V.; Stefíková, K.; Valachovicová, M.; Blazícek, P and Němcová, T.2010. The association between high plasma homocysteine levels and lower bone mineral density in Slovak women: the impact of vegetarian diet. , Apr; 49(3):147-53.

Larrosa,M.;Gonzalez-Sarrias,A.;Yanez-Gascon,M.J.;Selma,M.V.;Azorin-Ortuno,M;Toti,S.;Tomas-Barberan,F.;Dolara,P.andEspina,J.C.2010.Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. J Nut Biochem., 21(8):717-725.

Lee,C.J.;Chen,L.G.;Laing,W.L. and Wang,C.C.2010. Anti-inflammatory effects of *Punica granatum* Linne *in vitro* and *in vivo*. Food chem., 118:315-322.

Lewis , A.and Baley, H. (1995). Amino acid bioavailability of nutrients. J. Agric .Food Chem. , 35-65.

Lucas, R.L; Lentz, K.D and Hale, A.S 2004. Collection and preparation of blood products. Clin. Tech.in Small Ani. Prac. 19(2), 55-62.

Maldonado, C.; Soni, C.V.; Todnem, N.D.; Pushpakumar, S.; Rosenberger, D.; Givvimani, S.; Villafane, J and Tyagi, S.C.2010. Hyperhomocysteinemia and sudden cardiac death : potential arrhythmogenic mechanisms.

Madrigal-Carballo S, Rodriguez G, Krueger CG, Dreher M, Reed JD.2009. Pomegranate (Punica granatum L.) supplements: authenticity,antioxidant and polyphenol composition. J. Funct. Foods., 1:324–9.

Malinow, M.; Kang, S.; Talyor, L.; Wong, P.; Coull, B.; Inahara, T.; Mukerjee, D.; Sexton, G and Upson, B.1989. Prevalence of hyperhomocyst(e) inemia in patient with peripheral arterial occlusive disease, Circulation ;79:1180-1188.

Metzger J.M and Westfall M.V. 2004. Covalent and noncovalent modification of thin filament action. The essential role of troponin in cardiac muscle regulation. Circ. Res., 94:146–158.

Mohan, M.; Patankar, P.; Ghadi, P and Kasture S.2010. Cardioprotective potential of *Punica granatum* extract in isoproterenol-induced myocardial infarction in Wistar rats. J. Pharmacol. Pharmac.,1:32-7.

Monsefi, M.; Parvin, F.; Tahereh, T and Talaei-Khozani.2012. Effect of pomegranate extracts on cartilage , bone and mesenchymal cells of mouse fetuses. Bri. J Nut., 107. (5): 683-690.

Moshal, K.S. . 2008. Mitochondrial matrix metalloproteinase activation decreases myocyte contractility in hyperhomocysteinemia. Am. J. Physio. Heart Circ. Physiol., 295(2):H890–97.

Moshal, K.S.; Kumar, M.; Tyagi, N.; Mishra, P.K.; Metreveli, N.; Rodriguez, W.E. and Tyagi, S.C. 2009.Restoration of contractility in hyperhomocysteinemia by cardiac–specific deletion of NMDA-R1. Am. J. Physio. Heart Circ. Physiol., 296(3):H887–92.

Naji, F.; Suran, D.; Kanic, V.; Vokac, D and Sabovic, M.2010.High homcystein levels predict the recurrence of atrial fibrillation after successful electrical cardioversion .Int Heart J., 51 (1):30-3.

Naveena, B.M.; Sen, A.R.; Kingsly, R.P.; Singh, D.B.and Kondaiah, N.2008.Antioxidant activity of pomegranate powder extract in cooked chicken patties. Int. J. Food Sci. Technol., 43:1807–12.

Noda, Y.; Kaneyuka, T.; Mori, A.and Packer, L. 2002. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, andpelargonidin. J. Agric. Food Chem., 50:166–71.

Noguchi, T.; Hunlich, M.; Camp P.C.; Begin, K.J.; El-Zaru, M. and Patten, R.l 2004. Thin filament-based modulation of contractile performance in human heart failure. Circulation. 110:982–987.

Oz , H.S.; Chen T.S and Neuman, M .2008. Methionine deficiency and hepatic injury in a dietary steatohepatitis model", Digestive Diseases and Sciences 53 (3): 767–776.

Pedriali,C.A.;Fernandes,A.U;Santos,P.A.;Silva,M.M.;Severino,D. and Silva,M.B. 2010. Antioxidant activity, cito-and phototoxicity of Pomegranate (*Punica granatum* L.) seed pulp extract. Cienc. Tecnol. Aliment., Gampinas., 30(4):1017-1021.

Rosenberger, D.; Moshal, K.S.; Kartha, G.K.; Tyagi, N.;Sen, U.;, Lominadze, D.;Maldonado, C.;Roberts, A.M and Tyagi, S.C.2006. Arrhythmia and neuronal /endothelial myocyte uncoupling in hyperhomocysteinemia. Arch Physiol Biochem., 112(4-5):219-27.

Rosenberger,D.; Tyagi,N.; Rodriguez,W. and Steed, M.2007. Hyperhomocysteinemia, oxidative stress and arrhythmia-risk factors for cardiac evevts:4AP4-2. Clinical and Experimental Circulation., 24: p44.

Rosenberger, D.; Gargoum, R.; Tyagi, N.; Metreveli, N.; Sen, U.; Maldonado, C and Tyagi, s.2011. Homocysteine enriched diet leads to prolonge QT interval and reduced left ventricular performance in telemetric monitored mice. Nutr Metab Cardiovasc Dis. ,21(7):492-498.

Sahi , A.; Pan ,X .; Paul, R.; Malladi , P .; Kahli, R and Whitington, F.2006. Roles of phosphotidylinsitol 3- kinase and osteopontin in steatosis and aminotransferase roles release by hepatocytes treated with methionine –choline deficient medium. Am. J .Physiol. Gastro. Intest .physiol.liver Physiol., 291(1)55-62.

Suematsu, N. ;Ojaimi ,C.; Kinugawa ,S.; Wang,Z. ;Xu, X.; Koller ,A.; Recchia , F.A. , and Hintz ,T.H. 2007. Hyperhomocysteinemia alters cardiac substrate metabolism by impairing nitric oxide Bioavailability through oxidative stress. Circulation. 115:255-262.

Snedecor, G.W and Cochran, W.G. 1973 .Statistical Methods. 6th the Iowa State University Press. 238-248.

Solaro, R.J and Rarick, H.M. 1998. Troponin and tropomyosin. Proteins that switch on and tune in the activity of cardiac myofilaments. Circ. Res. 83:471–480.

Solaro R.J.2001.Modulation of cardiac myofilament activity by protein phosphorylation. In: Hand book of Physiology .Oxford University Press, New York: pp 264–300.

Soni, C.V.2012. Hyperhomocysteinemia alters sinoatrial and atrioventricular nodal function : role of the cardiac NMDA receptor. http://digital. Library.louisville. edu/cdm/landingpage/collection/etd/.

Szabo, Z.; Harangi, M. and Lorincz, I. 2005. Effects of hyperlipidemia on QT dispersion in patients with ischemic heart disease. Can. J. Cardiol ., 21:847–850.

Tezcan,F.;Guletkin,-Ozguven,M.;Diken,T.Ozcelik,B. and Erim,F.B. 2009. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices.Food Chem., 115(3):873-877.

Tounyz, R and Schiffrin, E. 2008. Reactive oxygen species and hypertension antioxidants and redox signaling . Eng. J . Med., 10(6):1041-1044.

Tyagi N, Vacek JC, Givvimani S, Sen U, Tyagi SC.2010. Cardiac specific deletion of N-methyl-d-aspartate receptor 1 ameliorates mtMMP-9 mediated autophagy/mitophagy in hyperhomocysteinemia. J. Recept Signal Transduct Res., 30(2):78–87.

Vacek,T.P.;Vacek,J.C. and Tyagi,S.C.2012.Mitochondrial mitophagic mechanisms of myocardial matrix metabolism and remodelling. Arch. Physio. Biochem.,118(1):31-42.

Viladomiu, M.; Hontecillas, R .;Lu, P and Bassaganya-Riera1, J. 2013. Preventive and Prophylactic Mechanisms of Action of Pomegranate Medicine, Article ID 789764, 18 pages.

Viuda-Martos,M.;Fernandez-Lopez,J.and Perez-Alvarez, J.A.2010. Pomegranate and its many functional components as related to human health : Areview. Comprehensive Reviews in Food Science and Food Safety. 9: 635-654.

Vroegrijk, O.C.M.; Van Diepen, J.A.; Vvan den Berg, S and et al. 2011.Pomegranate seed oil, a rich source of punicic acid, prevents diet-induced obesity and insulin resistance in mice," Food and Chemical Toxicology. 49, (6): 1426–1430.

Zhang,L.H.;Li,L.L.;Li,Y.Z.and Zhang,Y.H.2008.In vitro antioxidant activities of fruits and leaves of pomegranate. Acta Hort., 765:31-34.

Zhou, J.and Austin, R.C. 2008. Contributions of hyperhomocysteinemia to atherosclerosis: Causal relationship and myocyte contractility in hyperhomocysteinemia. Am. J. Physiol Heart Circ. Physiol., 295(2):H890–97.

White, A. ; Hang, X and Jabling, M. 2001. Homocysteine pontentiates copper and amyloid, beta peptide – mediated toxicity in primary neural cultures , possible risk factors in the Alzheimer – type neural generative pathways . J. Neuro. Chem., 78: 1509-1520.