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DETERMINE THE PREVENTIVE EFFECT OF POMETONE (POMEGRANATE SEED OIL) ON CARDIAC FUNCTION IN METHIONINE OVERLOAD IN TREATED FEMALE RABBITS

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ABSTRACT

This study was designed to investigate the 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 in each group) and treated for 42 days daily as follows: The first group were drenched corn oil, serving as control (group C), the second group (group T_1) were intubated orally with methionine 100mg/kg. B.W, while thethird groups (groupT_2) were intubated orally with methionine 100mg/kg. B.Wand pomegranate seed oil 30 mg /Kg. B.W, while theanimals in the groupT_3were intubated orally with pomegranate seed oil 30 mg /Kg. B.W. The electrocardiographs (ECG) were recorded for rabbits in allexperimental groups at the same interval of the experiment, The analysis of ECG in rabbits treated with methionine (T_1) 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. On the other hand, the cardioprotective role of pomegranate seed oil was clarifiedin animals of groupT_3, but it was less effective in methionine treated rabbits, where the oil fail to restore P, P-Q, Q-T wave in group T_2 to normal level. In conclusion, the results of this study clarified the deleterious effect of methionine overload on electrical conduction of the heart represented by abnormality of ECG component, as well as the present study confirms the cardioprotective role of pomegranate seed oil as antioxidant by ameliorating effect of methionine and keeping the normal shape of QRS wave and prevent prolongation of Q-T interval.

KEYWORDS: Pomegranate seed oil, Methionin overload, Antioxidant, oxidativee stress, cardiac muscle.

INTRODUCTION

Methionine is an essential amino acid found in both animal and plant proteins converted via enzymatic trans] methylation to homocysteine(Hcy) Methionine is a lipotropic and protective factor against various types of liver damage.Hcy level in the circulation will be elevated **(Oz *etal*.,2008).** The active form of methionine S-adenosyl methionine(SAM)reported as the major biological methyl donor required for numerous cellular processes, including the formation of proteins, nucleic acids, epinephrine, melatonin, phosphatidylcholine and creatine**(Stipanuk, 2004)**. 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), New suggestion revealed the role of HHcy in the induction lipid peroxidation and oxidative damage through way of hydrogen peroxide (H_2O_2) 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, peripheral vessels and deep venous thrombosis **(Sundstrom and Vasan, 2004;Suematsu*et al*., 2007and Hillenbrand *et al*.,2008)**Many of the diseases are mainly linked to oxidative stress due to free radicals **(Dayanand*aet al*., 2010)**. Oxidative stress is defined as an imbalance between higher cellular levels and reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide and nitric oxide (NO) **(Carmeli*et al*., 2009)**. The ROS-induced oxidative stresses in cardiac and vascular myocytes have been linked with cardiovascular tissue injury, ROS-induced oxidative stress plays a role in various cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure**(Kukreja and Hess, 1992)**.Pomegranate seeds are by-products of pomegranate juice industry are about 20% (w/w) of the whole fruit. **(Tehranifar *et al*., 2010 and Mohagheghi *et al*., 2011)**. Recent studies found that pomegranate seed may have the potential to be a good source of nutrients and antioxidants.

(Strohacker and Kueht., 2009). One of the main causes that lead to atherosclerosis is the development of an abnormal lipid profile. In one study, fifty-one patients with abnormal lipid profiles consumed **400 mg** of pomegranate seed oil twice daily for four weeks, by the end of the trial, not only were their triglyceride levels lower by **62 mg/dL**, but their HDL cholesterol levels had risen by more than **5 mg/dL**; control patients' beneficial HDL levels fell **(Mirmiran*et al.*, 2010).** Animal studies show that pre-treatment with pomegranate extracts can protect cardiac cells from death that arises from excessive adrenaline-like compounds and from damage by the potent oxidant-inducing chemotherapy drug doxorubicin **(Mohan*et al.*, 2010 and Hassanpour Fard *et al.*, 2011)**.

Pomegranate extracts improve EKG abnormalities and elevation of serum markers of heart muscle injury It's hardly surprising that*para-oxonases* activity,(PON1), which reflects serum antioxidant activity, should be closely correlated with endothelial function**(Pasqualini*et al.*,2005; Cakatay*et al.*,2008 ; Irace*et al.*,2008 andYildiz*et al.*,2008)**

MATERIALS AND METHODS

Materials

Chemicals

All chemicals and reagents used in this study are shown in Table(1) with their suppliers.

Chemicals	Suppliers
Electrode gel	Naturel(Turkey)
L-Methionine	Segma company, U. K.
Pometone (pomegranate seed oil) capsuls	VitanPharma (Germany)

Instruments

Instruments	Manufactures
Electrocardiograph	Nihonkohden (Japan)

Animals

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 Department of Anatomy/ College of Veterinary Medicine -University of Baghdad for the period from November 2012 up toFebruary2013.They were left for two weeks for adaptation with the experimental conditions. Animals had free access to water and standard pellet diet along the experimental period.

Experimental design

Thirty two adult rabbits were randomly divided in to four equalgroups (8/ in each group), and they were treated daily as follows for 42 days as follows:-

1-Group C: -rabbit in this wereadministrated ordinary corn oil orally, serving as control **(C)**

2-Group T_1:-rabbit in this group were treated with methionine(100 mg/kg BW) orally **(Seshadri& Robinson, 2000**).**(Segma)**

3-Group T_2:- Rabbit in this group were treated with methionine (100mg/kg BW) plus pometon(30mg/kg BW)

4-Group T_3:-rabbit in this group were treated with pometone(30 mg/kg BW).

Methed

Preparation of Animals for Recording ECG

The rabbits were placed on a table and then immobilized by ligation the four limb and they were left about 5-10 minutes to get calm. The rabbits were not anesthetized. All recordings were made on the same day. Electrodes were attached to the skin at the triceps brachial muscle (coputlongum and copout lateral) of the right and left limbs and biceps femoral muscle of the right and left hips. Electrode gel was rubbed into the skin in the area where the alligator clips were attached. ECGs were recorded by a direct writing electrocardiogram (NihonKohden,Co., LTD Germany). All ECGs were standardized at 1mV=10mm, with a chart speed of 50mm/sec. Lead I were recorded at 0,21,24days.

Statistical analysis

Statistical analysis of data were performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using Least Significant Differences (LSD) as described by **Snedecor and Cochran (1973).**

RESULTS AND DISCUSSION

P-Wave interval and amplitude

Data pertaining to the mean value of P-wave interval (sec) and P- wave amplitude (mv) of control group (C) and three treated groups (T_1),(T_2) and (T_3) were explained in table (8),Statistical differences were absent between groups during pretreated period. However, after 21and 42 days of intubation of rabbits with methionine (T_1),methionine plus pomegranate seed oil (T_2) and pomegranate seed oil (T_3), there was non-significant differences in mean values of P-wave interval and amplitude 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 T_1 (0.11±0.01) at 42 days of the experimental comparing to control and T_2 groups with the mean value (0.16±0.02) and (0.18±0.01).

Effect of pomegranate seed oil on P wave interval(sec) and amplitude(mv) in electrocardiogram lead II of methionine overload treated female rabbits

Groups days	C		T1		T2		T3	
	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 Aa
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

L.S.D for p interval: 0.02L.S.D for p m.v: 0.05,Value express as mean ± SE,- n=8
C: control group given corn oil, T1: Animal receivedmethionine only 100mg /gm BWT2: Animal receivedmethionine 100mg / kg BW + pometone 30 mg / kg BWT3: Animal recived30 mg / kg BWpometone
Small letters denote within group difference p<0.05.
Capital letters denote between groups difference p<0.05

QRS complex interval (sec) and amplitude (mv)

The mean value of QRS complex wave interval (sec.)and amplitude (mv) of ECG of four experimental groups are depicted, Statistical analysis indicated that the mean value of this parameter in all experimental groups was not significantly differ at pretreated period. The data in the table illustrated a significant ($p<0.05$) decrease in the mean values of QRS complex Amplitude (mv)in experimental groups T_1 and T_2, after 42 days of the experiment as compared with other treated group (T_3) and control group. The QRS-complex amplitude values were 0.31±0.01, 0.40±0.02, 0.46±0.02 and 0.44±0.08 for T_1, T_2 T_3 and control respectively. While no significant differences in the mean values of QRS-complex between control and group T_3 at 21 days of the experiment.On the other hand treatment of rabbits with methionine overload (group T_1) and methionine plus pomegranate seed oil (groupT_2) at 42 days 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 groups T_3. The results have also clarified that treatment rabbits with methionine plus pomegranate seed oil did not cause significant 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 T_3 at 42 days as compared to control group. A significant($p<0.05$) increase were manifested with in the time in the mean value of QRS interval and a significant($p<0.05$) decrease in QRS amplitude in T1 and T2 groups after 42 days of the experiment as compared to pretreated period.

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

Groups Days	C		T1		T2		T3	
	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 a	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

L.S.D for QRS interval: 0.002, L.S.D for QRS m.v: 0.01, Value express as mean ± SE,- n=8
C: control group given corn oil, T1: Animal receivedmethionine only 100mg /gm BW T2: Animal receivedmethionine 100mg / kg BW + pometon 30 mg / kg BW T3: Animal received 30 mg / kg BWpometon.
Small letters denote within group difference p<0.05.
Capital letters denote between groups difference p<0.05

T-Wave interval (sec) and amplitude (mv)

T- wave interval (sec) and amplitude (mv) in rabbits after daily intubation with 100mg/kg B.W of methionine overload (T_1), methionine overload in combination with pomegranate seed oil (T_2) and pomegranate seed oil for 42 days as well as in the control group(C) was clarified in table (10).The result manifested that there were no significant differences in the mean value of these parameters at zero timeas compared to each other. After 21,42 days from started 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 of in methionine overload treated (group T_1) when compared to control and other treated group(T_2 and T_3).Besides significant ($p<0.05$) decrease after21 and 42 days in the mean value of this parameter was observed in group treated with pomegranate seed oil(T_3), compared with the same time of the experiment. Also there were no significantdifferences in the mean values of T-wave amplitude for T_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. Within the time, and at

the end of the experiment significant ($p<0.05$) increase in the mean value of T-wave interval (sec) were observed (0.116±0.02), and (0.94±0.02), for $T_1$and T_2groups ascompared to the pretreated period whilesignificant ($p<0.05$) decrease in the mean value ofwave interval (sec) was observed in (T_3) when compared with pretreatedperiod with mean values0.058±0.001 and 0.081±0.001 respectively.

Effect of pomegranate seed oil on T wave interval(sec) and amplitude(mv) in electrocardiogram lead II of methionine overload treated female rabbits

Groups days	C		T1		T2		T3	
	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

L.S.D for T interval: 0.01, L.S.D for T m.v: 0.02, Value express as mean ± SE,- n=8
C: control group given corn oil, T1: Animal recivedmethionine only 100mg /gm BW T2: Animal revivedmethionine 100mg/kg BW + pometon 30 mg / kg BW T3: Animal received 30 mg / kg BWpometon only
Small letters denote within group difference p<0.05.
Capital letters denote between groups difference p<0.05.

P-Q and Q-T interval

Illustrated the mean values of P-Q and Q-T interval in the control and treated groups. Statistical analysis indicated that the mean value of the experimental groups was not significantlydifferat pretreated period. After 21 and 42 days of the treatment with methionine (T_1 group) a significant($p<0.05$) increase in P-Q interval was observed compering to control and group which treated with pomegranate seed oil (group T_3),while Q-T interval show a significant($p<0.05$) in T1 group as compared to control, T_2 and T_3 groups at 42 days of experiment.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 (T_2) after 42 days of the experimental as compared to group T_1 with mean valuesof 0.106±0.001, 0.140±0.004, 0.121±0.001 and 0.170±0.004 for T_2 and T_1 respectively. Depending on statistical results, each of pomegranate seed oil (group T_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.Within the time a significant ($p<0.05$) increase in these parameters (P-Q and Q-T) were observed in T_1 and T_2 treated groups at the end of the experimental compared to pretreated period. Besides, there was no significant difference in the mean values of P-Q and Q-T interval in T3 treated group at 21 and 42 days of the experiment compering to pretreated period.

Table (4-11): Effect of pomegranate seed oil on P-Q and Q-T wave interval(sec) in electrocardiogram lead II of methionine overload treated female rabbits.

Group days	C		T1		T2		T3	
	P- Q int	Q-T int	Q-T int	P- Q 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	0.130±0.003 A b	0.078±0.001 A c	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	0.130±0.004 A b	0.089±0.003 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	0.170±0.004 A a	0.121±0.001 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

L.S.D for P-Q interval: 0.005, L.S.D for Q-T interval: 0.006, Value express as mean ± SE,- n=8
C: control group given corn oil, T1: Animal receivedmethionine only 100mg/gm BW T2: Animal receivedmethionine 100mg / kg BW + pometon 30 mg / kg BW T3: Animal received 30 mg / kg BWpometon
Small letters denote within group difference p<0.05.
Capital letters denote between groups difference p<0.05

DISCUSSION

Cardiovascular disease is the cause number one of cardiac death. One causes of cardiac death is the struck, cardiac arrhythmia and vascular embolism, spasm and dementia due to de-arrangement in the Hcymetabolism and its clearance. Defective metabolism of the essential amino acid methionine, result in HHcy or situation HHcy(after a methionine overload), and is established as independent risk factor for atherosclerotic heart disease **(Den *et al*., 1996 and Matthias *et al*., 1996).**

The facts obtained from this in vivo study have exposed statistically significant ECG and heart rate alterations following methionine overload treatment. In explanation of these changes, it is essential to note the results that emphasize the relation between the protective role of

PSO, methionine overload and significant cardiac risk factors. **(Guyton, 1991).** 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).**

The results clarified that there was an increase in T-interval and Q-T interval in methionine overload treated group (T1) compared to control, T2 and T3 groups. Besides, the results showed morphological changes in ECG diagram (figure) in group T1 at 21 and 42 days of experiment manifested by decrease QRS interval and increase in QRS mv. 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; Rosenberger *et al*., 2011 and Acampa*et al*., 2011).**Changes in Q-T interval observed in methionine overload in group T1 may be have a direct effect of HHcy and hypercholesterolemia on ventricular repolarization by delay the activation of potassium channels as result of oxidation of cortical SH- group of channel protein resulting in hypokalemia and then Q-T intervalprolongation (**Kumar*et al*., 2009)** or due to altering the contractile property of rabbits myocardium **(Peterson *et al*., 1979).**The induced HHcyafter methionine overload maycause activation of matrix metalloproteinase and reduction of cardiac gap junction play an important role in the path mechanism of cardiac remodeling in moderate HHcy.

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).**Q-T interval represented the repolarization of myocardium and K+ channels set the membrane potential as well as the excitability of most living cells. NO has been established as an important molecules in the regulation of cardiovascular function. The ability of NO to regulate myocardial O2 consumption through the inhibition of cytochrome C oxidase in mitochondria has been well established (**Cleeter*et al*., 1994 and Trochu*et al*.,2000).**

Becker***et al*., (2005)** demonstrated that HHcy impairs the regulation of cardiac O2 consumption by NO and this effect proportional to plasma Hcy level and indicate that the inactivation of NO by elevated of NADPH oxidase activity has major impact on cardiac metabolism beyond that caused by impaired vasodilation. It seems reasonable that methionine overload induce Q-T prolongation in this study may be due to change in K^+ conduction in myocardium. The increase in Q-T interval in group T2 was lesser than Q-T interval in rabbits treated with methionine (group T1), suggesting the preventive and beneficial role of pomegranate seed oil to attenuation the repolarization characteristics of rabbits exposed to methionine overload. The $K^+$is responsible for the long Q-T. NO, is essential for the proper functioning of cardiovascular system, is derived from L-arginine by NOS in endothelial cells, NOS inhibition produces various cardiovascular abnormalities and ventricular contractile dysfunction **(Kojda*et al*., 1999).**The absence of eNOSinhomocysteinemic rats precedes activation of matrix metalloproteinase (MMP), reduce left ventricle load and increase the mean arterial pressure (MAP)**(Soode*t al*., 2002).** The mechanism for reduction in Q-T interval in rabbits in group T_2 in this experiment is not known. But it can be speculated that pomegranate seed oil may be increased NO and in turn alter the activity of ATP dependent K^+ channels hence repolarization. So, the reduction in Q-T prolongation in T_2 treated group may lead to reduction in the rate of arrhythmias.Meanwhileexplained that Hcy enriched diet lead to prolongation of Q-T interval. The increase in Q-T interval was lesser in methionine supplemented pomegranate seed oil (group T_2), suggesting the preventive and beneficial role of pomegranate seed oil in methionine overload rabbits induced repolarizations characteristics and reduce left ventricular performance **(Rosenberger *et al*., (2011)**

CONCLUSIONS

From the results obtained and discussed in this study, it can be conclude the following:

1- Intubation of female rabbits with 100mg/kg. B.W of methionine caused a case of cardiovascular dysfunction and abnormality in electrocardiograph pattern manifested by:

A- Significant prolongation in Q-T interval.

B- Significantprolongation of QRS interval and significant shorting in QRS mv.

2- Intubation the animals with pomegranate seed oil 30 mg/kg B.W. ameliorate the oxidative damage induced by methionine owing to its antioxidant properties.

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