The Role of Pometone (Pomegranate seed oil) in Ameliorating the Deleterious Effect of Methionine Overload on Some Histological Aspects of heart and aorta in Female Rabbits (Part-II)

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Summary

This experiment was aimed to investigate the role of pomegranate seed oil (PSO) in ameliorating the deleterious effects of methionine overload on some histopathological structure of heart and aorta in adult female rabbits. Thirty-Two female rabbits divided into four groups eight animals each, and treated for 42 days daily as follows: the first groups were drenched drinking corn oil, serving as control (group C), second group (group T1) were intubated orally with methionine 100mg/kg. B.W, while the third group (groupT2) were intubated orally with methionine 100mg/kg. B.W and pomegranate seed oil (PSO) 30 mg /Kg. B.W, and the animals in group T3 were intubated orally with pomegranate seed oil 30 mg /Kg. B.W. At the end of the experiment rabbits were sacrificed. Serial sections from the heart and aorta were prepared and examined microscopically. Histological examination of heart and aorta of methionine overload treated group (T1) showed edema, RBCs and few neutrophils infiltration, with vacuolar degeneration of cardiac muscle cells, fragment of muscle fiber, congested blood vessels between muscle fibers. An increase in thickness of intima, erosion and mononuclear cells infiltration in sub intima of aorta were also observed. Histological sections of heart and aorta in T2 and T3 groups showed the absence of histopathological lesions in aortic tissue with moderate edema between muscle fiber of T2 group as comparing to group T1. In conclusion, the results confirm the cardioprotective role of pomegranate seed oil by ameliorating the effect of methionine overload on cardiac muscle and aorta.

Keywords: Pomegranate seed oil, Methionine overload, Cardiac muscle, Aorta.

Introduction

Methionine is required for much physiological process. In principle excess could lead to generation of toxic sulfur metabolites (1). The richest sources of methionine is proteins of animal origin (2 and 3). It is used to prevent liver damages due to different agents like acetaminophen poisoning, methotrexate, lipid accumulation in the liver and improve the flow of bile and hepatic lipidosis (4-6). Homocysyeine (Hcy) is a sulfur containing amino acid extensively formed as an intermediate product in metabolism of methionine. Methionine in the diet is the main source of hyperhomocysyeinemia (HHcy) in the blood (7). HHcy is a condition in which the regulation of intracellular Hcy level is disrupted and Hcy export to the plasma compartment is accelerated (8). 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 (9-11). Chronic alcohol intake (12 and 13), smoking cigarettes (14 and15), hypothyroidism and malignancies (16 and 17) and coffee consumption (18and19) are seems to be linked with elevation in Hcy levels.

Many evidence demonstrated that there is a link between oxidative stress and cardiovascular disease, ROS-induced oxidative stress and plays a role in various cardiovascular diseases such as atherosclerosis, ischemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure (20-23) and abnormalities in myocyte function (24 and 25).

Pomegranate seed oil (PSO) is a potent source of punicic acid, an Omega 5 conjugated fatty acid, beneficial phytoestrogen and a rare plant based source of CLA (conjugated
linoleic acid). The CLA in pomegranate seed oil is an especially potent anti-inflammatory and anti-carcinogenic agent (26). Mirrman et al., (27) explained that patients consumed pomegranate seed oil caused a significant decrease in their serum triglycerides and LDL-cholesterol level with risen in HDL-cholesterol concentrations (28). Consumption of pomegranate juice reduced progression of arterial wall thickening among patients with the highest degree of oxidative stress and the most severe disturbances in their lipid profiles (29). The role of PSO against methionine overload has not so far been studied. Therefore, this study was carried out to investigate: The histological alteration in heart and aorta induced by methionine overload in female rabbits and the role of PSO in protecting the heart and aorta against the induced changes.

**Materials and Methods**

Thirty-two adult female local rabbit with a body weight ranging from 1250 to 2000 g. Rabbits were housed in cages in air 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 February 2013. They were left for two weeks for adaptation with the experimental conditions. Rabbits were fed the standard balanced pellets and supplied with tap water *ad libitum*. Thirty-Two female rabbits were divided into four equal groups, eight animals each, and treated for 42 days daily as follows: the first group were administered corn oil, serving as control (group C), second group (group T1) were intubated orally with methionine 100mg/kg. B.W, while rabbits in the third group (group T2) were intubated orally with methionine 100mg/kg. B.W plus pomegranate seed oil (PSO) 30 mg /Kg. B.W, and the animals in (group T3) were intubated orally with pomegranate seed oil 30 mg /Kg. At the end of the experiment rabbits were sacrificed. The abdominal wall was opened longitudinally, the heart and aorta were taken and fixed tissue sections were processed by using histological techniques of tissue preparation for histopathological examination changes (30). Histological sections 5 μm thick were stained with hematoxyline and eosin. Light microscope, photographs of histological sections and digital camera was used.

**Results and Discussion**

Comparing to control group (fig.1) histological sections of heart of rabbits exposed to methionine (group T1) showed edema, RBCs, and inflammatory cell particularly neutrophils between bundle of cardiac muscle in addition to vacuolation in the cytoplasm of the muscle cell of myocardium (fig. 2), sever congestion and fragment of the myocardium were observed (fig.3). Congestion of the capillary blood vessels between muscle fiber in addition to facular degeneration and fragmentation of muscle fiber (fig. 4, 5 and 6) were also observed in group T1. While microscopic examination of the cardiac muscle of rabbits treated with methionine plus PSO for 42 days (group T2) showed moderate edema between muscle fiber (fig.7) and no clear lesion were observed in the same group (fig.8) comparing to the methionine treated group Heart of the rabbits belongs to group of animal treated with PSO only (group T3) along the experimental period manifested normal structure of heart, besides no clear lesions were noted (fig. 9).

The microscopic study of the aorta in group T1 which received methionine for 42 days showed histological changes represented by increase thickness of intima due to aggregation of facular cell in subintema (Fig.11), and an erosion of intima and inflammatory cells infiltration in subintema of the aorta (Fig. 12) comparing to the control (Fig.10). Section of aorta in rabbits of group T2 received methionine plus pomegranate seed oil (Fig.13 and 14) manifested normal structure of aorta and no clear lesion were observed comparing to the control group (Fig. 10). Besides, section of rabbits treated with pomegranate seed oil (group T3) showed no clear lesion (Fig. 15) comparing to the aorta of control group (Fig. 10). From the above results for histological section of the heart and aorta of rabbits of group T2 it should be manifested that pomegranate seed oil decrease the deleterious effect of the methionine over load on heart and aorta.
Figure 1: Section in the cardiac muscle of rabbits of control group. Note: no clear lesions (H&E stain 40X).

Figure 2: Section in the cardiac muscle of rabbit treated with methionine over load (T1) for 42 day show edema, RBCs and few neutrophils infiltration between cardiac muscle bundle, in addition to vacuolar degeneration of muscle cells (H &E stain 40X).

Figure 3: Section in the cardiac muscle of rabbit treated with methionine over load (T1) for 42 days show edema and severe congestion of blood vessels between cardiac muscle cells (H &E stain 40X).

Figure 4: Section in the cardiac muscle of rabbit treated with methionine over load for 42 days (group T1). Note: inflammatory cells infiltration particularly neutrophils and macrophages and congestion capillaries blood vessels in addition to vacuolar degeneration and fragment of muscle fiber (H &E stain 40X).

Figure 5: Section in the cardiac muscle of rabbit treated with methionine over load at the end of experiment (group T1). Note: congestion of blood vessels with inflammatory cells in their lumen as well as vacuolar degeneration of muscle cells (H &E stain 40X).

Figure 6: Section in the cardiac muscle of rabbit after 42 day of treatment with methionine over load (group T1). Note: congestion of blood vessels with inflammatory cells infiltration and fragment of muscle fiber (H &E stain 40X).
Figure 7: Section in the cardiac muscle of rabbit after 42 days of treatment with methionine overload and pometon (group T2). Note: moderate edema between muscle fiber (H &E stain 40X).

Figure 8: Section in the cardiac muscle of rabbit after 42 day of treatment with methionine overload and pometon (group T2). Note: congestion and no clear lesions (H &E stain 40X).

Figure 9: Section in the cardiac muscle of rabbit treated with pometon (PSO) only for 42 day (group T3). Note: no clear lesions (H &E stain 40X).

Figure 10: Section in the aorta of rabbit of control group shows no clear lesions (H&E stain 40X).

Figure 11: Section in the aorta of rabbit after 42 day of treatment with methionine overload (group T1). Note: increase thickness of intima due to vacuolar cells aggregation in subintima (H &E stain 40X).

Figure 12: Section in the aorta of rabbit after 42 of treatment with methionine overload (group T1). Note: increase thickness of intima, erosion and mononuclear cells infiltration in subintima (H &E stain 40X).
The present study pointed that intubation of rabbits with methionine (group T1) for 42 days, resulted in the appearance of athermanous lesion with structural changing in the aorta, suggesting that methionine overload in rabbits may be evoked HHcy, which impairs endothelium –dependent relaxation of vessels produced by mechanism associated with increased oxidative stress. The functional role of oxidative stress induced by free ROS like H2O2 in endothelial dysfunction and the resultant atheroma was reported (31-33). Besides, the occurrence of oxidative stress with methionine overload and corresponding a state of HHcy induced ROS (34).

The development of typical atherosclerotic or pre-atherosclerotic in all homocysteinemic animals is composed of proliferation of smooth muscle cells surrounded by large amount of collagen, elastic fibers, glycosaminoglycan and sometimes lipid (35 and 38), whereby HHcy leads to atherosclerosis and increased collagen production in a dose-dependent manner (37). In addition to an increase the arterial wall thickness, lead to loss of elasticity (38). Similar results are seen with HHcy which caused elastinolysis and decrease elastic compliance of the vessels (39), through an increase in inducible NOS activity as a key contributor to HHcy – mediated collagen / elastin switch and leading to decrement in aortic compliance. Additionally, HHcy is associated with vascular disease in general, but particularly in subjects with significant carotid stenosis (40).

HHcy stimulates the expression of myocytes to chemotactic protein -1, vascular cell adhesion molecule -1 and E-selection, this lead to increase monocyte adhesion to the aortic endothelium, which may contribute significantly to the development of atherosclerosis (41). It has been reported that...
HHcy in rats produced by folate depletion impairs endothelium-dependent relaxation and is accompanied by increased arterial permeability and arterial stiffening (42). In contrast, the lesion observed in aorta and heart of rabbit supplementation with pomegranate seed oil for 42 days showed a lesser extent than in methionine overload rabbits (group T2).

Pomegranate seed oil may had a potential antiatherogenic effect and reduce the size of lesion due to its antioxidant properties (43 - 45) which cuased significant reduction in oxidative stress by inhibiting lipid peroxidation (43 and 46). The antioxidant capacity of PSO to alleviate the lesions in aorta and heart in group T3 may be due to photochemistry and pharmacological actions of pomegranate compounds which suggest a wide range of clinical application for the treatment and prevention of disease (47). Pomegranate reduced oxidation of serum LDL and increased PON-1 activity (48) due the high concentrations of polyunsaturated fatty acids (PUFA) such as linoleic acid and linoleic acid, as well as other lipids (49 - 51). As well as, both punicalagin and punicalin can be hydrolyzed to ellagic acid; a natural phenol with high antioxidant activity, thus prolonging the release of this acid into the blood (46) may increase the antioxidants properties of PSO against atherosclerosis (52).

Furthermore, pomegranate fruit Pomegranate extract (PFE) rich in polyphenolic antioxidant, reduces the expression of oxidation – sensitive genes and increasing endothelial NO synthase (ENOS) (53), reduction in an aortic sinus and coronary artery atherosclerosis in mice by PSO has been documented (54). As well as it’s a meliorative effect on abnormality of ECG components induced by methionine over load has been proved in rabbits (55).

Recently, consumption of Pomegranate juice (PJ) by atherosclerotic mice caused significantly reduced cholesterol accumulation, foam cell progression of atherosclerosis. This may be due to the impressive inhibitory effect of pomegranate juice on macrophage cholesterol uptake and accumulation of cholesterol ester as evidence by attenuation of cholesterol influx and by enhanced macrophages cholesterol efflux (56).

References


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