

Ellagic Acid (Pomegranate Extract) as Potent Antioxidant for
Myocardial Infarction (MI) *in vitro* Study

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Abstract:

The production of reactive oxygen species in most diseases including heart disease is confirm to be the most destructive process to human cell. Potent antioxidant is needed to overcome the lipid peroxidation and oxidative stresses. The effect of ellagic acid (EA) [pomegranate extract] on some metabolic enzymes were investigated in patients with myocardial infarction(MI).

Two ml of venous blood was obtained from thirty patients (10 male&20 female) with MI and 30 healthy individuals (10 male&20 female) as control group, the age of both genders range from 40-65 years. Serum used to determined activities of creatine kinase(CK), aspartate transaminase (AST) and alanine transaminase(ALT).

Elevated values of CK, AST and ALT activities in patient's were found (368.66± 66.63 , 90.93±13.8 , 97.83± 32.76) respectively. These activities returned to about normal values after addition of 10 µM ellagic acid (250± 72.07 , 58.3 ±14.48 , 64.77± 20.26) respectively.The results demonstrated no significant difference in results between the both genders.

A conclusion of the present study revealed the effectiveness of ellagic acid in treatment of patients with MI *invitro* study which ellagic acid act by different mechanisms to protect and prevent heart diseases.

Key word :Ellagic Acid , Pomegranate Extract , Myocardial Infarction

Introduction

Heart disease is any disorder that affects the heart's ability to function normally. Various forms of heart disease include : arrhythmias, cardiogenic shock, congenital heart disease, coronary artery disease, heart tumor, heart attack and heart failure [1].

The most common heart disease in the world is coronary heart disease, which can lead to heart attack[2] .

Some enzymes are commonly used in the diagnosis and follow up of heart disease as creatine kinase (CK), aspartate transaminase (AST) , alanine transaminase (ALT) and lactate dehydrogenase (LDH)[3].

Creatine Kinase (CK) is more abundant in cells of cardiac and skeletal muscle and in brain .Plasma enzyme activity are raised in about 95 percent of cases myocardial infarction . The degree of rise is a very rough indicator of the size of infarct[4,5].

Aspartate transaminase(AST) and alanine transaminase(ALT) release are found in many acute pathologies affecting the liver and heart. Determination of AST and ALT used in the diagnoses of heart disease, because the enzyme escapes into the serum from damaged heart cells . Increased of ALT is found only in a minority of patients in the period following a myocardial infarction [6].

Ellagic acid (EA) (3,4,3',4' - tetra) hydroxyl benzopyrano [5,4,3-c,d,e] benzo pyran -6,6- dione is a complex planer molecule,which containing four hydroxyl groups and two lactone groups [7].

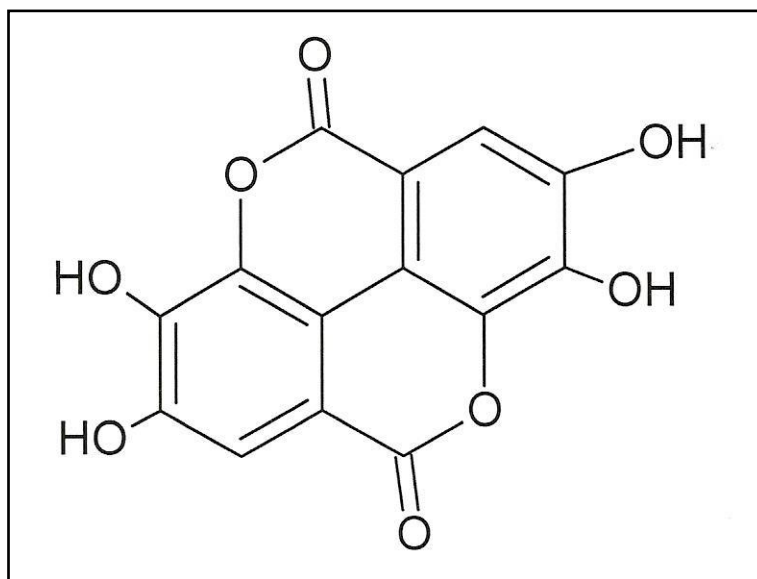


Figure (1): The Structure of Ellagic Acid[7].

There are relationship between the hydroxyl and lactone groups of ellagic acid and its role in reducing the risk of many diseases which lactone cyclic involved carbonyl group that is in resonance with hydroxyl group of ellagic acid which led to easily losing of a hydrogen atom from hydroxyl group,so ellagic acid acts as a free radicals scavenger [8].

Ellagic acid is antioxidant polyphenol found in numerous fruits and vegetables including raspberries strawberries, cranberries, walnuts, pecans and pomegranates[9]. Ellagic acid and glucose combine to form ellagitannins, which are water soluble compounds that are easier for animals and humans to absorb into their diets[10].

Researches indicate that Ellagic acid can prevent, treat, or cure specific disease as cancer, heart disease, liver disease, high blood pressure and bone disease [11].

Ellagic acid, a polyphenol extracted from natural products (i.e. pomegranate), has been identified to have potent antioxidant [12 ,13]. Dozens of recent studies reveal the pomegranate's surprising array of benefits. Pomegranate juice was found to exhibit three times antioxidant activity more than other sources of antioxidants. The active constituent that appears to be responsible is ellagic acid, a naturally occurring polyphenolic compound in pomegranates [14].

Scientific research groups tested pomegranate juice on both humans and laboratory mice which were genetically predisposed to develop heart disease. They found that pomegranate juice decreased LDL (bad cholesterol) and increased HDL (good cholesterol) by 20% in the humans. The oxidation of LDL which is believed by many researchers to be a major cause of heart disease[15].

Some researches concluded that pomegranate can offer a wide protection against cardiovascular disease which may be attributable to its antioxidant properties[16].

Materials & Methods :

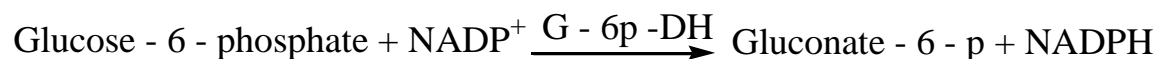
***Selection of subjects and Blood Sampling**

Two ml of venous blood was obtained from thirty patients(10 male&20 female) with myocardial infarction and 30 healthy individuals as control group(10 male&20 female), the age ranged from (40-65) years for the both groups.

Blood samples were transferred into plain tube, allowed to stand for (15) minutes at room temperature, centrifuged at 3500 rpm for (10) minutes. The resulting serum was separated and used for the estimation of CK, AST and ALT

***Determination of Creatine Kinase (CK) :**

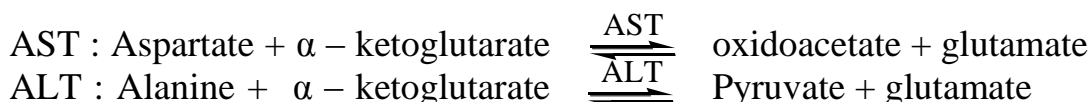
Creatine kinase was determined by coloric method according to the Rendox method [17], as the following reactions :



The blue/violet diformazan, which has an absorption maximum around 560 nm. The reaction is stopped by the addition of hydrochloric acid.

***Determination of AST and ALT :**

Coloric determination of AST and ALT activates were used by Redman and Fronkel method according to the following reactions [17].



The pyruvate or oxaloacetate formed were measured in its derivative form, 2,4 – dinitro phenyl hydrozone. The absorbance of samples were recorded against the reagent blank at 546 nm.

***Addition of Ellagic Acid (EA) in Determination of CK, AST and ALT as Treatment :-**

In this study , the effects of ellagic acid (EA) was investigated *in vitro* by addition of different concentrations of polyphenolic compound to human serum taken from patients of heart disease.

The concentration of EA was chosen to be 10 μ M among other concentrations studied for its effectiveness and this was compatible with previous study [18].

***Statistical Analysis :-**

Data presented as means and standard deviation. Study of T-Test (p) was used to compare the significance of the difference in the mean values of any groups ($p \leq 0.05$) were considered statistically significant. The overall predictive values for the results in all studied groups were performed according to biostatistics by Daniel in 1987[19].

Results and Discussion :

Table (1) summarizes the levels of CK, AST and ALT activities expressed as (mean \pm SD) before and after addition of ellagic acid (pomegranate extract) in sera of normal control and patients with myocardial infarction, *in vitro* study .

Table(1) : levels of CK ,AST,ALT activities in sera of normal control and patients with MI before and after addition of ellagic acid (pomegranate extract)

Subject	Control No.=30	Patient (Before addition) No.=30	Treatment (After addition)	T-Test
CK(U/L)	119.3 \pm 34.78	368.66 \pm 66.63	250 \pm 72.07	P\leq0.05
AST(U/ml)	34.83 \pm 7.64	90.93 \pm 13.8	58.3 \pm 14.48	P\leq0.05
ALT(U/ml)	44.83 \pm 4.74	97.83 \pm 32.76	64.77 \pm 20.26	P\leq0.05

The present study showed a significant high levels of CK, AST and ALT activities in sera of MI patients (368.66 ± 66.63), (90.93 ± 13.8) and (97.83 ± 32.76), ($p \leq 0.05$) respectively compared to control group for each enzyme (119.3 ± 34.78), (34.83 ± 7.64) and (44.83 ± 4.74), ($p \leq 0.05$).

Damage to tissues of heart, or proliferation of the cell from which these enzymes arise (CK, AST and ALT), will lead to an increase in the activity of these enzyme in serum. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance [4].

After addition of 10 μ M of ellagic acid (pomegranate extract) to serum of myocardial infarction patients *in vitro*, the levels of CK, AST and ALT activities were decreased (250 ± 72.07), (58.3 ± 14.48) and (64.77 ± 25.26) respectively, but not to the average control value.

Ellagic acid (pomegranate extract) were shown to process significant antioxidant activity in various *in vitro* models [11].

The decrease in the levels of CK, AST and ALT activities in MI patients after addition of ellagic acid can be attributed to many factors, as protects against cell damage, can lower levels of unhealthy cholesterol [10].

Some researches demonstrated that EA from pomegranate can offer wide protection against cardiovascular diseases, which could be related to its antioxidant potential, which act in different levels in the oxidative sequence involving lipid molecules. They act by decreasing oxygen concentration, intercepting single oxygen preventing first γ -chain initiation by scavenging initial radicals such as OH^\cdot , decomposing primary products to non-radical compounds, and chain breaking to prevent continued hydrogen obstruction from substrates [12,14,20].

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استخدام حامض اللاجيك (مستخلص الرمان) كمضاد قوي للأكسدة لمرضى احتشاء العضلة القلبية في دراسة خارج جسم الكائن الحي

د. زينب منيب مالك

قسم الكيمياء / كلية التربية - ابن الهيثم / جامعة بغداد

الخلاصة :-

لقد أصبح انتاج اصناف الاوكسجين الفعال نتيجة لمختلف الامراض ومن ضمنها امراض القلب دليلاً على انه من اكثر العمليات الضارة بالجسم لذا فإن الحاجة لمضادات الاكسدة أصبح ضرورياً للتغلب على هذه العملية من خلال منع عملية الاكسدة الفوقية للدهون والشد التأكسدي .

تم جمع نماذج الدم (2 مل) من ثلاثون مريضاً (10 ذكر، 20 انثى) مصابين بمرض احتشاء العضلة القلبية و ثلاثين شخصاً اصحاء (10 ذكر، 20 انثى) كمجموعة سيطرة . تتراوح اعمار المجاميع المدروسة (مجموعة المرضى والسيطرة) بين (40-60) سنة .

تم استخدام المصل في قياس فعالية كل من الانزيمات الكرياتين كينيز (CK) ،الانين ترانس امينيز (ALT) والاسبارتيت ترانس امينيز (AST) . أظهرت النتائج الزيادة في فعالية الانزيمات المذكورة عند مجموعة المرضى مقارنة بمجموعة السيطرة . تراجمت فعالية الانزيمات الى مستويات قريبة من المستويات الطبيعية في معظم الحالات بعد اضافة 10 مايكرومول حامض اللاجيك .

استنتج من هذه الدراسة فعالية حامض اللاجيك في علاج مرضى احتشاء العضلة القلبية حيث يعمل بميكانيكيات مختلفة على الحماية ومنع الاصابة بالامراض القلبية .