

"The effect of garlic vinegar on the blood of patients with high blood viscosity and biochemical variables through oxidation-antioxidant system"

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Keywords: garlic, blood viscosity, biochemical parameters, Free radicals, oxidants, antioxidants, hematological parameters.

Abstract:

Recent years have seen an increase focus on foods and its components in disease prevention. Garlic (*Allium sativum* L.), one of the best herbal remedies in researches which studied, holds important position in history. usually used to treat infection, diabetes, colds, heart disease, and a host of other disorders.

In this research, have been measurements the biochemical parameters such as lipids profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triglyceride), and hematological parameters such as PCV (packed cells volume "blood viscosity") , Hb (hemoglobin), WBC (white blood cells), platelets and MCHC (mean cell hemoglobin concentration). Also measurement the per-oxidant such as MDA (malondialdehyde) and antioxidant such as GSH (glutathione), SOD (superoxide dismutase) and catalase for the patients which have blood viscosity higher than normal levels before and after treated with the Garlic extract (Garlic vinegar).

Where it is found significant lowering in the values of PCV= 46.20, Hb= 14.66, Cholesterol= 151.4, Triglyceride= 149.04, VLDL= 25.64 and MDA= 1.73. and significance elevated in the values of SOD= 4.76, CAT= 77.44 and GSH= 25.08, where the treatment continue for 1 month in Baghdad/Iraq.

" تأثير خل الثوم على دم المرضى المصابين بارتفاع لزوجة الدم والمتغيرات البايوكيميائية من خلال نظام الأكسدة - مضادات الأكسدة "

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الخلاصة:

لوحظ مؤخرًا ازدياد التركيز على الأطعمة والمكونات الغذائية في الوقاية من الأمراض. لذا يعتبر الثوم (الآليوم بيسوم L)، أحد أهم العلاجات العشبية المأخوذة بنظر الاعتبار من حيث الدراسات البحثية، كونه حاصل على مكانة فريدة من نوعها منذ القدم كعلاج مستخدم للالتهابات، ونزلات البرد، والسكري، وأمراض القلب، ومجموعة من الاضطرابات الأخرى.

هذا البحث تضمن قياس المتغيرات البايوكيميائية مثل مجموعة الدهون الكاملة وهي: (الكوليسترول الكلي، الكوليسترول-LDL، الكوليسترول-HDL، الكوليسترول-VLDL والدهون الثلاثية TG)، والمتغيرات الدموية مثل PCV (حجم خلايا الدم الحمراء المضغوط "لزوجة الدم")، و Hb (هيموجلوبين الدم)، WBC (خلايا الدم البيضاء)، والصفائح الدموية و MCH (يعني تركيز الهيموغلوبين الخلايا). أيضا قياس المؤكسدات مثل MDA (المالون ثنائي الالديهيد) ومضادات الأكسدة مثل GSH (الجلوتاثيون)، و SOD (إنزيم السوبر اوكسيد) وإنزيم الكاتليز للمرضى المصابين بارتفاع لزوجة الدم قبل وبعد المعالجة بمستخلص الثوم (خل الثوم).

وجد إن هنالك انخفاض معنوي بعد المعالجة في قيم كل من $PCV = 46.20$ و $Hb = 14.66$ والكوليسترول = 157.40 والدهون الثلاثية = 129.72 والكوليسترول-VLDL = 25.64 والمالون ثنائي الالديهيد = 1.73 . وارتفاع معنوي بعد المعالجة قيم كل من $SOD = 4.76$ و $CAT=77.44$ و $GSH= 25.84$ ، حيث استمرت فترة العلاج لمدة شهر واحد في العراق/بغداد.

INTRODUCTION:

Diet is a main factor in the development and induce of some diseases, including cardiovascular disease. Therefore, must be suggesting consumption healthy foods such as, fruit and vegetable, because have the anti-inflammatory characteristics due to their phytochemical components [1]. One of the important these food is garlic (*Allium sativum*). The majority of garlic components is water (65%), and the most part of the dry weight is composed of fructose-containing carbohydrates, followed by sulfur compounds, protein, fiber, and free amino acids [2].

Moreover, its contains on high levels of phosphorus, potassium, saponins, sulfur, zinc, moderate levels of selenium and Vitamins A and C, and low levels of calcium, magnesium, sodium, iron, manganese, and B-complex vitamins; garlic also has a high phenolic content [3]. Newly, has been noticed increase the interest use of garlic as a preventive factor in cardiovascular diseases. Some studies showed an effect of garlic in lowering serum lipids concentration and preventing progressive atherosclerotic and consequently reducing the incidence of cardiovascular events [4-6].

The etiology of heart diseases is multi-factorial, for example, hypercholesterolemia, hyper blood viscosity, platelet aggregations hypertension, diabetes mellitus, heredity, hyperhomocysteinemia, increase in oxidative damage, and smoking as well demonstrated risk factors [7].

Also, cardiovascular disorders are related with increase production and release of inflammatory mediators, such as reactive oxygen species (ROSs), and nitric oxide [7].

Sulfur compounds are the main responsible about garlic's signature scent. When a garlic is crushing or cutting, alliin, in “odorless, sulfur-containing amino acid derivative” reacts with the enzyme alliinase to form alliin and other sulphur compounds [8,9]. Then, alliin will breaking up into diallyl disulfide, which is cause for garlic’s odor [10]. In addition, from the healthy benefits of garlic's alliin is antioxidant, anti-microbial, cholesterol-lowering and blood-thinning properties and is likely to play a role in garlic’s anti-cancer effects [11].

Oxidative stress plays active role in many diseases including arthritis, atherosclerosis, heart disease, stroke, AIDS, cancer, aging, and in programmed cell death (apoptosis) of neurons, that leads to Alzheimer’s disease and other neurodegenerative conditions [12, 13, 14].

Mammalian cells continuously produced reactive oxygen species (ROS) through various metabolic pathways. ROS are molecules that contain oxygen and have higher reactivity than ground-state molecular oxygen. These species include not only the oxygen radicals (like $O_2^{\bullet-}$, $\bullet OH$, and peroxy radicals), but also non-radical molecules such as hydrogen peroxide (H_2O_2) and oxygen (O_2). Superoxide is one from the commonest ROS and formed from the reduction of O_2 by the mitochondrial electron transport system [15]. Antioxidant systems of eukaryotic cells have the ability to convert ROS to H_2O via different cytosolic enzymes.

Oxidative stress results imbalance between the production of ROS and antioxidant capability of the target cell. In general, the lower levels of ROS are not harmful to cells, and indeed even perform useful signaling functions, but the higher levels of ROS are deleterious through covalent reactions with cellular proteins, lipids, and DNA that results in altered target molecule function. The accumulation of ROS lead to damage in biomolecule component of cells, then cause acute and chronic cell injury (procancer factors) [16,17]. Therefore, to maintenance of Cells survival must be have protection lines against oxidative stress including different intracellular antioxidant compounds, mainly Glutathione (GSH) and thioredoxin, and by other antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and heme oxygenase[18,19,20].

Garlic extracts (garlic vinegar), have antioxidant activities, and protection against free radical damage in the body. Through antioxidant properties of garlic compounds representing the four main chemical classes, alliin, allyl cysteine, allyl disulfide and allicin, prepared by chemical synthesis or purification [21].

METHODOLOGY:

Preparation of the extracts

The extraction performed by macerating 200 g from garlic in 600 mL of vinegar for one month. The macerated mixture was filtered by Whitman filter paper no. 42 (125 m) and kept in glass bottles for uses [22].

Packed cells volume (PCV):

The packed cell volume is that proportion of whole blood occupied by red cells, expressed as a ratio (liter/liter). Anticoagulated blood in a glass

capillary of specified length, bore size, and wall-thickness is centrifuged in a microhaematocrit centrifuge at RCF 12 000–15 000 xg for 3–5 minutes to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a microhaematocrit reader or calculated by dividing the height of the red cell column by the height of the total column of blood [23].

Hemoglobin (Hb):

Photometric techniques In photometric techniques the absorbance of haemoglobin in a blood sample is measured electronically using a filter colorimeter or a direct read-out haemoglobin meter. Techniques include dilution techniques in which blood is measured into a measured volume of diluting fluid, and nondilution techniques which do not require prior dilution of the blood. Haemoglobin values are expressed in grams per litre (g/l) or grams per deciliter (g/dl) [23].

Mean cell hemoglobin concentration (MCHC):

The MCHC gives the concentration of haemoglobin in g/l in 1 litre of packed red cells [23].

White blood cells (WBC):

Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells (not the nucleus of nucleated red cells), leaving the white cells to be counted. White cells are counted microscopically using an Improved Neubauer ruled counting chamber (haemocytometer) and the number of WBCs per liter of blood calculated [23].

Platelets count:

Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets are counted microscopically using an Improved Neubauer ruled counting chamber and the number of platelets per liter of blood calculated [23].

Determination of Malondialdehyde (MDA): The principle of the following method was based on the spectrophotometric measurement of the color, occurred during the reaction between thiobarbituric acid (TBA) and MDA, [24].

Determination of reduced glutathione (GSH):

More than one type of analytical methods are used to determine serum glutathione (GSH) depending on the action of sulfhydryl groups. Thus methods include photometric, enzymatic, fluourometric and HPLC are used [25].

Determination of Superoxide dismutase activity (SOD):

Pyrogallol (1,2,3-benzenetriol) has long been known to autoxidize rapidly, especially in alkaline solution and the reaction has been employed for the removal of oxygen from gases [26].

Determination of Catalase (CAT):

The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 , with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 570-610 nm [27].

Triglyceride Determination (TG):

Triglyceride in the serum was measured enzymatically [28].

Total cholesterol Determination:

This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine due proportional to the concentration of cholesterol in sample [29].

High-density lipoprotein (HDL):

High density lipoprotein concentration was measured enzymatically which based on the fact that LDL and VLDL precipitated with phosphatuntic acid in the presence of magnesium ions at room temperature, while HDL remains in the supernatant [30].

RESULTS:

Before the treatment by Garlic vinegar, on the patients, we make the following statistical analysis
 Table 1:

Table 1. Demographic characteristics of different study groups.

Parameter	Control Subjects	Patients without treatment	Patients with treatment by Garlic vinegar
Total number of subjects (n)	25	25	25
Sex (male)	100%	100%	100%
Smokers (%)	12%	64%	59%
Alcohols (%)	4%	12%	8%
Mean age (mean ± SD; years)	41.2±9.72	42.20±10.94	40.84±10.48
Body mass index (kg/m ²)	26.08±4.37	27.08±3.59	28.04±3.98
hours of Sleeping (Hour/Day)	9±1.5*	7±2.1**	8±1

Values are given as mean ± S.D from 75 subjects in each group.

* Patients without treatment compared with control, subjects ($p < 0.05$ and 0.001). ** Patients without treatment compared with control, subjects ($p < 0.05$).

Parameter	Control Subjects	Patients without treatment	Patients with treatment by Garlic vinegar
Hb (gm/dl)	14.22±0.64*	15.95±0.46**	14.66±0.44****
PCV%	44.76±1.56*	52.92±1.55**	46.20±1.35****
MCHC (gm/dl)	31.76±0.43*	30.15±0.08**	31.80±0.42
WBC (Cell/mm ³)	7560.00±1213.46	7148.00±1271.52	7544.00±1300.66
Platelets (x10 ³ /mm ³)	320.68±69.70	305.84±63.06	312.28±66.21
GSH (µmol/L)	31.28±4.68*	8.08±2.44**	25.84±3.13
SOD (IU/L)	5.04±0.58*	2.17±0.38**	4.76±0.16
CAT (IU/L)	81.08±5.14*	56.36±8.10**	77.44±5.75
MDA (µmol/L)	1.54±0.02*	5.84±0.60**	1.73±0.14
Triglyceride (mg/dl)	123.72±9.51*	149.04±10.56**	129.72±8.40****
Total Cholesterol (mg/dl)	153.16±5.87*	189.76±10.87**	157.40±9.30
HDL-Cholesterol (mg/dl)	60.36±3.56*	44.80±4.93**	56.96±3.20****
LDL-Cholesterol (mg/dl)	68.24±8.10*	115.16±10.39**	75.68±8.90****
VLDL-Cholesterol (mg/dl)	25.20±1.25*	30.04±2.60**	25.64±1.86

After that has been to start in the collection the blood sample of the patients before and after treatment by Garlic vinegar and controls. Then, begin to measurement the following parameters Table 2:

Table 2: levels of plasma MDA and enzymatic antioxidant status in SOD, CAT and GSH, in control and patients with and without treatment by Garlic vinegar.

Values are given as mean \pm S.D from 75 subjects in each group.

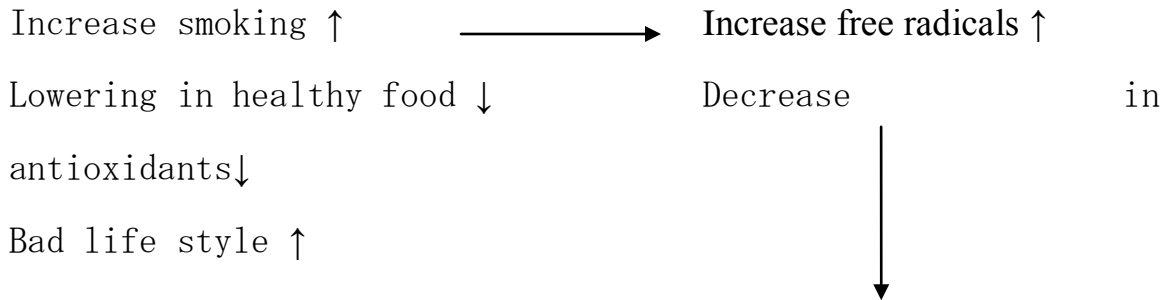
* Patients without treatment compared with control, subjects ($p < 0.05$ and $p < 0.001$). ** Patients without treatment compared with patient with treatment, subjects ($p < 0.05$ and $p < 0.001$). *** Patients without treatment compared with control, subjects ($p < 0.001$). **** Patients with treatment compared with control, subjects ($p < 0.05$).

DISCUSSION:

This research involves the study of the effect of garlic vinegar on patients with high blood viscosity (PCV higher than normal value), and some biochemical parameters through oxidative stress system - antioxidants. By the results shown in Table 2, the causes of high blood viscosity is due to high oxidizers (peroxidant such as MDA Table 2) more than normal levels (control group levels) with lower in antioxidants. Anyway, through the stats Table 1 can note that lack of sleep, smoking is one of the most important factors influencing the decline in antioxidants [13,33]. In addition to this, men are the most likely to have high blood viscosity than women, therefore the study is focus on men only Table1.

From the results (Table 2), we can suggest the significance elevated in blood viscosity or in blood viscosity (PCV = 52.93) compare with control. This is may be retrained to increase the oxidative stress in patients, as the following schemes:

Lowering in sleeping hours ↓



Elevate in blood viscosity
Increase thickness cell coat of RBC_s↑

The evidence on the increase thickness of cell coat is the value of MCHC (the ratio of Hb to PCV) for the patients without treatment (30.15) is lowering than control (31.76) while for treatment is (31.8). At the same time, the antioxidant GSH, SOD and catalase for the patients without treatment is lower than control and treatment groups. In contrast, elevation of MDA in patients without treatment (MDA=5.84).

Therefore, the patients treated with garlic vinegar; because contain on antioxidant agents such as selenium, zinc, vitamins (A and C) and phenolic compounds. These antioxidant agents make as supplements for human body antioxidant (GSH, SOD, catalase). Therefore it is raised in treatment groups (GSH= 25.84, SOD= 4.76 and catalase= 77.44).

At the same time the garlic vinegar activated macrophages to release ROSs, scavenge oxidized low-density lipoprotein (oxLDL), become foam cells, and lead to the development of the fatty streak in the early stage of atherosclerosis [31,32]. This explains why phytochemicals with anti-chronic inflammation, hypolipidemic, and antioxidative properties are thought to be capable of decreasing the incidence of atherosclerosis.

In addition, garlic vinegar can consider as a potent anti-atherogenic food [33]. And lowering effect for blood cholesterol is believed largely may be due to a reduction in LDL cholesterol [34,35], which may be due to inhibition of hepatic hydroxymethylglutaryl-CoA reductase activity by alliin and allicin [36, 37,38].

Finally, garlic is regarded as a safe food, and this treatment do not affect on platelet and white blood cells counts. Also the patients not have any problem in platelet counts before treatment.

CONCLUSIONS:

In briefly, the modern studies focus on the importance of medical herbs, especially in vitro and in animal models, addressing the protective effect of garlic against cardiovascular disease and cancer. This protection can arise from its diverse biological activities: enhanced antioxidant defense, lowering of blood lipids, inhibition of blood aggregation (such as elevated PCV), enhancement of cancer cell cycle arrest/apoptosis, inhibition of invasion

and/or metastasis, and modulation of drug metabolism and/or the immune response.

However, the results observed in human clinical and intervention studies have been inconsistent. The risk of garlic drug interactions is attracting increasing interest, especially in the elderly and in those with chronic diseases. Further experiments are warranted to understand the actual health benefits and impact of garlic.

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