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RESEARCH ARTICLE

Inhibitory Effect of *Allium Cepa* Extract on LDL Oxidation Induced By $Cuso_4$ *in Vitro* Compared With *Allium Sativum* and *Allium Ascalonicom*

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ABSTRACT

Oxidation of low-density lipoprotein (LDL) has been strongly suggested as a key factor in the pathogenesis of atherosclerosis. Thus, the inclusion of some anti-oxidant compounds in daily dietary food stuff may inhibit the production of oxidized LDL and may decrease both the development and the progression of atherosclerosis. The present work investigated the inhibitory effects of extract of *Allium cepa* (onion) on LDL oxidation induced by $CuSO_4$ quantitatively in vitro and compared with extract of *Allium ascalonico (shallot)* and *Allium sativum* (garlic). LDL was incubated with CuSO4 and the formation of conjugated dienes and thiobarbituric acid reactive substances (TBARS) were monitored as markers of LDL oxidation. Inhibition of this Cu-induced oxidation was studied in the presence of extracts of *Allium ascalonicom, Allium cepa* and *Allium sativum*. It was demonstrated that extract of *Allium cepa* as well as *Allium ascalonicom* and *Allium sativum* were able to inhibit LDL oxidation and increase the resistance of LDL against oxidation in vitro. The pattern of inhibition was in this order: *Allium sativum>Allium ascalonicom>Allium cepa.* This study showed that extract of *Allium ascalonicom, Allium cepa* and *Allium sativum* prevented the oxidation of LDL *in vitro* and it may suggest that they have the similar effect *in vivo*.

Keywords: *Allium cepa, Allium sativum, Allium ascalonicom, LDL oxidation*

Cardiovascular disease is one of the leading causes of mortality in our society. Although an increased concentration of plasma low density lipoprotein (LDL) is believed to be a major risk factor in this regard, the underlying mechanisms remain unclear and needs more investigations. To date, considerable evidence support a role for oxidatively modified LDL in the pathogenesis of atherosclerosis [1,2]. The uptake of oxidized LDL (Ox-LDL) by macrophages results in the formation of foam cells and cellular cholesterol accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesions. Ox-LDL is cytotoxic to arterial wall cells, where it stimulates haemostatic and thrombotic process and promotes secretion of cytokines and growth factors from cells of the arterial wall [3-6]. Ox-LDL has already been detected in human and animal atherosclerotic lesions, adding support to the contributory role of Ox-LDL in this clinical condition. Ox LDL has also been reported to compromise endothelial integrity, a silent feature of atherosclerosis. It has already been shown that Alliums such as *Allium cape* and garlic could decrease the formation of atherosclerotic lesions in animal models and epidemiological data suggest that an inverse relationship exist between the intake of antioxidant *Allium cepa* and garlic and the risk of coronary artery disease [7-9].

According to the oxidation hypothesis, LDL is protected against oxidative stress by using antioxidants, thereby delaying the formation of modified LDL [10-12]. The regular consumption of onions has, like garlic, been shown to lower high cholesterol levels and high blood pressure [13], both of which may help to prevent atherosclerosis and diabetic heart disease, and reduce the risk of heart attack or stroke [14,15]. Clinical trials revealed that *Allium cepa* and *Allium sativum* have profound beneficial effects in the following areas: Antilipidemic, inhibition of cyclooxygenase activity and thromboxane B_2 synthesis, anticancer properties, inhibition of platelet aggregation, hydroxyl scavenging properties, antioxidant, hemostatic, and hemodynamic

activities, anti-atherosclerotic, antibiotic, antiviral and antifungal activities [13-19].

Allium ascalonicom, Allium cepa and *Allium sativum* are the important Allium species commonly used in Iranian diets and they are widely consumed. There is no information about the compare beneficial effects of Allium *ascalonicom, Allium cepa* and *Allium sativum on inhibition of LDL oxidation* .Thus, this work is undertaken to investigate the effect extract of *Allium ascalonicom, Allium cepa* and *Allium sativum* on the modification of LDL induced by $CuSO_4$ in vitro by monitoring the formation of conjugated dienes, the formation of thiobarbituric acid reactive substances (TBARS).

MATERIALS AND METHODS

Chemicals

Disodium ethylene diamine tetraacetat (Na_2EDTA), Potassium bromide (Ker), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO4) and oil red O were purchased from Sigma chemical co. *Allium Cepa*, *Allium sativum* and *Allium ascalonicom* were purchased from the local market.

Blood sampling

The protocols for the blood sampling were approved by the Lorestan University of Medical Sciences ethics committee, and all subjects of the study provided informed consent for participation. Fasting blood samples after an overnight fasting were collected in EDTA containing tubes (1.6 mg EDTA/mL blood). To obtain fresh plasma, blood samples were centrifuged (3000 rpm for 10 min at 4°C) as soon as the samples were collected to avoid auto oxidation. To minimize oxidation *in vitro,* sodium azide (0.06% wt/vol) was added to plasma samples immediately after separation.

Isolation of LDL

The LDL fraction was isolated from fresh plasma by single vertical discontinuous density gradient ultracentrifugation [20]. The density of the plasma was adjusted to 1.21 g/ml by the addition of solid KBr (0.365 g/ml). Centrifuge tubes were loaded by layering 1.5 ml of density-adjusted plasma under 3.5 ml of 0.154 mol/L NaCl, and centrifuged in a Beckman L7-55 ultracentrifuge at 40000 rpm at 10 °C for 2.5 hours. The yellow LDL band, located in the upper middle portion of the tube, was collected into a syringe by puncturing the tube. The isolated LDL was dialyzed for 48h at 4°C against three changes of deoxygenated-PBS (0.01 mol/L Na_2HPO_4, 0.16 mol/L NaCl, pH 7.4) containing 0.01% NaN_3 and 0.01% EDTA.

Preparation of aqueous extracts of Allium cepa, Allium sativum and Allium ascalonicom

Allium cepa, *Allium sativum* and *Allium ascalonicom* were weighted (0.6 g) and homogenized with 6 mL of distilled water for 20 min. These solutions were centrifuged at 10000 × g for 10 min at 4°C and the supernatants were recovered and used at the final concentration of 5 and 10 mg/ml [21].

Oxidation of LDL

Continuos monitoring of formation of conjugated dienes in LDL

After isolation of total LDL, the protein content of LDL was measured [22]. LDL was adjusted to 150 µg/ml of LDL protein with 10 mM PBS, PH7.4 and then aliquots of *Allium cepa, Allium sativum,* and *Allium ascalonicom* extracts were added to the solution. The oxidative modification of LDL was initiated by addition of freshly prepared 10 µM CuSO4 solution at 37°C in a water bath for 5 h. The kinetics of LDL oxidation was monitored every 10 minutes by removing an aliquot, measuring its absorbance at 234 nm. The lag phase was calculated from the oxidation profile of each LDL preparation by drawing a tangent to the slope of the propagation phase and extrapolation into intercept the initial-absorbance axis. The lag phase represented the length of the antioxidant-protected phase during LDL oxidation by extract of *Allium cepa*, *Allium sativum*, and *Allium ascalonicom* in vitro. The lag time was measured as the time period until the conjugated dienes began to increase [23]. The formation of conjugated dienes was calculated as conjugated dienes equivalent content (nmol/mg-protein) at 5 h. The conjugated dienes concentration was calculated by using the extinction coefficient for diene conjugates at 234 nm (29500 L/mol.cm).

Assay of the formation of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation end products were determined as TBARS according to modified method of Buege and Aust. After initiating the oxidation process with $CuSO_4$, the sample mixtures were incubated at 37°C for 5 h in a water bath and the reaction was terminated by adding. EDTA (2 mM). TBARS formation was measured in a spectrophotometer at 532 nm. The results were recorded as malondialdehyde (MDA) equivalent content (nmol/mg LDL-protein) [24,25].

Statistical analysis

Each data value was presented the mean ± S.D. of three experiments performrd in duplicate. The variables used to describe the difference between the oxidation curves were lag time, conjugated dienes and MDA. These parameters were obtained using Student's t-test for independent data and differences were considered significant when $p<0.05$.

RESULTS

The effects of *Allium cepa*, *Allium sativum*, and *Allium ascalonicom* on LDL oxidation are shown in Fig 1. It clearly shows that $CuSO_4$ dramatically increased oxidation of LDL. The formation of conjugated dienes, a marker of LDL oxidation decreased by extract of

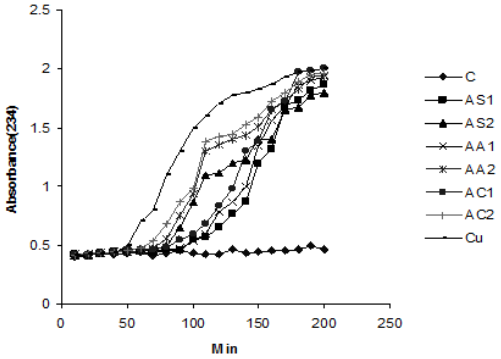

Fig 1. The effects extract of *Allium cepa*, *Allium ascalonicom* and *Allium sativum* on the kinetic of LDL oxidation in l0 mM PBS, pH 7.4 at 37°C for 5 h. (C) n-LDL (LDL without copper and extract), (Cu) n-LDL + copper, (AC1) n-LDL + *Allium cepa* extract (10mg/m1), (AC2) n-LDL + *Allium cepa* extract (5mg/m1), (AA1) n-LDL + *Allium ascalonicom* extract (10mg/m1), (AA2) n-LDL + *Allium ascalonicom* extract (5mg/m1), (AS1) n-LDL + *Allium sativum* extract (10mg/m1), (AS2) n-LDL + *Allium sativum* extract (5mg/m1). Each point represents the mean of three experiments.

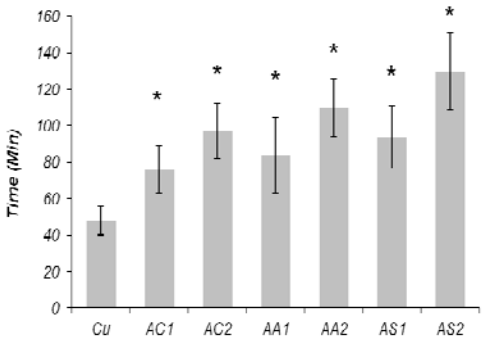

Fig 2. The effects extract of Allium *cepa*, *Allium ascalonicom* and *Allium sativum* on lag time (The lag phase represented the length of the antioxidant-protected phase during LDL oxidation by extract). . (C) n-LDL (LDL without copper and extract), (Cu) n-LDL + copper, (AC1) n-LDL + *Allium cepa* extract (10mg/m1), (AC2) n-LDL + *Allium cepa* extract (5mg/m1), (AA1) n-LDL + *Allium ascalonicom* extract (10mg/m1), (AA2) n-LDL + *Allium ascalonicom* extract (5mg/m1), (AS1) n-LDL + *Allium sativum* extract (10mg/m1), (AS2) n-LDL + *Allium sativum* extract (5mg/m1). Each point represents the mean of three experiments. (*) is the symbol for significant difference compared to Cu.

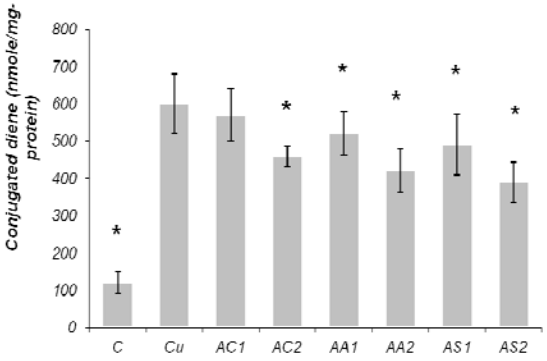

Fig 3. The effects extract of *Allium cepa*, *Allium ascalonicom* and *Allium sativum* on the formation of conjugated dienes of LDL oxidation. (C) n-LDL (LDL without copper and extract), (Cu) n-LDL + copper, (AC1) n-LDL + *Allium cepa* extract (10mg/m1), (AC2) n-LDL + *Allium cepa* extract (5mg/m1), (AA1) n-LDL + *Allium ascalonicom* extract (10mg/m1), (AA2) n-LDL + *Allium ascalonicom* extract (5mg/m1), (AS1) n-LDL + *Allium sativum* extract (10mg/m1), (AS2) n-LDL + *Allium sativum* extract (5mg/m1). Each point represents the mean of three experiments. (*) is the symbol for significant difference compared to Cu.

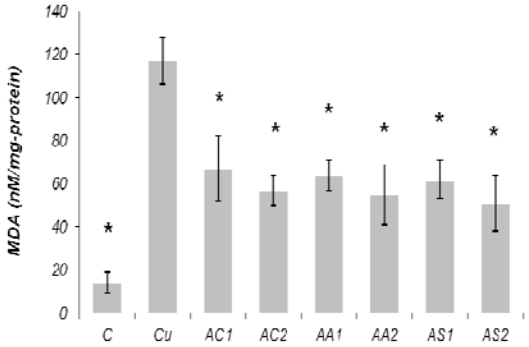

Fig 4. The effects extract of *Allium cepa*, *Allium ascalonicom* and *Allium sativum* on the formation of MDA. (C) n-LDL (LDL without copper and extract), (Cu) n-LDL + copper, (AC1) n-LDL + *Allium cepa* extract (10mg/m1), (AC2) n-LDL + *Allium cepa* extract (5mg/m1), (AA1) n-LDL + *Allium ascalonicom* extract (10mg/m1), (AA2) n-LDL + *Allium ascalonicom* extract (5mg/m1), (AS1) n-LDL + *Allium sativum* extract (10mg/m1), (AS2) n-LDL + *Allium sativum* extract (5mg/m1). Each point represents the mean of three experiments. (*) is the symbol for significant difference compared to Cu.

Allium cepa, *Allium sativum*, and *Allium ascalonicom*. Figs 2 and 3 show the levels of conjugated dienes at 5 h and lag time of all experimental groups. Thus extract of *Allium sativum* decreased the level of conjugated dienes and was significantly different from control. Extract of *Allium cepa* and *Allium ascalonicom* decreased final levels of conjugated dienes in the medium respectively. The antioxidative effect extract of *Allium cepa*, *Allium sativum*, and *Allium ascalonicom* on LDL was determined and expressed by measurement of MDA equivalent content. The levels of MDA after 5 h of incubation in all experiment groups are shown in Fig 4. Addition extract of *Allium sativum* decreased TBARS formation about eleven-fold and was statistically significant. The xtract of *Allium cepa* and *Allium ascalonicom* also decreased TBARS formation significantly.

DISCUSSION

The oxidative modification of LDL (Ox-LDL) is the major factor that stimulates the development and progress of atherosclerosis [2]. Therefore, the major objective of this study was to compare the antioxidant effects extract of *Allium cepa with Allium ascalonicom* with and *Allium sativum* using in vitro model.The oxidative modification of LDL induced by copper ions is shown to be related to free radical reaction, though

the exact mechanism has not been elucidated yet. It is suggested that LDL oxidation may require the generation of super oxide anion and probably the ultimate generation of hydroxyl radicals by the Fenton reaction [2]. After oxidation by copper ions, polyunsaturated fatty acids of LDL resulted in an elevation of lipid peroxides and depletion of vitamin E in Ox-LDL [1-3,26]. Previous study showed that extract of *Allium ascalonicom* had good antioxidant activity and various concentrations of extract of *Allium ascalonicom* had a dose-dependent antioxidant activity against LDL oxidation by inhibiting the formation of conjugated dienes and TBARS and increase lag time [27]. Our results clearly showed that extract of *Allium cepa as* well as *Allium ascalonicom* and *Allium sativum* had antioxidant activity against LDL oxidation by inhibiting the formation of conjugated dienes and TBARS. They also increased lag time for oxidation. The present study indicate that extract of *Allium ascalonicom, Allium cepa* and *Allium sativum* are potent antioxidants and protect plasma LDL against oxidation. The protective effect extract of *Allium cepa* and *Allium ascalonicom* on LDL oxidation was less when compared with extract of *Allium sativum*. The antioxidant effect of garlic has already been investigated and is shown to be due to the prevention of free radical oxidation [9,11,28].

Wide-ranging claims have been made for the effectiveness of onions against conditions ranging from the common cold to diabetes, osteoporosis, heart disease, and other diseases [14]. They contain chemical compounds believed to have anti-inflammatory, anticholesterol, anticancer, and antioxidant properties, such as quercetin. Preliminary studies have shown increased consumption of onions reduces the risk of head and neck cancers [29,30]. Onions decreased serum lipids, lipid peroxidation and glucose in the diabetic rats [14]. Onion itself was also reported to have a serum lipid lowering effect in hyperlipidemic experimental animals [31,32].

Allium sativum has been used in folk medicine of many cultures for the prevention of cardiovascular diseases and other disorders [3,9,11]. It has been shown in many cases that the protective effect of *Allium sativum* is associated with its antioxidant properties [14,33]. Chronic administration of raw garlic homogenate increased catalase and superoxide dismutase activities in rat heart and protected heart against oxidative damage induced by adriamycin or ischemia and reperfusion [9]. Aqueous extract of garlic powder is also able to scavenge hydroxyl radicals and superoxide anions [34]. This activity is shown to be heat resistant as the heated aqueous extract of garlic powder maintained its ability to scavenge hydroxyl radicals [35,36].

In conclusion, we showed that extract of *Allium cepa* such as *Allium ascalonicom* and *Allium sativum* is affective in the prevention of LDL oxidation and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease.

REFERENCES

1. Kendler BS. Garlic (Allium sativum) and onion (Allium cepa): a review of their relationship to cardiovascular disease. *Prev Med* 1987; 16:670-85.
2. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997; 272:20963-6.
3. Alkreathy H, Damanhouri ZA, Ahmed N, Slevin M, Ali SS, Osman A. Aged garlic extract protects against doxorubicin-induced cardiotoxicity in rats. *Food Chem Toxicol* 2010; 48:951-6.
4. Singh UP, Singh DP, Maurya S, Maheshwari R, Singh M, Dubey RS, Singh RB. Investigation on the phenolics of some spices having pharmacotherapeuthic properties. *J Herb Pharmacother* 2004; 4:27-42.
5. Ani M, Moshtaghie AA, Ahmadvand H. Comparative Effects of Copper, Iron, Vanadium and Titanium on Low Density Lipoprotein Oxidation in vitro. *Iran Biomed J* 2007; 11:113-8.
6. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 1991; 88:1785-92.
7. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic Biol Med* 2009; 47:1673-706.
8. Banerjee SK, Maulik M, Mancahanda SC, Dinda AK, Gupta SK, Maulik SK. Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life Sci* 2002; 70:1509-18.
9. Banerjee SK, Dinda AK, Manchanda SC, Maulik SK. Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury. BMC Pharmacol 2002; 16:2-16.
10. Borek C. Garlic reduces dementia and heart-disease risk. *J Nutr* 2006; 136:810S-812S.
11. Rahman K. Historical perspective on garlic and cardiovascular disease. *J Nutr* 2001; 131:977S-979S.
12. Lau BH. Suppression of LDL oxidation by garlic compounds is a possible mechanism of cardiovascular health benefit. *J Nutr* 2006; 136:765S-768S.
13. Alder R, Lookinland S, Berry JA. A systematic review of the effectiveness of garlic as an anti-hyperlipidemic agent. *J Am Acad Nurse Pract* 2003; 15:120-9.
14. Bang MA, Kim HA, Cho YJ. Alterations in the blood glucose, serum lipids and renal oxidative stress in diabetic rats by supplementation of onion (Allium cepa. Linn). *Nutr Res Pract* 2009; 3:242-6.
15. Ostrowska E, Gabler NK, Sterling SJ, Tatham BG, Jones RB, Eagling DR, Jois M, Dunshea FR. Consumption of brown onions (A. cepa var. cavalier and var. destiny) moderately modulates blood lipids, haematological and haemostatic variables in healthy pigs. *Br J Nutr* 2004; 91:211-8.
16. Nishimura H, Higuchi O, Tateshita K. Antioxidative activity of sulfur-containing compounds in Allium species for human LDL oxidation in vitro. Biofactors 2004; 21:277-80.
17. Ali M, Thomson M, Afzal M. Garlic and onions: their effect on eicosanoid metabolism and its clinical relevance. Prostaglandins Leukot Essent Fatty Acids 2000; 62:55-73.
18. Lin GI, Frishman WH. Herbal medicine for the treatment of cardiovascular disease: clinical considerations. *Arch Intern Med* 1998; 158:2225-34.
19. Sohn DW, Han CH, Jung YS, Kim SI, Kim SW, Cho YH. Anti-inflammatory and antimicrobial effects of garlic and synergistic effect between garlic and ciprofloxacin in a chronic bacterial prostatitis rat model. *Int J Antimicrob Agents* 2009; 34:215-9.
20. Richard SC, Sunder RM, Robert RH, Alan C. Inhibition of LDL oxidation in vitro but not ex vivo by troglitazone. Diabetes 1999; 48:83-90.
21. Pedraza-Chaverrí J, Gil-Ortiz M, Albarrán G, Barbachano-Esparza L, Menjívar M, Medina-Campos ON. Garlic's ability to prevent in vitro Cu2+-induced lipoprotein oxidation in human serum is preserved in heated garlic: effect unrelated to Cu2+-chelation. *Nutr J* 2004; 3:10-4.

22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1979; 72:248-54.
23. Navder KP, Baraona E, Leo MA, Lieber CS. Oxidation of LDL in baboons is increased by alcohol and attenuated by polyenylphosphatidylcholine. *J Lipid Res* 1999; 40:983-7.
24. Kaya A, Uzunhasan I, Baskurt M, Ozkan A, Ataoglu E, Okcun B, Yigit Z. Oxidative status and lipid profile in metabolic syndrome: gender differences. *Metab Syndr Relat Disord* 2010; 8:53-8.
25. Sheu JY, Chen PH, Tseng WC, Chen CY, Tsai LY, Huang YL. Spectrophotometric determination of a thiobarbituric acid-reactive substance in human hair. *Anal Sci* 2003; 19:957-60.
26. Obata T, Yonemoti H, Aomine M. The protective effect of fluvastatin on hydroxyl radical generation by inhibiting low-density lipoprotein (LDL) oxidation in the rat myocardium. *Microvasc Res* 2009; 77:163-5.
27. Ahmadvand H, Khosrowbeygi A, Ghasemi M. Inhibitory effect of Allium ascalonicom hydroalcoholic extract on low-density lipoprotein (LDL) oxidation induced by CuSO4 in vitro. *J Med Plants Res* 2011; 5:1012-7.
28. Lewin G, Popov I. Antioxidant effects of aqueous extract. 2nd communication: Inhibition of the Cu (2+)-initiated oxidation of low density lipoproteins. Arzneimittelforschung 1994; 44:604-7.
29. Park J, Kim J, Kim MK. Onion flesh and onion peel enhance antioxidant status in aged rats. *J Nutr Sci Vitaminol* (Tokyo) 2007; 53:21-9.
30. Lanzotti V. The analysis of onion and garlic. *J Chromatogr A* 2006; 1112:3-22.
31. Bordia A, Verma SK, Vyas AK. Effect of essential oils of onion and garlic on experimental atherosclerosis in rabbits. Atherosclerosis 1977; 26:375-86
32. Carson JF. Chemistry and biological properties of onion and garlic. *Food Rev Int* 1987; 3:71-103.
33. Kourounakis PN, Rekka EA. Effect on active oxygen species of alliin and Allium sativum (garlic) powder. *Res Commun Chem Pathol Pharmacol* 1991; 74:249-52.
34. Prasad K, Laxdal VA, Yu M, Raney BL. Evaluation of hydroxyl radical-scavenging property of garlic. *Mol Cell Biochem* 1996; 154: 55-63.
35. Efendy JL, Simmons DL, Campbell GR, Campbell JH. The effect of the aged garlic extract, 'Kyolic', on the development of experimental atherosclerosis. Atherosclerosis 1997; 132:37-42.
36. Agarwal KC. Therapeutic actions of garlic constituents. *Med Res Rev* 1996; 16:111-24.

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