

Effect of garlic-apple vinegar mixture on hyper-cholesterimia induced rabbits

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تأثير خليط الثوم وخل التفاح على الأرانب التي تعاني من فرط الكوليسترول

الخلاصة:

استخدم الثوم وخل التفاح لكون الثوم يحتوي على عناصر مؤكسدة و خافضة للدهون في الوقاية من أمراض القلب والأوعية الدموية وغيرها من أمراض التمثيل الغذائي، مثل تصلب الشرايين والسكري. أجريت هذه الدراسة لتقييم فعالية خليط الثوم وخل التفاح للوقاية من ارتفاع الدهون في الدم high-lipidaemia المسبب لتصلب الشرايين، ودراسة آثار جرعة مخفضة من هذا الخليط لمدة ستين يوماً، كذلك جسر الهوة بين الدراسات التي أجريت على الخل من ناحية و الثوم من ناحية أخرى للحصول على فوائد الجمع بينهما (خليط الثوم وخل التفاح).

أجريت الدراسة على أرانب محلية أعطيت عليه أضيف إليها الكوليسترول بتركيز ١ % لمدة ٨ أسابيع، حيث قورنت مع مجموعة أخرى أعطيت عليه عالية الكوليسترول بالإضافة إلى خليط الثوم وخل التفاح. العليقة عالية الكوليسترول أدت إلى ارتفاع معنوي بالكوليسترول الكلي (TC) والكوليسترول منخفض الكثافة (LDL) وتركيز الكليسيريدات الثلاثية (الدهون الثلاثية TG) مع انخفاض معنوي في الكوليسترول عالي الكثافة (HDL). أظهرت نتائج المجموعة المعالجة انخفاض ملحوظاً بتركيز كل من الكوليسترول الكلي (TC) والكوليسترول منخفض الكثافة (LDL) والكليسيريدات الثلاثية (TG) مع ارتفاع معنوي في الكوليسترول عالي الكثافة (HDL) والفحوصات النسيجية أثبتت التحسن في جدار الشريان البطني للمجموعة المعالجة مما دعم

النتائج:

نستنتج من ذلك إن لخليط الثوم وخل التفاح دور واضح في تقليل مستوى الدهون في مصل الدم وبالتالي تقليل خطر الإصابة بتصلب الشرايين حيث إن الجرعة القليلة من الخليط أعطت نتائج جيدة وبآثار جانبية قليلة لمكونات الخليط. كذلك الحصول على منافع المؤازرة المدعمة بينهما مقارنة لما أظهرته نتائج الفحوصات النسيجية للأرانب المصابة من جهة والمعالجة بخليط الثوم وخل التفاح من جهة أخرى.

ABSTRACT

Garlic contains hypolipidemic and antioxidant compounds acts on prevention of cardiovascular and other metabolic diseases, such as atherosclerosis high-lipidemia, thrombosis hypertension and diabetes .This study have investigated the efficacy of garlic-apple vinegar mixture intake for prevention of dys-lipidaemia which considered as the major cause of arterial dysfunction. Study the effects of low dose of mixture for a period of sixty days, and attempt to bridge the gap between studies on apple vinegar alone and garlic studies to get benefits of their combination.

The study carried on male rabbits received hyper-cholesterolemic diet (1% cholesterol) with treatment (mixture group) for 8 weeks which compared with control and hyper-cholesterolemic groups reveal significant decrease in total

cholesterol(TC)low density lipoproteins(LDL)and triglyceride (TG) with significant increase in high density lipoproteins(HDL).Moreover the histological changes sustenance these results.

Conclusion: the mixture of garlic apple-vinegar have a clear role in reducing the level of lipids in the serum and thus reduce the risk of atherosclerosis, as the low dose therapy of the mixture gave an excellent results with low side effects of both components. The present study prove okay results after obtaining their synergistic benefits as a mixture compared to the results discovered by histological examinations of abdominal aorta in the hyper-cholesterolemic group on one hand and the treated group with mixture on the other hand.

INTRODUCTION

Atherosclerosis is an inflammatory disease[1],leads to endothelial dysfunction[2]. Garlic competence in cardiovascular diseases encourage experimental studies, and many of these studies showed a positive effect also conversely a number of negative studies make suspicion on the efficacy of garlic especially its cholesterol lowering effect. Several studies have shown that a brief increase in blood concentrations of triglycerides (TG) and fatty acids can affect endothelium-dependent vasodilatation [3,4].The early stage of the atherosclerotic lesion involves formation of oxidized low density lipoprotein, where lipolytic process takes place both at the luminal surface and in the sub endothelial space of the vessels implying lipoprotein lipase activity[5].Eruption of the inflammation and platelet aggregation during endothelial dysfunction can decrease plaque stability and encourage the formation of atherosclerotic lesions and thrombi [6]. Many adverse reactions to garlic can be certified to an excess of oil-soluble organo-sulfur constituents. For example, the lipid lowering effects of some oil-soluble sulfur compounds in hepatocytes correspond with cytotoxicity, as revealed by increased lactate dehydrogenase release from cells [7],therefore release of acetone in the breath after oral consumption by human [8], on the other hand water soluble sulfur compounds although of its effect on reducing cholesterol, were not cytotoxic .Several clinical reports including meta analyses, have revealed a cholesterol lowering effect of garlic in humans [9,10,11]. Other reports have promoted public knowledge about the cholesterol lowering effects of garlic [12–17].However, recent negative publications have caused confusion within both the public and academic domains. Although the reason for these inconsistencies remains unknown, it likely relates to components occurring in the preparation, the quantity of the preparation provided and/or the duration of the study [18,19]. Atherogenic and thrombogenic changes due to increased triglyceridemia after meals encounter in all subjects throughout the day [20].Epidemiological studies resulted in an association between consumption of apples and reduced risk of cardiovascular disease, lipid oxidation, and cholesterol [21].Plasma cholesterol and (HDL)reduced due to consumption of antioxidants (as phenolic compounds that found in apple), acts to prevent the development of atherosclerosis in hamsters [22] while Vinegar improve endothelial function and enhance the resistance to oxidative modification of(LDL) [23]. The main component of vinegar is acetic acid [24] and polyphenolics constituents such as flavones (e.g. quercetin, kaempferol), flavones (Catechin, epicatechin) and anthocyanins (e.g. Cyanidin-3-glucoside) [25] also Organic acids (e.g. tartaric, citric, lactic and malic),amino acids vitamins, and mineral [26]. Flavonoids and phenolic acids have anti-inflammatory properties and antioxidant

activity [27]. Acetic acid is one of the short chain fatty acid (SCFA) which are products of the caecal and colonic fermentation that is stimulated by the ingestion of dietary fiber. Feeding highly fermentable dietary fiber is reported to reduce the increments of serum cholesterol [28]. Dietary acetic acid reduced serum (TC) and (TG) ; first due to the inhibition of lipogenesis in liver second due to the increase in fecal bile acid excretion in rats fed a diet containing cholesterol [29]. However, very little is known about the effect of dietary acetic acid on lipid metabolism. Abramoviz et.al. investigate the effect of allicin as an active component of garlic on decrease in the formation of fatty streaks in aorta and lipid profile in mice[30].

MATERIALS and METHODS

Animals and experimental design

Twelve local Adult male rabbits, weighing between 1870-1925 g were used in this study. Animals were housed in a temperature-controlled room (23–25°C) with a 12 h light–dark cycle. The light period began from 07.00 for two weeks, The animals had free access to water and adequate diet of standard pellets. Rabbits were randomly divided into three groups, each of four : Control group (normal diet); induced untreated group (normal diet with 1% cholesterol) and the treated group (hyper-cholesterolemic diet with garlic vinegar).

Venous blood were collected from all rabbits before starting experiment as a baseline values (day zero). On the first day each rabbit of the third group receive one drop (50.00 µl) from the mixture orally, and then increased daily 50.00 µl up to 25 drop(1.250 ml) from day twenty five, to day 60 (the end of experiment).

Blood samples again collected from all groups on the 2nd, 4th and 8th week. All blood samples were centrifuged at 3500 rpm for 20 minutes to get serum. The (TC)(TG),and (HDL-C) were determined by using standard enzymatic kits (Gesam,s.r.I. Italy) while (LDL-C) was determined as a result of equation $LDL = TC - (HDL + VLDL)$ [31]. Two rabbits from each group killed by i/v injection of an overdose of sodium phenobarbitone and their abdominal aorta removed, dissected, processed, and 5-6µ paraffin sections were stained with haematoxylin and eosin to examine the histological changes [32,33] .

Preparations of the garlic vinegar mixture:

Composition: 350g raw garlic and 200ml of apple vinegar 10-15%.

Preparation method: The vinegar added to peeled smashed garlic in a pottery jar, firmly closed the jar cover. The jar left in dark cool place for fifteen days, and then the mixture squeezed by using muslin cloth. The viscous outcome liquid placed in dark colored glass jars for more three days to provide adequate time for complete reaction of the components.

Statistical analysis

Analysis ANOVAs Repeated measures design were used to determined the significant level of difference in groups and times.

RESULTS

TC in induced and treated groups increased significantly compared with control (P<0.001) while treated group significantly decreased compared with induced group (P<0.001) (872.235± 604.73 , 482.751± 312.41 ,80.418± 1.47) respectively . Figure(1).The same result shown with changes in(TG)concentration(p<0.001) (355.283±213.29, 221.392±106.51, 88.419± 11.36) as shown in Fig(2) and (LDL)(788.403±4.07, 414.943±1.644 , 43.232±1.924).Fig (3).

The (HDL) concentration in treated group increased significantly compared with induced

and control group (p<0.001) it turn up to (23.702±1.644, 12.799±4.072), 19.252±1.924) respectively, while its concentration in induced group decreased significantly (p<0.001) compared with control group.(Fig (4)).

No pathological changes in normal diet group (Fig 5) Sections from high-cholesterol group prove intimal thickening in aorta wall, while many fat-loaded macrophages were seen in the intimal surface. (Fig 6). Sections from mixture group show a few number of foam cell and macrophages and decreased intimal coat thickness (Fig 7)

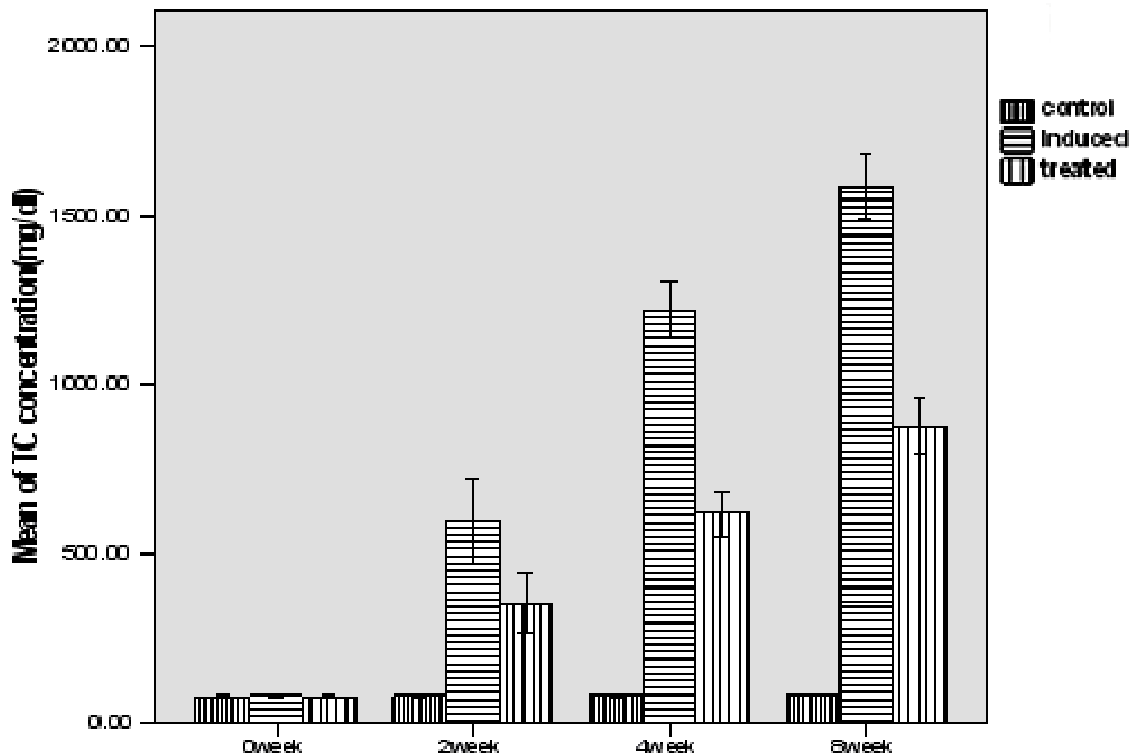
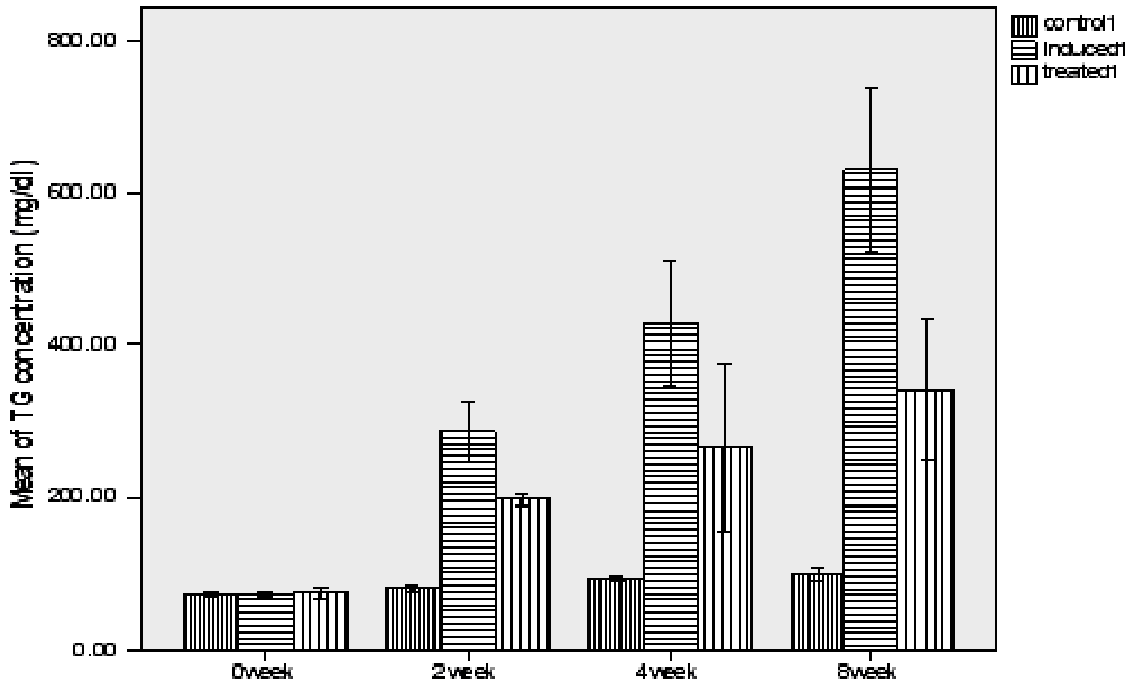


Figure (1) Changes in TC concentration within groups and times

Error bars: +/- 2 SD



Figure(2) Changes in TG concentration within groups and times

Error bars: +/- 2 SD

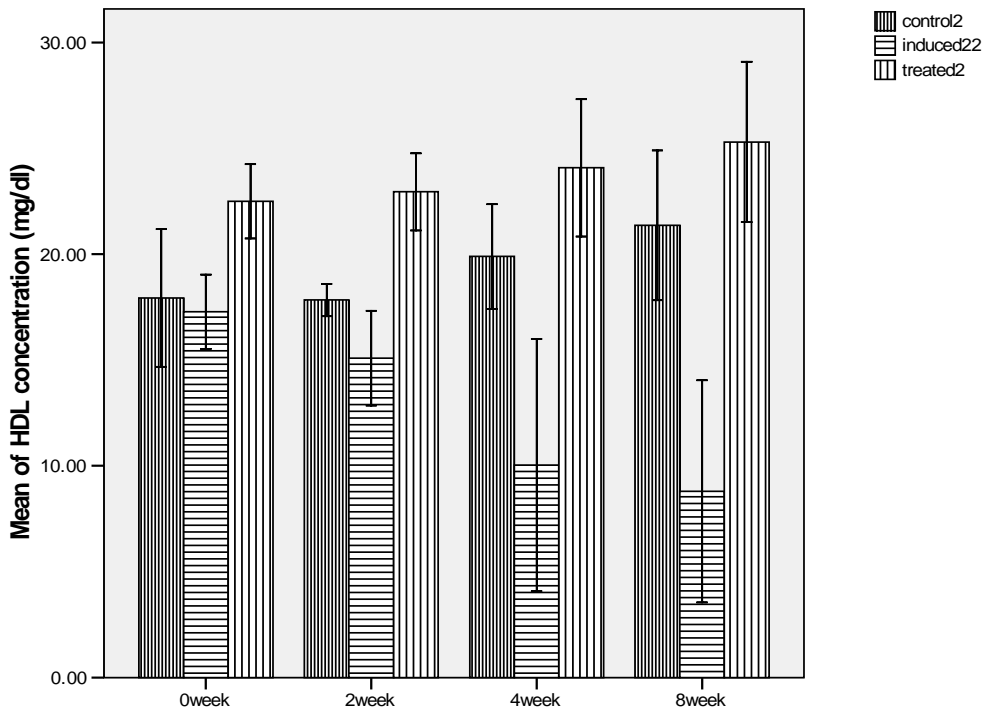


Figure (3) Changes in HDL concentration within groups and times

Error bars: +/- 2 SD

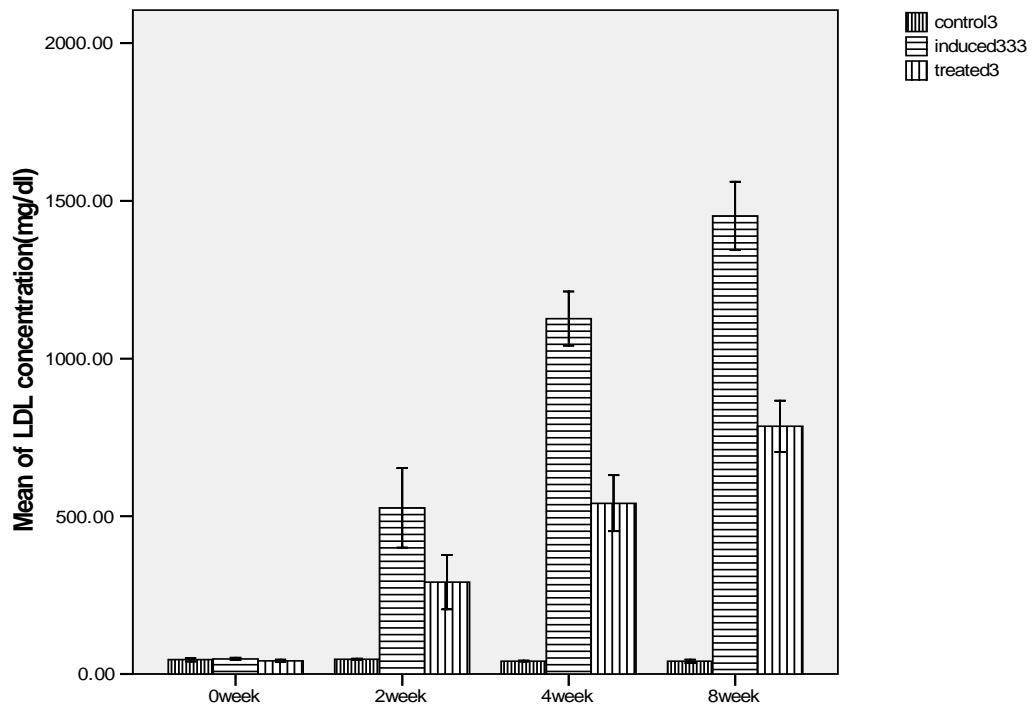


Figure (4) Changes in LDL concentration within groups and times

Error bars: ± 2 SD

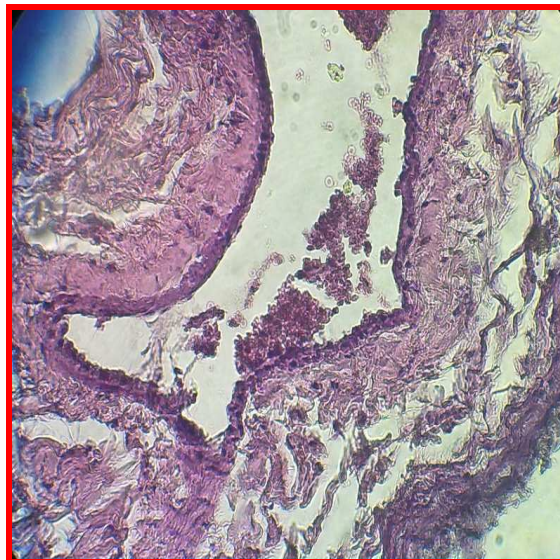


Fig 5 Vessel wall with normal histology

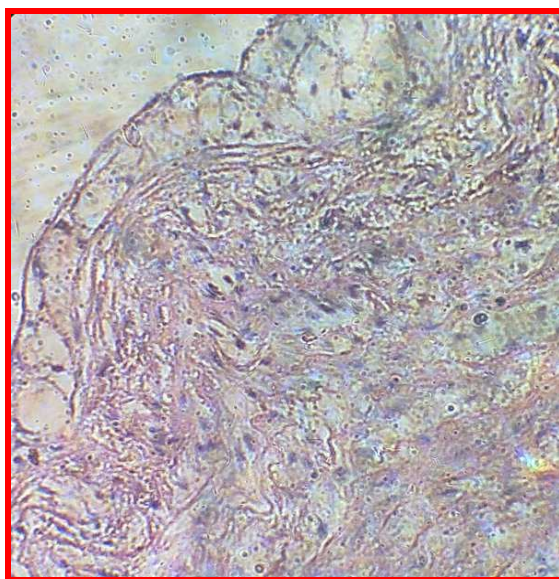


Fig 6 intimal thickening in aorta wall, foam cell, macrophages in the intimal surface

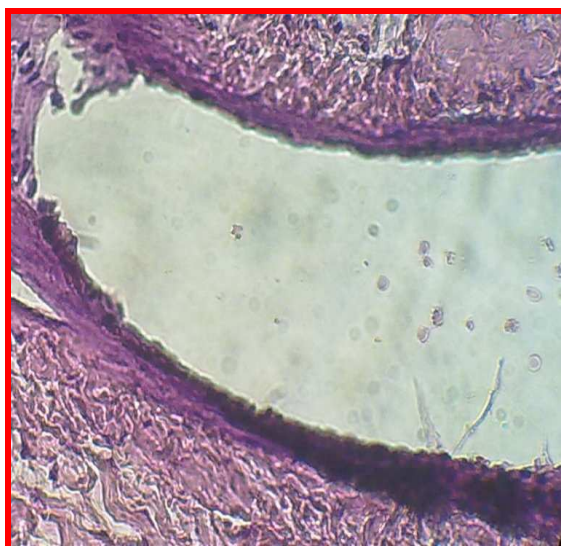


Fig 7 decreased intimal coat thickness with Few foam cell

DISCUSSION

Apple can in fact prevent the progress of atherosclerosis, due to antioxidant and anti-inflammatory component (vitamin C and of various phenolic compounds and quercetin) [29]. Some study suggest that vinegar, might have some acute effects on biochemical risk factors of atherosclerosis and a probable protective value can be considered [34]. Protective effect of garlic on atherosclerosis has been attributed to its capability to reduce lipid content in arterial wall.

Garlic causes direct anti-atherogenic (preventive) and anti-atherosclerotic (causing regression) effects at the level of artery wall [35], also increased the excretion of cholesterol, as manifested by enhanced excretion of acidic and neutral steroids after garlic feeding [36]. Garlic depressed the hepatic activities of lipogenic and cholesterologenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG, CoA) reductase [37]. Raw garlic homogenate augmented endogenous antioxidants along with reduction of basal lipid peroxidation in rat heart, liver and kidney in a dose dependent style [38,39]. Our study which reveal a clear reduction in (TC, TG, LDL) levels, it may be due to; first allicin which decrease the formation of fatty streaks in aorta and lipid profile [30]. Moreover the used 10-15% acetic acid reduced serum (TC and TG); first due to the inhibition of lipogenesis in liver; second due to the increase in faecal bile acid excretion in rats fed a diet containing cholesterol [29]. Our results disagree with [30] who found no effect of garlic on lipid concentration in circulation. Cured lesions in the intimal coat support the significant lowering effect of mixture.

REFERENCES

1. Braunwald, E., (1997). Effects of apple juice on risk factors of lipid profile, inflammation and coagulation, endothelial markers and atherosclerotic lesions in high cholesterolemic rabbits. *Hearth disease*. Vol 3, 1625.
2. Nitenberg A, Cosson E, Pham I (2006). Postprandial endothelial dysfunction: role of glucose, lipid and insulin. *Diabetes Metab*. 32: 28-33.
3. Vogel RA, Corretti MC, Plotnick GD: Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* (1997), 79:350-354.
4. Creager MA, Cooke JP, Mendelsohn ME: Impaired vasodilatation of forearm resistance vessels in hyper-cholesterolemic humans. *J Clin Invest* 1990, 86: 224-228
5. Léger CL, Kadri-Hassani N. (1995); [Modulation by some fatty acids of protein kinase C-dependent NADPH oxidase in human adherent monocyte: mechanism of action, possible implication in atherogenesis]. *C R Seances Soc Biol Fil*. 189(5):765-79 (French abst.).
6. Vane JR, Anggard EE, Botting RM (1990): Regulatory functions of the vascular endothelium. *N Engl J Med* 323:27-36
7. Liu, L. & Yeh, Y. (1999) Organosulfur compounds of garlic inhibit fatty acid biosynthesis in cultured rat hepatocytes. *FASEB J*. 13: A556 (abs.). Lybarger, J. A., Gallagher, J. S., Pulver, D. W., Litwin, A., Brooks, S. & Bernstein
8. Lawson LD (1998): Garlic: a review of its medicinal effects and indicated active compounds. In: *Phytomedicines of Europe. Chemistry and Biological Activity*. Series 691 (Edited by: Lawson LD & Bauer R) American Chemical Society, Washington, DC 176-209.

9. Lau, B.H.S., Lam, F. & Wang-Cheng, R. (1987) Effect of an odor-modified garlic preparation on blood lipids. *Nutr. Res.* 7: 139–149.
10. Silagy, C. A. & Neil, H. A. (1994) A meta-analysis of the effect of garlic on blood pressure. *J. Hypertens.* 12: 463–468.
11. Warshafsky, S., Kamer, R. S. & Sivak, S. L. (1993) Effect of garlic on total cholesterol, a meta-analysis. *Ann. Intern. Med.* 119: 599–605.
12. Berthold, H. D., Sudhop, T. & Bergmann, K. V. (1998) Effect of a garlic oil preparation on serum lipoproteins and cholesterol metabolism. *J. Am. Med. Assoc.* 279: 1900-1902.
13. Breithaupt-Großgler, K., Ling, M., Boudoulas, H. & Belz, G. G. (1997) Protective effect of chronic garlic intake on elastic properties of aorta in the elderly. *Circulation* 96: 2649-2655.
14. Isaacsohn, J. L., Moser, M., Stein, E., Dudley, K., Davey, L., Liskov, E. & Black, H. (1998) Garlic powder and plasma lipids and lipoproteins. *Arch. Intern. Med.* 158: 1189–1194.
15. McCrindle, B. W., Helden, E. & Conner, W. T. (1998) Garlic extract therapy in children with hypercholesterolemia. *Arch. Pediatr. Adolesc. Med.* 152: 1089-1094.
16. Neil, A., Silagy, C., Lancaster, T., Hodgeman, J., Vos, K., Moore, J., Jones, J., Cahill, J. & Fowler, G. (1996) Garlic powder in the treatment of moderate hyperlipidaemia: a controlled trial and meta-analysis. *J. R. Coll. Physicians Lond.* 30: 329–334.
17. Simons, L. A., Balasubramaniam, S. von Konigsmark, M., Parfitt, A., Simons, J. & Peters, W. (1995) On the effect of garlic on plasma lipids and lipoproteins in mild hypercholesterolemia. *Atherosclerosis* 113: 219–225.
18. Asgary S, Jafari DN, Madani H, Mahzoni P, Naderi GH (2007) Effect of *Glycyrrhiza glabra* extract on aorta wall atherosclerotic lesion in hypercholesterolemic rabbits. *Pak. J. Nutr.* 4: 313-317.
19. Julie H, Campbell M, Johnny L, Efendy J, Gordon R (2001). Molecular Basis by which garlic suppresses atherosclerosis *J. Nutr.* 131: 1006-1009.
20. Cohn, J.S (1998). Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can. J. Cardio.* 5: 18-27.
21. Boyer J, Rui HL (2004). Apple phytochemicals and their health benefits. *Nutr. J.* 12: 3-5.
22. Décordé K, Teissèdre PL, Auger C, Cristol JP, Rouanet JM (2008). Phenolics from purple grape, apple, purple grape juice and apple juice prevent early atherosclerosis induced by an atherogenic diet in hamsters.. *Mol Nutr. Food Res.* 52(4):400-7 (France abs.).
23. Stein JH, Keevil JG, Wiebe DA :Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 1999,100: 1050–1055.
24. Ren H, Endo H, Watanabe E & Hayashi. T (1997) Chemical and sensory characteristics of Chinese, Korean and Japanese vinegars. *J Tokyo Univ. Fish* 84, 1-11.
25. Shahidi F, McDonald J, Chandrasekara A, Zhong Y: Phytochemicals of foods, beverages and fruit vinegars: chemistry and health effects. *Asia Pac J Clin Nutr.* (2008);17Suppl 1: 380-382.

26. Johnston CS, Gaas CA(2006): Vinegar: Medicinal uses and antiglycemic effect. *Med. Gen. Med*,8:61.
27. Havsteen B: Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* (1983), 7:1141-1148.
28. Aritsuka T, Tanaka K & Kiriyama S (1989) Effect of beet fiber on lipid metabolism in rats fed a cholesterol-free diet in comparison with pectin and cellulose. *J Jpn Soc Nutr Food Sci* 42, 295-304.
29. Fushimi T, Suruga K, Oshima Y (2006): Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br J Nutr*, 95:916-924.
30. Abramovitz D, Gavri S, Harats D, Levkovitz H, Mirelman D, Miron T, Eilat-Adar S, Rabinkov A, Wilchek M, Eldar M, Vered Z(1999): Allicin induced decrease in formation of fatty streaks (atherosclerosis) in mice fed a cholesterol-rich diet. *Coron. Artery Dis*,10:515-519.
31. Friedwald w.t, et.al (1972), Estimation of concentration of LDL- cholesterol in plasma without use the preparative ultracentrifuge .*Clin. Chem.* 18:499-502.
32. Luna,L.G.(1968).Manual of Histological staining methods of Armed Forces Institute of Pathology.3rd Ed. McGraw-Hill book company New York.
33. Humason,G.L. (1967) *Animal Tissue Techniques*. W. H. Freeman and Company. San Francisco.
34. Mahbubeh Setorki , Sedighe Asgary , Akram Eidi , Ali Haeri rohani and Nafiseh Esmaeil (2009). Effects of apple juice on risk factors of lipid profile, inflammation and coagulation endothelial markers and Athero-sclerotic lesions in high cholesterol-emic rabbits *Lipids in Health and Disease*, 8:39.
35. Orekhov AN, Grunwald J:(1997) Effects of garlic on atherosclerosis. *Nutrition* 13:656-663.
36. Chi MS, Koh ET, Stewart TJ (1982): Effect of garlic on lipid metabolism in rats fed cholesterol or lard. *J Nutr*, 112:241-24.
37. Yu-Yan Yeh, Liu L(2001): Cholesterol lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. *J Nutr*,131:989-993.
38. Banerjee SK, Maulik M, Mancahanda SC, Dinda AK, Gupta SK, Maulik SK: (2002) Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life Sciences*, 70:1509-1518.
39. Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK:(2001) Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status *Food Chem. Toxicol.* 39:793-797.