Aged Garlic Extract™

Research Excerpts from Peer Reviewed Scientific Journals & Scientific Meetings

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AGED GARLIC EXTRACT

Kyolic® Aged Garlic Extract™ is truly the only odorless garlic supplement in the market. The production of Kyolic Aged Garlic Extract begins with garlic grown on Wakunaga farms under strictly controlled organic conditions without the use of chemical fertilizers, herbicides or pesticides. Wakunaga's garlic crop is cultivated with time-tested procedures to enhance its beneficial constituents. Quality control begins when the harvested raw garlic is tested for conformity to specified quality standards. Only the finest garlic is used for the production of Kyolic. The unique aging process applied in manufacturing Aged Garlic Extract distinguishes Kyolic from other garlic products available on the market. First, the garlic cloves are cleaned and sliced. Then, under carefully controlled conditions, the sliced garlic is stored in an aqueous ethanol solution in stainless steel tank and naturally aged, without heating, for up to 20 months. Through this unique process, the harsh and unstable organosulfur compounds are converted into mild and effective compounds including the sulfur-containing amino acids (e.g. *S*-allylcysteine, *S*-allylmercaptocysteine) and Maillard reaction products that are responsible for Kyolic's health benefits, and the extract is reported to increase the antioxidant effects during the natural aging process. This conversion eliminates odor-causing components, resulting in the truly odorless Kyolic Aged Garlic Extract that contains safe, stable, bioavailable and beneficial compounds. Kyolic Aged Garlic Extract is manufactured and quality controlled under International Standard Organization (ISO) 9001 and Good Manufacturing Practices (GMP) as detailed in Part 211 of Title 21 of The Code of Federal Regulations. Aged Garlic Extract also complies with the specifications established in the US Pharmacopeia/National Formulary (USP/NF) monograph. Aged Garlic Extract, and its various constituents, has been the subject of almost 600 scientific studies around the world including Japan, the United States and Europe since its development in 1955. The following are short summaries of published data, which confirm the safety and effectiveness of this unique garlic preparation.

PHARMACOLOGICAL PROPERTIES OF AGED GARLIC EXTRACT

Cardioprotective Effects Seen in Clinical Studies

The following clinical studies have shown the reduction of cardiovascular disease risk factors including reduction of blood lipids or fats, such as serum cholesterol, and triglycerides, inhibition of platelet aggregation or blood-thinning effects, enhancement of circulation and reduction in blood pressure following the intake of Aged Garlic Extract:

Inhibits Plaque Formation in the Coronary Artery

Budoff, M., Takasu, J. et al. FASEB J. 2003, April 15, 2003, San Diego.

A randomized double-blind placebo controlled pilot clinical study has shown significant inhibition of plaque formation in the coronary artery of heart surgery patients by consuming 4ml of Kyolic® Aged Garlic Extract™ a day, for about a year. Approximately 65% of significant reduction in plaque formation was observed in the Kyolic goup compared to placebo. Blood homocysteine and LDL have a tendency to reduce via Kyolic supplementation as well. In addition, HDL has a tendency to increase. Thus, Kyolic AGE may be very beneficial and useful for prevention of stopping blood flow to the heart, especially in high-risk people including heart surgery patients.

Budoff, M., Takasu, J. et al. 2004. Prev. Med. 39(5): 985-91.

Budoff, M. et al. FASEB J. April 2003, San Diego.

Budoff et al. found in their one year, placebo-controlled, double-blind, randomized pilot study that patients with known coronary artery disease who were given 4 ml of Kyolic® Aged Garlic Extract™ for one year have a significantly lower calcium score (mean change: 7.5 ± 9.4%) than the placebo group (22.2 ± 18.5%). This pilot study indicates the potential ability of AGE to inhibit the rate of progression of coronary calcification.

Budoff M. Aged Garlic Extract Retards Progression of Coronary Artery Calcification. J. Nutr. 2006;136 Suppl 3:S741-4.

Using electron beam computed tomography (EBCT) in a placebo-controlled, double-blind, randomized pilot study, aged garlic extract (AGE) has been shown to inhibit coronary plaque formation by 7.5% in patients on statin therapy.

Amagase, H., Budoff, M. et al. FASEB J. April 2004, 18(6): 600: 6.

Amagase, H., Budoff M. and Rosen, R.T. ICNPR. July 31-August 4, 2004.

A randomized, double-blind, placebo-controlled clinical study has shown more than 65% significant inhibition of plaque formation of cardiac patients consuming 4ml of Kyolic® Aged Garlic Extract™ a day for over a year compared to the placebo. Significant reductions of blood pressure, platelet aggregation, LDL oxidation and homocysteine level were also observed in the Kyolic AGE group in many other clinical studies, which was done in a same -blind, placebo-controlled manner. This suggests that Kyolic AGE may be useful and beneficial in the treatment and prevention of cardiac atherosclerosis through multiple risk reduction mechanisms.

Lipid Lowering Effects

Lau, B., Lam, F. et al. 1987. Nutr. Res. 7:139-149.

Lau et al. (1987) administered four capsules of Kyolic® Aged Garlic Extract liquid daily to subjects with elevated levels of cholesterol (220-440 mg/dl) while another group received a placebo indistinguishable from the Kyolic capsules. Serum levels of cholesterol and triglycerides in the Kyolic-taking group significantly dropped, while the placebo group showed no change. After six months, 11 of 15 subjects or 73% experienced a significant reduction in serum cholesterol compared to baseline (↓12-31%), while 10 of 15 subjects experienced drops of greater than 10% in triglycerides. Moreover, subjects' serum levels of LDL cholesterol, a major risk factor for heart disease, were decreased by Kyolic supplementation. Concurrently, an increase in HDL cholesterol, which is known to protect against heart disease, was observed in the Kyolic-taking group.

Silagy, C. and Neil, A. 1994. J. Royal Coll. Physic. Lond. 28(1): 39-45.

Lau's paper (1987) met the stringent criteria established for the meta-analysis of various clinical studies conducted by Silagy et al. (1994) to determine the effects of garlic on cholesterol. Pooling Lau's data in with a total of 16 trials including more than 900 subjects confirmed that garlic-treated subjects experienced a 12% reduction in cholesterol beyond the effect of placebos alone.

Warshafsky, S., Kamer, R. et al. 1993. Ann. Intern. Med. 119: 599-605.

Another meta-analysis conducted by Warshafsky et al. (1993) included Lau's paper (1987) in the pooled data and found a 9% reduction in cholesterol from garlic intake.

Kawashima, Y., Ochiai, Y.. et al. 1989. Shinryou To Shinyaku (Treat. New Med.) 26: 377-388.

After taking four capsules of a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B_1, vitamin B_{12} and liver extract, each day for 16 weeks, a 9% reduction in serum cholesterol (234±10 mg/dl → 213±10 mg/dl), a 17% reduction in triglycerides (229±20 mg/dl → 190±16 mg/dl) and a 9% reduction in LDL cholesterol (152±10 mg/dl → 138±10 mg/dl) were seen in 19 patients studied by Kawashima et al. (1989).

Yeh, Y., Lin, R. et al. 1995. J. Am. Coll. Nutr. 13:545

Yeh, Y., Lin, Ret al. 1997. In Food Factors for Cancer Prevention. Ohigashi, et al. eds., p 226-230. Springer-Verlag, Tokyo.

In a five-month, double-blind, randomized, placebo-controlled intervention study of men with high cholesterol (241±21 mg/dl) by Yeh et al. (1995, 1997), Aged Garlic Extract was found to reduce total plasma and LDL-cholesterol by 7% and 10%, respectively. During the study, the men maintained their normal diets.

Steiner, M.., Lin, R. et al. 1996a. Shinyaku To Rinsho (New Drug Clin.) 45(3): 456-466.

Steiner, M.. and Lin, R. 1994. J. Amer. Coll. Nutr 13(5): 524.

Steiner et al. (1994, 1996a) in a six-month, double-blind, crossover study found that aged garlic extract powder supplementation led to a modest reduction in LDL cholesterol (8%) in free-living men with high cholesterol levels (230-290 mg/dl) on a lowfat/low cholesterol diet. No change in HDL was noted. In participants, who had good compliance in their regimes, triglycerides were significantly reduced 18.9% compared to baseline and 6.7% compared to placebo.

Steiner, M.., Lin, R. et al. 1996b. Am. J. Clin. Nutr. 64: 866-870.

In this double-blind, crossover study of hyperlipidemic patients (cholesterol 220-290 mg/dl) on the NCEP[1] Step I Diet, Steiner et al. (1996b) found a 6-7% reduction in total serum cholesterol and a 4-5% reduction in LDL cholesterol following six month oral intake of Aged Garlic Extract powder.

Lee, YY, YY Yeh. 2003. FASEB J 17(4): A752, # 455.1

The lipid-lowering effect of garlic in humans is explored via the effects of aged garlic extract and water-soluble sulfur containing compounds of garlic in HepG-2 cells, a human hepatomoa cell line. Kyolic contains *S*-allyl cysteine (SAC), *S*-ethyl cysteine (SEC), and *S*-propyl cysteine (SPC) and gamma glutamyl *S*-alk(en)yl cysteines. Kyolic inhibits cholesterol synthesis due to these water-soluble organosulfur compounds. The results of this study show that the maximal inhibition on cholesterol synthesis by garlic requires a concerted action of these various compounds in HepG-2 cells.

Allison GL, Lowe GM, Rahman K. Aged Garlic Extract and Its Constituents Inhibit Platelet Aggregation through Multiple Mechanisms. J. Nutr. 2006;136 Suppl 3:S782-8.

Aged Garlic Extract (AGE) and its constituents inhibits platelet aggregation in a concentration-dependent manner by working in a synergistically and exerting multiple effects on biochemical pathways. Calcium movement from/into the cells will be the key mechanism.

Thomson M. et al. 2006. J Nutr. 136(3S): 800S-802S

In a study by Thomson et al. (2006), raw and boiled aqueous extracts of garlic were administered to subjects orally and intraperitoneally for 4 weeks. A significant reduction in the cholesterol level (11-14%) of subjects was observed in the group that received a low dose of raw aqueous extract of garlic. A significant reduction in triglyceride levels (38%) was also noted in subjects who received garlic orally and intraperitoneally. Glucose, cholesterol, and triglyceride levels were also significantly reduced in subjects treated with a high dose of raw garlic.

Circulation-Enhancing/Blood-Thinning Clinical Effects

The following studies showed that Aged Garlic Extract decreases platelet aggregation and adhesion to the vessels and improves blood circulation:

Weiss N, Ide N, Abahji T, Nill L, Keller C, Hoffman U. Aged Garlic Extract Improves Homocysteine-Induced Endothelial Dysfunction in Macro- and Microcirculation. J. Nutr. 2006;136 Suppl 3:S750-4.

In a placebo-controlled, blinded, crossover pilot study by Weiss et al., pretreatment with Aged Garlic Extract (AGE) for 6 weeks significantly reduced the adverse effects associated with acute hyperhomocysteinemia in macro- and microvascular endothelial dysfunction. AGE may be partly responsible in preventing a decrease in bioavailable nitric oxide (NO) and endothelium-derived hyperpolarizing factor during acute hyperhomocysteinemia.

Abahji T, Ide N, Nill L, Keller C, Hoffmann U, Weiss N. 2005. 5th International Conference on Homocysteine Metabolism. Milano (Italy), June 26-30. Haematologica Reports. 1: 39#PO36

Abahji T, Weiss N. 2005. Med Report. 31:11 (German)

Weiss N. Abahji T. Ide N. et al. 2006. XIV International Symposium of Atherosclerosis. Rome (Italy). June 18-22. Atherosclerosis. 7: Abst# P-7: 143

Hyperhomocysteinemia leads to endothelial dysfunction and decreased bio-available nitric oxide due to increased vascular oxidant stress. Subjects were involved in a placebo-controlled, double-blinded crossover study where their brachial artery was studied by ultrasound and laserdoppler fluxmetry (acetylcholine-induced skin profusion) with treatment of AGE before and after 6 weeks of treatment. It was found that AGE improves endothelium dysfunction with the increase in intracellular thiol antioxidants.

Williams MJA. et al. 2005. Phytother Res. 19: 314-319.

A randomized, placebo controlled, cross-over design study supplemented 15 men with AGE. All men had angiographically proven coronary artery disease (CAD). When supplemented with AGE, brachial artery flow mediated endothelium-dependent dilation (FMD) significantly increased (44 percent, P=0.04). At the end of AGE treatment, FMD levels were significantly higher (p=0.03) when compared with the placebo treatment. This study has shown that AGE may help in the improvement in endothelial function in men with CAD treated with aspirin and a statin.

Rahman K. and Billington, D. 2000. J. Nutr. 130: 2662-2665.

K. Rahman et al., 3rd International Congress on Phytomedicine (3rd ICP), Munich (Germany) October 11-13, 2000.

By means of a randomized double-blind clinical study, Rahman et al. (2000) found that Aged Garlic Extract (5 ml of plain liquid Kyolic) taken for 13 weeks by human subjects with normal cholesterol, reduced clotting as seen by a significant inhibition of the ability of ADP, a clotting agent, to cause platelet aggregation. Both the rate of clotting and the amount of clotting were significantly inhibited.

Steiner, M.. and Lin, R. 1998a. J. Cardiovascular Pharmacol. 31: 904-908.

Steiner and Lin (1998) found in their 10-month placebo controlled study of men with high cholesterol that supplementation of 7.2 g of Aged Garlic Extract could improve blood flow by reducing platelet aggregation or the ability of platelets to cluster and make clots. Blood samples were drawn from subjects taking either AGE or a placebo and compounds (epinephrine and collagen) were added to the blood samples to induce aggregation or clumping. After three months, blood samples from subjects taking AGE showed 33% less platelet aggregation and after six months showed 43% less aggregation than blood from the placebo group. Further, the ability of the platelets to adhere to fibrinogen, a clotting agent in the blood, was reduced by approximately 30% in subjects taking AGE compared to placebo.

Steiner, M. 1998b. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998b.

*Steiner, M. and W. Li, J. Nutr., **131**: 980S-984S (2001).*

Steiner, M. et al., 3rd International Congress on Phytomedicine (3rd ICP), Munich (Germany) October 11-13, 2000.

Steiner et al. (1998b, 2000, 2001) presented the reduction of cardiovascular risk factors by AGE administration (2.4 and 4.8 g/day) to moderately hypercholesteolemic men in a randomized double-blind clinical trial. AGE reduced blood levels of total and LDL cholesterol by 5-7%. AGE also reduced platelet aggregation induced by collagen and epinephrine, and inhibited its adhesion to fibrinogen and collagen. In addition, blood pressure was reduced by AGE. Steiner indicated the fact that AGE reduced so many different risk factors for cardiovascular disease made it unique and potent.

Steiner, M., Lin, R. et al. 1996a. Shinyaku To Rinsho (New Drug Clin.) 45(3): 456-466.

Steiner M. and Lin, R.I. .1994. J. Amer. Coll. Nutr 13(5): 524.

Steiner et al. (1994, 1996a) found in their six-month, double-blind, crossover study that Aged Garlic Extract powder supplementation led to a significant reduction in platelet adhesion to fibrinogen (34-58%) and a 10-25% reduction in platelet aggregation (platelets sticking together and clumping). The subjects studied were free-living men with high cholesterol levels (230-290 mg/dl) on a lowfat/low cholesterol diet (NCEP)[1].

Kikuchi, N., Nishimura, Y., et al. 1994. Shinyaku To Rinsho (Jpn. J. New Remedies Clin.) 43(1): 146-158.

Via skin temperature thermography (colored photographs reflecting temperature changes in the body), Leopin-5®, an Aged Garlic Extract preparation, showed a greater enhancement in microcirculation than cooked garlic juice in patients who had demonstrated a deficiency in microcirculation. Various symptoms associated with insufficient microcirculation, such as headaches, dizziness, vertigo, weariness, lumbago, etc., were also improved (Kikuchi et al., 1994).

Okuhara, T. 1994. Jpn. Pharmacol. Therapeut. 22(8): 3695-3701.

Okuhara (1994), also using skin thermography, showed that following both single administration and continuous administration for 14 days, a greater degree of improvement in blood flow was found in subjects receiving liquid Aged Garlic Extract than those receiving heated garlic juice.

Williams, M. J. A., et al. 2005. Aged Garlic Extract Improves Endothelial Function in Men with Coronary Artery Disease. Phytother. Res. 19: 314-319.

A randomized, placebo controlled, cross-over design study supplemented 15 men with AGE. All men had angiographically proven coronary artery disease (CAD). When supplemented with AGE, brachial artery flow mediated endothelium-dependent dilation (FMD) significantly increased (44 percent, p=0.04). At the end of AGE treatment, FMD levels were significantly higher (p=0.03) when compared with the placebo treatment. This study has shown that AGE may help in the improvement in endothelial function in men with CAD treated with aspirin and a statin.

Mizuno I, Sumioka I, Ushijima M, Yasuda K, Mouri Y, Ono M, Ogasawara K ,Kyo E. 2004. Oyo Yakuri (Pharmacometrics) 67:371-8.

Mizuno et al. found that AGE combined with Leopinroyal (LER) improved the condition of peripheral blood circulation by a four-week human trial, where 1 ml of LER was taken twice a day after meals in the morning and evening. The condition of the peripheral blood circulation was estimated 0, 2, and 4 weeks after taking LER. LER was found to improve the peripheral blood circulation index and the width of small veins.

Weiss N, Abahji T, Ide N, Keller C, Hoffman U. 2006. XIV International Symposium of Atherosclerosis. Rome (Italy), June 18-22. Atherosclerosis. 7: Abst# P-7:143.

Hyperhomocysteinemia leads to endothelial dysfunction and decreased bio-available nitric oxide due to increased vascular oxidant stress. Subjects were involved in a placebo-controlled, double-blinded crossover study where their brachial artery was studied by ultrasound and laserdoppler fluxmetry (acetylcholine-induced skin profusion) with treatment of AGE before and after 6 weeks of treatment. It was found that AGE improves endothelium dysfunction with the increase in intracellular thiol antioxidants.

Hypotensive/Blood Pressure Lowering Effects

Steiner, M. Lin. R. .et al.1996a. Shinyaku To Rinsho (New Drug Clin.) 45(3): 456-466.

Compared to baseline values, significant reductions in both systolic and diastolic blood pressure (9% decrease, respectively) were noted in this double-blind, crossover study by Steiner et al. (1996a). Hyperlipidemic patients consuming the NCEP Step I diet[1] supplemented with placebo for six months and followed by Aged Garlic Extract powder for four months showed the most significant results.

Steiner, M., Lin, R. et al. 1996b. Am. J. Clin. Nutr. 64: 866-870.

Another double-blind, placebo-controlled, crossover study of hyperlipidemic patients (cholesterol 220-290 mg/dl) on the NCEP step I diet[1] by Steiner et al. (1996b) showed a 5.5% decrease in systolic blood pressure following six-month oral intake of Aged Garlic Extract powder.

Tokunaga A, Hayashi T. 1996. Shinyaku to Rinsho (J New Remedies & Clinics), 45: 2015-20 (Japanese)

An open clinical trial was performed on the effects of Aged Garlic Extract (AGE) with vitamin B1, oriental benzoar, and ginseng extract (Leopin 5; LE-5) on blood pressure. Patients were given 6mL of LE-5 for 8 days. Blood pressure was monitored at all times. Results of this study indicate that supplementation with LE-5 was effective in lowering the blood pressure in patients with high blood pressure, but was not effective in those with normal blood pressure. According to this and previous studies, AGE may be useful in maintaining normal blood pressure.

Sickle Cell Anemia (Pilot Study)

Ohnishi, S.T., Ohnishi, T., et al. 2000. Nutrition 16: 330-338.

Ohnishi et al. (2000) found that Aged Garlic Extract (4.0 mg/ml) could inhibit dense cell formation by 50%. Other effective nutrients included black tea extract, green tea extract, pycnogenol, α-lipoic acid, vitamin E, coenzyme Q10, and ß-carotene. A pilot clinical trial demonstrated that a cocktail consisting of daily doses of 6 g of Aged Garlic Extract, 4-6 g of vitamin C and 800-1200 I.U. of vitamin E may indeed be beneficial to patients with sickle cell anemia.

Sickle Cell Anemia

Takasu J, Uykimpang R, Sunga MA, Amagase H, Niihara Y. Aged Garlic Extract Is a Potential Therapy for Sickle Cell Anemia. J. Nutr. 2006;136 Suppl 3:S803-5.

Aged garlic extract (AGE) was administered to five patients with sickle-cell anemia at a dose of 5 mL daily. It is suggested that AGE exerts a significant antioxidant activity on sickle RBCs by decreasing the number of Heinz bodies in all patients over a 4-week period.

Takasu, J.MD, PhD, et al. Aged Garlic Extract for Sickle Cell Anemia Patients, BMC Blood Disorders, June 2002, 2:3

Takasu et al. (2002) examined the potential role of Aged Garlic Extract as an antioxidant for sickle red blood cells (RBC). Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study. These data suggest the significant antioxidant activity of Aged Garlic Extract on sickle cell anemia, and may represent a potential therapy to combat complications of the disease.

Ohnishi, S.T. Therapeutic Use of Specially Processed Garlic for Sickle Cell Disease. US Patent # 6,254,871; July 3, 2001.

Kyolic was recently patented by the U.S. government for its ability to reduce painful crisis of sickle cell anemia.

Decreased the Levels of Serum Prostaglandins, PGE_2 and PGF_{2a}

Dimitrov N. Bennink M. 1997. Ch 21. In: Nutraceuticals: Designer Foods III Garlic, Soy, and Licorice. Paul P. Lanchance (ed). Food & Nutrition Press. Trumbell, Ct. pp. 199-202.

In this pilot study, Dimitrov et al. (1997) found that 10 ml of Aged Garlic Extract liquid taken daily for three months decreased the levels of the serum prostaglandins, PGE_2 and PGF_{2a}.

Cardiovascular Review

Banerjee SK, and Maulik, S.K. Effect of garlic on cardiovascular disorders: a review. Nutrition Journal, 2002, 1: 1-14.

Garlic has benefits on cardiovascular system in humans. This review article cites many articles including the scientific papers of Aged Garlic Extract (AGE) and concluded garlic is beneficial for cardiovascular health, but dependent upon the preparation.

Rahman K. 2001. Historical Perspective on Garlic and Cardiovascular Disease. J Nutr. 131(3S):977S-979S.

Rahman, K. reported that many studies show the cardioprotective benefits of aged garlic extract. Since aged garlic has been shown to help lower serum cholesterol, triglyceride, LDL, blood pressure levels, and inhibit platelet aggregation, it may play an important role in the prevention of cardiovascular disease.

Rahman K, Lowe GM. Garlic and Cardiovascular Disease: A Critical Review. J. Nutr. 2006;136 Suppl 3:S736-40.

Garlic has multiple effects on parameters involved in cardiovascular disease, as confirmed by numerous studies. Aged garlic extract (AGE) has been shown to be cardioprotective in a number of trials including those conducted in our laboratory. Dietary supplementation with AGE decreased plasma and urinary oxidative stress marker levels in smokers and non-smokers and increased the antioxidant status of smokers. Thus AGE may prevent or delay free radical mediated diseases such as atherosclerosis.

Loy MH, Rivlin RS. 2000. Nutr Clin care. 3: 145-52.

Loy and Rivlin (2000) review studies of garlic and its effect on cardiovascular health. Although most studies produce positive results (e.g. reduced serum cholesterol, reduced blood pressure, and decreased LDL) there are studies that have not demonstrated these lipid-lowering effects. Still even with positive indications, Loy and Rivlin (2000) address concerns about cooked versus raw garlic and raw garlic versus supplements, and the effects on cardiovascular health. Studies have indicated that fresh/raw garlic is necessary in high doses (5-28 cloves/day) to view health benefits, whereas supplement dosage is much lower. One study found no beneficial effects with cooked/boiled garlic compared to fresh garlic. Even with such limitations garlic proves a potential combatant for cardiovascular disease.

Overview of Cardioprotective Effects of Aged Garlic Extract Found in Various Clinical Studies	
Anemia (RBC, Hemoglobin)[1]	↓ in 61% of patients
Blood Pressure[2,3]	↓ 6-9%
Chest Pain[4]	↓ in 90% of patients
Chill (limbs)[4-8]	↓ in 40-100% of patients
Cholesterol Oxidation[9,17, 19, 25]	↓ 38%
Circulation (Hands/Feet)[8,10]	↑ in 67% of patients
Dizziness[4,5,7,8]	↓ in 50-85% of patients
Endothelial Function[22,23]	↑ in 44-66% of patients
Headaches[4,5,7,8]	↓ in 50-80% of patients
Homocysteine[22]	↓ in 27% of patients
Heart Palpitations[1,4,5,7,8]	↓ in 45-75% of patients
LDL Cholesterol[2,11-13]	↓ 5-12%
Numbness in Limbs[5,7]	↓ in 40-80% of patients
Oxidative Stress by Smoking[24]	↓ in 290-37% of patients
Platelet Aggregation[9,12, 20]	↓ 10-25%
Platelet Adhesion[9,12]	↓ 30-58%
Plaque formation in artery[21]	↓ 67%
Safety with Warfarin[14, 26]	Confirmed
Shortness of Breath[5,7]	↓ in 45-80% of patients
Sickle Cell Anemia[15, 18,20]	↓ dense rbc by 30%
	↓ Heinz bodies by 30%
Total Serum Cholesterol[2,3,7,11-13,16,17]	↓ 6-31^{+}%
Triglycerides[3,4,11]	↓ 10-19%

1. Hasegawa Y, et al. 1983. Japanese J. New Remedies Clinic 32:365-76.
2. Steiner M, et al. 1996 Am J Clin Nutr. 64:866-70.
3. Steiner M, et al. 1996. Shinyaku to Rinsho. 45(3):456-66.
4. Kawashima H, et al. 1985. Shinryou to Shinyaku. (Treatment and New Medicine). 22:3012-24.
5. Miyoshi A, et al. 1984. Shinryou to Shinayku. 21:1806-20.
6. Kohno M, et al. 1976. Yakuri to Chiryo 4:700-8.
7. Hasegawa Y, et al. 1984. Shinryo to Shinyaku. (New Drug Clin.). 21:2021-35.
8. Kikuchi T, et al. 1994. Shinyaku to Rinsho. (Jpn J New Remedies Clin). 43(1):146-58.
9. Steiner M, et al. 1998. J Cardiovascular Pharmacol. 31:904-8.
10. Okuhara T. 1994. Jpn Pharmacol Therapeut. 22(8):3695-3701.
11. Lau B, et al. 1987. Nutr Res. 7:139-49.
12. Steiner M, et al. 1994. J Am Coll Nutr. 13(5):524.
13. Yeh Y, et al. 1995. J Am Coll Nutr. 14(5):545.
14. Rozenfeld V, et al. 1999. 18th Annual Eastern States Conference for Pharmacy Residents and Preceptors. Baltimore, MD. April 21-24.
15. Ohnishi ST, Ohnishi T, et al. 2000. Nutrition. 16:330-8.
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19. Lau. BHS, 2001. J. Nutr. 131:985S-8S
20. Rahman K, Billington D. 2000. J. Nutr. 130:2662-5.
21. Budoff M, Takasu J, et al. 2004. Prev Med. 39(5):985-91.
22. Weiss N, et al. 2006. J Nutr. 136(3S):750S-4S.
23. Williams, et al. 2005. Phytother. Res. 19(4):314-9.
24. Dillon SA, et al. 2002. J. Nutr. 132:168-71.
25. Lau B, et al. 2001. J Nutr. 131:168-71.
26. Macan H, et al. 2006. J. Nutr. 136(3S):793S-5S.

Cardioprotective Effects Seen in Preclinical Studies

The following preclinical studies have shown the reduction of cardiovascular disease risk factors including lowering of serum cholesterol and triglycerides, inhibition of platelet aggregation, lowering of serum homocysteine levels and inhibition of fatty streak development and smooth muscle cell proliferation[2] following intake of Aged Garlic Extract.

Lipid Lowering Effects

The following studies showed that Aged Garlic Extract and *S*-allyl cysteine,[3] a key compound in Aged Garlic Extract, demonstrated lipid lowering effects in models and cell cultures:

Khalid, Q., Sultana, L., et al. 1994. Pak. J. Sci. Ind. Res. 37(12): 524-527.

The avian model is an ideal model for cholesterol because its lipoprotein profile is more closely related to the human than that of others. Moreover, according to Khalid et al. (1994), atherosclerosis of at type closely resembling that found in humans can be produced easily and consistently in models fed a high cholesterol diet.

Qureshi, A., Lin, R. et al. 1990a. First World Congress on the Health Significance of Garlic and Garlic Constituent., Washington, D.C. August 28-30, p. 16.

Abuirmeileh, N.., Lin, R. et al. 1991. FASEB J. 5: A1756.

Yu, S., Qureshi, N., et al. 1991. National Conference on Cholesterol and high blood pressure. Sponsored by Cholesterol Education Program of the National Institutes of Health. April 8-10, 1991, Washington, D.C.

Research by Qureshi et al. (1990a) Abuirmeileh et al. (1991) and Yu et al. (1991) found that Aged Garlic Extract and, a key constituent in Aged Garlic Extract, lowered total serum cholesterol and LDL cholesterol in hypercholesterolemic models. Cholesterol reduction was achieved by inhibition of the activity of key enzymes involved in cholesterol synthesis (ß-hydroxy-ß-methylglutaryl CoA synthetase and reductase) in the liver. According to Qureshi and Yu et al. (1991), among garlic preparations tested, maximum inhibition of cholesterol-producing enzyme activities was observed in this order:

Kyolic® > SAC > commercial garlic oil > garlic powder

Singh DK, Porter TD. Inhibition of Sterol 4ά-Methyl Oxidase Is the Principle Mechanism by Which Garlic Decreases Cholesterol Synthesis. J. Nutr. 2006;136 Suppl 3:S759-64.

Using chromatography-mass spectrometry, it is found that garlic-derived compounds containing an allyl-disulfide or allyl-sulfhydryl group are most likely responsible for decreasing cholesterol synthesis by inhibiting the sterol 4ά-methyl oxidase.

Slowing K, Ganado P, Sanz M, Ruiz E, Tejerina T. 2001. J. Nutr. 131: 994S-9S

Slowing et al. (2001) found that intake of garlic can prevent diet-induced hypercholesterolemia and vascular alterations in the endothelium-dependent relaxation associated with atherosclerosis using a model study system. Subjects were fed a cholesterol-enriched diet for 16 weeks and were divided into 10 groups. Plasma total cholesterol decreased in all groups treated with garlic and its fractions. LDL decreased significantly in the hypercholesterolemic group.

Water-Soluble Sulfur Compounds Best Candidates for Lipid Reduction

Liu, L. and Yeh, Y.Y. 1998. FASEB J. 12(4):A261 #1523.

Liu, L. and Yeh, Y. 1999. FASEB J. 13(4): A556; #442.7.

Yeh, Y. 1999. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

Liu, L. and Yeh, Y. 2000. Lipids 35(2): 197-203.

*Yeh, Y.Y. and L. Liu, J. Nutr., **131**: 989S-993S (2001).*

Yeh and Liu (1998, 1999, 2000, 2001) determined the active organosulfur compounds of garlic responsible for inhibiting fatty acid synthesis. The cultured hepatocytes were treated with [2-^{14}C] acetate in the presence or absence of the sulfur compounds at 0.05 to 4.0 mmol/L. The rate of [2-^{14}C]acetate incorporation into fatty acid of the non-treatment group was used for comparison. Among water-soluble compounds, *S*-allyl-cysteine (SAC),[3] *S*-ethyl cysteine, *S*-propyl cysteine (SPC), γ-glutamyl-*S*-allyl cysteine, γ-glutamyl-*S*-methyl cysteine, γ-glutamyl-*S*-propyl cysteine and *S*-allyl mercaptocysteine (SAMC) inhibited fatty acid synthesis by 20% to 99% with IC_{50} of 0.27 to 1.72 mmol/L. Alliin, *S*-allyl-N-acetyl cysteine, *S*-allylsulfonyl alanine, and *S*-methylcysteine did not inhibit fatty acid synthesis. All tested water-soluble compounds except SAMC did not alter cellular release of lactate dehydrogenase (LDH) [4] into medium. Lipid-soluble compounds such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, dipropyl sulfide, dipropyl disulfide reduced fatty acid synthesis by 10% to 98%, which was accompanied by markedly increased LDH release (indicator of cell toxicity). In addition, SAC and SPC depressed triacylglycerol (TG) and phospholipid (PL) synthesis. It is shown that the TG lowering effect of garlic may stem from the impairment of fatty acid and TG synthesis by water-soluble sulfur compounds. Judging from the maximal inhibition and the IC_{50}, SAC, SEC, and SPC are equally potent in inhibiting cholesterol synthesis.

Liu, L. and Yeh, Y. 2001. Regulation of HMG-CoA reductase by organosulfur compounds of garlic in primary cultures of rat hepatocytes. Orange County Convention Center, Exhibit Hall A4. April 4, 2001.

Liu and Yeh (2001) tried to elucidate the mechanism by which water-soluble organosulfur compounds of garlic (*S*-allyl cysteine, *S*-ethyl cysteine and *S*-propyl cysteine) inhibit hepatic cholesterol biosynthesis. Their results strongly indicated that they inhibit cholesterol synthesis by decreasing HMG-CoA reductase activity due to posttranslational but not pretranslational modification of HMG-CoA reductase.

Gupta, N. and Porter, T. 2001. Garlic and garlic-derived compounds inhibit human squaline monooxygenase. J. Nutr. 131: 1662-1667.

Gupta (2001) from the College of Pharmacy at the University of Kentucky sought to elucidate effective compounds and mechanisms whereby such compounds may reduce cholesterol synthesis. After studying garlic extract as a whole, and 16 water or lipid-soluble compounds (compounds that dissolve in either water or fat), they found that only the extract itself, selenocysteine, *S*-allyl cysteine, alliin, diallyl trisulfide and diallyl disulfide substantially inhibited squaline monoxygenase, an enzyme that catalyzes or facilitates the second step in the generation of cholesterol.

Liu, L. and Yeh, Y. 2001. Water-soluble organosulfur compounds of garlic inhibit fatty acid and triglycerides synthesis in cultured rat hepatocytes. Lipids 36(4): 395-400.

Previous work by Dr. Yeh at the Pennsylvania State University found that water-soluble compounds in garlic could inhibit cholesterol synthesis. This current paper concluded that the water-soluble compounds SAC and *S*-propyl cysteine (SPC) could also inhibit triglycerides synthesis. These compounds, as well as *S*-ethyl cysteine, and γ-glutamyl-*S*-methyl cysteine were also effective at inhibiting fatty acid synthesis. On the contrary, precursor compounds (ones that are used to generate the active compounds) in garlic (alliin, γ-SAC, γ-SPC), were ineffective, alliin, at inhibiting fatty acid synthesis, suggesting processing such as aging (via long-term extraction) is necessary for garlic to yield its maximal benefits. SAC and SPC also inhibited the activity of a lipogenic or fat-producing enzyme.

Liu. L. and Yeh, Y. 2002., S-Alk9en)yl Cysteines of Garlic Inhibit Cholesterol Synthesis by Deactivating HMG-CoA Reductase in Cultured Rat Hepatocytes., J. Nutr. 132: 1129-1134.

The researchers studied the effects of water-soluble organosulfur compounds of garlic on hepatic cholesterol biosynthesis in cultured hepatocytes. The conclusions of the study suggest that *S*-alk(en)yl cysteines inhibit cholesterol synthesis by deactivating HMG-CoA reductase through enhanced phosphorylation leaving the levels of mRNA, or the amount of enzyme, unchanged. In addition, only SAC appeared to further decrease the activity of HMG-CoA reductase by increasing sulfhydryl oxidation of the enzyme.

Qureshi, A. , Lin, R. Et al. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. August 28-30, p. 37.

In another study by Qureshi, A. et al. (1990), reductions in both serum and LDL cholesterol in both normolipemic and hypercholesterolemic models were noted. Results were more pronounced for Kyolic (14-32%) than for commercial garlic powder or garlic oil (11-16%). Significant reductions in triglycerides were also observed following Aged Garlic Extract intake and the key enzymes of lipogenesis (acetyl CoA carboxylase and fatty acid synthetase) were significantly inhibited.

Yeh, Y. and Yeh, S. 1994. Lipids 29(3): 189-193.

Yeh et al. (1994) found that Aged Garlic Extract and one of its key constituents, SAC, inhibited the synthesis of cholesterol and fatty acids in cultured liver cells.

Yeh, Y. and Yeh, S.1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. August 28-30, p. 37.

Yeh et al. (1990, 1994) further found that Aged Garlic Extract lowered the plasma levels of cholesterol and triglycerides. In models fed a diet supplemented with 2% Aged Garlic Extract powder, plasma triglyceride and cholesterol levels were 30% and 15% lower, respectively, than control models.

Efendy, J.., Campbell, J. et al. 1996. J. Vasc. Res. 33(S1): 23.

Efendy et al. (1996a) found that eight weeks oral intake of "Kyolic" liquid Aged Garlic Extract significantly reduced elevated levels of ßVLDL induced by cholesterol feeding in models. Elevated ßVLDL is a greater risk factor for atherosclerosis than HDL or LDL in models.

Circulation-Enhancing/Blood-Thinning Preclinical Effects

Amagase, H., Moriguchi T., and Kasuga, S. 2000. Comparison of oxidative damage of garlic preparations including enteric-coated garlic powder preparation and allicin-derived compounds on erythrocyte duodenum. Phytomed. 7(2): 118.

Amagase et al. (2000) indicated that allicin and its derived oil-soluble compounds contribute to some of the toxicity found from various forms of garlic, such as anemia and gastrointestinal problems. AGE, on the other hand, prevents hemolysis and loss of flexibility of red blood cells caused by lipid peroxidation. Raw garlic and enteric-coated garlic products (Garlicin®, Garlique® and Garlinase 4000®), which deliver allicin directly to the gut, caused severe damage to the epithelial mucosa or cells lining the intestinal tract.

Moriguchi, T., Itakura, Y. et al. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

*Moriguchi, T, N. Takasugi, and Y. Itakura,. J. Nutr., **131**: 1016S-1019S (2001).*

Moriguchi et al. (1998, 2001), found that Aged Garlic Extract significantly prevented the loss of erythrocyte deformability (flexibility of red blood cells to move through small microvessels) caused by lipid peroxidation (oxidative damage). AGE also significantly reduced the generation of TBA-RS[5] (markers of free radical oxidation) and suppression of intercellular ATP (cell energy) caused by lipid peroxidation. Moreover, AGE significantly suppressed not only the hemolysis (rupturing of red blood cells) induced by peroxidation but also hemolysis due to non-peroxidation (naturally occurring). These results suggest the possibility that AGE improves microcirculation and rheological blood properties (blood flow) and preserves the structure and function of red blood cells by stabilizing the cell membranes and supporting cell metabolism.

Qureshi, N., Lin, R., et al. 1990b. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. August 28-30, p. 17.

Yu, S., Qureshi, N., et al. National Conference on Cholesterol and high blood pressure. Sponsored by Cholesterol Education Program of the National Institutes of Health. April 8-10, 1991, Washington, D.C.

Abuirmeileh, N., Lin, R. et al. 1991. The FASEB J. Journal, 5: A1756.

Qureshi, N. et al. (1990b) and Yu et al. (1990) and Abuirmeileh et al. (1991) found that Aged Garlic Extract and SAC lowered the levels of plasma thromboxane B_2, and factor 4 (blood clotting factors) in hypercholesterolemic models up to 30%. Aged Garlic Extract and SAC also decreased platelet aggregation, or blood clotting, induced by the potent clotting agents, collagen and adenosine diphosphate.

Al-Qattan KK, Thomson M, Al-Mutawa'a S, Al-Hajeri D, Drobiova H, Ali M. Nitric Oxide Mediates the Blood Pressure Lowering Effect of Garlic in the Rat Two Kidney, One-Clip Model of Hypertension. J. Nutr. 2006;136 Suppl 3:S774-6.

Al-Qattan et al. (2006) found that garlic reduces the blood pressure by enhancing the concentration and activity of many vasodilatory agents including nitric oxide (NO).

Harauma A, Moriguchi T. Aged Garlic Extract Improves Blood Pressure in Spontaneously Hypertensive Rats More Safely than Raw Garlic. J. Nutr. 2006;136 Suppl 3:S769-73.

Harauma A, Moriguchi T. 2005. Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease. Washington DC, April 9-11, pp48.

Aged garlic extract (AGE) or raw garlic (RW) was administered to hypertensive models for 10 weeks. Both (AGE) and (RW) reduced the increase of systolic blood pressure (SBP) compared with the control group after 4 weeks of administration. AGE also showed a decrease of pulse pressure (PP) suggesting blood-vessel extensibility while harmful effects were observed in RG group, which includes a decrease in erythrocytes, an increase in reticulocytes, and generation of polyps were found in the forestomach. Results suggest that AGE may be safe in improving circulatory diseases related to blood vessel than raw garlic.

Allison GL, Lowe GM, Rahman K. Aged Garlic Extract May Inhibit Aggregation in Human Platelets by Suppressing Calcium Mobilization. J. Nutr. 2006;136 Suppl 3:S789-92.

Repeated experiments of platelet-rich plasma (PRP) in the presence of Aged Garlic Extract (AGE) were found to suppress platelet aggregation and calcium mobilization. Furthermore, the metal-chelating properties of AGE was confirmed when platelets were preincubated with AGE significantly reduced the initial concentration of intracellular calcium.

Allison GL, Lowe GM, Rahman K. 2005. Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease. Washington DC, April 9-11, pp45.

One of the risk factors of cardiovascular disease is an increase of platelet aggregation; calcium mobilization plays an important role in stimulating platelet aggregation. Allison et al. (2005) found that with the addition of AGE to platelet-rich plasma; there was inhibition of platelet aggregation due to the immobilization of calcium.

Yokoyama K. Fuwa T. et al. 1988. Oyo Yakuri (Appl. Pharmacol.) 36: 301-308.

Yokoyama et al. (1988) found that a Kyolic Liquid Aged Garlic Extract preparation containing vitamin B_1, vitamin B_{12}, and liver extract (KLE), and Leopin- (LE-5), another Aged Garlic Extract preparation with assorted nutritional factors, improved peripheral blood circulation. Specifically, I.V. administration of KLE and LE-5 increased peripheral blood flow in the hind limbs of models immersed in cool water (15°C) for 10 minutes significantly more than control (saline and vehicle). Oral administration of KLE and LE-5 shortened the average time for re-warming in these models. Further, *in vivo* administration of KLE and LE-5 inhibited norepinephrine-induced contraction of an isolated artery (demonstrating an α-antagonistic effect) and LE-5 casued relaxation of a depolarized vascular smooth muscle.

Morihara N. et al. 2002. Life Sci. 71: 509-17.

Morihara et al (2002) observed that Aged Garlic Extract increased NO production by activating cNOS, but not iNOS. The researchers noted that Aged Garlic Extract increased NO production roughly 30-40% after administration and it was returned to the basal value after 2hr. one of the many curative properties of garlic has been attributed to its ability to increase physiological levels of NO, thereby acting as an anti-arteriosclerotic, anti-inflammatory, compound while possibly reducing damage in diseased blood vessel walls.

Morihara N. Sumioka I. Ide N. et al. 2006. J Nutr. 136(3S): 777S-781S.

The effect of Aged Garlic Extract (AGE) was investigated by administering a single dose of AGE to subjects resulting in a 30-40% increase in nitric oxide (NO) production by activating constitutive NOS (cNOS), but not inducible NO synthase (iNOS). Another experiment found that AGE suppressed the rate of peroxynitrite-induce hemolysis in a dose-dependent manner, which suggests that AGE could be useful for long-term prevention of cardiovascular diseases associated with oxidative stress or dysfunctions of NO production.

Reduction of Serum Homocysteine

Yeh, Y., Yeh, S. et al. 1999. FASEB J. 13(4): A232; #209.12

Hyperhomocysteinemia (high blood level of homocysteine (Hcy) is a well-established risk factor for arteriovascular diseases and folate deficiency contributes to this condition. Yeh et al. (1999) found that Aged Garlic Extract effectively reduced hyperhomocysteinemia caused by severe folate deficiency. When succinysulfathiazole, an antibiotic that destroys bacteria which produce folate, was added to an already folate deficient diet, homocysteine levels increased significantly. However, the addition of Aged Garlic Extract reduced plasma Hcy by 30%, and plasma free homocysteine by 24%.

Amagase, H. 2000. Method and pharmaceutical composition for reducing serum homocysteine concentrations. Patent #6,129,918.

Kyolic Aged Garlic Extract is patented by the U.S. government for its ability to reduce homocysteine.

Yeh, Y-Y., et al. 2005. Garlic extract attenuates hyperhomocysteinemia caused by folic acid deficiency status. Nutrition Research. 25: 93-102.

The supplementation of AGE was found to decrease homocysteine levels by 28 to 33 percent in models severely deficient in folate.

Yeh Y-Y, Yeh, S-M. Homocysteine-Lowering Action Is Another Potential Cardiovascular Protective Factor of Aged Garlic Extract. J. Nutr. 2006;136 Suppl 3:S745-9.

The supplementation of Aged Garlic Extract (AGE) to hyperhomocysteinemic subjects (severely induced by folate deficiency) decreased plasma total homocysteine concentration by 30%. Increased *S*adenosylmethionine and decreased *S*-adenosylhomocysteine concentrations in the liver were also noted. The hypohomocysteinemic effect from AGE is most likely due to impaired remethylation of homocysteine to methionine and enhanced transsulfuration of homocysteine to cystathionine.

Mizuno I, Ushijima M, Ichikawa M, Ishii S, Nishihama T, Morihara N, Kashimoto N, Hayama M. 2006. Oyo Yakuri (Pharmacometrics), 71:61-6

AGE products combined with Kyoleopin Neo (KLEN) has been found to extenuate short- or long-term hyperhomocysteinemia induced by L-methionine loading in models, and as a result of improvement of homocysteine metabolism, may function as a useful agent for prevention of cardiovascular disease.

Anti-atherogenic / Anti-atherosclerotic Effects

According to the following studies, Aged Garlic Extract and its various constituents were found to inhibit the progression of heart disease by preventing smooth-muscle cell proliferation[2] (the growth of smooth muscle cells over accumulated scar tissue in blood vessels):

Lee, E., Steiner, M. et al. 1994. Biochim. Biophys. Acta 1221:73-77.

Lee, E. et al. (1994) found that *S*-allyl mercaptocysteine and *S*-allyl cysteine, two constituents in Aged Garlic Extract, could inhibit vascular smooth-muscle cell (SMC) and umbilical endothelial cell proliferation. SMC proliferation[2] constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subjected to angioplasty.[6]

Efendy, J.., Campbell, J. et al. 1997. Atherosclerosis 132: 37-42.

Efendy, J.., Campbell, J. et al. 1996a. J. Vasc. Res. 33(S1): 23.

Efendy, J.., Campbell, J. et al. 1996b Proceedings of the 1996 Conference of the Anatomical Society of Australia and New Zealand., Brisbane, Australia, p. 19.

Efendy JL, Simmons DL, Campbell GR, Campbell JH. 1996. IX International Vascular Biology Meeting. Seattle, Sept. 4-8, Abst# 090.

Efendy et al. (1997, 1996a,b) investigated the direct effect of "Kyolic" on the development of atherosclerosis (heart disease) in hypercholesterolemic conditions. After being fed a high cholesterol diet in combination with "Kyolic" for eight weeks, models developed 45% fewer fatty streak lesions following angioplasty[6] than those fed a high cholesterol diet alone. "Kyolic" significantly reduced neointimal formation (thickening of the artery wall) and thoracic aortae fatty streak development (accumulation of fat/cell/tissue on the arterial wall) in hyperlipidemic conditions. Further, "Kyolic" inhibited smooth muscle cell proliferation,[2] providing protection against the onset of atherosclerosis.

Campbell, J.H., Efendy, J.L. et al. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

*Campbell, J.H, J.L. Efendy, N.J. Smith, and G.r. Campbell, J. Nutr., **131**: 1006S-1009S (2001).*

Campbell et al. (1998, 2001) found that "Kyolic" significantly reduced the development of atherosclerosis (heart disease) in models with high cholesterol. After being fed a high (1%) cholesterol diet in combination with "Kyolic" (0.8mg/kg body wt/day) or alone, "Kyolic" significantly reduced the percent surface area covered by fatty streaks (25±3% versus 70±8%) and reduced aortic arch cholesterol (1.7±0.2 versus 2.1±0.1mg chol/g tissue). "Kyolic" also significantly inhibited the development of thickened, lipid filled lesions in pre-formed neointimas produced by balloon catheter injury of the right carotid artery in cholesterol-fed models (23.8±2.3% versus 42.6±6.5%).

"Kyolic" completely prevented vascular smooth muscle phenotypic change from the contractile, high volume fraction of myofilament (Vvmyo) state in test in an *in vitro* model, and potently inhibited smooth muscle cell proliferation[2] with an ED_{50} of 0.01%. "Kyolic" also inhibited the accumulation of lipid in cultured macrophages and smooth muscle.

Immune Enhancement and Anti-Infection Effects

Aged Garlic Extract has been shown to mitigate infectious diseases through enhancement of the immune system. As seen in the following studies, it has been found to enhance various immune factors such as the phagocytic (cell-killing) activity of macrophages, T-lymphocyte activity, natural killer cell activity and antibody generation. It has also demonstrated antiviral and anti-fungal activities and has been shown to modify, both directly and indirectly, the function of immune cells, which play a leading role in allergic cascade reactions including inflammation. In addition, Aged Garlic Extract has been shown to improve age-related deterioration of the immune response.

Immune Enhancement Seen in Clinical Studies

Abdullah, T., Kirkpatrick, D. et al. 1989. Onkologie 21:52-53.

Abdullah et al. (1989) found that Aged Garlic Extract enhanced natural killer cell activity and improved helper/suppressor T cell ratios in AIDS patients. After only six weeks intake, natural killer cell activity was within the normal range for all subjects. Further, patients in this study noted improvements in diarrhea, candidiasis, pansinusitis with recurrent fever and interruption of recurrent cycles of genital herpes.

Kandil, O., Abdullah, T. et al. 1988. Potential role of Allium sativum in natural cytotoxicity. Arch. AIDS Res 1:230-231.

Kandil, O., Abdullah, T. et al. 1987. Fed. Proc. 46(3): 441.

Abdullah, T., Kandil, O., et al. 1988. J. Nat. Med. Assoc. 80(4): 439-445.

After a three-week period of garlic intake, Kandil et al. (1987, 1988) and Abdullah et al. (1988) found that Aged Garlic Extract (1800 mg of Kyolic® capsules) was effective at enhancing Natural Killer cell activity. Further, Aged Garlic Extract was more effective than a high dosage of raw garlic (0.5 mg/kg body weight -for a 70 kg/154 lb. man this would be equivalent to 35 g or almost 10 cloves). Natural Killer cell activity was enhanced 140% by raw garlic and 160% by garlic capsules.

Kohno, M., Shirakura, T. et al. 1976. Yakuri To Chiryo 4: 700-708.

In patients 46-50 years of age participating in the Omiya Camp of the Japanese Land Self-Defense Force, daily consumption of 4 mls of Leopin-5®, an Aged Garlic Extract preparation containing ginseng, B vitamins and other nutritional factors, significantly increased leukocyte, or white blood cell, count after 50 days intake.

Ushirotake, T. et al. 2004. Clin. Drug and Pharmacol (Rinsho Iyaku). Suppl. 20(7):785-793.

Ishikawa H. et al. 2006. J Nutr. 136(3S): 816S-820S.

Following a 2 month examination period between the group taking AGE for more than one year and the control group, Ushirotake (2004) observed that the frequency of catching colds was significantly lower in the AGE group than the control group. Those in the AGE group who caught the cold recovered from the symptoms quicker as compared to the control group.

Immune Enhancement Seen in Pre-Clinical Studies

Kyo E. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Jan. 28, Hiroshima (Japan), pp43 (Japanese)

Kyo E, Uda N, Kasuga S, Itakura Y. 1999. 6th Annual Meeting on Japanese Association for Cancer Prevention. Tokyo (Japan), July 16-17, Abst#P-38 (Japanese)

The various biological activities of Aged Garlic Extract (AGE) have been discussed in previous conferences. In this conference, the immune modulation effect of AGE was introduced. AGE inhibited histamine release from RBL-2H3 cells, and decreased ear swelling, indicating an IgE-mediated skin reaction. AGE also inhibited the growth of Sarcoma-180 and LL/2, and enhanced the number of natural killer (NK) cells and NK activity. Furthermore, AGE inhibited the decrease in spleen weight, cell numbers, anti-SRBC antibody production, and NK activity induced by psychological stress. All of which indicate that AGE may be useful for maintaining homeostasis of immune function as an immune modulator.

Immune Enhancement Seen in Cell Culture Studies

Kyo, E., Uda, N., et al. 1998. Phytomed. 5(4): 259-267.

Kyo E, Uda N. 1999. 58th Annual Meeting of Japanese Cancer Association. Hiroshima (Japan), Sep 29-Oct. 1, Abst #2170 (Japanese)

Kyo et al. (1998) found that Aged Garlic Extract stimulated the proliferation of model spleen cells and the release of cytokines. Aged Garlic Extract strongly enhanced phagocytosis of peritoneal macrophages (the ability of immune cells to engulf foreign agents) and increased natural killer cell activity both *in vitro* and *in vivo*. After 24 hours, Aged Garlic Extract doubled the ability of Natural Killer cells to destroy YAC-1 (a cancer cell line).

Lau, B. 1989. Nutr. Rev. 9:27-31.

In Lau's study (1989) leukocytes (white blood cells) were taken from the peritoneal cavity, spleen and lymph nodes of models following intake of Kyolic Aged Garlic Extract. Using chemiluminescence, he found that the phagocytic activity (ability to engulf toxins and pathogens) of these cells was significantly enhanced.

Horie, T., Awazu, S. 1989. Planta Med. 55: 506-508.

When free radicals attack membranes, which are high in lipids (fat), they form lipid peroxides. Lipid peroxidation seems to be closely related to toxicity, disease and aging. TBA-RS[5] are used as markers of lipid peroxidation and when Aged Garlic Extract was added to liver cells, Horie et al. (1989) found a reduction in TBA-RS suggesting an antioxidant effect of the Aged Garlic Extract. Specifically, at 40 mg/ml garlic extract completely inhibited the oxidant ascorbic acid/$FeSO_4$-induced lipid peroxidation of liver tissue.

Horie, T. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents. Washington, D.C. August 28-30, 1990, p. 23.

Horie (1990) found that Aged Garlic Extract added to liver tissue (microsomal membranes) exposed to peroxidation (oxidized by oxidants ascorbic acid and Iron-Fe^{2+}) prevented oxidation as indicated by prevention of the formation of TBA-RS[5] (fluorescent substances that indicate oxidation). Aged Garlic Extract also prevented the decrease in membrane fluidity (flexibility and ability to transport nutrients and wastes across the membrane) caused by peroxidation.

Horie, T., Awazu, S. et al. 1992. Planta Medica 58: 468-469.

Additional research by Horie et al. (1992) found that the polysulfide fraction of Aged Garlic Extract also significantly prevented lipid peroxidation of liver microsomes.

Amagase, H. et al. 2000. Ch. 6. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, pp. 62-78.

Ide, N., Itakura, Y. et al. 1996b. 2nd International Congress on Phytomedicine. September 11-14, 1996. Munich, Germany.

Amagase et al. (1999) and Ide et al. (1996b) also found that Aged Garlic Extract and two of its constituents, SAC and *S*-allyl mercaptocysteine (SAMC), decreased emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. On the other hand, water extracts of raw and heat-treated garlic enhanced such emissions. AGE reduced emissions by 30% whereas a water extract of raw garlic enhanced emissions by 110% (Amagase et al. 1999). Aged Garlic Extract, SAC and SAMC scavenged hydrogen peroxide *in vivo* and Aged Garlic Extract was more effective than raw garlic juice.

Ohnishi, S. and Kojinra, R. 1997. Ch. 12. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT , pp. 105-115.

Ohnishi and Kojima (1997) found that Kyolic® and *S*-allyl cysteine (SAC), one of its key ingredients, could inhibit the oxidation or rancidity of vegetable oil (a linoleic acid micelle suspension) caused by the radical generator or oxidant 2,2'-azo-bis(2-amidinopropatne HCl (AAPH). When Aged Garlic Extract or SAC were added to this oil, they prevented the oxidizing effects of AAPH. In this biologically relevant reaction, Kyolic was found to be more effective than pure SAC alone at inhibiting lipid peroxidation.

Yang, C., Yasaei, P. and Page, S. 1993. J. Food Drug Anal. 1(4): 357-364.

Yang et al. (1993) from the FDA found that Kyolic (50 mg) afforded protection from lipid oxidation. When Kyolic was added to a standard lipid/fat (methyl linoleate) held at 60°C (140°F), it reduced the heat induced oxidation of this fat as indicated by a decrease in methyl linoleate hydroperoxide, an intermediate in the lipid oxidation process. Aged Garlic Extract also scavenged peroxide radicals.

Dillon S, Rhaman K. 2003. Life Sci 72: 1583-1594.

Oxidation of LDL has been recognized as playing an important role in the development and progression of atherosclerosis. Human LDL was isolated and challenged with a range of oxidants either in the presence of AGE or its diethyl ether extract. Results of this study indicate AGE's ability to inhibit the *in vitro* oxidation of LDL by scavenging Superoxide and inhibiting the formation of lipid peroxides. AGE also reduced LDL oxidation by chelating copper ions. Thus, AGE may have a role to play in preventing the development and progression of atherosclerotic disease.

Dillion, S., Rahman, K. et al. 2003. Life Sciences. 72, 1583-1594.

Dillion et al. (2003) found that Aged Garlic Extract inhibits *in vitro* oxidation of isolated LDL by scavenging superoxide and inhibits the formation of lipid peroxides in cell free assays. AGE was also shown to reduce LDL oxidation by the chelation of Cu^{2+}. Thus, AGE may have a significant role in preventing the development and progression of atheroschlerotic disease.

Horie T. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Jan 28, Hiroshima (Japan), pp42. (Japanese).

Since ancient times, garlic has been used as both food and medicine. Recently, it has been determined that reactive oxygen species and lipid peroxidation cause changes in biological functions, which is associated with disease and aging. Preventing these changes is important for maintaining a healthy body. In this work, the antioxidant effects of garlic are introduced. Incubation with isolated microsome, iron and ascorbic acid caused lipid peroxidation in *in vitro* systems. Co-incubation with the aforementioned compounds and garlic compounds, such as diallyl polysulfides, has shown strong antioxidant effects, and the ability to inhibit lipid peroxidation.

Ohnishi, S. and Kojinra, R. 1997. Ch. 12. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT , pp. 105-115.

Ohnishi et al. (1997) found that both liquid Aged Garlic Extract (0.05 ml/ 20 g body weight intraperitoneally) and Kyolic tablets (0.01-0.02 g/20 g body weight orally), given six times weekly, protected the heart against the cardiotoxicity of DOX (1.5 mg/kg body weight given three times a week for 40 days). Aged Garlic Extract assisted in maintaining a normal QRS width. Further, Aged Garlic Extract inhibited lipid peroxidation as seen by a decrease in TBA-RS.[5] DOX alone increased TBA-RS in heart tissue by 76% whereas liquid Aged Garlic Extract reduced TBA-RS to 17%. Aged Garlic Extract also ameliorated the decrease in body weight and survival rate caused by the DOX administration.

Mostafa, M., Mima, T., Ohnishi, S.T., Mori, K. 2000. S-Allylcysteine Ameliorates Doxorubicin Toxicity in the Heart and Liver in Mice. Planta Medica, 66: 148-151.

Doxorubicin (Adriamycin), a potent anticancer drug, is effective against a wide range of human cancers. However, the clinical uses of doxorubicin have been limited due to its serious cardiotoxic adverse effects, which are likely the result of generation of free radicals and lipid peroxidation. Dr. Ohnishi's team found that *S*-allylcysteine (SAC), an antioxidative, organosulfur compound from Aged Garlic Extract, significantly reduced doxorubicin-induced mortality, heart and liver damage. The authors suggested that SAC research may ultimately lead to a resolution of the adverse effects of doxorubicin treatment in cancer chemotherapy.

Awazu, S. 1997. Ch. 14. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT , pp. 131-138.

Diallyl pentasulfide, a constituent in Aged Garlic Extract, inhibited the production of oxidants by DOX. It completely inhibited both TBA-RS5 and chemiluminescence (indicators of oxidation) in heart tissue incubated with DOX and NADH.

— Prevented Oxidation Of LDL Cholesterol in Pre-Clinical Studies

Ide, N. and Lau, B.H.S. . 1997a. FASEB J. 11(3): A122/#713.

Ide, N., Lau B.H.S. et al. 1997a. Planta Med. 63: 263-264.

Oxidative modification of low-density lipoprotein (LDL) has been recognized as playing an important role in the initiation and progression of atherosclerosis. Ide and Lau (1997a) from Loma Linda University found that Aged Garlic Extract and its various constituents could inhibit copper-induced peroxidation of LDL cholesterol in a concentration dependent manner. Lipid oxidation was determined by measuring TBA-RS.[5] Further, it was found that when Aged Garlic Extract and its various constituents were preincubated with pulmonary artery endothelial cells (cells which line the lungs), cell damage caused by oxidized LDL was prevented, as indicated by prevention of lactate dehydrogenase release,[4] loss of cell viability and TBA-RS formation. Thus, these researchers found that Aged Garlic Extract and its constituents could not only prevent the oxidation of LDL, but also prevent oxidized LDL from damaging membranes, oxidizing lipids (fats) and damaging or killing cells which may contribute to the initiation/progression of cardiovascular disease.

Ide, N. and Lau, B.H.S. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

Ide N, Lau BHS. 1998. 5th Annual Meeting of the Oxygen Society. Washington DC, November 19-23, Abst# 68, Free Rad Biol Med. 25: S33.

*Ide, I and. Lau, B.H.S J. Nutr., **131**: 1020S-1026S, 2001.*

Ide and Lau (1998, 2001) determined the effects of aged garlic extract (AGE) and its major compound, *S*-ally cysteine (SAC), on oxidized LDL (Ox-LDL)-induced injury in endothelial cells (EC). Lactase dehydrogenase (LDH) release[4] as an index of membrane damage, methiazol tetrazoium (MTT) assay for cell viability, and thiobarbituric acid reactive substances (TBA-RS)[5] indicating lipid peroxidation were measured. OX-LDL caused an increase of LDH release, loss of cell viability and TBA-RS formation. AGE or SAC prevented all these changes. To elucidate the mechanism, effects of AGE or SAC on intracellular glutathione (GSH) level in EC, and release of peroxide from EC and macrophages (MΦ) were determined. OX-LDL resulted in intracellular GSH depletion and in of peroxide release. AGE or SAC inhibited these changes. Effects of SAC on hydrogen peroxide (H_2O_2) or tumor necrosis factor, alpha (TNF-α)-induced nuclear factor kappa B (NF-κB) activation was determined. Pretreatment of EC with SAC inhibited NF-κB activation. AGE and SAC protected EC from Ox-LDL-induce injury preventing intracellular GSH depletion in EC and by releasing peroxides from EC and MΦ. SAC also inhibited H_2O_2 or TNF-α-induced NF-κB activation. These data suggest that AGE, and its main compound, SAC, may be useful for prevention of atherosclerosis.

Lau, B.H.S. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

*Lau, BHS, J. Nutr., **131**: 985S-988S, 2001.*

Oxidized LDL, but not native LDL, promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, by increasing chemotactic properties in monocytes, and by transforming macrophages to foam cells via scavenger-receptors; all these events are recognized to contribute to atherogenesis. Lau (1998) presented experimental data from his own laboratory and those from other investigators showing that several garlic compounds can effectively suppress LDL oxidation. Short-term supplementation of garlic in subjects has demonstrated an increased resistance of LDL oxidation. These data suggest that suppressed LDL oxidation may be one of the mechanisms accounted for the anti-atherosclerotic properties of garlic.

Reduced Physiological Stress in Pre-Clinical Studies

Ushijima, M., Sumioka, I. et al. 1997. Phytother. Res. 22: 226-230.

Amagase, H. et al. 2000. Ch. 6. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, pp. 62-78.

Ushijima et al. (1997) and Amagase et al. (1999) found that in various stress tests Kyolic was more effective than raw garlic, heated garlic or powdered garlic at reducing both physiological and psychological stress. Subjects given Kyolic swam 82% (2ml) and 90% (4ml) of the time they were in water, whereas those not given garlic swam only 50% of the time. Except at a low dosage of raw garlic, only Kyolic significantly enhanced swimming time. Subjects given Kyolic were also found to run longer than those given a placebo (control), raw garlic (RGJ), heated garlic (HGJ) or garlic powder (PG) in a mechanical treadmill running test. Control ran for 929 seconds whereas those given Kyolic ran for 1611 seconds, almost twice as long.

Ishii S,Ushijima M, Mizuno I, Nishihama T, Kashimoto N, Morihara N, Hayama M. 2006. Oyo Yakuri (Pharmacometrics), 71:101-9

AGE products with Kyoleopin Neo(KLEN) attenuates the reduction of skin surface conductance (SSC), induced by immobilization stress, and increases skin pigmentation induced by UV-B in models, suggesting that it may function as a useful agent for improvement of skin condition deterioration caused by various internal or external factors.

Reduced Physiological Stress in Clinical Studies

Ishii S, Nishihama T, Sumioka I, Morihara N, Ushijima M, Mizuno I, Kashimoto N, Hayama M. 2006. Oyo Yakuri (Pharmacometrics), 70: 97-105.

Ishii et al. (2006) examined a four-week human trial, where 1 ml of Kyoleopin Neo (KLEN) was administered to subjects twice a day after meals in the morning and evening. Fatigue was evaluated at 0, 2, and 4 weeks after taking KLEN and using an Advanced Trail Making Test (ATMT); a tool used to measure the performance of brain function associated with mental fatigue. KLEN decreased "brain-age" time-dependently and significantly. Demonstrating that KLEN maybe useful for ameliorating daily fatigue or for preventing the accumulation of fatigue.

Yaguchi S, Tokoro k, Tada M. 2005. Shinyaku to Rionsho (New Drug Clin.). 42: 189-96 (Japanese).

Yaguchi et al. (2005) performed clinical evaluations of Aged Garlic Extract combined with ginseng, oriental bezoar, antler velet, cuscuta see, and epimedium herb (Leopin Royal; LER) on various unexplained complaints (malaise). Subjects (n=19) were given 2mL of the AGE product daily for 4 weeks. Improvement in symptoms was evaluated. Supplementation with the AGE product reduced stress indexes and improved symptoms such as chill, stiffness in the shoulders, fatigue lassitude, decline of physical strength/willpower, headache, abnormal bowel movement, and lumbago. The efficacy of the AGE product was felt by 87.5% of subjects, indicating that the AGE product is useful for various unexplained complaints and malaise.

Improved Recovery from Athletic Performance in Pre-Clinical Studies

Kasuga S. Morihara N. et al. 1998. Hokkaido J Sports Med Sci. 6: 29-36.

The effect of AGE for endurance performance, SDH activity in the models gastrocunemius muscle and soleus muscle and plasma NO metabolic concentrations increased significantly. The results showed that AGE would facilitate the skeletal muscle oxidative enzyme activity and contribute to enhancement of physical strength and endurance training.

Improved Recovery from Athletic Performance in Clinical Studies

Kohno, M. , Shirakura, T. et al. 1976. Yakuri To Chiryo (Jpn. J. Pharmacol. Ther.) 4(3): 700-708.

In a double-blind study by Kohno et al. (1976), a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B_1, vitamin B_{12} and liver extract (KLE) was given to personnel of the Omiya Camp of the Japanese Land Self-Defense Force. Subjects taking KLE recovered from exhaustion faster and made fewer complaints of exhaustion and tiredness after manual labor than those taking placebos. Results were more marked in the 45-50 year-olds than in the 25-35 year-olds.

Kawashima, H., Ochiai, Y.. et al. 1986. Kiso to Rinsho (Preclinical and Clinical Report) 20(16): 8229-8245.

Kawashima et al. (1986) studied 20 healthy male college students and found that Aged Garlic Extract (2 ml, twice per day) improved subjective and objective symptoms of fatigue following 22 days of intense physical training. Aged Garlic Extract also improved patellar reflex and promoted serum levels of GOT, GPT, triglycerides, and lactic acid, which favor relief from fatigue. These latter effects provided further objective measurements of the anti-fatigue effects of Aged Garlic Extract. The authors concluded that Aged Garlic Extract is an effective medicine for the prevention of and recovery from fatigue.

Kimoto R, Kambayashi, Ishimura N, Nakamura T, Uchida E, Kyo E, Fuji H, Takeda H. 2005. Hokkaido J Sports Med Sci. 10:17-26.

Kimoto et al. (2005) investigated AGE's influence on the change of urinary 8-OHdG content, which is thought to be a marker of oxidative stress during daily regular and temporary intense exercise. Twelve healthy males were divided into two groups: AGE supplementation group and the control group. The AGE supplementation group was given AGE for 2 weeks. Urinary 8-OHdG content was found to be significantly lower with the AGE group than with the control. Also, AGE supplementation significantly increased the sum total of oxygen uptake during intense exercise.

Inhibits Stress-Induced Peptic Ulcer Formation

Nagai, K. 1972. Kiso To Rinsho (Preclin. Clin. Reports) 6: 1536-1538.

Nagai (1972) found that a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B_1, vitamin B_{12} and liver extract, orally administered at dosages of 2.0, 0.2, and 0.02 ml clearly prevented stress-induced ulcer formation. The authors attributed the anti-ulcer effects to strengthening of the gastric mucosa rather than to a decrease in aggravating agents such as gastric acid and pepsin.

Anticancer and Cancer-Preventive Effects

You, W., Blot, W. et al. 1988. Cancer Res. 48: 3518-3523.

Buiatti, E., Palli, D. et al. 1989. Int. J. Cancer 44: 611-616.

Case control epidemiological studies in northeast China (You et al., 1988) and Italy (Buiatti et al., 1989) showed that there are strong reverse trends in stomach cancer risk with dietary intake of garlic. Further, a number of studies have reported inhibitory effects of garlic and its constituents on the development and growth of cancer. Specifically, Aged Garlic Extract and its constituents have demonstrated anti-cancer effects in an array of cancer models including bladder tumors, melanoma cells, neuroblastoma cells, skin cancer, breast cancer, colon cancer, prostate cancer, esophageal cancer, stomach and lung cancer, erythroleukemia and aflatoxin induced mutagenesis as seen in the following studies:

Singh, S. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement held in Newport Beach, CA. November 15-17, 1998.

*Singh,, S.V., J. Nutr., **131**: 1046S-1048S, 2001.*

Oral administration of diallyl disulfide (DADS) resulted in a dose-dependent and statistically significant inhibition of H-ras oncogene transformed tumor growth. The tumor growth inhibitory effect of DADS was apparent in terms of delay in the appearance of measurable tumors, tumor volume as well as tumor weight. DADS suppresses the growth of H-*ras* oncogene transformed tumors by inhibiting the membrane association of tumoral p21H-ras, and that the allyl group is an important determinant in tumor growth inhibitory effect of DADS.

Thomson M, Muslin A. 2003. Curr Cancer Drug Targ, 3: 67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations; including fresh garlic extract, aged garlic extract, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE, but not the fresh garlic extract, exhibits radical scavenging activity. The two major compounds in aged garlic, SAC & SAMC have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Chu Q, Ling MT, Feng H, Cheung HW, Tsao SW, Wang X, Wong YC. 2006 Carcinogenesis. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related death since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, Chu et al. found that two main water-soluble constituents of the garlic, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), were able to suppress Pca cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Inhibited the Growth of Bladder Tumors

Lau, B.H.S., Woolley, J. et al. 1986. J. Urol. 136: 701-705.

Lau et al. (1986) found Aged Garlic Extract to be more effective than *bacillus Calmette-Guerin (BCG)* [11] at inhibiting the development and growth of bladder tumors from implanted transitional cell carcinoma. Delivering the Aged Garlic Extract directly into the tumor was more effective than through the intraperitoneal route.

Riggs, D., Lamm, D. et al. 1995. J. Urol. 153(4): 1029.

Aged Garlic Extract was found to be a "highly impressive nontoxic oral treatment modality" in a bladder cancer model. The effect was similar to that of BCG. At a low dosage (50 mg) Aged Garlic Extract significantly reduced tumor volume, but not mortality. At a higher dosage (500 mg) it significantly reduced both tumor volume and mortality. No adverse effects were noted in any Aged Garlic Extract groups (Riggs et al., 1995).

Lau, B., Barker, G et al. 1985. 85th Annual Meeting of the American Society for Microbiology. March 3-7, 1985, Las Vegas, Nevada, E-95.

Marsh, C., Lau, B. et al. 1987. J. Urol. 137: 359-362.

Lau and Marsh et al. (1985, 1987) observed that Aged Garlic Extract was equally or slightly more effective than BCG at inhibiting the development and growth of intravesically transplanted bladder tumors. Aged Garlic Extract, given one and six days after transplantation of bladder tumor cells, yielded the lowest final tumor incidence.

Reeve V., □ Bosnic, M. et al. 1993a. Photochem. Photobiol. 58(6):813-817.

Reeve et al. (1993a) found that Aged Garlic Extract protected bald models from photocarcinogenesis (skin carcinogenesis induced by ultraviolet radiation). Aged Garlic Extract-treated models exposed to DMBA, a cancer causing agent, then exposed to UV radiation for six weeks also developed significantly fewer tumors than untreated models exposed to the same regiment.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Song K ; Milner, J. 1999. J. Nutr. 129(3):657-661.

Song et al (1999) found that providing either 0.105 micromol diallyl disulfide or *S*-allyl cysteine, constituents in Aged Garlic Extract, by gastric gavage thrice weekly for 2 weeks was effective in retarding DMBA bioactivation. Isomolar alliin was not effective.

Pinto, J. and Rivlin, R. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

*Pinto, J.T and R.S. Rivlin, J. Nutr., **131**: 1058S-1060S, 2001.*

Garlic constituents have been shown to inhibit both initiation and promotion of cancer and have attracted wide interest for their anticancer potential. Pinto and Rivlin (1998, 2001) conducted studies with the human breast cancer cell lines, MCF-7 (estrogen-sensitive) and the MCF-ras (estrogen-insensitive) and found that Aged Garlic Extract, SAC and SAMC, at increasing concentrations inhibit cell growth progressively.

Tiwari, R., Pinto, J. et al. 1993. Breast Cancer Res. Treat. 27:(1-2): 80.

Tiwari et al. (1993) found that *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine in Aged Garlic Extract inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione-*S*-transferase[13] and peroxidase levels in the non-transformed cells. Glutathione-*S*-transferase is critical for detoxification and gene expression.

Li, G., Tiwari, R. et al. 1995. Oncology Rep. 2: 787-791.

Li et al. (1995) also reported that the *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine in Aged Garlic Extract inhibited the growth of transformed human breast cells and increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells. Glutathione-*S*-transferase is critical for detoxification and gene expression.

Milner, J. and Liu, J. 1990. First World Congress on the Health Significance of Garlic and Garlic Constituents p. 25.

Milner and Liu (1990) found that DMBA treatment while receiving a diet of 4% Aged Garlic Extract powder, mammary cancer incidence was 35% compared to 90% in controls receiving no Aged Garlic Extract. The 4% diet was given two weeks prior to and two weeks following DMBA treatment. Aged Garlic Extract also significantly inhibited *in vivo* formation of adducts[14] which were 96% correlated to ultimate tumor number. This research suggested that Aged Garlic Extract could alter the mammary tissue's ability to convert DMBA into an active cancer-causing agent, which can then bind to DNA.

Zoumas, C., Milner, J. et al. 1992. FASEB J. 6(4): 2641.

In a study by Zoumas et al. (1992), a diet supplemented with 2% Aged Garlic Extract depressed DMBA-DNA binding by 30-40%. Aged Garlic Extract given in accordance with a high fat diet (20% of total calories) depressed binding by 56%. An increase in DMBA-DNA binding is associated with an increase in fat content of the diet.

Schaffer, E. and Milner, J. 1997. 16th International Congress of Nutrition. IUNS July 27-August 1, 1997, p. 67.

Subjects fed additional linoleic acid or oleic acid (to a standard 5% corn oil diet) had approximately 60 or 35% more ($P<0.05$) total mammary DNA adducts,[14] respectively, than those fed the basal diet; but only 10 or 20% more adducts, respectively, when 2% Aged Garlic Extract was also supplemented in the diet. This research by Schaffer and Milner (1997) concluded that Aged Garlic Extract could block the enhancement by specific fatty acids of the initiation of DMBA-induced mammary carcinogenesis.

Amagase, H. and Milner, J. 1992. FASEB J. 6(4): 3229.

Amagase H., and Milner, J. 1993. Carcinogenesis 14: 1627-1631.

Amagase et al. (1992, 1993) found that the majority of the garlic preparations tested could reduce the binding of the carcinogen DMBA to mammary cell DNA. However, among the effective garlic preparations, only Aged Garlic Extract did not cause any side effects. The other garlic preparations, including commercial garlic powder, significantly decreased food intake and body weight gain. SAC, a constituent in Aged Garlic Extract, also dose-dependently reduced DMBA-DNA adduct[14] formation in the mammary gland.

Liu J. and Milner, J. 1990. FASEB J. 4: A1175.

Liu and Milner (1990) found that treatment with Aged Garlic Extract (4% of diet) and selenium (1mcg/g) resulted in a synergistic 30% reduction in DNA adduct[14] formation exposed to the carcinogen DMBA.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

Shirin H, Pinto JT, Kawabata Y, Soh JW, Delohery T, Moss SF, Murty V, Rivlin RS, Holt PR and Weinstein lB. 2001. Antiproliferative effects of S-allylmercaptocysteine on colon cancer cells, when tested alone or in combination with sulindac sulfide. Cancer Res. 61(2):725-731.

Dr. Weinstein's group at Columbia and Cornell Universities compared two water-soluble derivatives of Aged Garlic Extract, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), to sulindac sulfide (SS), a well-established colon cancer chemopreventive agent, for their effects on two human colon cancer cell lines, SW-480 and HT-29. SAMC, but not SAC, inhibited the growth of both cell lines at doses similar to that of SS. SAMC also induced apoptosis (cell death). These affects of SAMC were accompanied a marked increase in endogenous levels of reduced glutathione (a potent antioxidant). SAMC inhibited growth progression of both cell lines. SAMC, co-administered with SS enhanced the growth inhibitory and apoptotic effects of SS. These findings suggest that SAMC may be useful in colon cancer prevention when used alone or in combination with SS or other chemopreventive agents.

Wargovich, M. 1987. Carcinogenesis 8: 487-489.

Wargovich (1987) found that diallyl sulfide, a constituent in Aged Garlic Extract, significantly reduced the incidence of colon cancer, by 74%, induced by 20 weekly injections of the carcinogen, 1,2-dimethylhydrazine (DMH).

Sumiyoshi, H. and Wargovich, M. 1990. Cancer Res. 50: 5084-5087.

Sumiyoshi and Wargovich (1990) found that two constituents in Aged Garlic Extract, diallyl sulfide and SAC, significantly reduced the incidence of colon cancer induced by a carcinogen, dimethylhydrazine (DMH). Both these compounds, as well as various other organosulfur compounds in Aged Garlic Extract, stimulated the activity of glutathione *S*-transferase, an enzyme known to assist in the detoxification of carcinogens in the liver and colon.

Sumiyoshi, H. and Wargiovich, M. 1989. Proc. Am. Assoc. Cancer Res. 30:181.

Two constituents in Aged Garlic Extract, diallyl sulfide and SAC, significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Further, both compounds also significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon (Sumiyoshi and Wargovich, 1989).

Sundaram, S. and Milner, J. 1996. J. Nutr 126: 1355-1361.

Diallyl disulfide, a compound in Aged Garlic Extract, was found to be more effective than 5-fluorouracil (5-FU), a common anti-cancer drug, at inhibiting the growth of human colon tumor cells, especially when injected intraperitoneally. Further, diallyl disulfide given simultaneously with (5-FU) prevented some of the side effects induced by 5-FU such as: depression of white blood cells, spleen weight and elevated plasma urea (Sundaram and Milner, 1996).

Hatono, S., Wargovich, M. et al. 1996. Carcinogenesis 17(5): 1041-1044.

Aberrant crypt foci are considered to be the most likely precursors of colon cancer. SAC administration inhibited development in the colon of one third to one half of the foci induced by DMH when given prior to this carcinogen (initiation phase). Further, SAC was found to significantly enhance GST (glutathione *S*-transferase) activity not only in the liver, but also in the proximal and middle small bowel. GST is a detoxification enzyme system in the body. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens (Hatono et al., 1996).

Hatono, S. and Wargovich, M. 1997. Ch. 15. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT , pp. 139-151.

The administration of 0.4 and 0.8 maximum tolerated dose of SAC incorporated into the experimental diet significantly decreased the number of aberrant crypt foci when given during initiation but not promotion induced by the carcinogens dimethylhydrazine or azoxymethane. Models given *S*-ethylmercaptocysteine, *S*-propyl-mercaptocysteine and *S*-propaglylcysteine, exhibited increased foci, determined to be due to decreased food intake caused by these compounds.

Uda, N., Wargovich, M., et al. 1996. Proc. Annu. Meet Am. Assoc. Cancer Res. 37: 1871.

Additional research by Wargovich et al. team found that the Aged Garlic Extract could change the metabolism of the carcinogen azoxymethane (AOM) and thus inhibit AOM-induced aberrant crypt foci (Uda et al., 1996). Models (F344 male) were given AOM (15 mg/kg /wk, i.p.) for two weeks. In the initiation study, they were also given Aged Garlic Extract (120, 600 or 3000 mg/kg) for three consecutive days one week prior to and including the two weeks exposure to AOM. Aged Garlic Extract significantly inhibited AOM-induced aberrant crypt foci. When Aged Garlic Extract was taken 2 weeks after AOM treatment for four weeks (post-initiation) it had only a mildly preventive effect.

Knowles, L. and Milner, J.1997. FASEB J. 11(3): A422/#2445

Knowles and Milner (1997) found that DADS and SAMC, constituents in Aged Garlic Extract are effective at suppressing the growth of cultured human colon tumor cells. At equimolar amounts, DADS was most effective. Twenty-five mcg of DADS and 300 mcg of SAMC caused a 23% suppression in cell growth. DADS also assisted cells in converting from a mutated to a normal state (from G1 to S phase).

Ishikawa H. 2002. Jpn J Cancer Chemother. 29:545-9.

Ishikawa H. 2005. Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease. Washington DC, April 9-11, pp53.

Tanaka S, Haruma K, Kunihiro M, Nagata S, Kitadai Y, Manabe N, Sumii M, Yoshihara M, Kajiyama G, Chayama K. 2005. Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease. Washington DC, April 9-11, pp 57.

Ishikawa (2002) established a protocol for a randomized controlled trial for prevention of colorectal cancer. The study will include 100 patients with hereditary non-polyposis colorectal cancer that will consume either aged garlic extract or placebo, both in capsule form. The main end point of this trial is the number and size of colorectal adenomas after 2 years. Subject recruitment began in March 2002, and the trial will be completed in September 2005.

Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis in Clinical Studies

Tanaka S. Haruma K. et al. 2006. J Nutr.136(3S): 821S-826S.

In a preliminary double-blind randomized clinical trial, a high dose (2.4 mL/d) and low dose (0.16 mL/d) Aged Garlic Extract (AGE) was administered to patients with colorectal adenomas for 12 months. Following the 12 months, there was a significant suppression on both the size and number of colon adenomas in patients of the high-dose treatment whereas the number of adenomas increased in the control group. Results suggest that AGE has a suppressive effect on the progression of colorectal adenomas in humans.

Increased Natural-Killer Cells and Cell Activity

Ishikawa H, Saeki T, Otani T, Suzuki T, Shimozuma K, Nishino H, Fukuda S, Morimoto K. Aged Garlic Extract Prevents a Decline of NK Cell Number and Activity in Patients with Advanced Cancer. J. Nutr. 2006;136 Suppl 3:S816-20.

In a randomized double-blind trial by Ishikawa et al. (2006), Aged Garlic Extract (AGE) was administered to patients with inoperable colorectal, liver or pancreatic cancer. It was found that both the number of natural-killer (NK) cells and the NK cell activity increased significantly in the AGE group.

Inhibited the Development of Liver Cancer

Uda N, Kashimoto N, Sumioka I, Kyo E, Sumi S, Fukushima S. Aged Garlic Extract Inhibits Development of Putative Preneoplastic Lesions in Rat Hepatocarcinogenesis. J. Nutr. 2006;136 Suppl 3:S855-8.

Using the medium-term bioassay system based on the 2-step model of liver cancer, a significant reduction was observed in the specific chemical-labeling indices in the livers of the Aged Garlic Extract (AGE) group than in the control group, indicating that AGE inhibited the development of early stage of liver cancer.

Belloir C, Singh V, Daurat C, Siess MH, Le Bon AM. 2006. Food Chem Toxicol. 44:827-34.

SAC and allyl mercaptan (AM) significantly decreased DNA breaks in HepG2 cells treated with dimethyl-nitrosamine. Additionally, all the garlic organosulfur compounds studied were shown to decrease the genotoxicity of the direct-acting compounds, hydrogen peroxide and methyl methanesulfonate. This study demonstrated that garlic organic sulfur compounds (OSC) displayed antigenotoxic activity in human metabolically competent cells.

Fukushima S. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Jan. 28, Hiroshima (Japan), pp45 (Japanese)

The effects of Aged Garlic Extract (AGE)on the development of glutathione *S*-tranferase pacental form (GST-P) positive foci was determined using a diethylnitrosamine-induced hepatocarcinogenesis model system. AGE significantly inhibited GST-P positive hepatocellular foci in a dose-dependent manner. These findings indicate that AGE inhibited the development of putative preneoplastic lesions in heptocarcinogenesis, by reducing the proliferation rate of liver cells after partial hepatectomy.

Hori T, Wei M, Ichihara T, Takada N, Taniyama T, Fukushima S. 1997. 56th Annual Meeting on Japanese Cancer Association. Kyoto (Japan), Sep. 26, Abst# CCO-08. (Japanese)

The effects of *S*-methylcysteine and cysteine on the development of glutathione *S*-transferase pacental form (GST-P)-positive foci were determined using a diethylnitrosamine-induced hepatocarcinogenesis model system. Both *S*-methylcysteine and cysteine significantly inhibited GST-P positive hepatocellular foc. These findings indicate that *S*-methylcysteine and cysteine inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis.

Overview of Inhibition of Cancer Growth by Aged Garlic Extract and Its Constituents

Cancer	*In vivo* (Model)	*In vitro* (Cancer Cell Culture)
Bladder	Model[1-5]	
Breast	Model[9-21, 22,23]	Human[6-8]
Colon	Model[24-27, 28-30]	Human[31]
Erythroleukemia		Human[32-33]
Esophagus	Model[34]	
Liver		Model[35-37]
Lung	Model[38-40]	Human[51]
Melanoma		Human[41,42] Model[42]
Neuroblastoma		Human[43]
Prostate		Human[44-47]
Skin	Model[48-50]	
Stomach	Model[38-40]	

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12. Amagase, H. and Milner, J. 1992. FASEB J. 6(4): 3229.
13. Amagase, H., and Milner, J. 1993. Carcinogenesis 14: 1627-1631.
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Inhibition of Vascular Smooth-Muscle Cell and Umbilical Endothelial Cell Proliferation

Yamasaki, T., Lau, B. et al., 1994. Phytother. Res. 8:408-412.

Yamasaki et al. (1994) found that SAC can protect cells that line the blood vessels of the lungs from oxidant injury. SAC, in this *in vitro* test, protected bovine pulmonary endothelial cells from hydrogen peroxide (H_2O_2)-induced oxidant injury. Pretreatment of cells overnight with SAC (4 mg/mL) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase release (LDH)[4] and lipid peroxidation induced by H_2O_2. The authors suggesting that these compounds may be effective in hampering the aging process and for prevention of atherosclerosis.

Lee, E., Steiner, M. et al. 1994. Biochim. Biophys. Acta 1221:73-77.

SAC was found to inhibit vascular smooth-muscle cell (SMC) and umbilical endothelial cell proliferation. SMC proliferation constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subjected to angioplasty.[6]

Sickle Cell Anemia

Ohnishi, S. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998.

*Ohnishi, S.T and T. Ohnishi, J. Nutr, **131**: 1085S-1092S, 2001.*

Sickle cell anemia is a genetic disease caused by abnormal hemoglobin. In Africa, one out of 80 people suffers from this and the patients die before reaching the age of 20. In the African American population, one out of 500 suffers. Although hydroxy urea was found to have some efficacy, still there is no cure. The patients are known to have a decreased vitamin E level, suggesting oxidative stress may be involved in the disease process. By exposing sickle red blood cells to a deoxy-oxy cycling *in vitro*, Ohnishi (1998, 2001) found that dense red cells were formed. The dense cells can be found in the patients, and they may cause blood vessel occlusion. Using this method, the Ohnishi found that SAC inhibited the formation of dense cells *in vitro*. The inhibition was 30 % at the concentration of 1 mg/ml.

Takasu, J., et al. 2002. BMC Blood Disorders, 2:3.

Takasu et al. (2002) examined the potential role of Aged Garlic Extract as an antioxidant for sickle red blood cells (RBC). Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study. These data suggest the significant antioxidant activity of Aged Garlic Extract on sickle cell anemia, and may represent a potential therapy to combat complications of the disease.

Antioxidative Effects of SAC

Ide, N. Lau, B et al. 1997a. Planta Med. 63: 263-264.

Ide and Lau (1997b) from Loma Linda University found that SAC could prevent copper, a potent oxidant, from oxidizing LDL cholesterol in an *in vitro* system.

Ide, N., Itakura, Y. et al. 1996. 2nd International Congress on Phytomedicine. September 11-14, 1996a. Munich, Germany.

Amagase, H. et al. 2000. Ch. 6. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, pp. 62-78.

Ide et al. (1996a) and Amagase et al. (1999) found that SAC decreased the emission of low level chemiluminescence (LLC) initiated by t-butyl hydroperoxide. SAC inhibited LLC emissions 33% at 5 mmol/L and 45% at 10mmol/L. SAC also demonstrated radical and hydrogen peroxide scavenging activities *in vivo*.

Ide, N., Matsuura, H. et al. 1996b. Phytother. Res. 10: 340-341.

SAC demonstrated a scavenging effect on hydrogen peroxide and also inhibited the chain oxidation induced by a hydrophilic radical initiator in another study by Ide et al. (1996b).

Imai, J., Itakura, Y. et al. 1994. Planta Medica 60:417-420.
Ide, N. Lau, B et al. 1997a. Planta Med. 63: 263-264.

SAC inhibited the emission of low level chemiluminescence and the early formation of TBA-RS[5] (markers of oxidation), whereas water extracts of raw and heat-treated garlic enhanced such emissions. Imai et al. (1994) suggested that SAC has antioxidative efficacy.

Banerjee SK, et al. 2003. Phytother Res. 17; 97-106.

Various preparations of garlic, mainly aged garlic extract (AGE), have been shown to have promising antioxidant potential. SAC, a major compound in AGE but not in raw garlic, has been reported to have powerful antioxidant and radical scavenging effects. This review touches on several of these areas.

Hsu, C. 2004. Five Cysteine-Containing Compounds Have Antioxidative Activity Balb/cA Mice Nutrient Interactions and Toxicity Research Communication. 149-152.

Five cysteine-containing compounds derived from garlic, including SAC, were added to drinking water at 1 g/L for a 4 week treatment, while cysteine was used as a comparison. At the end of treatment, glutathione (GSH) levels were higher ($p<0.05$) in the kidney and liver than in controls. SAC and the other cysteine-containing compounds were also found to increase catalase and glutathione peroxidase (GPX) activities in the kidney and liver. When compared with the control and cysteine-treated groups, the cysteine-containing

Sumiyoshi, H. and Wargivich, M. 1989. Proc. Am. Assoc. Cancer Res. 30:181. (abs. #718)

SAC significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Further, both compounds significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon. GST is an enzyme known to assist in the detoxification of carcinogens.

Hatono, S., Wargovich, M. et al. 1996. Carcinogenesis 17(5): 1041-1044.

Aberrant crypt foci are considered to be the most likely precursors of colon cancer. SAC administration inhibited development in the colon of one third to one half of the foci induced by DMH when given prior to this carcinogen (initiation phase). Further, SAC was found to significantly enhance GST (glutathione *S*-transferase) activity not only in the liver, but also in the proximal and middle small bowel. GST is a detoxification enzyme system in the body. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens (Hatono et al., 1996).

Knowles, L. and Milner, J. 1998. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, 1998b.

*Knowles, L.M. and J.A. Milner, J. Nutr., **131**: 1061S-1066S (2001).*

Knowles and Milner (1998, 2001) found that diallyl disulfide (DADS), a constituent in Aged Garlic Extract and garlic oil, suppresses proliferation of human colon tumor (HCT-l5) cells by reducing apoptosis and altering cell division through a block in the G2/M phase of the cell cycle. A marked suppression in p34cdc2 kinase activity and depressed protein tyrosine phosphatase (PTPase) activity accompanied the observed G2/M phase arrest. Western blot analysis revealed that 25 and 50 µM DADS decreased cdc25C phosphatase expression, by 21 and 45%, respectively. DADS exposure caused a dose dependent decrease in $p34^{cdc2}$ protein expression. Suppression of $p34^{cdc2}$ and cdc25C expression likely accounts for the ability of DADS to inhibit $p34^{cdc2}$ kinase activity. Other sulfur compounds found in processed garlic may alter tumor cell proliferation by a similar mechanism.

Sustained Circulatory Antioxidants Depleted by Cancer-Causing Agent

Balasenthil, S and Nagini, S. 2000. J. Biochem. Med. Biol. & Biophys. 4: 35-39.

7,12-Dimethylbenz(a)anthracene (DMBA) enhances lipid peroxidation in the circulation. In addition, it significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase. Administration of SAC significantly decreased DMBA-induced lipid peroxidation and enhanced the levels of antioxidants.

Sundaresan, S, and Subramanian, P. 2003. S-allylcysteine inhibits circlulatory lipid peroxidation and promotes anti-oxidants in N-nitrosodiethylamine-induced carcinogenesis. Pol. J. Pharmacol. 55: 37-42.

In a study where SAC was administered to N-nitrosodiethylamine (NEDA)-induced hepatocarcinogenesis, SAC was found to decrease tumor incidence and lipid peroxidation. SAC was also found to increase antioxidant levels by decreasing the formation of free radicals.

Liver Protective Effects of SAC

Inhibited Both the Formation and Bioactivation of a Liver Carcinogen

Dion, M. and Milner, J. 1996. Exp. Biol. 96 (abs.)

SAC inhibited both the formation and bioactivation of the liver carcinogen nitrosomorpholine (NMOR). Adding SAC to a solution of sodium nitrite and morpholine prevented these two compounds from generating nitrosomorpholine. SAC also prevented NMOR's ability to mutate a cell model.

Fukushima, S. Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement held in Newport Beach, CA. November 15-17, 1998.

*Fukushima, S., et al., J. Nutr., **131**: 1049S-1053S, 2001.*

S-methylcysteine, a constituent in Aged Garlic Extract, suppressed chemically-induced (sodium nitrite and morpholine) liver cancer.

Protected Liver Cells from the Liver Toxins: Paracetamol (Acetaminophen), Carbon Tetrachloride and Bromobenzene

Nakagawa, S., Kasuga, S. et al. 1988. Phytother. Res. 1:1-4.

Nakagawa et al (1988) found that SAC protected the liver cells from the liver toxins, Paracetamol and carbon tetrachloride. These compounds induce acute hepatitis. SAC appeared to enhance the activity of glutathione, a detoxifying enzyme, and to act as chemical scavengers. Thus, SAC was found to be more effective than the other chemicals used.

Peng Q, et al. Neuroprotective effect of garlic compounds in amyloid-B peptide-induced apoptosis in vitro. Med Sci Monit. 8(8):BR328-337, 2002.

AGE and SAC were reported to decrease apoptosis by enhancing endogenous antioxidant defenses in a study determining their effects on Aß-induced apoptosis and reactive oxygen species (ROS) generation in a pheochromocytoma (PC12) cell line.

Kosuge, Y. et al. 2003, Neuroscience. 122(4): 885-95.

Kosuge et al. (2003) found that *S*-allyl-L-cysteine (SAC) protected differentiated PC12 cells against Aβ- and tunicamycin-induced neuronal death and also attenuated the Aβ-induced increase of intracellular reactive oxygen species.

Kim JM, Chang N, Kim WK, Chun HS. 2006. Biosci Biotechnol Biochem. 70:1969-71.

S-Allyl-L-cysteine (SAC), an active organosulfur compound derived from garlic, was found to reduce mortality with lesser incidence of stroke and also to lower the overall stroke-related behavioral score in stroke-prone spontaneously hypertensive (SHRSP) models by dietary administration.

Kosuge Y, Koen Y, Minami K, Ishige K, Edagawa Y, Saito H, Ito Y. 2001. 74th Annual Meeting of the Japanese Pharmacological Society. Yokohama (Japan), March 21-23, Abst# P-849.

SAC selectively protects amyloid β (Aβ) -induced neuronal death in hippocampal neurons due, at least in part, to suppression of A β-induced oxidative stress. Mechanistic differences exist between A β-induced cell death in hippocampal neurons and in cerebellar granule neurons.

Kosuge Y, Sakikubo T, Ishige K, Ito Y. 2006. Neurochem Int. 49: 285-93.

Kosuge et al. (2006) determined that hippocampal neurons (HPN) were protected from neuronal cell death in the presence of SAC. In contrast, SAC did not exhibit any protective effects on cerebellar graule neurons (CGN). Both neurons found in the endoplasmic reticulum (ER) are vulnerable to neuronal cell death with ER stress. Research has shown ER stress to be an important factor in amyloid β peptide (Aβ)-induced neurotoxicity and Alzheimer's disease pathology.

Lung Protective Effects of SAC

Protected Cells from Toxic Carbon Tetrachloride in the Lungs

Mizuguchi S, Takemura S, Minamiyama Y, Kodai S, Tsukioka T, Inoue K, Okada S, Suehiro S. 2006. Biofactors. 26:81-92.

SAC was found to be effective in reducing CCl_4-induced lung injury through intraperitoneal injection of CCl_4 into models twice a week for 8 weeks; SAC, N-acetyl cysteine or L-cysteine was orally administered everyday for 8 weeks. SAC significantly reduced the increases of transforming growth factor beta, lipid peroxides, AST, and ALT in plasma induced by CCl_4. SAC dose-dependently and significantly attenuated CCl_4-induced systemic inflammation and fibrosis of lung. SAC also prevented the decline of thiol levels, the increase of inducible nitric oxide synthase expression, the infiltration of leukocytes, and the generation of reactive oxygen species in lungs.

SAC Has Been Confirmed to be Bioavailable and Active

Amagase, H. 2000. Inconsistent cholesterol-lowering effects of garlic powder products due to inappropriate standardization. Phytomed. 7(2): 51.

Amagase (2000) indicated that SAC is one of the water-soluble sulfur compounds in garlic derived from γ-glutamyl peptides that increases during extraction. SAC is utilized to standardize AGE. SAC is contended to be the only compliance marker compound for clinical studies involving garlic consumption. Since SAC is present in garlic preparations, has many biological effects, and is confirmed to be bioavailable, it can be considered one of the active principles in garlic preparations.

Nagae, S., Itakura, Y. et al. 1994. Planta Med. 60: 214-217.

SAC is a biologically active transformation product from garlic. Nagae et al. found that SAC was rapidly and easily absorbed in the gastrointestinal tract and, then distributed mainly in the plasma, liver, and kidney after oral consumption. Its bioavailability was between 87.2% through 103% in models.

Rosen, R. Identification of metabolites of garlic and garlic products in human body fluids, 2nd International Conference on Food Factors (2nd ICoFF), Kyoto (Japan) December 12-17, 1999.

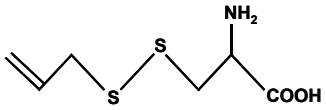
Rosen, R. 2000a. Phytomed. 7(2): 51.

Rosen (1999, 2000a,b) established a procedure for measuring SAC in blood and urine of human subjects after consumption of garlic and garlic products. SAC was easily detected in plasma and urine by HPLC-MS using negative ion atmospheric pressure chemical ionization mode (APcI)-MS. SAC levels in the blood reach levels of 800 ppb in the blood after consumption of three Aged Garlic Extract capsules (Steiner, M., East Carolona University, personal communication, 1998).

Rosen, R., Hiserodt, R. et al. 2000b. Biofactors13(1-4): 241-249.

Rosen (2000b) showed that allicin is decomposed in stomach acid into various allyl sulfides, disulfides and other volatiles. Thus allicin is not detected anywhere.

S-ALLYLMERCAPTOCYSTEINE (SAMC): A CONSTITUENT UNIQUE TO AGED GARLIC EXTRACT



Aged Garlic Extract contains a unique sulfur-containing compound, known as *S*-allylmercaptocysteine (SAMC). SAMC is produced only during aging and is not present in fresh raw garlic (Koch, 1996; Heber, 1997) or in various garlic preparations (Kodera, 1997). SAMC is a water-soluble compound that has shown an array of effects, as seen in the following studies, including anti-oxidative, liver-protective and anti-carcinogenic effects:

Koch, H. and Lawson, L.D. 1996. Garlic the Science and Therapeutic Application of Allium sativum L. and Related Species. Williams & Wilkins, Baltimore, MD, pp. 104.

Heber, D. 1997. Am. J. Clin. Nutr. 66: 425 (editorial).

Kodera, Y. 1997. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice (Lachance, P.A. ed.) Ch. 11, pp. 95-103. Food and Nutrition Press, Inc. Trumbell, CT.

Antioxidative and Radioprotective Effects of SAMC

Pedraza-Chaverri, J. 2004. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo. BMC Clinical Pharmacology. 4.

In a study by Pedraza-Chaverri, SAMC treatment was found to weaken the gentamicin-induced oxidative and nitrosative stress as well as the destruction to the kidneys (nephrotoxicity) *in vivo*. SAMC was found to scavenge hydroxyl radicals and singlet oxygen *in vitro*.

Protected Cells from Oxidant Injury

Ide, N., Matsuura, H. et al. 1996a. Phytother. Res. 10: 340-341.

Ide et al. (1996a) found that SAMC demonstrated a scavenging effect on hydrogen peroxide. They also inhibited the chain oxidation induced by a hydrophilic radical initiator. Hydrogen peroxide yields a free radical by reacting with iron or copper (a process called the Fenton reaction). This free radical damages both membranes and DNA and/or induces lipid peroxidation.

Amagase, H. et al. 2000. Ch. 6. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, pp. 62-78.

Ide, N., Itakura, Y. et al. 1996b. 2nd International Congress on Phytomedicine. September 11-14, 1996. Munich, Germany.

Amagase et al. (1999) and Ide et al. (1996b) found that SAMC , decreased emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. SAMC inhibited LLC emissions 60% at 5 mmol/L and 82% at 10mmol/L, nearly the same effect as the potent antioxidant glutathione. SAMC also scavenged hydrogen peroxide *in vivo.*

Ide, N. and Lau, B. 1997. FASEB J. 11(3): A122/#713

Ide and Lau (1997) found that SAMC could inhibit copper-induced peroxidation of LDL in a concentration dependent manner. Lipid oxidation was determined by measuring TBA-RS.[5] Further, it was found that when SAMC was preincubated with pulmonary artery endothelial cells, cell damage caused by oxidized LDL was prevented, as indicated by prevention of lactate dehydrogenase release, loss of cell viability and TBA-RS formation.

Anticancer Effects of SAMC

Thomson M, Muslim, A. 2003. Curr Cancer Drug Targ, 3: 67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations; including fresh garlic extract, aged garlic extract, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE, but not the fresh garlic extract, exhibits radical scavenging activity. The two major compounds in aged garlic, SAC & SAMC have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Borek, C. 2005. Antioxidants and the prevention of hormonally regulated cancer. jmhg. 2(3): 346-352.

SAC and SAMC found in aged garlic extract have been shown to destroy cancer cells by inducing apoptosis, or programmed cell death. SAC and SAMC have been shown to decrease the growth of prostate cancer cells by 80 percent while SAMC has also been shown to inhibit the growth of breast cancer cells, erythroleukemia and colon cancer cells.er cells.

Borek, C. 2004. Int. Cancer Ther. 3(4): 333-341.

Borek, C. 2004. J. Nutr. 134: 3207S-3209S.

SAMC induces apoptosis in human prostate cancer cells and in breast cancer cells, as well as colon cancer cells by activating caspase 3, inhibits antiapoptotic protein Bcl-2, and disrupts microtubules in cancer cells, preventing further growth.

Chu Q, Ling MT, Feng H, Cheung HW, Tsao SW, Wang X, Wong YC. 2006 Carcinogenesis. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related death since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, Chu et al. found that two main water-soluble constituents of the garlic, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), were able to suppress Pca cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Tiwari, R., Pinto, J. et al. 1993. Breast Cancer Res. Treatment 27:(1-2): 80.

Tiwari et al. (1993) found that SAMC inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells.

Li, G., Qiao, C., et al. 1995. Oncol. Rep. 2: 787-791.

Li et al. (1995) also reported that SAMC in Aged Garlic Extract inhibited the growth of transformed human breast cells and increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

Shirin H., Pinto J. et al. 2001. Cancer Res. 61(2):725-731.

Pinto et al. (2001) found that SAMC inhibited the growth of two human colon cancer cell lines (SW-480 and HT-29) at doses similar to that of sulindac sulfide, a well-established colon cancer chemopreventive agent. SAMC also induced apoptosis[17]. In addition, SAMC cause a marked increase in endogenous levels of reduced glutathione. SAMC also augmented the growth inhibitory and apoptotic effects of SS when co-administered.

Knowles, L. and Milner, J. 1997. FASEB J. 11(3): A422/#2445

Xiao D, Pinto JT. Et al. FASEB J, 2003, 17(4): A1152, # 7511.1.

Xiao D, Pinto JT, et al. 2003. Cancer Res 63: 6825-6837

Experimental carcinogenesis studies indicate that components of garlic (i.e. allyl sulfides) inhibit both the initiation and promotion stages of tumorigenesis for various types of cancer, including colorectal. These researchers previously reported that SAMC inhibits growth, arrests cells in G2-M, and induces apoptosis in human colon cancer cells. This study concludes that the garlic-derived compound SAMC exerts antiproliferative effects by binding directly to tubulin and disrupting the MT assembly, thus arresting cells in mitosis and triggering JNK1 and capase-3 signaling pathways that lead to apoptosis.

Xiao D, Pinto JT, Palazzo A, Gundersen GG, Shirin H, Weinstein IB. 2002. International Research Conference on Food, Nutrition and Cancer. Washington DC, July 11-12. J Nutr 132: 3549S.

Xiao et al. had previously reported that *S*-allyl mercaptocysteine (SAMC) inhibited growth, arrested cells in G_2/M and induced apoptosis in SW480 and HT29 human colon cancer cells. Upon examination of this mechanism, in this current study, Xiao et al. (2002) concluded that antiproliferative effects exerted by SAMC work to disrupt microtubule assembly, and cells in mitosis are arrested. Jun kinase (JKN1) and caspase-3 are then triggered, which signals pathways leading to apoptosis.

OTHER CONSTITUENTS IN AGED GARLIC EXTRACT

Oil-soluble and Water-soluble Organosulfur Compounds

Weinberg, D., Manier, M., et al. 1992. J. High Resolut. Chromatogr. 15: 641-654.

Weinberg, D., Manier, M., et al. 1993. J. Agric. Food Chem. 41: 37-41.

Aged Garlic Extract is rich in water-soluble compounds and contains small amounts of oil-soluble compounds. Weinberg et al. (1992, 1993) developed methodology to detect, identify and quantify nine oil-soluble organosulfur compounds in Aged Garlic Extract: allyl sulfide, allyl disulfide (diallyl disulfide), allyl trisulfide, allyl methyl sulfide, allyl methyl disulfide, allyl methyl trisulfide, methyl disulfide, methyl trisulfide and ethyl 2 propenesulfinate. It was found that the concentration of most of these constituents increased with time. Some compounds nearly tripled in concentration and others increased by an order of magnitude. The results were confirmed by an independent study of the extract from the National Cancer Institute.

Dimitrov, N. and Bennink, M. 1997. Modulation of arachidonic acid metabolism by garlic extracts. Ch. 21. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT, pp. 199-202.

Gwilt, P. et al. 1994. Cancer Epidemiol. Biomarkers Prev. 3:155-160.

Dimitrov et al. (1997) and Gwilt et al. (1994) also quantified various organosulfur compounds in Aged Garlic Extract.

Ichikawa M, Ide N, Yoshida J, Yamaguchi H, Ono K. 2006. J. Agric Food Chem. 54:1535-40.

Ichikawa et al. (2006) developed and validated a simple, rapid, and precise analytical method to determine seven organosulfur compounds that are found in garlic: alliin, isoalliin, methiin, cycloalliin, gamma-L-glutamyl-*S*-methyl-L-cysteine (GSMC), gamma-L-glutamyl- *S*-(2-propenyl)-L-cysteine (GSAC), and gamma-L-glutamyl-*S*-(trans-1-propenyl)-L-cysteine (GSPC). The method consisted of using a one-step sample preparation procedure with NP and RP high performance liquid chromatography, with overall recoveries of all seven organosulfur compounds of 97.1-102.3%.

Vijayaraghavan M, Wanibuchi H, Takada N, Yano Y, Otani S, Yamamoto S, Fukushima S. 2000. Jpn J Cancer Res. 91: 780-5.

Vijayaraghavan et al. determined the water-soluble components of garlic, *S*-methylcysteine (SMC) and cysteine provides inhibitory effects on diethylnitrosamine (DEN)-induced hepatocarcinogenesis, promoted with sodium phenobarbitol (NaPB), at the promotion stage. However, only SMC was able to significantly reduce ODC enzyme activity.

Inhibited the Activity of Human Cytochrome-P450 (CYP) Enzymes

Greenblatt DJ, Leigh-Pemberton RA, von Moltke LL. In Vitro Interactions of Water-Soluble Garlic Components with Human Cytochromes P450. J. Nutr. 2006;136 Suppl 3:S806-9.

Two water-soluble components of aged garlic extract (AGE), *S*-methyl-L-cysteine and *S*-allyl-L-cysteine, at 100 μmol/L reduced CYP3A activity to 20-40% of control.

Inhibited the Levels of Hepatic CYP2E1 Protein

Wargovich MJ. Diallylsulfide and Allylmethylsulfide Are Uniquely Effective among Organosulfur Compounds in Inhibiting CYP2E1 Protein in Animal Models. J. Nutr. 2006;136 Suppl 3:S832-4.

Research results indicate that two organosulfur compounds diallylsulfide and allylmethylsulfide, are effective in significantly reducing levels of hepatic CYP2E1 protein.

Wargovich MJ. 1998. Recent Advances of the Nutritional Benefits Accompanying the Use of Garlic as a Supplement. Newport Beach, CA. November 15-17, pp32.

Organosulfur compounds in Alliums (AOSC) are effective in inhibiting carcinogenesis at the initiation stage of esophageal and colon cancers. Inhibitory effects are primarily due to AOSCs ability to reduce CYP2E1 enzyme which activates carcinogens nitrosomethylbenzylamine and azoxymethane responsible for inducing esophageal and colon cancers.

Enhanced Nerve Survival

Nishiyama, N., Moriguchi, T. et al. 1996. Int. Acad. Biomed. Drug Res. Basel. Karger. 11: 253-258.

Moriguchi, T, Nishiyama, N. et al.1996. Phytother. Res. 10: 468-472.

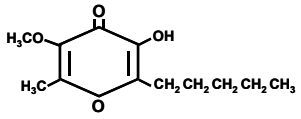
Nishiyama et al. (1996) and Moriguchi et al. (1996) found Aged Garlic Extract's F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, the first clear evidence that Aged Garlic Extract interacts with brain neurons.

Enhanced Growth of Friendly Bacteria B. bifidum and L. acidophilus

Matsuura, H. 1997. In: Nutraceuticals: Designer Foods III Garlic, Soy and Licorice (Lachance, P.A. ed.), Ch 7., pp. 55-69. Food and Nutrition Press, Inc. Trumbell, CT.

Kokai Tokyo Koho, Japanese Patent H1-252276.

Matsuura (1997) found that the F-4 protein fraction in Aged Garlic Extract enhanced the growth of the beneficial bacteria, *B. bifidum* and *L. acidophilus*. There is an international patent (*#H1-252276*) for this effect.



Allixin

Antimicrobial Activity

Kodera, Y., Matsuura, H. et al. 1989. Chem. Pharm. Bull., 37: 1656-1658.

In 1989, Kodera et al. Isolated a phenolic stress compound from garlic and termed it allixin. Allixin was found to possess weak antimicrobial activity.

Kodera, Y. et al. 2002. Chem.. Pharm. Bull. 50(3); 405-407.

Kodera et al. found that allixin, a *de novo* synthesized substand categorized as a phytoalexin, may pose an prohibitory, inhibitory, or post-inhibitory antimicrobial function in garlic. The basis of this conclusion comes from the observation of high accumulation of allixin in necrotic tissue areas after long-term storage.

In vivo Anti-tumor Activity

Nishino, H., Nishino, A., et al. 1990. Cancer J. 3: 20-21.

Nishino et al. found that allixin demonstrated *in vitro* anti-tumor activity and suppressed the promotion of two-stage carcinogenesis *in vivo*. Allixin, inhibited the development of skin cancer induced by the carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), and the promoter, 12-O-tetradecanoyl (TPA). Nishino et al. suggested that since allixin seems to have no side effects, it might be useful for the prevention of human cancer.

Antimutagenic and Chemopreventive Activity

Yamasaki, T., Lau, B. et al. 1991. Cancer Lett. 59: 89-94.

Allixin showed a dose-related inhibition of histidine+ revertants induced by AFB_1. (AFB_1 is an aflatoxin produced by the molds found on peanuts). Allixin prevented the binding of this carcinogen to calf thymus DNA and reduced the formation of AFB_1-DNA adducts. Allixin also inhibited the formation of carcinogenic metabolites of this aflatoxin and, therefore, the authors suggested that this compound "may thus be useful in the chemoprevention of cancer."

Enhanced Nerve Survival

Nishiyama N, Moriguchi T, Katsuki H, Saito H, Matsuura H, Itakura Y. 1996. 116th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa (Japan), March 27-29. Abst# 27(J1) 10-2. (Japanese)

Nishiyama (1996) has reported the effect of allixin, one of the stress compounds in garlic, on survival of neuron cells using a cell culture system. The neuron cells cannot survive without serum and began to die in 72h. Neuron cells were conincubated with allixin for 48h, and the survival rate and condition of the cells was examined. Results indicate incubation with allixin demonstrated a significant improvement in survival rate in a dose dependent manner, and promoted the branching of cells. In order to reduce the side effects, garlic's derivatives were chemically synthesized and the survival rate of nerve cells was studied. As a result, 2,6-dimethyl-3-hydroxy-4-pyrone (DHP) was found to show similar activity with less toxicity. These two compounds may be useful as external neurotrophics.

Chemistry of AGE

Morihara, N., et al. 2005. Aged garlic extract inhibits peroxynitrite-induced hemolysis. Redox Report. 10(3): 159-165.

Morihara, N., et al. found that when AGE was combined with a suspension of erythrocytes, it decreased peroxynitrite-induced hemolysis in a concentration-dependent manner. SAC, found in AGE, was also found to decrease hemolysis. Since peroxynitrite is a strong oxidant, it has been shown to cause vascular or tissue damage. Therefore, AGE and its constituents may be helpful for preventing cardiovascular diseases and may help prevent the damage of membranes in erythrocytes.

Amagase H, Rosen R. Garlic is Effective without Allicin (1) Current Marker Compound for Herbs is Not Marker. ICNPR, July 31-August 4, 2004.

Allicin is not active or marker compound in garlic products. AGE uses *S*-allyl cysteine (SAC) instead, since SAC is bioavailable and active in the body. It is reasonable to use such compound for standardization.

Ide N, et al. Antioxidants in processed garlic. 1. Fructosyl arginine identified in aged garlic extract. International Congress Series 1245m 447-448, 2002.

Ide, et al. used several in vitro assay systems and high-performance liquid chromatography (HPLC) to determine the antioxidant effects of fructosyl arginine (Fru-Arg), a compound in AGE. The study reported that Fru-Arg forms and is increased during the aging process and plays an important role as an antioxidant.

Ide, N, et al. 2003. Antioxidants in Processed Garlic: Tetrahydro-ß-carboline Derivative in Aged Garlic Extract. American Chemical Society, Ch 22 pg. 250-263.

Ide, et al. used liquid chromatography mass spectrometry (LC-MS) to determine the antioxidant effects of four tetrahydro-ß-carboline derivatives. These compounds were found to increase during the aging process and may be an important antioxidant in AGE.

Ide N, Ichikawa M, Ryu k, Ogasawara K, Yoshida J, Yoshida S, SasaokaT, Sumi S, Sumiyoshi H. 2002. In International Congress Series 1245, The Maillard Reaction in Food Chemistry and Medical Science: Update for The Postgenomic Era. Pp449-50.

Kodera, Y, et al. 2003. Garlic Chemistry: Chemical and Biological Properties of Sulfur-Containing Compounds Derived from Garlic. American Chemical Society, Ch. 30, pg. 346-357.

Garlic chemistry has been overviewed by this article. It explains unique characteristics of AGE with a cascade of chemical reactions of garlic. Many unique chemical constituents in AGE were listed and reviewed with pharmacokinetic behavior *in vivo* (in the actual body), which is essential for the true active compounds. AGE has many of these bioavailable compounds in it.

Thomson M, Ali M. 2003. Garlic [Allium sativum]: A Review of its Potential Use as an Anti-Cancer Agent, Curr Cancer Drug Targets, Feb 3(1): 67-81.

In a review on garlic, SAC and SAMC, present in aged garlic extract, were found to have the highest activity of scavenging free radicals. SAC, in several disease models, was also shown to decrease the growth of both chemically induced and transplantable tumors. This review suggests that garlic consumption may play an important role in the protection from the development of cancer.

Ryu, K, and Ide, N., et al 2003. Liquid chromatography-mass spectrometry method for fructosylarginine, an antioxidant in the aged garlic extract. American Chemical Society, Ch 23, pg. 264-273.

Fructosyl-arginine (Fru-Arg) is a unique compound only found in AGE and no other garlic products have this kind of compounds in it. Fruct-Arg has a strong anti-oxidant effect and in this article, state-of-the-art analytical technology, new and sensitive liquid chromatography-mass spectrometry (LC-MS) method has been developed for this unique compound and reported.

Kodera Y, Matsuura H, Sumiyoshi H, Sumi S. 2001. Joint ACS-ISoFF Symposium on Food Factors for Health Promotion. San Diego, CA April 1-5 Abst #AGFD 108.

The biological activities of water-soluble compounds derived from garlic, such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), has become the center of attention because they are odorless and safe. *S*-allyl groups play a significant role in the pharmacological activities of organosulfur compounds derived from garlic based on studies on structure-activity relationships.

Ichikawa M, Ide N, Ono K. 2006 J. Agric Food Chem. 54: 4849-54 9

Ichikawa et al. (2006) studied the changes in organosulfur compounds in garlic cloves during storage at different temperatures. Results indicated that gamma-glutamyl peptides undergo marked conversion to sulfoxides when garlic cloves are stored at 4 degrees Celsius. They also demonstrated that isoalliin produced enzymatically from γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC) is chemically converted to cycloalliin and that the cycloalliin content increases when garlic cloves are stored at higher temperatures.