### Cardioprotective Effects Seen in Clinical Studies

- Inhibits Plaque Formation in the Coronary Artery
- Lipid Lowering Effects
- Circulation-Enhancing/Blood-Thinning Clinical Effects
- Hypotensive/Blood Pressure Lowering Effects
- Sickle Cell Anemia (Pilot Study)
- Sickle Cell Anemia
- Decreased the Levels of Serum Prostaglandins, PGE\textsubscript{2} and PGF\textsubscript{2α}
- Cardiovascular Review
- Overview of Cardioprotective Effects of Aged Garlic Extract Found in Various Clinical Studies

### Cardioprotective Effects Seen in Preclinical Studies

- Lipid Lowering Effects
- Water-Soluble Sulfur Compounds Best Candidates for Lipid Reduction
- Circulation-Enhancing/Blood-Thinning Preclinical Effects
- Reduction of Serum Homocysteine
- Anti-atherogenic / Anti-atherosclerotic Effects
- Sickle Cell Anemia

### Liver-Protective/Detoxification Effects

- Protected Cells from Toxic Carbon Tetrachloride and Paracetamol (Acetaminophen)
- Enhanced Metabolism of Acetaminophen in Humans
- Prevented the Mutagenic Effects of the Liver Toxin Aflatoxin B\textsubscript{1}
- Prevented the Toxic Effects of Phenobarbital and Bromobenzene
- Enhanced Detoxification of Acetaldehyde
- Enhanced Detoxification Enzymes in the Liver
- Inhibited Cholesterogenesis in HepG-2 Cells

### Immune Enhancement and Anti-Infection Effects

- Immune Enhancement Seen in Clinical Studies
- Immune Enhancement Seen in Pre-Clinical Studies
- Immune Enhancement Seen in Cell Culture Studies
- Overview of Immune-Enhancing Effects of Aged Garlic Extract (AGE) and Its F-4 Protein Fraction
- Superior Immunostimulatory Properties
- Indirect Anti-tumor Effects Via Immune Enhancement
- Increased Natural-Killer Cells and Cell Activity in Clinical Studies
- Inhibition of UV-Induced Immunosuppression
- Antiviral Effects Against Influenza
- Improved Immune Function and Depressed State Post-Menopause
- Improved Age-Related Deterioration of Immune Responses

### Antioxidative and Radioprotective Effects

- Protection from Radiation and Chemotherapy
- Protection from Gentamicin-Nephrotoxicity
- Reduces Antioxidative Damage in Smokers in Clinical Studies
- In vitro Protection from Lipid Peroxidation
- In vivo Protection from Lipid Peroxidation
- Enhanced Antioxidant Systems In the Body
- Showed Scavenging Effects
- Superior Antioxidant Activity Compared to Other Forms of Garlic
- Cardioprotective Antioxidant Effects
  - Protected Vascular System and Red Blood Cells from Oxidant Injury
  - Decreased Cardiotoxicity of Anti-Cancer Drug Doxorubicin
  - Prevented Oxidation Of LDL Cholesterol in Pre-Clinical Studies
- Prevented Oxidation of LDL Cholesterol in Clinical Studies
  - Prevented Damage to Cells Caused by Oxidized LDL Cholesterol
  - Ameliorated damaging effects of nitric oxide
- Antioxidant Effects Attenuating Ischemic Brain Damage
- Reduced Depletion of Circulatory Antioxidants Caused by a Cancer-Causing Agent
- Antioxidative Effect: Review

### Anti-stress and Anti-fatigue Effects

- Reduced Fatigue in a Fatigue-Model in Mice
- Reduced Stress in a Stress-Model in Rats
<table>
<thead>
<tr>
<th>Chapter Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved Recovery from Subjective Symptoms from Various Internal Diseases</td>
<td>27</td>
</tr>
<tr>
<td>Reduced Physiological Stress in Pre-Clinical Studies</td>
<td>28</td>
</tr>
<tr>
<td>Reduced Physiological Stress in Clinical Studies</td>
<td>28</td>
</tr>
<tr>
<td>Improved Recovery from Athletic Performance in Pre-Clinical Studies</td>
<td>28</td>
</tr>
<tr>
<td>Improved Recovery from Athletic Performance in Clinical Studies</td>
<td>28</td>
</tr>
<tr>
<td>Improved Recovery from Chemical and Physical Stress</td>
<td>29</td>
</tr>
<tr>
<td>Improved Recovery from Oscillation Stress (Dizziness)</td>
<td>29</td>
</tr>
<tr>
<td>Prevented Stress-Induced Hypertrophy of the Adrenal Gland and Hyperglycemia</td>
<td>29</td>
</tr>
<tr>
<td>Improved Stress-Induced Immunosuppression</td>
<td>29</td>
</tr>
<tr>
<td>Reduced Stress-Induced Activation of the Peripheral Sympathetic System</td>
<td>29</td>
</tr>
<tr>
<td>Inhibits Stress-Induced Peptic Ulcer Formation</td>
<td>30</td>
</tr>
<tr>
<td><strong>SAFETY OF AGED GARLIC EXTRACT</strong></td>
<td>50</td>
</tr>
<tr>
<td><strong>OTHER PHARMACOLOGICAL EFFECTS</strong></td>
<td>47</td>
</tr>
<tr>
<td>Chronic Toxicity</td>
<td>50</td>
</tr>
<tr>
<td>Improved Male Reproduction Function</td>
<td>49</td>
</tr>
<tr>
<td>Protection from Heavy Metals</td>
<td>48</td>
</tr>
<tr>
<td>Anti-bacterial Properties</td>
<td>47</td>
</tr>
<tr>
<td>Brain, Neurotrophic, Anti-aging, and Anti-depression Effects of Aged Garlic Extract</td>
<td>42</td>
</tr>
<tr>
<td>Increased Serotonin Level</td>
<td>45</td>
</tr>
<tr>
<td>Enhanced Nerve Growth</td>
<td>44</td>
</tr>
<tr>
<td>Sustains Levels of Antioxidants Depleted by a Carcinogen</td>
<td>38</td>
</tr>
<tr>
<td>Decreased the Levels of Serum Prostaglandins, PGE2 and PGF2a</td>
<td>38</td>
</tr>
<tr>
<td>Reduced Incidence of Precancerous Gastric Lesions in Clinical Studies</td>
<td>39</td>
</tr>
<tr>
<td>Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis</td>
<td>39</td>
</tr>
<tr>
<td>Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis in Clinical Studies</td>
<td>40</td>
</tr>
<tr>
<td>Inhibited the Development of Liver Cancer</td>
<td>40</td>
</tr>
<tr>
<td>Anticancer and Cancer-Preventive Review</td>
<td>41</td>
</tr>
<tr>
<td>Overview of Inhibition of Cancer Growth by</td>
<td>42</td>
</tr>
<tr>
<td>Aged Garlic Extract and Its Constituents</td>
<td>42</td>
</tr>
<tr>
<td><strong>ANTICANCER AND CANCER-PREVENTIVE EFFECTS</strong></td>
<td>30</td>
</tr>
<tr>
<td>Inhibited the Growth of Bladder Tumors</td>
<td>30</td>
</tr>
<tr>
<td>Inhibited the Growth of Melanoma Cells</td>
<td>31</td>
</tr>
<tr>
<td>Inhibited the Growth of Neuroblastoma Cells</td>
<td>31</td>
</tr>
<tr>
<td>Inhibited the Growth of Sarcoma Cells</td>
<td>31</td>
</tr>
<tr>
<td>Inhibited the Development of Carcinogen-Induced Skin Cancer</td>
<td>32</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of the Breast</td>
<td>32</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of the Colon</td>
<td>34</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of the Colon in Clinical Studies</td>
<td>35</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of Esophagus</td>
<td>35</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of the Stomach and Lung</td>
<td>35</td>
</tr>
<tr>
<td>Inhibited the Growth of Prostate Cancer Cells</td>
<td>36</td>
</tr>
<tr>
<td>Inhibited Growth of Erythroleukemia Cell Lines</td>
<td>36</td>
</tr>
<tr>
<td>Inhibited Aflatoxin B1- and Benzo[a]pyrene-induced Mutagenesis</td>
<td>37</td>
</tr>
<tr>
<td>Suppressed Activity of Procarcinogen-Activating Enzymes</td>
<td>37</td>
</tr>
<tr>
<td>Potential Mechanisms</td>
<td>37</td>
</tr>
<tr>
<td>Inhibited Both the Formation and Bioactivation of Carcinogenic Nitrosamines</td>
<td>38</td>
</tr>
<tr>
<td>Inhibited the Growth of Carcinogen-Induced Tumors of the Breast</td>
<td>32</td>
</tr>
<tr>
<td>Reduced Levels of Serum Prostaglandins, PGE2 and PGF2a</td>
<td>38</td>
</tr>
<tr>
<td>Sustains Levels of Antioxidants Depleted by a Carcinogen</td>
<td>38</td>
</tr>
<tr>
<td>Decreased the Levels of Serum Prostaglandins, PGE2 and PGF2a</td>
<td>38</td>
</tr>
<tr>
<td>Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis</td>
<td>39</td>
</tr>
<tr>
<td>Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis in Clinical Studies</td>
<td>40</td>
</tr>
<tr>
<td>Inhibited the Development of Liver Cancer</td>
<td>40</td>
</tr>
<tr>
<td><strong>BRAIN, NEUROTROPIC, ANTI-AGING, AND ANTI-DEPRESSION EFFECTS OF AGED GARLIC EXTRACT:</strong></td>
<td>43</td>
</tr>
<tr>
<td>Improved Survival, Memory Retention, Learning Deficits and Immune Response</td>
<td>43</td>
</tr>
<tr>
<td>Enhanced Nerve Growth</td>
<td>44</td>
</tr>
<tr>
<td>Improved Serotonin Level</td>
<td>45</td>
</tr>
<tr>
<td>Anti-Alzheimer’s Effect</td>
<td>45</td>
</tr>
<tr>
<td>Enhancement of Human Growth Hormone</td>
<td>46</td>
</tr>
<tr>
<td>Brain, Neurotrophic, Anti-aging, and Anti-depression Effects: Review</td>
<td>47</td>
</tr>
<tr>
<td><strong>OTHER PHARMACOLOGICAL EFFECTS</strong></td>
<td>47</td>
</tr>
<tr>
<td>Anti-fungal Properties</td>
<td>47</td>
</tr>
<tr>
<td>Anti-bacterial Properties</td>
<td>47</td>
</tr>
<tr>
<td>Improved Intestinal Conditions</td>
<td>47</td>
</tr>
<tr>
<td>Protection from Heavy Metals</td>
<td>48</td>
</tr>
<tr>
<td>Nutritional Support for Genital and Oral Herpes</td>
<td>48</td>
</tr>
<tr>
<td>Effects of Aged Garlic Extract on Sugar Metabolism</td>
<td>48</td>
</tr>
<tr>
<td>Blood Building Effects of Aged Garlic Extract Preparation</td>
<td>48</td>
</tr>
<tr>
<td>Anti-Allergy</td>
<td>48</td>
</tr>
<tr>
<td>Improved Male Reproduction Function</td>
<td>49</td>
</tr>
<tr>
<td>Reducing the Side Effects</td>
<td>49</td>
</tr>
<tr>
<td>Overview of Various Effects</td>
<td>49</td>
</tr>
<tr>
<td><strong>SAFETY OF AGED GARLIC EXTRACT</strong></td>
<td>50</td>
</tr>
<tr>
<td>General Toxicity</td>
<td>50</td>
</tr>
<tr>
<td>Acute Toxicity</td>
<td>50</td>
</tr>
<tr>
<td>Chronic Toxicity</td>
<td>50</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td>50</td>
</tr>
</tbody>
</table>
Acute and Subacute Toxicity .................................................................................................................. 51
Safety During Pregnancy .......................................................................................................................... 51
Drug Interactions with Aged Garlic Extract ............................................................................................. 51
— Coumadin .............................................................................................................................................. 51
— Statins .................................................................................................................................................. 52
— Acetaminophen ................................................................................................................................ 52
— Saquinavir ........................................................................................................................................... 52
Dosages in Studies .................................................................................................................................... 52
Side Effects ............................................................................................................................................... 52

5-ALLYL CYSTEINE (SAC): A KEY COMPOUND IN AGED GARLIC EXTRACT ........................................ 53

CARDIOVASCULAR EFFECTS OF SAC ............................................................................................... 53
Cholesterol Lowering Effect .................................................................................................................... 53
Kyolic® > SAC > commercial garlic oil > garlic powder .......................................................................... 53
Blood-Thinning Effect .............................................................................................................................. 53
Inhibition of Vascular Smooth-Muscle Cell and Umbilical Endothelial Cell Proliferation .................... 54
Sickle Cell Anemia .................................................................................................................................... 54

ANTIOXIDATIVE EFFECTS OF SAC ..................................................................................................... 54
Inhibits Advanced Glycation Endproduct (AGEP) Formation in Diabetics .............................................. 55
Attenuating Ischemic11 Brain Damage ..................................................................................................... 55
Inhibits the Activation of NF-κB which Mediates Inflammatory Reactions ........................................... 56

ANTICANCER AND CANCER-PREVENTIVE EFFECTS OF SAC ....................................................... 56
Ameliorates Anticancer Drug, Doxorubicin, Cardiotoxicity ........................................................................ 56
Inhibited the Growth of Carcinogen-Induced Tumors of the Breast ....................................................... 57
Inhibited the Growth of Melanoma Cells ................................................................................................ 57
Inhibited the Growth of Neuroblastoma Cells ....................................................................................... 57
Inhibited Colon Cancer Progression ....................................................................................................... 57
Sustained Circulatory Antioxidants Depleted by Cancer-Causing Agent ................................................. 58
LIVER PROTECTIVE EFFECTS OF SAC .............................................................................................. 58
Inhibited Both the Formation and Bioactivation of a Liver Carcinogen ................................................. 58
Protected Liver Cells from the Liver Toxins: Paracetamol (Acetaminophen), Carbon Tetrachloride and Bromobenzene ......... 58

BRAIN, NEUROTROPIC, ANTI-AGING, AND ANTI-DEPRESSION EFFECTS OF SAC .................... 59

LUNG PROTECTIVE EFFECTS OF SAC .............................................................................................. 60
Protected Cells from Toxic Carbon Tetrachloride in the Lungs. .............................................................. 60

SAC HAS BEEN CONFIRMED TO BE BIOAVAILABLE AND ACTIVE .................................................. 60

SAC HAS CONFIRMED SAFETY ........................................................................................................... 61
Pharmacokinetics ................................................................................................................................ 61
Chemistry ................................................................................................................................................ 61

5-ALLYLMERCAPTOCYSTEINE (SAMC): A CONSTITUENT UNIQUE TO AGED GARLIC EXTRACT ...... 62

ANTIOXIDATIVE AND RADIOPROTective EFFECTS OF SAMC ..................................................... 62
Protected Cells from Oxidant Injury ........................................................................................................ 62

LIVER PROTECTIVE EFFECTS OF SAMC ............................................................................................ 63
In vivo Protection from Carbon Tetrachloride and Paracetamol ............................................................. 63

ANTICANCER EFFECTS OF SAMC ....................................................................................................... 64
Inhibited the Growth of Carcinogen-Induced Tumors of the Breast ...................................................... 64
Inhibited the Growth of Carcinogen-Induced Tumors of the Colon ...................................................... 64
Inhibited the Growth of Prostate Cancer Cells ...................................................................................... 65
Inhibited Growth of Erythroleukemia Cell Lines .................................................................................. 65

OTHER CONSTITUENTS IN AGED GARLIC EXTRACT ................................................................... 66

OIL-SOLUBLE AND WATER-SOLUBLE ORGANOSULFUR COMPOUNDS ........................................ 66
Inhibited the Activity of Human Cytochrome-P450 (CYP) Enzymes ..................................................... 66
Inhibited the Levels of Hepatic CYP2E1 Protein ..................................................................................... 66
Reduced the Incidence of Chemically-Induced Cancers ..................................................................... 67
Organosulfur Compounds ........................................................................................................................ 67
in Aged Garlic Extract ........................................................................................................................... 67

MALLARD REACTION PRODUCTS ...................................................................................................... 67
N-Fructosyl Glutamate and N⁰-Fructosyl Arginine (Fru-Arg) ................................................................. 67
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**

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 group 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 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.

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.

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 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.

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.

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.

After taking four capsules of a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 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).

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 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.

In this double-blind, crossover study of hyperlipidemic patients (cholesterol 220-290 mg/dl) on the NCEP 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.

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 hepatoma 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.

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.

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:

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.

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 laser Doppler 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.

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.

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 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 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 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) 

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 (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.

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 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.

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 laser Doppler fluxmetry (acetylcholine-induced 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.

Hypotensive/Blood Pressure Lowering Effects

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 supplemented with placebo for six months and followed by Aged Garlic Extract powder for four months showed the most significant results.

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

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)

Ohashi 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 β-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

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 et al. (2002) examined the potential role of Aged Garlic Extract as an antioxidant for sickle cell anemia patients. 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.

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$_{2a}$

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

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. 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.

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 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.
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 following intake of Aged Garlic Extract.

Lipid Lowering Effects

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

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

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

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 [2,14C]acetate in the presence or absence of the sulfur compounds at 0.05 to 4.0 mmol/L. The rate of [2,14C]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-propyl cysteine and S-allyl mercaptocysteine (SACM) inhibited fatty acid synthesis by 20% to 99% with IC50 of 0.27 to 1.72 mmol/L. Alliin, S-allyl-N-acetyl cysteine, S-allylsulfonfyl alanine, and S-methylcysteine did not inhibit fatty acid synthesis. All tested water-soluble compounds except SACM did not alter cellular release of lactate dehydrogenase (LDH) 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 IC50, SAC, SEC, and SPC are equally potent in inhibiting cholesterol synthesis.

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 (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 squalene monooxygenase, an enzyme that catalyzes or facilitates the generation of cholesterol. Using chromatography-mass spectrometry, it is found that garlic-derived compounds containing an allyl-disulfide or allyl-sulphhydryl group are most likely responsible for decreasing cholesterol synthesis by inhibiting the sterol 4α-methyl oxidase.

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.

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

**Circulation-Enhancing/Blood-Thinning Preclinical Effects**

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.

Morishuchi, T., Itakura, Y. 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. 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 et al. (2006) found that garlic reduces the blood pressure by enhancing the concentration and activity of many vasodilatory agents including nitric oxide (NO).
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.

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.

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 et al. (1988) found that a Kyolic Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12, 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 vivno 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 et al (2002) observed that Aged Garlic Extract increased NO production by activating constitutive NOS (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 2 hr. 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.

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-induced 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**

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%.

Kyolic Aged Garlic Extract is patented by the U.S. government for its ability to reduce homocysteine.
The supplementation of AGE was found to decrease homocysteine levels by 28 to 33 percent in models severely deficient in folate.

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.

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 (the growth of smooth muscle cells over accumulated scar tissue in blood vessels):

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 constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subject to angioplasty.

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 than those fed a high cholesterol diet alone. “Kyolic” significantly reduced neointimal formation (thickening of the artery wall) and thoracic aorta fatty streak development (accumulation of fat/cell/tissue on the arterial wall) in hyperlipidemic conditions. Further, “Kyolic” inhibited smooth muscle cell proliferation, providing protection against the onset of atherosclerosis.
Ide et al. (2000) reported that the effects of AGE on vascular homeostasis associated with atherosclerosis using several in vitro and in vivo systems. AGE inhibited Cu²⁺-induced LDL oxidation dose-dependently. AGE inhibited LDH release indicating cell membrane damage and intracellular GSH, as an index of intracellular antioxidant, depletion. The effect of AGE on nitric oxide (NO) production was examined in vivo (10 ml/kg, p.o.). The data suggest that AGE stimulates cNOS specifically. In conclusion, AGE inhibits Cu²⁺-induced LDL oxidation, protects endothelial cells from Ox-LDL-induced injury by preventing intracellular GSH depletion. AGE also modulates NO production in vivo, suggesting that AGE may be useful for prevention of athrogenesis and thrombin formation.

Ide et al. (2006) determined that aged garlic extract (AGE) may prove useful in preventing atherosclerotic lesions in monocytes incubated with homocysteine, a risk factor for cardiovascular diseases.

Ohnishi et al. (2000) found that conincubation of monocytes/macrophages (THP-1) with Aged Garlic Extract (AGE) inhibited homocysteine (Hey)-induced CD36 expression by 61.8 ± 13.9%, compared with control conditions. AGE also slightly inhibited oxidized low-density lipoprotein (OxLDL) uptake into THP-1 cells by 85.6 ± 2.8% of control conditions, which suggest that AGE could modulate the formation of early atherosclerotic lesions.

**Sickle Cell Anemia**

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, it has severe, toxic side effects and still there is no cure. The patents 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. Dense cells that possess an abnormal membrane can be found in the patients, and they may cause blood vessel occlusion since they have a tendency to adhere to neutrophils, platelets, and vascular endothelial cells. Using this method, Ohnishi found that S-allyl cysteine (an anti-oxidant component of the aged garlic extract) inhibited the formation of dense cells in vitro. The inhibition was 30% at the concentration of 1 mg/ml. If orally taken aged garlic extracts reduce the dense cell formation in the patients, then, the extracts may have a beneficial effect for this disease.

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 β-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.

Dense cells, which have an elevated density and possess an abnormal membrane, have a tendency to adhere to blood components such as neutrophils, platelets, and endothelial cells, which line blood vessels. Thus, they could trigger vasoocclusion, closing off of the blood vessels restricting blood flow, and the subsequent painful crisis from patients with sickle cell anemia suffer. Dr. Ohnishi (2001) at Philadelphia Biomedical Research Institute found that nutritional antioxidant supplements, hydroxyl radical scavengers, and iron-binding agents could inhibit the formation of dense cells in test tubes. Specifically, aged garlic extract, black tea extract, green tea extract, Pycnogenol, α-lipoic acid, vitamin E, coenzyme Q10, and β-carotene effectively inhibited dense cell formation by 50% at varied concentrations.
Oral consumption of Aged Garlic Extract before carbon tetrachloride (CCl₄) injection significantly reduced the amount of pentane in the breath by 80% compared to control models showing a suppression of lipid peroxide formation.

Dr. Yamada’s team at Tottori University in Japan found that S-allylmercaptocysteine (SAMC), a constituent in Aged Garlic Extract, could ameliorate the toxicity of acetaminophen-induced liver damage in their preclinical study. SAMC reduced liver cell death and mortality. The authors suggested that SAMC might be useful as an antidote for acetaminophen overdose.

Nakagawa et al. (1985) found that Aged Garlic Extract and its constituents, S-allyl cysteine (SAC), S-allyl mercaptocysteine (SAMC) and S-propyl cysteine, completely suppressed the cytotoxicity (cell-killing power) of the potent liver toxin carbon tetrachloride (CCl₄), whereas four positive control drugs (vitamin E, piperonyl butoxide, glycyrrhizin and glutathione) were found to be less effective at protecting liver cells.

Sumioka et al. (1998) found that S-allyl mercaptocysteine (SAMC; 100mg/kg, p.o.), a constituent only found in Aged Garlic Extract, given to models 2 and 24 hours before administration of acetaminophen (APAP; 500 mg/kg, p.o.) prevented liver damage as shown by a reduction in alanine aminotransferase (ALT) activity, which is enhanced by APAP. ALT was shown to decrease by 79%, 97% and 100% when APAP was given in conjunction with 50, 100 and 200 mg/kg of SAMC. SAMC also prevented the reduction in glutathione (important for the detoxification of APAP) induced by APAP administration. One mechanism proposed for liver protection was inhibition of cytochrome P450 2E1 activity since SAMC suppressed an enzyme representative of P450 2E1 activity. P450 2E1 is a major enzyme responsible for bioactivation of APAP. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation (oxidation of liver tissue) and the decrease in liver COQ9H2 suggesting an antioxidative effect.

Nakagawa et al. (1988) found that Aged Garlic Extract and its constituents, SAC and SAMC, protected the liver cells from the liver toxins Paracetamol (acetaminophen) and CCl₄ that induce acute hepatitis. Both SAC and SAMC appeared to enhance the activity of glutathione, a detoxifying enzyme, and acted as chemical scavengers. These garlic constituents were found to be more effective than the other chemicals used.

Nagai and Yamawaki (1974) studied the protective effects of a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B₁, vitamin B₁₂ and liver extract, on liver tissue damage induced by inhalation of CCl₄. At dosages of both 0.2 and 2.0 ml for 10 days preceding lung inhalation of CCl₄, Kyolic® Liquid B1 Formula treated models maintained normal healthy liver tissue, as evidenced by various parameters of liver tissue analysis, whereas control models exposed to CCl₄ alone experienced significant liver damage.

Enhanced Metabolism of Acetaminophen in Humans

Gwilt et al. (1994) found that Aged Garlic Extract enhanced the metabolism/detoxification of the pain killer acetaminophen (1 g), as shown by increased sulfate conjugation and glucuronic acid formation of this drug for excretion from the kidneys, in sixteen subjects taking 10 mls of Liquid Aged Garlic Extract daily for three months. Since the metabolism of acetaminophen is very similar to that of carcinogens, and the effects of Aged Garlic Extract were only slight, the results suggest that Aged Garlic Extract inhibits carcinogenesis through a mechanism other than modification of drug metabolism. Potential theories include the fact that AGE increased glucuronidation, and some evidence of enhanced sulfate conjugation may explain another mechanism.
Prevented the Mutagenic Effects of the Liver Toxin Aflatoxin B1

Lau et al. (1990, 1991) found that Aged Garlic Extract protected liver tissue from damage caused by aflatoxin B1. Specifically, it inhibited binding of AFB 1’s to DNA and mutatation of liver cells. Aged Garlic Extract also significantly decreased toxic metabolites of AFB 1 and increased nontoxic metabolites, such as glucuronide and glutathione.

Prevented the Toxic Effects of Phenobarbital and Bromobenzene


Billington et al. (1998) from Liverpool John Moores University in UK found that Aged Garlic Extract protected liver cells from phenobarbital and bromobenzene exposure. Phenobarbital is a sedative drug. It exacerbates the destructive effects of bromobenzene, an industrial solvent. Together, phenobarbital and bromobenzene elicit the generation of a potent liver toxin (bromobenzene-3,4-oxide) that causes liver damage in vitro. This model is used to assess liver damage and/or potential chemicals or drugs that may be liver-protective. Aged Garlic Extract (at a concentration of 1-5% (v/v)) reduced the toxicity of bromobenzene in a concentration dependent manner as judged by all of the parameters of viability (cell life) studied. Lipid peroxidation, on the other hand, was reduced to control levels even at the lowest concentration of Aged Garlic Extract. The mechanism appeared to be sparing of the protective enzyme, reduced glutathione, by the extract.

Enhanced Detoxification of Acetaldehyde

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


Kasuga (1998, 2001) investigated detoxifying activities of four garlic preparations including raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (PGP) and aged garlic extract (AGE) on intoxication by acetaldehyde. HGJ and DGP stimulated acetaldehyde detoxification but RGJ was ineffective. AGE showed significant beneficial effects.

Enhanced Detoxification Enzymes in the Liver

Blakely et al. (1993) found that Aged Garlic Extract enhanced the activity of detoxification and antioxidant enzymes in the liver of models consuming garlic and cholesterol or garlic and high levels of iron. Activity was more pronounced in female than in male models. Specifically, levels of benzphetamine demethylase, aniline hydroxylase, and Glutathione-S-transferase8 were enhanced.

Inhibited Cholesterogenesis in HepG-2 Cells

Lee et al. (2003) discovered that there is maximal inhibition on cholesterol synthesis when Kyolic (which contains S-allyl-cysteine [SAC], S-ethyl-cysteine [SEC], S-propyl-cysteine [SPC] and gamma-glutamyl S-alk(en)yl cysteines) is used in addition with various water-soluble sulfur compounds (SAC and/or SPC) in HepG-2 cells.
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 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.

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.

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.

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

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 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).

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.

Tadi, P., Lau, B. et al. 1990. Nutr. Rev. 10: 423-429. Tadi et al. (1990), also using chemiluminescence, confirmed the phagocytic enhancing activity of Aged Garlic Extract and attributed the control of Candida albicans in a living model to this effect.

Overview of Immune-Enhancing Effects of Aged Garlic Extract (AGE) and Its F-4 Protein Fraction

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>AGE</th>
<th>F-4 FRACTION</th>
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</thead>
<tbody>
<tr>
<td>Natural Killer Cells</td>
<td>↑ 160%</td>
<td>↑ 160%</td>
</tr>
<tr>
<td>Macrophages</td>
<td>↑ 110-290%</td>
<td>↑ 110-290%</td>
</tr>
<tr>
<td>T-Cells</td>
<td>↑ 60%</td>
<td>↑ 60%</td>
</tr>
<tr>
<td>Phagocytes</td>
<td>↑ 30-900%</td>
<td>↑ 30-900%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>↑ 230-300%</td>
<td>↑ 230-300%</td>
</tr>
</tbody>
</table>

↑ indicates an increase in activity.

Kasuga (1998, 2001) investigated pharmacological activities of four garlic preparations including raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (PGP ) and aged garlic extract (AGE). Though all four garlic preparations enhanced NK and Killer activities of the spleen cells of tumor bearing models, only AGE and HGJ inhibited the growth of inoculated tumor cells. These results suggest that different types of garlic preparations have different pharmacological properties, and among the four garlic preparations studied, AGE could be the most sophisticated and beneficial garlic preparation.

Kyo et al. (1999) reviewed the immunostimulatory properties of Aged Garlic Extract. Aged Garlic Extract was shown to enhance proliferation of spleen cells and to augment the immune-stimulating activity of various well-known immunostimulatory agents (ConA, phytohemagglutinin, lipopolysaccharide and IL-2). Aged Garlic Extract also increased cytokine production (IL-2, tumor necrosis factor $\alpha$, and interferon $\gamma$) from spleen cells. It enhanced the activity of NK against lymphoma (YAK-1). Aged Garlic Extract also prevented immunosuppression induced by removal of the thymus and showed various anti-tumor activities via immunomodulation. Restoration of stress-induced immune suppression and anti-allergy effects were also noted. Suggested actives such as a protein fraction and low-weight sugar fractions were mentioned.

Kyo et al. (1998, 2001) found that Aged Garlic Extract could be a significant immuno potentiator, and could exhibit anti-tumor activities through immune modulation. After three weeks oral intake, Aged Garlic Extract inhibited the growth of Sarcoma-180 by 50% and LL/2 (Lewis Lung cancer cell line) by 20%. Killer cell activity of spleen cells against Sarcoma-180 cells was also significantly enhanced by AGE ($p<0.01$), but not PSK,9a positive control drug, and Natural Killer cell activity against YAC-1 (cancer cell line) was enhanced by both Aged Garlic Extract ($p<0.01$), and PSK ($p<0.05$). Aged Garlic Extract had no direct effect on LL/2 and Sarcoma-180 $\text{in vitro}$ confirming that its effects were via immune enhancement and not cytotoxicity.

Lamm et al. and his group at West Virginia University School of Medicine found that Aged Garlic Extract may help to reduce the development of tumors in the bladders implanted with MBT2 (human bladder cancer cells). Aged Garlic Extract was also found to have some ability to directly kill MBT2 cells in test tubes. Aged Garlic Extract given orally was effective without toxicity or side effects. It reduced tumor incidence by as much as 40% and tumor growth by as much as 60% ($P=.0002$) and enhanced survival by 50%. Its effects were similar to that of Bacillus calmette-Guerin (BCG), a potent immunomodulatory treatment widely used in human cancer therapy. The authors suggested the Aged Garlic Extract might be an excellent adjuvant to traditional treatment.

Morioka et al. (1993) found that a protein fraction isolated from Ag ed Garlic Extract enhanced the cytotoxicity (cell killing ability) of human peripheral blood lymphocytes against tumor cells. Moreover, the protein fraction significantly stimulated the lymphokine (interleukin-2)-activated killer activity. The protein fraction also enhanced the proliferation of lymphocytes induced by interleukin-2 and concanavalin-A, suggesting a possible reduction of the dosage of interleukin-2 in cancer immunotherapy.
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.

Reeve et al. (1997) found that Aged Garlic Extract at 4% of the diet provided significant protection from edema and photoimmunosuppression induced by moderate exposure to UVB radiation.

Reeve et al. (1993a, b) found that UV light exposure caused a 58% suppression of systemic contact hypersensitivity (SCH, an immune response). When the models were also fed 4% Aged Garlic Extract, the immune response was suppressed by only 19%. Thus, the Aged Garlic Extract helped to ameliorate the immunosuppressive effects of UV exposure. When UV exposure was replaced by topical application of cis urocanic acid-containing lotions, which also suppress the SCH, Aged Garlic Extract totally protected the models from this form of immunosuppression.

Nagai et al. (1973a) found that in models that were per nasally inoculated with influenza virus (AO/PR 8), Aged Garlic Extract was found to enhance the effectiveness of an influenza vaccine and when used alone was found to be as effective as the vaccine.

Nagai et al. (1973) also found that giving either Aged Garlic Extract alone or a Kyolic ® Liquid Aged Garlic Extract preparation containing vitamin B 1, vitamin B 12 and liver extract to models for 15 days prior to per nasal inoculation with influenza virus significantly improved their outcome.

Kyo et al. (1997) demonstrated an allergic-type reaction by adding compounds (anti-TNP monoclonal antibody and TNP-BSA hapten carrier complex) to immune cells (basophile cell line RBL-2H3) that cause them to release histamine. When Aged Garlic Extract was additionally added at increasing dosages (1.25, 2.5 and 5.0 v/v %) it significantly inhibited the histamine release by 50, 80, and 90%, respectively. The anti-allergy drug oxatomide (10 mcg/ml) inhibited histamine release by 80%.

In another experiment by these authors, AGE given orally (10 ml/kg) showed anti-inflammatory effects by decreasing the ear swelling induced by the topical administration of a known skin irritant (picryl chloride) and intravenous administration of an antibody (anti-TNP Ig E antibody) that induces skin reactions, a type I allergic reaction. AGE reduced swelling by 25-45% thus decreasing the allergic reaction triggered by immune (mast) cells.

When AGE and oxatomide were administered directly into the stomach one-hour following a picryl chloride application to the ears, oxatomide reduced swelling by 47% and AGE reduced it by 19%. However, four and 24 hours following application, AGE out-performed oxatomide.

In a final experiment conducted by these authors, AGE was found to inhibit a type IV allergic reaction. Picryl chloride was first applied to abdominal skin and then subsequently to the ears seven days later. Either dexamethasone, a known immunosuppressor, or AGE was then administered orally 0, 4, and 16 hours (three times or one time at each respective hour). Ear thickness was measured 24 hours after the secondary challenge. Repeated oral administration of the drug dexamethasone suppressed ear swelling by 65% and AGE inhibited ear swelling by 55%.

Thus, Aged Garlic Extract may reduce allergic-type reactions.
In an IgE mediated allergic model, Kyo et al. (1998, 2001) found that AGE significantly decreased the antigen specific ear swelling which was induced by an application of picryl chloride ointment to the ear and an intravenous administration of anti-trinitrophenyl antibody IgE ascites.

Improved Immune Function and Depressed State Post-Menopause

The pharmacological effects of Aged Garlic Extract combined with ginseng, oriental bezoar, antler velvet, cuscuta seed, and epimedium herb (Leopin Royal; LER) were evaluated using post-menopausal models. Removal of the ovaries caused a state of depression and immune function disorder. However, when supplemented with the AGE preparation the models showed improvement in the depressed state and cytokine production (IFN-gamma), an index of immune function. This data indicates that AGE improves the disorder of nerve-internal secretion-immune network induced by the ovary dysfunction, and suggests it may be useful for women during menopause.

Improved Age-Related Deterioration of Immune Responses

Zhang et al. (1997) found that chronic oral administration of Aged Garlic Extract significantly improved the suppression of antibody production caused by removal of the thymus gland from two different models genetically prone to accelerated aging. Aged Garlic Extract also restored to control levels the amounts of hypothalamic norepinephrine, 3,4-dihydroxyphenylacetic acid and homovanilic acid, and the hypothalamic choline acetyltransferase activity, which were significantly increased by removal of the thymus. Chronic ingestion of Aged Garlic Extract significantly enhanced white blood cell production activated by the immune-stimulating agents concanavalin A or lipopolysaccharides in both SAMP8 and SAMR1, a normal substrain of SAM not genetically prone to accelerated aging.

Antioxidative and Radioprotective Effects

Oxygen radical injury and lipid peroxidation have been suggested as major causes of atherosclerosis, cancer, liver disease and the aging process. Aged Garlic Extract and its various constituents have demonstrated an array of antioxidant and radioprotective effects in studies. They have been shown to protect white blood cells from radiation damage, liver cells from lipid peroxidation and vascular endothelial cells from oxidant injury. Further, they have been shown to enhance antioxidative enzyme systems in cells. They have been shown to scavenge hydrogen peroxide, to inhibit the formation of TBA-RS, to protect the heart from cardiotoxic, anti-cancer drug doxorubicin, to protect the kidneys from the antibiotic gentamicin, and to attenuate ischemic brain damage as seen in the following studies:

Oxidative modification of DNA, proteins and lipids by reactive oxygen plays a role in aging and including cardiovascular, inflammatory disease and cancer. Garlic and garlic extracts contain antioxidant nutrients and phytochemicals that prevent oxidant damage via different and complementary mechanisms; scavenging reactive oxygen and increasing inherent defenses. Aging the garlic extracts increases their antioxidant potential, in part by amplifying stable bioavailable organosulfur compounds. Aged garlic extracts (AGE) contain antioxidants that protect DNA against free radical mediated damage and mutations, defend against radiation and sunlight damage and inhibit multi-step carcinogenesis. AGE inhibits lipid peroxidation, maintains blood vessel integrity, increases blood flow and reduces the risk of atherosclerosis and cardiovascular diseases that are accelerated by LDL peroxidation. AGE supplementation amplifies and strengthens the antioxidant network in cells. AGE has wide reaching health potentials in defending the body against age related ailments, including inflammatory conditions and oxidant-mediated damage to the brain, helping preserve brain function and a healthy life.

AGE is helpful in reducing risk factors of cardiovascular disease, including high cholesterol; which is associated with dementia and Alzheimer's disease. AGE is able to scavenge oxidants, inhibit cholesterol synthesis, and help prevent cognitive decline by protecting neurons from 

beta-amyloid (Abeta) neurotoxicity and apoptosis.


As a Supplement. Newport

Consumption of KLE assisted in alleviating anorexia and fatigue and enhanced their will to fight their disease. Twelve of 30 patients, on either radio- or chemotherapy, suffering from head and neck tumors found that

Damage.

The poorly absorbable compound, fluoracin isothiocyanate-labeled dextran (average molecular weight, 4400) (FD-4) was examined to evaluate damage to the intestine using the in vitro intestinal loop technique. The FD-4 absorption through the small intestine increased in the anti-tumor drug-

Protection from Gentamicin-Nephrotoxicity

Anti-tumor drugs like 5-fluorouracil (5-FU) and methotrexate (MTX) induce intestinal damage, a serious side

Induced toxicity could not be prevented with SAC at different concentrations (0.5-8 mM); however SAC had no protective activity. Although SAC elicited a significant increase in the renal levels of oxidative stress markers: nitrotyrosine and protein carbonyl groups and the decrease in manganese superoxide dismutase (Mn-SOD), GPx, and glutathione reductase (GR) activities.

Aged Garlic Extract and its constituents protected liver membranes from lipid peroxidation (oxidative damage). In vitro oxidative stress in humans.

In vitro antioxidative activities as protective compounds against free radical damage in the body. Alliin and allyl cysteine was shown as a hydroxyl radical scavenger. Allicin had no effect.

Aged Garlic Extract protected cultured lymphocytes from irradiation damage. It prevented both the formation of lipid peroxides and the physical damage they cause to membranes, such as decrease of membrane fluidity or ability to exchange nutrients and wastes across the membrane.

Protection from Radiation and Chemotherapy

Fresh garlic, on the other hand, had no protective effect. Rather, it worsened irradiation damage more so than

Protection from Lipid Peroxidation

Dr. Rahman and his team of researchers at Liverpool John Moores University found dietary supplementation with Kyolic Aged Garlic Extract (AGE) (5ml per day) for 14 days reduced plasma and urine concentrations of 8-iso-PGF2α by 29% and 37% in nonsmokers and by 35% and 48% in smokers. 8-iso-PGF2α is a marker of

Depressed uric acid excretion levels were improved after dietary supplementation with AGE: increase in plasma creatinine, increase in plasma glutathione peroxidase (GPx) activity and urinary decrease in N-acetyl-

Protection from Gentamicin-Nephrotoxicity

AGE, GPE, SAC, DAS, or DADS may be administered along with Gentamicin to alleviate Gentamicin-

Protection from Gentamicin-Nephrotoxicity without interfering with its antibiotic activity.

These results suggest that the aged garlic extract protects the small intestine from anti-tumor drug-induced intestinal damage. However, absorption decreased in vivo compared to the in vitro data for the models fed with the standard diet with and without the aged garlic extract. The small intestine absorption of a poorly absorbable compound, fluoracin isothiocyanate-labeled dextran (average molecular weight, 4400) (FD-4) was examined to evaluate damage to the intestine using the in vitro intestinal loop technique. The FD-4 absorption through the small intestine increased in the anti-tumor drug-

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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/Fe\(^{2+}\)-induced lipid peroxidation of liver tissue.

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.

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 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 \textit{in vivo} and Aged Garlic Extract was more effective than raw garlic juice.

Ohnishi and Kojima (1997) found that Kyolic\textsuperscript{®} 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 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.

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 \textit{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 et al. (2003) found that Aged Garlic Extract inhibits \textit{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 atherosclerotic disease.

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 \textit{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.
The antioxidant effects of Aged Garlic Extract (AGE) and its compounds were determined using several in vitro systems. Incubation of human LDL with copper ion for 24h increased lipid peroxidation, however, AGE and its compounds were able to inhibit lipid peroxidation. Incubation of endothelial cells with oxidized LDL for 24h caused an increase in LDH release, an index of cell membrane damage, TBARS, an indication of lipid peroxidation, and a loss in cell viability. However, the opposite effect was seen when endothelial cells were pre-treated with AGE or its compounds, suggesting that it may be useful for the prevention of cardiovascular disease due to its antioxidant effects.

Cell membrane damage in myocardial infarction-induced models increased enzymatic leakage, lipid peroxidation, and free radical formation. Oral pretreatment with SAC (100 mg and 150 mg/kg) improved superoxide dismutase, catalase, glutathione reductase, and ascorbic acid enzymatic activities. End measures of lipid peroxidation, TBARS, were decreased with SAC oral pretreatment (100 mg and 150 mg/kg).

Padmanabhan and Prince (2006) conclude improvements made in lipid peroxide markers (decrease) and antioxidant status (increase) are due to the anti-oxidant effect of SAC.

Antioxidant effects of garlic preparation including raw garlic extract, boiled garlic extract, and Aged Garlic Extract (AGE) were determined using several in vitro systems. Incubation with microsome and t-butyl hydroperoxide enhanced ultra weak chemiluminescence indicating lipid peroxidation. Out of these three garlic preparations, only AGE inhibited lipid peroxidation in this system. AGE and its constituents have shown scavenging effects on hydrogen peroxide. Fructosyl-arginine, a component of AGE, proved 1/10 as strong as the common antioxidant ascorbic acid.

The effect of Aged Garlic Extract (AGE) on H2O2-induced oxidant injury was studied, and cell viability (MTT assay), lactate dehydrogenase (LDH) release, and lipid peroxidation (TBA-RS) were assessed. Results indicate that in a dose-dependent manner S-allylcysteine (SAC) and AGE inhibited LDH release and TBA-RS production with 50µM of H2O2, demonstrating that SAC and AGE have antioxidant properties that can protect vascular endothelial cells from oxidant injury.

In vivo Protection from Lipid Peroxidation

Ide, N., Itakura, Y. et al. 1996. 2nd International Congress on Phytomedicine. September 11-14, 1996. Munich, Germany. Ide et al. (1996) found that Aged Garlic Extract inhibited in vivo lipid peroxidation as indicated by an 80% decrease in pentane production following carbon tetrachloride-induced lipid peroxidation. When Horie (1990) used Aged Garlic Extract prior to treatment of carbon tetrachloride (CCl4), a potent liver toxin, it protected liver tissue from oxidative damage as indicated by a reduction in TBA-RS.

Enhanced Antioxidant Systems In the Body

Wei, Z and Lau, B.H.S. 1998. Nutr. Res. 18(1): 61-70. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play an important role in scavenging oxidants and preventing cell injury. Lau et al. (1998) from Loma Linda University found that Aged Garlic Extract enhanced the activity of these three enzymes, and thus suppressed the generation of the free radicals hydrogen peroxide and the superoxide anion in pulmonary artery endothelial cells (cells which line the blood vessels of the lungs).

Geng, Z. and Lau, B. 1997. Phytother. Res. 11: 54-56. Geng and Lau (1997) from Loma Linda University found that Aged Garlic Extract could protect cells which line the veins and arteries from damage caused by free radicals by enhancing the activity of antioxidant enzymes (the glutathione (GSH) redox cycle and superoxide dismutase (SOD) in these cells). GSH is a potent intracellular antioxidant and detoxifier. SOD is another potent intracellular antioxidant. Aged Garlic Extract was found to time- and dose-dependently enhance intracellular GSH level, glutathione disulfide reductase and SOD and thus may help to protect the cardiovascular system.
New antioxidative compounds were found in the garlic skin. These compounds are also found in AGE. Six phenylpropanoids from garlic formed in the extraction process. Ichikawa et al. (2004) demonstrated that four alkaloids 1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acids (MTCC) and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (MTCC) were isolated and identified in Aged Garlic Extract (AGE) and not in raw or other processed garlic preparations. These compounds show potent antioxidant properties in in-vitro systems using the hydrogen peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay. In particular, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline (MTCdiC) in both diastereoisomers were identified in fractionated AGE using a hydrogen peroxide peroxide scavenging assay.
In a study that used liquid chromatography mass spectrometry (LC-MS), four tetrahydro-ß-carboline derivatives were found to have strong hydrogen peroxide scavenging activities. These compounds found in AGE, were shown to increase during the aging process and were not detected in raw garlic. This study suggests that these compounds in AGE are potent antioxidants, and may play an important role in preventing disorders that are associated with oxidative stress.

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.

Cardioprotective Antioxidant Effects
— Protected Vascular System and Red Blood Cells from Oxidant Injury

Yamasaki et al. (1994) found that both Aged Garlic Extract (AGE) and S-allyl cysteine (SAC) can protect cells which line the blood vessels of the lungs from oxidant injury. AGE and SAC, in this in vitro test, protected bovine pulmonary endothelial cells from hydrogen peroxide (H2O2)-induced oxidant injury. Pretreatment of cells overnight with AGE (2-4 mg/ml) or SAC (4 mg/ml) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase (LDH) release and lipid peroxidation induced by H2O2. The authors suggesting that these compounds may be effective in hampering the aging process and for prevention of atherosclerosis.

When t-butylhydroperoxide, a free radical generator and oxidant, is used to oxidize red blood cells, it results in rupturing of the cells and darkening of the hemoglobin. Lin (1990) found that Aged Garlic Extract added to the red blood cell suspension prior to the addition of the oxidant minimizes such injuries. On the other hand, fresh garlic extract and raw garlic powder products enhanced the oxidative effects of this oxidant.

Morihara, 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.

— Decreased Cardiotoxicity of Anti-Cancer Drug Doxorubicin

Kojima, R., Ohnishi, S. et al. 1994. Nutr. Cancer 22:163-173. Doxorubicin (DOX), a cardiotoxic drug used to treat cancer, causes vacuolization in muscle cells, disruption of myofibrils, swollen mitochondria, and lipid peroxidation in heart tissues. Kojima et al. (1994a) found that Aged Garlic Extract in conjunction with doxorubicin showed significantly less lipid peroxidation and no significant pathological lesions in their hearts compared to those treated with doxorubicin alone. Models receiving Aged Garlic Extract in conjunction with DOX had better weights, survival and ECG's than those receiving DOX alone. Though the hearts treated with DOX alone exhibited significant pathology, those treated with DOX and Aged Garlic Extract were almost normal (Kojima et al., 1994b).
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. 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.

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.

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

— Prevented Oxidation Of LDL Cholesterol in Pre-Clinical Studies

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. 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, loss of cell viability and TBA-RS formation. AGE or SAC inhibited these changes. Effects of SAC on hydrogen peroxide (H$_2$O$_2$) or tumor necrosis factor, alpha (TNF-$\alpha$) activation was determined. Pretreatment of EC with SAC inhibited NF-$\kappa$B activation. OX-LDL resulted in intracellular GSH depletion and in of peroxide release. AGE or SAC inhibited these changes. Effects of SAC on hydroperoxide (H$_2$O$_2$) or tumor necrosis factor, alpha (TNF-$\alpha$)-induced nuclear factor kappa B (NF-$\kappa$B) activation was determined. Pretreatment of EC with SAC inhibited NF-$\kappa$B activation. AGE and SAC protected EC from OX-LDL-induced injury preventing intracellular GSH depletion in EC and by releasing peroxides from EC and NF-κB activation was determined. Pretreatment of EC with SAC inhibited NF-$\kappa$B activation. AGE and SAC inhibited H$_2$O$_2$ or TNF-$\alpha$-induced NF-$\kappa$B activation. These data suggest that AGE, and its main compound, SAC, may be useful for prevention of atherosclerosis.

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.

Dr. Lau (2001) at Loma Linda University, further investigated the antioxidant activity of S-allyl cysteine (SAC), a water-soluble sulfur-containing compound in Aged Garlic Extract. SAC prevented copper-induced oxidation of LDL cholesterol (ox-LDL). SAC also prevented further damage caused by ox-LDL. Specifically, SAC prevented oxidative damage caused by ox-LDL in J774 macrophages (immune cells) and another sensitive cell line. Nuclear factor kappa B (NF-kB) is activated by oxidants, such as hydrogen peroxide and also by tumor necrosis factor (TNF-α). NF-kB in immune T cells is involved in immune and inflammatory reactions. When incubated together, SAC was shown to inhibit the activation of NF-kB by both TNF-α and by hydrogen peroxide. Since both ox-LDL and NF-kB are involved in atherogenesis, SAC may act via antioxidant mechanisms to inhibit the atherogenic process.


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 indicates 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.

Prevented Oxidation of LDL Cholesterol in Clinical Studies
A double-blind, placebo-controlled, cross-over study conducted by Lau found that ingestion of age garlic extract (AGE) significantly increased the resistance of plasma LDL to oxidation. Independent work by Munday et al. in New Zealand showed that 2.4g of Aged Garlic Extract given for seven days made the LDL of subjects significantly more resistant to oxidation by Cu2+. Lag time for oxidation increased 27% (86.5 to 119 minutes, p<0.01). Six grams of raw garlic, on the other hand, was ineffective. This work was consistent with the results of Lau's team from Loma Linda University and Steiner's team from Brown University.

— Prevented Damage to Cells Caused by Oxidized LDL Cholesterol


Lau and Ide (1999) found that pretreatment of bovine pulmonary endothelial cells (cells which line the lungs) with Aged Garlic Extract, prevented the cell damage (as measured by a decrease in lactate dehydrogenase, LDH) and depletion of the antioxidant/detoxification enzyme glutathione (GSH) caused by oxidized LDL (Ox-LDL). AGE also reduced Ox-LDL peroxides or free radicals generated from Ox-LDL.


SAC, a constituent in Aged Garlic Extract, in addition to preventing LDH release and depletion of GSH in pulmonary endothelial cells (PAEC) exposed to oxidized LDL, dose-dependently inhibited oxidized LDL-induced peroxide release from PAEC. SAC was also found to scavenge hydrogen peroxide. Thus, SAC protected endothelial cells from oxidized LDL-induced injury by removing peroxides and preventing GSH depletion.


Lau and Ide (1997c) found that Aged Garlic Extract could prevent damage to cell membranes caused by oxidized LDL cholesterol. LDH (lactate dehydrogenase) is an enzyme found inside of cells that leak out into the culture medium when cell membranes are damaged. A higher level of LDH in the solution indicates a greater level of membrane damage. When oxidized LDL was added to endothelial cells, it caused about 30% of the LDH to be released. Aged Garlic Extract, on the other hand caused only 18-22% to be released, inhibiting 35-51% of the membrane damage caused by oxidized LDL.


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 elicited a trend toward reduced oxidation of these subjects' cholesterol. Oxidized cholesterol is more likely to adhere to the lining of the veins and cause scarring than is cholesterol that has not been oxidized.
Ameliorated damaging effects of nitric oxide


Lau and Ide (1999) found that Aged Garlic Extract inhibited nitric oxide and peroxide production in J774 macrophage immune cells and scavenged the free radical hydrogen peroxide in a dose dependent manner.


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 2 hr. 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.

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-induced 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.

Antioxidant Effects Attenuating Ischemic Brain Damage


Numagami et al. (1996 ) found that when S-allyl cysteine, a constituent in Aged Garlic Extract was administered 30 minutes prior to ischemic insult there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in this middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species generated due to ischemia.

The efficacy of S-allyl cysteine (SAC) as a free radical scavenger using brain ischemia models has been studied (Numagami et al. 1998). In a middle cerebral artery occlusion model, pre-ischemic administration of SAC improved (i) motor performance and (ii) memory impairment, and reduced (iii) water contents and (iv) the infarct size. In a transient global ischemia model, (i) the production of free radicals (alkoxyl radicals) as studied by electron paramagnetic resonance spectroscopy (EPR) was biphasic; the first peak occurring at 5 min and the second peak at 20 min after reperfusion. SAC did not attenuate the first peak, but did the second peak. (ii) The lipid peroxidation as estimated by TBA-RS increased significantly at 20 min after reperfusion. SAC decreased TBA-RS to the levels found without ischemia. These results suggest that SAC would have beneficial effects in brain ischemia and that the major protective mechanism may be the inhibition of free radical-mediated lipid peroxidation.

Reduced Depletion of Circulatory Antioxidants Caused by a Cancer-Causing Agent

7,12-Dimethylbenz(a)anthracene (DMBA) significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase and enhances lipid peroxidation in the circulation of tumor-bearing models. Administration of S-allyl cysteine, a constituent in Aged Garlic Extract, significantly decreased lipid peroxidation and enhanced the levels of antioxidants when exposed to DMBA-induced oxidative stress.

Antioxidative Effect: Review

Banerjee, et al., reported that numerous studies show AGE's potential as an antioxidant. Many scientific research studies support that AGE may help protect against aging, radiation, chemical exposure, and many oxidant-induced disease conditions.
Aged Garlic Extract preparations have demonstrated an array of anti-stress and anti-fatigue effects. Numerous clinical studies have shown significant improvement in subjective symptoms from a wide range of internal diseases including colds and fatigue as well as improved recovery from athletic performance. In preclinical and clinical studies, these preparations have improved physical strength following chemical and physical stresses, stress-induced immunosuppression and, stress-induced activation of the peripheral sympathetic system. Intake of Kyolic ® Liquid Aged Garlic Extract containing vitamin B 1, vitamin B 12 and liver extract, was given to more than one thousand subjects suffering from unexplained complaints which often accompany internal diseases. Recently, chemically synthesized antioxidants and natural antioxidants such as Vitamin C and Vitamin E have been studied for their role in the prevention and treatment of life-style related diseases. In this review, the antioxidant effects of Aged Garlic Extract (AGE) were discussed. Previously, AGE has been shown to inhibit the formation of oxidized LDL. AGE is produced through the natural aging process of garlic, forming unique biological compounds during this time. Since these unique compounds have been reported to show strong antioxidant effects, they may play an important role in the antioxidant effects of AGE.

Oxidative stress is associated with initiation and progression of life style related diseases and the aging process. Recently, it has been recognized that reactive oxygen species and lipid peroxidation play an important role in the cardiovascular disease and aging processes. The antioxidant effects of Aged Garlic Extract (AGE) were reviewed. AGE has been shown to directly scavenge the reactive oxygen species, superoxide radical and hydrogen peroxide, and inhibit lipid peroxidation. AGE also demonstrates the ability to inhibit LDL oxidation, and protect red blood cells through antioxidant effects in vitro. In clinical studies, AGE has also been reported in vivo to inhibit the formation of oxidized LDL. AGE is produced through the natural aging process of garlic, forming unique biological compounds during this time. Since these unique compounds have been reported to show strong antioxidant effects, they may play an important role in the antioxidant effects of AGE.

In a clinical study by Hasegawa et al. (1983), 130 subjects experienced improvements in various subjective symptoms including systemic, neuromuscular, respiratory, cardiovascular and digestive complaints following intake of Kyolic ® Liquid Aged Garlic Extract containing vitamin B 1, vitamin B 12 and liver extract. In only 7 cases. Some noteworthy findings included healthy weight gain, improved anemia, a beneficial effect on cholesterol metabolism, and a tendency towards normalization of GOT, a liver enzyme. In chill, abdominal pain, general fatigue, general myalgia and physical disorders were observed. Patients also noted improvement in headaches, lumbago, joint and chest pain. Improved patellar reflex values provided an objective measurement to confirm the anti-fatigue effects of the Aged Garlic Extract preparation in this study.

In a clinical study by Hasegawa et al. (1984), of 132 subjects suffering from indefinite medical complaints, 71% who exhibited facial flush, 79% who exhibited palpitations and sweating, 95% who exhibited fatigue, 83% who exhibited stiff shoulders, 82% who exhibited lethargy and 81% who exhibited poor appetite. Total effectiveness (rated slightly effective or better) was 95%, and the medicine was ineffective in only 7 cases. Some noteworthy findings included healthy weight gain, improved anemia, a beneficial effect on cholesterol metabolism, and a tendency towards normalization of GOT, a liver enzyme. In chill, abdominal pain, general fatigue, general myalgia and physical disorders were observed. Patients also noted improvement in headaches, lumbago, joint and chest pain. Improved patellar reflex values provided an objective measurement to confirm the anti-fatigue effects of the Aged Garlic Extract preparation in this study.

Miyoshi et al. (1984) found moderate and apparent improvement in fatigue, dizziness, debility, anorexia and headache in the majority of the subjects. Kawanishi et al. (1985) noted that when Kyolic ® Liquid Aged Garlic Extract containing vitamin B 1, vitamin B 12 and liver extract was administered to 39 patients with colds and general fatigue, significant improvements were noted. Improvements in fatigue, weakness, and constipation were also noted.

Aged Garlic Extract also showed a reduction in stress-induced ulcer formation. In a clinical study, Okada et al. (1983) found Kyolic ® Liquid Aged Garlic Extract containing vitamin B 1, vitamin B 12 and liver extract to be moderately to highly effective in improving subjective symptoms in their patients. Subjective symptoms included non-specific complaints such as fatigue, headache, heavy head, stiff shoulders, eye fatigue etc. A trend toward stabilized blood pressure was also noted (i.e. increases for low blood pressure, decreases for high blood pressure).
Reduced Physiological Stress in Pre-Clinical Studies

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.

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 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.

Improved Recovery from Athletic Performance in Pre-Clinical Studies

In a double-blind study by Kohno et al. (1976), a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 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 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.

Improved Recovery from Athletic Performance in Clinical Studies

In a double-blind study of 12 subjects, Ushijima et al. (1997) and Amagase et al. (1999) found that Aged Garlic Extract provided significant improvements in physical 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.

Kimoto et al. (2005) investigated AGF'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: AGF supplementation group and the control group. The AGF supplementation group was given AGF for 2 weeks. Urinary 8-OHdG content was found to be significantly lower with the AGF group than with the control. Also, AGF supplementation significantly increased the sum total of oxygen uptake during intense exercise.
Improved Recovery from Chemical and Physical Stress


Takasugi et al. (1984) showed that Aged Garlic Extract accelerated recovery from fatigue induced by exposure to 4 hr oscillation movement, chronic rope climbing and prolonged cold stimulus (4°C). Further, Aged Garlic Extract improved learning test results exposed to 40% alcohol administration and accelerated recovery from alcohol induced disruption of motor activity. Garlic was also shown to accelerate the removal of blood alcohol in alcohol treated models.

The anti-stress effect of Aged Garlic Extract (AGE) combined with ginseng, oriental benzoar, antler velet, cuscuta seed, and epimedium herb (Leopin Royal; LER) were evaluated using a model system. Physical and social stresses have been determined to lower momentum and the ability for antibody production. The AGE preparation was able to improve this state in models. AGE supplementation has also been shown to promote spermatogenesis. This data indicates that the AGE preparation has positive effects on recovering from physical fatigue, modulating the immune system, and promoting spermatogenesis, thus suggesting, that AGE may be useful for reducing symptoms induced by various stressors.


Improved Recovery from Oscillation Stress (Dizziness)

Takasugi et al. (1986) found that Aged Garlic Extract preparations given 10 minutes prior to and immediately after exercise prevented physical and mental disorders induced by acute oscillation stress.

Prevented Stress-Induced Hypertrophy of the Adrenal Gland and Hyperglycemia


Amagase et al. (1999) reported that Aged Garlic Extract (10mL/kg) prevented stress-induced hypertrophy of the adrenal gland in models undergoing immobilization stress. Raw garlic juice, heated garlic juice and powdered garlic showed no protective effects.


Pretreatment with Aged Garlic Extract (5 and 10ml/kg, p.o.) significantly prevented adrenal hypertrophy, hyperglycemia and elevation of corticosterone, without altering insulin level, exposed to 2 days (16hr/day) of immobilization stress.

Improved Stress-Induced Immunosuppression


Yokoyama et al. (1986) found that Aged Garlic Extract preparations restored the stress-induced suppression of antibody production and atrophy of the spleen and provided anti-stress and anti-fatigue benefits.


Kyo et al. (1999) found that Aged Garlic Extract (AGE) prevented the suppression of the immune system caused by psychological stress. After four days exposure to psychological stress (communication box), a significant decrease in spleen weight and spleen cells was observed compared nonstressed condition. AGE significantly prevented these decreases, as well as a reduction of hemolytic plaque-forming-cells and anti-SRBC antibody titer in serum caused by this psychological stress. Further, AGE maintained Natural Killer (NK) immune cell activities almost to the level of non-stressed condition, whereas stressed models without AGE showed suppressed NK activity. Kyo et al. concluded that psychological stress qualitatively and quantitatively impairs immune function, and that AGE prevents such damage.

Reduced Stress-Induced Activation of the Peripheral Sympathetic System


Kvetnansky et al. (1990) found that Aged Garlic Extract administered acutely or repeatedly for 14 days (30, 100, 300 mg/kg p.o.) reduced the stress-induced activation of the peripheral sympathetic system without affecting the adrenal medulla or pituitary-adrenocortical system. Administration of a 30 mg dose of Aged Garlic Extract in unstressed models reduced norepinephrine (NE) and ACTH levels whereas higher dosages only increased epinephrine levels. Repeated administration reduced NE even more. Aged Garlic Extract in conjunction with immobilization stress reduced NE levels. Administration of a single 300 mg dose of Aged Garlic Extract slightly reduced ACTH, corticosterone, and plasma prolactin levels.
Inhibits Stress-Induced Peptic Ulcer Formation

Nagai (1972) found that a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B₁, vitamin B₁₂ 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


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:

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.

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.

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 et al. (1986) found Aged Garlic Extract to be more effective than bacillus Calmette-Guerin (BCG) 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. 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 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.
Aged Garlic Extract significantly inhibited (>50%) the growth of melanoma cell lines. Further, equivalent concentrations of Aged Garlic Extract did not inhibit the growth of beneficial lymphocytes (white blood cells). Immune effects of garlic reported in the literature include activation of the immune system, increased infiltration of macrophages and lymphocytes into tumor cells. Di-allyl disulfide inhibits leukocyte activity is likely the result of multiple mechanisms.

In vitro, Aged Garlic Extract inhibited tumor promoter TPA responsible for the earliest stages of cancer development (enhancement of phospholipid metabolism). Further, Aged Garlic Extract significantly decreased tumor (MBT2) incidence by 28.6, 44.4 and 28.6%, respectively, according to the work of Lamm et al. (1990). Aged Garlic Extract was as effective as BCG. Tumor volume was also significantly decreased by Aged Garlic Extract. The authors concluded that “the highly beneficial reduction in tumor growth with Aged Garlic Extract immunotherapy was comparable to that of BCG immunotherapy and was significantly better than the control group.”

In vivo, Aged Garlic Extract suppressed the growth of sarcoma cells. Referring to the work of Welch et al. (1997) reducing the movement of sarcoma cells would inhibit metastasis, thereby making surgical removal of tumors more effective.

Inhibition of the Growth of Melanoma Cells

According to Hoon et al. (1990) melanoma, a skin cancer, has one of the fastest increasing incidence rates of all cancers in humans. It divides and spreads rapidly and is difficult to treat when malignant. In the study, Aged Garlic Extract (AGE) significantly inhibited (>50%) the growth of melanoma cell lines.

Garlic and Garlic Constituents.


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 regimen.

**Inhibited the Growth of Carcinogen-Induced Tumors of the Breast**


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.

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 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 and peroxidase levels in the non-transformed cells. Glutathione-S-transferase is critical for detoxification and gene expression.


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


Schaffer et al. found that SAC and DADS are effective inhibitors of mammary carcinogenesis. Garlic powder, SAC and DADS supplementation significantly delayed the onset of mammary tumors compared to the control group. Tumor incidence 23 weeks after MNU treatment was reduced by 76, 41, and 53% in models fed garlic, SAC and DADS. Also the quantity of mammary DNA alkylation occurring 3 hours after MNU treatment was reduced; specifically, O 6-methylguanine adducts were reduced by ≥ 0.1 mM, SAMC ≥ 0.01 mM, N7-Methylguanine adducts decreased by 48,22, and 21%.

Garlic powder, SAC and DADS supplementation significantly delayed the onset of tumor incidence and reduced DNA adduct formation induced by the carcinogen DMBA. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism. Schaffer et al. (1997) at East Carolina University found that garlic powder, SAC and DADS supplementation significantly delayed the onset of tumor incidence and reduced DNA adduct formation induced by the carcinogen DMBA. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism.

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In vivo, it was shown that DMBA-DNA adducts were reduced by 44, 64, and 70% at 0.001, 0.01 and 0.1 mM Aged Garlic Extract, respectively. Additionally, DNA-DMBA adduct levels were reduced by 50-96% and N7-Methylguanine adducts decreased by 24-63% in the liver and 48, 22, and 21% in the mammary gland. Amagase et al. (1996) found that Aged Garlic Extract powder could reduce the development of DNA adducts 14 formation by reducing DMBA-DNA binding. glutathione transferase activity in liver and mammary tissues. This increase may be responsible for the reduced tumor incidence by 40, 80 and 75%, respectively. Further, Amagase et al. observed that glutathione transferase activity increased in both the liver and mammary tissues of models taking Aged Garlic Extract.

Chronic oral administration of Aged Garlic Extract inhibited the binding of various N-nitroso compounds to both liver and mammary DNA. Liu et al. (1995) showed that Aged Garlic Extract and two of its constituents, S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC) in Aged Garlic Extract could inhibit the growth of a hormone responsive breast cancer cell line (MCF-7). At 0.05 mM it still produced a 60% inhibition in growth. A substantial decrease in viability was shown at > 0.01 mM. Aged Garlic Extract inhibited the binding of various N-nitroso compounds to both liver and mammary DNA. Liu et al. (1995) showed that Aged Garlic Extract and two of its constituents, SAC and DADS, are effective inhibitors of N-methylnitrosourea-induced mammary tumors. Further, Aged Garlic Extract inhibited the binding of various N-nitroso compounds to both liver and mammary DNA. Liu et al. (1995) showed that Aged Garlic Extract and two of its constituents, SAC and DADS, are effective inhibitors of N-methylnitrosourea-induced mammary tumors. Final tumor incidence was 81% for the control, 19% for the Aged Garlic Extract group, 38% for the SAC group and 38% for the DADS group.

Diallyl sulfide and diallyl disulfide, are effective inhibitors of N-methylnitrosourea-induced mammary tumors. Final tumor incidence was 81% for the control, 19% for the Aged Garlic Extract group, 38% for the SAC group and 38% for the DADS group. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism. Schaffer et al. (1997) at East Carolina University found that garlic powder, SAC and DADS supplementation significantly delayed the onset of tumor incidence and reduced DNA adduct formation induced by the carcinogen DMBA. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism.

Amagase et al. (1996) found that Aged Garlic Extract powder could reduce the development of DNA adducts 14 formation by reducing DMBA-DNA binding. glutathione transferase activity in liver and mammary tissues. This increase may be responsible for the reduced tumor incidence by 40, 80 and 75%, respectively. Further, Amagase et al. observed that glutathione transferase activity increased in both the liver and mammary tissues of models taking Aged Garlic Extract.

In vivo, it was shown that DMBA-DNA adducts were reduced by 44, 64, and 70% at 0.001, 0.01 and 0.1 mM Aged Garlic Extract, respectively. Additionally, DNA-DMBA adduct levels were reduced by 50-96% and N7-Methylguanine adducts decreased by 24-63% in the liver and 48, 22, and 21% in the mammary gland. Amagase et al. (1996) found that Aged Garlic Extract powder could reduce the development of DNA adducts 14 formation by reducing DMBA-DNA binding. glutathione transferase activity in liver and mammary tissues. This increase may be responsible for the reduced tumor incidence by 40, 80 and 75%, respectively. Further, Amagase et al. observed that glutathione transferase activity increased in both the liver and mammary tissues of models taking Aged Garlic Extract.

Chronic oral administration of Aged Garlic Extract inhibited the binding of various N-nitroso compounds to both liver and mammary DNA. Liu et al. (1995) showed that Aged Garlic Extract and two of its constituents, SAC and DADS, are effective inhibitors of N-methylnitrosourea-induced mammary tumors. Final tumor incidence was 81% for the control, 19% for the Aged Garlic Extract group, 38% for the SAC group and 38% for the DADS group. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism. Schaffer et al. (1997) at East Carolina University found that garlic powder, SAC and DADS supplementation significantly delayed the onset of tumor incidence and reduced DNA adduct formation induced by the carcinogen DMBA. Further, selenium appeared to enhance the activity of each of these compounds suggesting synergism.

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Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

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. SAC also induced apoptosis (cell death). These effects of SAC were accompanied a marked increase in endogenous levels of reduced glutathione (a potent antioxidant). SAC inhibited growth progression of both cell lines. SAC, co-administered with SS enhanced the growth inhibitory and apoptotic effects of SS. These findings suggest that SAC may be useful in colon cancer prevention when used alone or in combination with SS or other chemopreventive agents.

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 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.

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).

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).

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).

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-propylmercaptocysteine and S-propaglylcysteine, exhibited increased foci, determined to be due to decreased food intake caused by these compounds.

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 and Milner (1997) found that DADS and SACMC, 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 SACMC 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).
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 caspase-3 signaling pathways that lead to apoptosis.

Using MIB-5 immunohistochemistry to assess cell proliferation of normal-appearing colonic mucosa, it was found that treatment with Aged Garlic Extract significantly decreased the mean MIB-5-labeling index suggesting that AGE has antiproliferative action on colorectal carcinoma cells and an inhibitory action on angiogenesis.

Models were provided a semi-purified, casein based diet with or without 57 or 570 µmole/kg of SAC, DADS or SAMC for 13 weeks prior to determination of aberrant crypt foci (ACF) and aberrant crypt number. All treatments, except 57 µmole/kg SAC, significantly lowered ACF compared to controls. ACF was significantly reduced by DADS and SAMC at both concentrations tested. This study revealed that all allyl sulfur compounds are not equivalent in retarding early preneoplastic markers for colon cancer.

Subjects were given weekly subcutaneous injections of dimethylhydrazine (DMH) for 20 weeks and were fed with either a normal or AGE-containing diet. The AGE significantly reduced in number the amount of colon tumors and aberrant crypt foci compared to the normal diet. Also, AGE treatment caused a significant decrease in the mean MIB-5 label index of normal appearing colonic mucosa.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon in Clinical Studies

In a double-blinded randomized study, 51 subjects diagnosed with colorectal adenomas by colonoscopy, were divided into two groups. One group received high-AGE doses (2.4 ml/day) and the other group received low-AGE doses (0.16 ml/day). By using colonoscopy, the number and size of the adenomas were measured before AGE intake (0 month) and after AGE intake (6 to 12 months). Out of the 37 subjects chosen for evaluation, there was a 50.0% decrease rate of at least one adenoma in the high-AGE group (6 to 12 months after intake of AGE). There was no decrease in adenomas found in the low-AGE group (p=0.02). This study also found that the total size of adenomas increased in the low-AGE group, while the increase of adenomas was suppressed in the high-AGE group after 6 to 12 months of intake (p=0.04).

Inhibited the Growth of Carcinogen-Induced Tumors of Esophagus


Wargovich et al. (1989) found that diallyl sulfide, a constituent in Aged Garlic Extract, totally suppressed the development of carcinogen (NMBA)-induced esophageal tumors.

Inhibited the Growth of Carcinogen-Induced Tumors of the Stomach and Lung


Sparnins et al. (1986), found that allyl methyl trisulfide (AMT) an organosulfur compound in Aged Garlic Extract, could increase glutathione S-transferase activity in the forestomach, small bowel mucosa, liver and lung of models when given orally. AMT also inhibited benzo-(a)-pyrene (BP) 15-induced neoplasia (cancer) of the forestomach as shown by a greater than 70% reduction in the number of tumors.


Sparnins et al. (1988) found that several organosulfur compounds in Aged Garlic Extract, allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), diallyl trisulfide (DAT), and diallyl sulfide (DAS), inhibited BP-induced neoplasia of the forestomach. DAS was more effective than AMT. DAS and AMD also inhibited pulmonary adenoma formation (lung cancer). From the study it appeared that the number of sulfur atoms in the organosulfur compound could determine the organosulfur compounds sites at which protection against carcinogenesis would occur. All four compounds induced glutathione S-transferase (GST) activity in the forestomach but varied in their capacity to induce GST activity in the lung, liver and small bowel.
In vitro studies suggested that diallyl disulfide (DADS) derived from garlic inhibits growth of prostate cancer cells 
by reducing testosterone. The garlic-derived cancer preventive compound diallyl disulfide (DADS) was explored.

Wattenberg et al. (1987) found that diallyl disulfide (DADS) and allyl methyl disulf ide, constituents in Aged 
Garlic Extract, produced a marked inhibition of N-Nitrosodiethylamine (NDEA)-induced tumors of the 
oral cavity. Treatment with DADS and allyl mercaptan (a metabolic product of DADS) inhibited forestomach tumor 
formation. When given 96 and 48 hours prior to NDEA, DADS reduced tumor formation by more than 90%.

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Sigounas et al. (1997) further confirmed that S-allylmercaptocysteine (SAMC) in Aged Garlic Extract could inhibit the growth of two erythroleukemia cell lines (HEL and OCIM-1). It induced a dose-dependent inhibition with a 50% lethal dose of 0.046 mM for CIM-1 cells and 0.093 mM for HEL cells. The authors concluded from analyses of [3H] thymidine incorporation and high molecular weight DNA fragmentation that SAMC is an effective antiproliferative agent against erythroleukemia cells that induces death by apoptosis.

Inhibited Aflatoxin B1- and Benzo[a]pyrene-induced Mutagenesis


Tadi et al. (1990) found that Aged Garlic Extract and its constituents inhibited aflatoxin B1 (AFB1) from binding to DNA, preventing the formation of AFB1-DNA adducts. Aged Garlic Extract prevented AFB1 from being converted into active cancer-causing compounds in the body by enhancing its conversion to non-toxic conjugates.

Aged Garlic Extract also prevented benzo[a]pyrene-induced mutagenesis. Benzo[a]pyrene is found in cigarette smoke, charcoal broiled meat and automobile exhaust and is a procarcinogen causing a number of model tumors.


Tadi et al. (1991) found that Aged Garlic Extract and one of its constituents, diallyl sulfide, inhibited mutagenesis induced by aflatoxin B1 (AFB1). These compounds inhibited AFB1 binding to DNA. Aged Garlic Extract also significantly decreased noxious metabolites of AFB1 and increased glucuronide and glutathione (detoxified metabolites of AFB1). Tadi et al. concluded that Aged Garlic Extract was antimutagenic and potentially anticarcinogenic.


Yamasaki et al. (1991) found that allixin, a constituent in Aged Garlic Extract, inhibited the binding of the carcinogenic aflatoxin AFB1 to calf thymus DNA and reduced the formation of AFB1-DNA adducts. Allixin inhibited the formation of carcinogenic metabolites of this aflatoxin and the authors suggested that this compound “may thus be useful in the chemoprevention of cancer.”

Suppressed Activity of Procarcinogen-Activating Enzymes


Milner et al. (1997) from Pennsylvania State University used a biochemical model to determine if Kyolic and/or one of its constituents, diallyl disulfide (DADS), could inhibit enzyme systems in the cell responsible for generating toxic metabolites from procarcinogens. Specifically, the cytochrome P450 system was tested, more specifically P450 2E1 (CYP 2E1) activity. CYP 2E1 is also capable of converting chlorzoxazone (CZX, a muscle relaxer) into its metabolite 6-OH hydroxychlorzoxazone (6-OHCZX). Measurements of this metabolite in urine following oral intake of CZX can be used to determine CYP 2E1 activity. Subjects were given Kyolic and DADS for two weeks and then given CZX. Over the following 24 hours, excretion of 6-OHCZX was reduced 15% in Kyolic treated models and 27% in DADS-treated models. Thus, Kyolic and its constituent DADS were found to suppress CYP 2E1 activity. The researchers concluded that since Kyolic contains only a small amount of DADS, other compounds present in the Kyolic, such as S-allyl cysteine, must have an ability to suppress CYP 2E1 activity.

Potential Mechanisms

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

Wargovich et al. (1998) indicated that organosulfur compounds act primarily during the initiation phase of carcinogenesis. Using two model systems, one that induces esophageal cancer, and the other, colon cancer, they found that diallyl sulfide, potently inhibits carcinogenesis. In the aberrant crypt model, this compound and other organosulfur compounds were only effective during initiation, not during the promotional phase of carcinogenesis, despite being relatively strong inducers of detoxification enzymes, such as glutathione-S-transferase. Many of the garlic organosulfur compounds are inhibitors of CYP2E1 the enzyme responsible for the metabolic activation of nitrosomethylbenzylamine and azoxymethane, two carcinogens used to induce esophageal cancer and colon cancer. Recent work suggests that CYP1E1 gene expression levels are sharply reduced in the presence of diallyl sulfide and work in progress is aimed at understanding whether this is a unique or common mechanism for cancer chemoprevention by garlic constituents.
Diallyl sulfide (DAS), a constituent found in small amounts in Aged Garlic Extract, is metabolically converted to diallyl sulfoxide and diallyl sulfone (DASO2). All these compounds are competitive inhibitors of cytochrome P450 (CYP)2E1. DASO2 is also a suicide inhibitor of CYP2E1. Therefore, these compounds are expected to prevent toxicity induced by many environmental chemicals, which are metabolically activated by CYP2E1. Indeed, this has been demonstrated with carbon tetrachloride- and N-nitrosodimethylamine-induced toxicity. DAS, DASO2, and fresh garlic homogenates also inhibited hepatotoxicity caused by a high dose of acetaminophen (APAP), a commonly used analgesic. The protective effect was observed when these events were given before, together with, or within an hour of APAP application. DAS and DASO2, also inhibited the bioactivation of a strong tobacco carcinogen 4-(methynitrosamino)-1-(3pyridyl)-1-butanone (NNK) and related lung tumorogenesis. DAS and DASO2, are likely to inhibit the activities of other CYP enzymes that catalyze the activation of NNK. DAS also has other biological effects such as the induction of certain CYP enzymes and phase II drug metabolism enzymes and the decrease of hepatic catalase activities. All these effects were observed at concentrations much higher than those resulted from dietary garlic consumption or supplementation. The biological activities of lower concentrations of DAS and garlic-derived compounds remain to be studied further.


The metabolism of acetaminophen is very similar to that of carcinogens. According to Gwilt et al. (1994), Aged Garlic Extract showed only slight increased sulfate conjugation and glucuronide formation suggesting that it inhibits carcinogenesis through a mechanism other than modification of drug metabolism.

Dion and Milner (1996) found that SAC could inhibit 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.


Further, Milner et al. (1996) found that SAC, diallyl sulfide, and diallyl disulfide, constituents in Aged Garlic Extract, were more effective than isomolar ascorbic acid in reducing NMOR mutagenicity (ability to damage the DNA of cells).


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, PGE2 and PGF2α, in most of the healthy women subjects.


7,12-Dimethylbenz(a)anthracene (DMBA) enhances lipid peroxidation in the circulation of tumor-bearing models. In addition, it significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase. Administration of S-allyl cysteine, a constituent in Aged Garlic Extract, significantly decreased lipid peroxidation and enhanced the levels of antioxidants in models exposed to DMBA-induced oxidative stress.

Borek (2001) of Tufts University reviewed an array of dietary antioxidants used for cancer prevention, including Aged Garlic Extract that contains antioxidants that protect against cancer and inflammatory prostaglandins that promote diseases, including cancer. Aged Garlic Extract does not contain the oxidizing allicin, which converts to antioxidants with limited bioavailability, like other forms of garlic. Aged Garlic Extract exerts various protective antioxidant actions from scavenging reactive oxygen species (free radicals) to enhancing internal antioxidants in the body, thus preventing free radical damage to DNA. It also shows various anticancer activities.
Dr. Pinto from Cornell University detailed numerous mechanisms whereby at least 20 constituents, including S-allyl cysteine and S-allylmercaptocysteine, in garlic, especially Aged Garlic Extract, reduce cancer risk. They are effective at each stage of cancer and show such effects as inhibiting uptake and formation of carcinogens (cancer-causing chemical agents), inactivating carcinogens, inhibiting genetic damage, increasing metabolic detoxification, protecting against oxidative damage, restoring normal cell growth/differentiation, stimulating immune surveillance and inhibiting angiogenesis (blood supply to the cancer). They function generally by the following three ways:

1. Impeding generation of carcinogens from their precursors
2. Preventing carcinogens from reacting with vulnerable cellular targets
3. Delaying or reversing expression of malignancy or preventing proliferation (growth) of tumor cells.

Reduced Incidence of Precancerous Gastric Lesions in Clinical Studies

A long-term, randomized, double-blind, placebo-controlled intervention study in Shandong Province, China, evaluated whether any of the 3 interventions, alone or in combination could reduce the prevalence of precancerous gastric lesions over 3,400 human subjects. The interventions are: treatment in late 1995 with amoxicillin and omeprazole to eradicate Helicobacter pylori; supplementation with Kyolic™ aged garlic extract (400mg)-based preparation twice daily from 1996-2003; and supplementation with a mixture of alpha-tocopherol (100 IU), vitamin C (250mg) and selenium (37.5 µg) twice daily from 1996-2003. A previous case-control study of gastric cancer in this region showed that persons in the highest quartile of intake of allium-containing vegetables (including garlic, scallions and onions) had only 40% of the risk of those in the lowest quartile. Similar results were found in a case-control study in Italy. This seven year intervention study will inform us benefits of garlic intake for cancer prevention in the humans based upon a good compliance to the interventions.

A randomized, placebo-controlled trial was conducted on 3,365 subjects aged 35-64 years in 13 randomly selected villages in Linqu County; who have a high prevalence of H. pylori infections and precancerous gastric lesions and gastric cancer mortality rates. The purpose of this study was to examine whether one-time treatment with amoxicillin/omeprazole or long-term supplementation with vitamin or garlic preparations could decrease the development of advanced precancerous gastric lesions in this county. Results indicated that one-time H. pylori treatment with amoxicillin and omeprazole had statistically significant reductions, while supplementation for 7.3 years with vitamin or garlic supplements had no such effect.

Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis

In a study by Matsuura et al., aged garlic extract (AGE) has been shown to have antiproliferative effects on colorectal carcinoma cells and inhibitory activity on angiogenesis through the suppression endothelial cell motility, proliferation, and tube formation.


Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease.


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

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

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

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.

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.

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.

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 foci. These findings indicate that S-methylcysteine and cysteine inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis.
The effect of Aged Garlic Extract (AGE) on the development of glutathione S-transferase placental 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 hepatocarcinogenesis, along with reducing the rate of proliferation of liver cells after partial hepatectomy.

Takada N. et al. (1997) found that S-methylcysteine (SMC) and cysteine can inhibit both the initiation and promotion stages of hepatocarcinogenesis. Suppression of polyamine metabolism and the transitory down-regulation of induction of c-jun expression may play important roles in this chemopreventive action. Uda N et al. (2005) found that AGE inhibited the development of putative preneoplastic lesions by hepatocarcinogenesis through treating the models with AGE 2, 5, 10 ml/kg, intragastrically 5 times per week, where GST-P positive foci were significantly decreased. Also, AGE slowed down the proliferation rate of liver cells after partial hepatectomy.

Reduced the Side Effects of Drugs Methotrexate (MTX), although highly effective as an anticancer drug, is non-specific making rapidly dividing cells, like those in the intestinal tract, susceptible to apoptosis, thus increasing the incidence of malabsorption syndrome. Yuncu et al. (2006) concluded when taken along with MTX aged garlic extract (AGE) was able to protect the intestine not only on a physiological and pharmacological level, but also at the cell level. The mechanism for this protection, however, remains to be discovered.

Aged Garlic Extract (AGE) (0.5%) inhibits methotrexate (MTX)-induced apoptosis of IEC-6 cells. These results indicate that AGE may be useful for cancer chemotherapy by reducing the intestinal damage induced by anti-tumor drugs.

Anticancer and Cancer-Preventive Review Many evidence points to the anticancer properties of garlic, especially Aged Garlic Extract, and a number of specific sulfur compounds from garlic. These prevention characteristics arise through both a dose and temporal related change in several cellular events including those involving cancer-causing chemicals' metabolism, immune system, cell control, and blood supply to the cancer cells. Garlic and its chemical compounds have many mechanisms to inhibit the growth of cancer cells. But there are differences in the efficacy among these various compounds and across tumor types. Our genetic background may influence such differences. Additional studies are needed with more modest exposures and over prolonged periods for these clarifications. Finally, additional research is needed to identify sensitive "effect" and "susceptibility" biomarkers that can ultimately be used to identify responders from non-responders.

Investigation of garlic studies reveals the many beneficial properties attributed to garlic and its constituents. The sulfur and thiol components have long been examined for their protective effects, such as inhibiting nucleotoxicity in the colon, enhancing the body's mechanism for eliminating exogenous substances (carcinogens) and altering Phase 1 and Phase 2 enzymes. Not all garlic processing allows for these beneficial compounds to surface, however, since heat ~60° C can destroy them. In contrast, the aged garlic preparation can help to keep these compounds intact to be fully absorbed.

Since 1981, cancer has been the No. 1 cause of death in Japan, and it is speculated these numbers will continue to rise in the future. Cancer prevention studies using food from all over the world, is one of the most important topics researchers focus on. Recently, the National Cancer Institute (NCI) has presented a designer food program for cancer prevention, and garlic is ranked at the top of the important foods. In this review, recent studies on anti-carcinogenesis and anti-tumor properties of garlic and its constituents using various model systems were introduced.
### Overview of Inhibition of Cancer Growth by Aged Garlic Extract and Its Constituents

<table>
<thead>
<tr>
<th>Cancer</th>
<th>In vivo (Model)</th>
<th>In vitro (Cancer Cell Culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Model 1-5</td>
<td>Human 6-8</td>
</tr>
<tr>
<td>Breast</td>
<td>Model 5</td>
<td>Human 21, 22, 23</td>
</tr>
<tr>
<td>Colon</td>
<td>Model 24-27, 28-30</td>
<td>Human 31</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>Model 4</td>
<td>Human 32-33</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Model 34</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Model 18-40</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>Model 15-37</td>
<td>Human 51</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Model 42</td>
<td>Human 41, 42</td>
</tr>
<tr>
<td>Prostate</td>
<td>Model 43</td>
<td>Human 43</td>
</tr>
<tr>
<td>Stomach</td>
<td>Model 44-47</td>
<td>Human 44-47</td>
</tr>
<tr>
<td>Skin</td>
<td>Model 46-50</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>Model 18-40</td>
<td></td>
</tr>
</tbody>
</table>

Brain, Neurotrophic, Anti-aging, and Anti-depression Effects of Aged Garlic Extract:

In the following studies, Aged Garlic Extract significantly improved survival, learning behaviors and memory ability in SAMP8 models, which are genetically prone to undergo accelerated aging and are often used as senile dementia models because of their genetic age-related learning deficit. Declines in both cognitive and immune function are predominant features of aging. Improvement of immune function and antioxidative effects were suggested as possible mechanisms for the ameliorating effects of Aged Garlic Extract. Aged Garlic Extract also demonstrated neurotrophic effects, an ability to enhance nerve growth, as well as an ability to enhance the release of serotonin, an anti-depression effect.

Improved Survival, Memory Retention, Learning Deficits and Immune Response


The thymus plays a key role in balancing the nervous and immune systems (neuroendocrine immunomodulation network or NIM). Thymectomy, or removal of the thymus gland, reduces immune function and causes an imbalance in NIM, further accelerating the aging process. Zhang et al. (1994) found that Aged Garlic Extract significantly improved the impairment in memory induced by thymectomy in SAMP8. A trend toward improved learning ability and immune function (improving number of plaque-forming splenocytes) was also noted suggesting the anti-aging effects of Aged Garlic Extract may be due to improvement of immune function.


Zhang et al. (1997) found that chronic oral administration of Aged Garlic Extract significantly ameliorated both thymectomy-reduced antibody production response and thymectomy-induced impairment of learning behaviors in two strains of models. Aged Garlic Extract also restored the significantly increased levels of hypothalamic norepinephrine, 3,4-dihydroxyphenylacetic acid and homovanilic acid, and the hypothalamic choline acetyltransferase activity to control levels. Enhancement of immune function was seen in that chronic ingestion of Aged Garlic Extract significantly potentiated lymphocyte proliferation (enhanced white blood cell production) induced by concanavalin A or lipopolysaccharides in both SAMP8 and SAMR1 (normal senescence-resistant substrain of SAM).


Moriguchi et al. (1994a) found that Aged Garlic Extract delayed the manifestation of learning and memory impairments in SAMP8. Further, regular ingestion of Aged Garlic Extract significantly improved the learning performance of normal (SAMR1) models. Aged Garlic Extract improved the survival of SAMP8 models by restraining their aging speed via multiple biological mechanisms such as anti-oxidation and immunomodulation.


In another study, Moriguchi et al. (1994b) found that Aged Garlic Extract increased the survival ratio of senescence accelerated models and enhanced their memory retention as indicated by various memory tests. The authors suggested that Aged Garlic Extract might be useful for treating physiological aging and age-related memory deficits in humans.


Moriguchi et al. (1996a) found that a diet containing 2% Aged Garlic Extract extended the survival ratio of a strain of senescence-accelerated-prone (SAMP8) models to that of a senescence-accelerated-resistant strain (SAMR1). Moreover, Aged Garlic Extract markedly improved learning deficits in the prone models. 


Nishiyama et al. (1996) found that Aged Garlic Extract supplementation improved the survival of SAMP8 to that of normal models (SAMR1) that are not prone to accelerated aging. Aged Garlic Extract decreased the total number of errors in two different passive avoidance tests showing an improvement in learning/memory.


In this study, Dr. Rahman proposes that the elderly can benefit from taking garlic, either for preventing or reducing chronic diseases associated with old age. The premise associated with this effect is due to the potent antioxidant properties of Aged Garlic Extract.
Enhanced Nerve Growth

**Neurotrophic Activity**

Aged Garlic Extract (AGE) possesses strong neurotrophic activity and for this reason may be useful for preventing age-related morphological changes in the central nervous system.

Moriguchi et al. (1996b) confirmed that Aged Garlic Extract could significantly prolong the survival of cultured hippocampal neurons. Aged Garlic Extract also demonstrated a neurotrophic effect in that it enhanced the axonal branching of nerve endings.

**Neural Survival and Branching**

The protein fraction from Aged Garlic Extract also showed a potent survival-promoting effect on hippocampal neurons and increase the number of branching points per axon when added to the culture medium. The protein fraction from Aged Garlic Extract also showed a potent survival-promoting effect on hippocampal neurons and increase the number of branching points per axon when added to the culture medium.

**Immunological Enhancement**

In addition to its neurotrophic activity, Aged Garlic Extract also improved the immune response, noradrenalin content, and learning impairment. Therefore, long-term supplementation with Aged Garlic Extract may be associated with memory and learning improvement through its beneficial effects on aging.

**Neurosensory Function**

Although age-related memory loss and learning impairments are common, significant improvements were seen with AGE supplementation. Moriguchi et al. (1996) found that Aged Garlic Extract and its F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, clear evidence that Aged Garlic Extract interacts with brain function.

**Gene Expression**

Zhang et al. (1994) found that Aged Garlic Extract prolonged the survival of cultured neurons from the hippocampus by at least 3-fold. The mRNA of neurotrophic gene SAC also promoted neuronal survival. Gamma-glutamyl-allyl-L-cysteine also exerted neurotrophic activity similar to SAC.

**Model of Senescence**

Moriguchi et al. (1997) found that Aged Garlic Extract prevented the decrease in brain weight and the atrophic changes in the frontal brain at 12 months of age in the SAMP 10 age-accelerated models. The authors suggested that these compounds in AGE may be helpful in the development of therapeutic and/or prophylactic drugs for neurodegenerative disorders.

**Embryonic Hippocampus**

Saito et al. (1996) found that Aged Garlic Extract improved memory acquisition and memory retention processes in the embryonic hippocampus, suggesting a direct neurotrophic effect.

**Antibody Production**

Reduced brain weight and atrophy of the forebrain caused models to become learning impaired and experience memory loss. However, significant improvements were seen with AGE supplementation. Saito et al. (1996) found that Aged Garlic Extract improved memory acquisition and memory retention processes in the embryonic hippocampus, suggesting a direct neurotrophic effect.

**Immune Function Disorders**

Immune function disorders may cause a change in the central nervous system through the immune-endocrine-nerve network. Senescent accelerated models caused a reduction in spleen proliferation and microglobulin-related protein gene. However, Aged Garlic Extract (AGE) supplementation inhibited these reductions. Also, AGE increased survival of cultured hippocampal neurons and the number of branching points per axon, showing that AGE has beneficial effects on aging, and that it contains multiple components with neurotrophic activity.
Apoptosis is induced by the activation of Caspase-3. Studies show that dietary AGE inhibits human Alzheimer's disease, which is characterized by the loss of neuronal cells, most likely due to apoptotic death. Alzheimer's disease is the most common cause of progressive intellectual failure and memory impairment among the elderly, the authors suggested that Aged Garlic Extract can be useful for the amelioration of this disease.

One of the pathological features of Alzheimer's disease (AD) is neuronal apoptosis. This condition is associated with senile plaques containing amyloid-B peptide, which are assumed to be involved with neurodegenerative disorders, migraines, Multiple Sclerosis and alcoholism. In cases of impulsivity, aggressivity, panic obsessive compulsive disorder, schizophrenia, suicidal behavior, autism, and possibly homeostasis or balance in the brain. When the serotonin system is faulty it results in anxiety, depression, including major depression and such pathologies, the serotonergic (5-HT) system is in deficit. Antidepressants are often given in an attempt to boost the serotonin system. MAO (monoamine oxidase) inhibitors, for example, block the enzyme that degrades serotonin. Other antidepressants attempt to make more serotonin available. [Specifically Aged Garlic Extract enhanced the binding of [3H] 5-HT to 5HT1 receptors, likely 5-HTIB/ID receptors in a non-competitive manner]. By making more serotonin available, researchers treated PC12 cells with Aged Garlic Extract and SAC for 24 hr prior to exposure to Aß. Cell viability using either the MTT (methylthiazol tetrazolium) assay or the MTS-based assay (Promega). They then added beta-amyloid (Aß), a neurotoxin that is a progenitor of oxidative stress. The addition of Aged Garlic Extract as Kyolic Liquid Aged Garlic Extract to the medium, protected neuronal PC12 cells against Aß (70 µg/ml) toxicity in a dose dependent manner. Since the accumulation of Aß is a hallmark for Alzheimer's disease, the most common cause of progressive intellectual failure and memory impairment among the elderly, the authors suggested that Aged Garlic Extract can be useful for the amelioration of this disease.
Further, researchers report that AGE inhibits caspase-3, and as such an inhibitor may be effective in reducing apoptotic death of neurons, since caspase inhibitors have been shown to inhibit neuronal cell death.

Another of AGE's constituents, S-Adenosyl-Methionine (SAM) induces apoptosis in PC12 cells. AGE provides protection from apoptosis, thereby protecting from Alzheimer's, due to activity by its SAM compound.

Nitric Oxide (NO) is an essential metabolite for normal physiological function; however, at higher concentrations it can be neurotoxic, perhaps by inducing apoptosis. The purpose of this study was to determine whether AGE can protect cells from the cytotoxicity of NO donors. Results show that AGE protects PC12 cells from the cytotoxicity of NO donors and may be helpful in the fight against Alzheimer's disease.

Review article of the neurotrophic effect of AGE based upon preclinical studies. AGE has been shown to possess neurotrophic effects and protect brain or reduce dementia in the Alzheimer model. SAC and other constituents may be active compounds in AGE.

Evidence suggests that risk factors for cardiovascular disease, such as high cholesterol, hypertension, high homocysteine, and inflammation, increases the risk of dementia, including its most common form, Alzheimer's disease (AD). Clinical studies have shown that Aged Garlic Extract (AGE) may help to prevent cardiovascular and cerebrovascular diseases and lower the risk of dementia and AD by inhibiting cholesterol, LDL oxidation, platelet aggregation, arterial plaque formation, decreasing homocysteine, lowering blood pressure, increasing microcirculation, protecting neurons from Abeta neurotoxicity and apoptosis.

AGE is helpful in reducing risk factors of cardiovascular disease, including high cholesterol; which is associated with dementia and Alzheimer's disease. AGE is able to scavenge oxidants, inhibit cholesterol synthesis, and help prevent cognitive decline by protecting neurons from [beta]-amyloid (Abeta) neurotoxicity and apoptosis.

Chauhan (2006) concludes that therapy for Alzheimer's patients may be improved by the many beneficial activities of AGE, e.g. anti-oxidant, anti-inflammatory, and anti-apoptotic. In this study, a reduction in cerebral plaques and inflammation, SDS-extractable detergent soluble and formic acid extractable detergent resistant [beta]-amyloid (Aβ) species, and tau phosphorylation was seen in models with Alzheimer's disease given aged garlic extract. All of which are characteristics found in the pathophysiology of Alzheimer's disease.

Enhancement of Human Growth Hormone

The amount of human growth hormone decreases significantly after the age of 30. This decrease has been implicated as one of the major causes in the signs of aging, such as thinning of the skin and bones, a decrease in lean muscle mass and an increase in adipose tissue. Supplementing the body's dwindling supply with recombinant human growth hormone has been shown to reverse the signs and symptoms of aging. However, it is quite costly, requires repeated injections and has side effects such as carpel tunnel syndrome, gynecomastia and insulin resistance. Lau and his colleagues (2002) report that a combination of equal amounts of L-arginine and L-lysine, aged garlic extract (Kyolic ®) S-allyl cysteine and Pycnogenol ® significantly increased secretion of HON in an in vitro model of genetically-engineered keratinocytes.

Buz'Zard et al. (2002) reported that the combination of equal amounts of L-arginine and L-lysine, Aged Garlic Extract (Kyolic), S-allyl cysteine (SAC) and pycnogenol has been shown to significantly increase the secretion of human growth hormone (HGH) in an in vitro model.
Brain, Neurotrophic, Anti-aging, and Anti-depression Effects: Review

Nishiyama et al. (1997) has studied the effects of Aged Garlic Extract (AGE) and its compounds on neuron cell survival, memory loss, and learning impairment using several aging model systems. In this review article, recent studies on the prevention of aging processes by AGE and its compounds were discussed.

The effects of Aged Garlic Extract (AGE) on brain function (i.e. neuronal cell survival, memory loss and learning, and mental stress) were reviewed. AGE and one of its main compounds, S-allylcysteine (SAC), have increased the survival rate of neuron cells, and have had a positive effect on establishing a new network between neuronal cells through improved branching. Structure-activity relationship studies determined the S-allyl group to be the most important moiety of the chemical structure. Physical and/or mental stress may cause a reduction in immunity. However, AGE is able to reduce stress and enhance natural killer (NK) activity. Also, inhibition of serotonin receptor (5-HT1B/1D) binding by AGE may enhance brain function via improved immune system response. Memory and learning improvement is also observed with AGE.

The effect of Aged Garlic Extract (AGE) and the prevention of aging were reviewed using recent brain and cardiovascular research. AGE and SAC, a main component, have increased neuronal cell survival and have had positive effects on establishing new networks between neuron cells through improved branching. Memory loss and learning was also improved by AGE using a model system. Several clinical studies demonstrated that AGE shows cholesterol lowering effects, inhibits LDL oxidation, and protects endothelial cells from oxidized LDL-induced cell injury. All indicate that AGE may be useful for the prevention of aging processes.

Other Pharmacological Effects

Anti-fungal Properties


Matsuura et al. (1988) found that a constituent in the Aged Garlic Extract, a furostanol glycoside, demonstrated anti-fungal effects by inhibiting the growth of Candida albicans in test tubes.


In a study by Tadi et al. (1990), Kyolic® Aged Garlic Extract hastened the clearance of Candida albicans from the circulation of subjects systemically infected with this organism. Further, Aged Garlic Extract reduced the number of these organisms growing in the kidneys where Candida albicans typically colonizes.


In this study by Abdullah et al. (1989), Aged Garlic Extract was found to improve candidiasis in AIDS patients.

Anti-bacterial Properties

The effect of Aged Garlic Extract (AGE) on H. pylori-induced gastritis was determined using a model system. Oral administration of H. pylori caused infection, observed dropsical swelling, and reddening of the mucosa. AGE intake of 4% for 6 weeks reduced the reddening and thickening of the mucosa, suggesting AGE may reduce H. pylori-induced gastritis.

Improved Intestinal Conditions


Matsuura et al. (1997) found that Aged Garlic Extract enhances the growth of Lactobacillus acidophilus and Bifidobacterium bifidum, friendly intestinal bacteria, whereas other forms of garlic inhibited the growth of Bifidobacterium bifidum. B. bifidum and L. acidophilus produce both acid and anti-bacterial factors and have demonstrated an ability to decrease the growth of pathogens and their production of toxic and cancer-causing compounds in the intestinal tract.


The effects of Aged Garlic Extract on the growth of the beneficial bacteria, B. bifidum and L. acidophilus can be attributed, in part, to the F-4 protein fraction in Aged Garlic Extract.
Aged Garlic Extract preparations were shown to be effective at improving the gastrointestinal motor disorders induced by stress, such as delayed emptying of the stomach and intestine. Gwilt et al. (1994) found that liquid Aged Garlic Extract can enhance gastrointestinal motility.

Protection from Heavy Metals

Lau (1989) added various heavy metals (copper, mercury, aluminum and lead) to two sets of blood samples and to one set he also added Kyolic Aged Garlic Extract. The heavy metals ruptured the red blood cells in each of the samples except for the cells to which Kyolic Aged Garlic Extract had been added. This experiment clearly demonstrated a protective effect from the Aged Garlic Extract.

Wong, S., D.D.S and Zhu, D.A.H., M.D. The Effectiveness of S.G.P. on Dental Patients with Mercury Restorations — A Pilot Study

In this unpublished study, Wong and Zhu found that 60 days administration of Aged Garlic Extract led to increased excretion of mercury as measured by hair analyses. The average content of mercury excreted in hair was 1.565 p.p.m. This pilot study suggests that Aged Garlic Extract also has an in vivo chelation effect.

Nutritional Support for Genital and Oral Herpes


Tsuei (1987) completed a patent for Aged Garlic Extract for oral and genital herpes. The Aged Garlic Extract can be used orally or topically, and delays and minimizes the symptoms of the virus, as well as increasing the period of time between recurrences of viral shedding.


In the study by Abdullah et al. (1989), AIDS patients noted interruptions of recurrent cycles of genital herpes during their six week course with Aged Garlic Extract powder.

Effects of Aged Garlic Extract on Sugar Metabolism


Nagai et al. (1975) found that the group receiving a Kyolic Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 and liver extract showed good control of their blood glucose level. Especially, the group that received 8 g/kg of glucose load showed a very good control of blood glucose.


Pretreatment with Aged Garlic Extract (5 and 10ml/kg, p.o.) significantly prevented adrenal hypertrophy, hyperglycemia and elevation of corticosterone, without altering insulin level, exposed to 2 days (16hr/day) of immobilization stress. From these results, the authors suggested that Aged Garlic Extract may prevent stress-induced hyperglycemia, which is the risk of suffering from diabetes mellitus and its progression.

Blood Building Effects of Aged Garlic Extract Preparation


In a study by Nagai et al. (1975), blood (1/200 volume of the body weight) was withdrawn from models to determine the blood building effects of a Kyolic Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 and liver extract. The group taking Kyolic, when compared to the control, showed a remarkably slower rate of decrease in the number of red blood cells, hematocrit, hemoglobin and body weight loss. The appearance of the reticulocytes was also decreased in the Kyolic group. Kyolic group also showed increased hematopoietic (red blood cell production) activity of the bone marrow.


In a clinical study by Hasegawa et al. (1984), of 132 subjects suffering from indefinite medical complaints, a noteworthy improvement in anemia was found.

Anti-Allergy

Three systems, the in vitro histamine release system, the in vivo IgE mediated skin reaction system and the in vivo late phase reaction system, were used to determine the effect AGE has on mast cell and activated T lymphocyte function. Kyo (1998) found that AGE could modify allergic cascade reactions (i.e. inflammation).
Morihara et al. (2001) studied the effects of tonic herbal medicines on male reproductive dysfunction induced by hyperthermic treatment. Aged garlic extract (AGE) demonstrated spermatogenesis promotion, while raw and boiled garlic extract did not. When observing the mechanism, researchers found that AGE acted differently from testosterone by improving the peripheral blood circulation which may play an important role in spermatogenesis promotion.

Kasuga (1998) investigated pharmacological activities of four garlic preparations including raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (PGP) and aged garlic extract (AGE) using testicular hypogonadism (hypospermatogensis and impotence) induced by warm water treatment. RGJ effectively recovered testicular functions, DGP restored recovery of spermatogenesis and AGE was also effective. HGJ had no effect on impotence.

Reducing the Side Effects

SAC was found to be effective in reducing CCl4-induced lung injury through intraperitoneal injection of CCl4 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 CCl4. SAC dose-dependently and significantly attenuated CCl4-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.

Overview of Various Effects

Kyo E. 2003. Igaku no Ayumi. 204: 74-9 (Japanese)

In his overview, Yeh (1996) presented various pharmacological effects of Aged Garlic Extract. These effects included its ability to stimulate phagocytic function of macrophages, proliferate lymphocytes and remove *Candida albicans* from the blood circulation of models (immunostimulatory properties), its antioxidative effects, its ability to prolong life and improve memory retention of senescence accelerated models and its potential as a functional food, food supplement and means of curbing health care costs.

Since ancient times, garlic has been used for the treatment and prevention of disease all over the world. Generally, sulfur compounds are the main constituents found in garlic. However, cooking and/or processing through boiling, cutting, and/or aging will change the content and species of these compounds. Previous reports indicate that different cooking and/or processing methods cause different pharmacological activities and safety. Among the different garlic products, Aged Garlic Extract (AGE), which is manufactured through a natural aging process, has been studied based on its various pharmacological activities, such as anti-tumor and anti-fungal effects, anti-oxidant properties, and preventing cardiovascular disease.

Garlic has been used as folk medicine for more than 5000 years. Its variety of efficacies has been reported, and more than 3000 scientific papers have been published on its chemistry, safety and biological activities. There are many different kinds of garlic products on the market, including Aged Garlic Extract (AGE). In this review article, the various pharmacological effects of AGE, including anti-tumor effects, were discussed.

Pharmacological findings suggest garlic has more preventive rather than therapeutic benefits. The anti-carcinogenic, cardioprotective, and immune stimulating effects of garlic (aged garlic extract) components such as SAC, have been demonstrated.
Clinical studies conducted on more than 1,000 subjects (Miyoshi et al, 1984) have reported no serious side effects from the long term of Aged Garlic Extract. Furthermore, the safety of Aged Garlic Extract has been confirmed by toxicological studies including the following acute toxicity, sub-acute toxicity, chronic toxicity and mutagenicity tests:

General Toxicity

Nakagawa, S., Masamoto, K. et al. 1980. (J. Toxicolog. Sci) 5: 91-112. Nakagawa et al. (1980) found that no growth retardation, stomach injuries, changes in red blood cell count, or morphological abnormalities were observed in models given 5 ml/kg Aged Garlic Extract for three to 21 days. On the other hand, consumption of 5 ml/kg raw garlic juice led to a decrease in total serum protein and albumin, acute inflammation of the stomach and stomach ulcers, decreases in red blood cells and hemoglobin, and increases in serum bilirubin. The authors concluded that when garlic is aged, the toxicity is greatly reduced since none of the side effects observed in the raw garlic group were observed in the Aged Garlic Extract group.

Kanezawa A., Nakagawa, S. et al. 1984. Oyo Yakuri (Applied Pharmacol.) 27: 909-929. Kanezawa et al. (1984) found that the LD 50 for a Kyolic ® Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 and liver extract, for oral and subcutaneous administration was over 30 ml/kg, the maximum amount physically possible to administer to models. One-time per oral administration caused inhibition of spontaneous movements due to abdominal distention. Subcutaneous (under the skin) administration led to thickening of the skin and granulation where administered. However, no death due to Kyolic® Liquid Aged Garlic Extract containing vitamin B1, vitamin B12 and liver extract occurred during the seven-day observation period. For long-term administration, adverse effects (such as decreased red blood cells, hemoglobin and slight increase in spleen weight) were noted only in dosages 250 times the usual dosage. Even in such cases, no abnormal pictures of red blood cells were observed.

Enteric-coated garlic supplements have been designed to deliver garlic powder directly to the intestinal tract. Using the newly developed endoscopic compressed air delivery system, Hoshino et al. (1998) assessed the effects of garlic powders on the gastrointestinal mucosa. Raw garlic cloves, boiled garlic cloves and aged garlic extract, freeze-dried and pulverized into powder, were administered into the stomach and their effects on the mucosa were endoscopically determined 24 hr after administration. Fresh garlic powder caused severe damage including erosion and ulcer-like lesions. Boiled garlic powder caused a reddening of the mucosa, whereas aged garlic extract powder caused no undesirable effects. These results suggest that the safety should be seriously considered when choosing a garlic supplement.

Acute Toxicity

Nakagawa, S. Masamoto, K. et al. 1984. (J. Toxicolog. Sci) 9: 57-60. In an acute toxicity test, Nakagawa et al. (1984) estimated the LD 50 of Aged Garlic Extract to be over 30 ml/kg. This was the maximum amount that could be physically delivered.

Chronic Toxicity


Mutagenicity

Various tests (Ames' test, Rec assay) showed no evidence of mutagenicity of the Aged Garlic Extract. Aged Garlic Extract also did not affect the incidence of micronucleated cells and polychromatic cells, whereas raw garlic juice increased the incidence of such damaged cells. Though cells cultured in fresh garlic juice showed signs of growth inhibition and morphological changes, those cultured in Aged Garlic Extract did not. No signs of cytotoxicity (cell death) were observed, and even at the highest concentration of Aged Garlic Extract, only slight signs were seen.
Acute and Subacute Toxicity


Nakagawa et al. (1984) noted that the oral LD$_{50}$ for Leopin-5® (LE-5), an Aged Garlic Extract preparation containing ginseng, B vitamins and other nutritional factors, was over 30 ml/kg. No toxic signs were seen within seven days after administration. At 10 ml/kg, a slight decrease in food intake was noted though there was no change in weight and no toxic signs were seen for three consecutive months. At excessively high dosage, red blood cell count and hemoglobin value decreased slightly while spleen and liver weights increased a little. There were no toxic signs observed in any of the tissues or organs examined.


Imada (1990) concluded the following in his presentation "Toxicity Aspects of Garlic":

I. Raw garlic, allicin, and diallyl disulfide (DADS) are toxic when large doses are taken.
II. When garlic is aged its toxicity is greatly reduced, and a commercially available Aged Garlic Extract is almost without toxicity even when a very large dose is taken.
III. The toxicity of oil-soluble garlic constituents is higher than water-soluble constituents.

Safety During Pregnancy


The safety of Aged Garlic Extract has been confirmed in preclinical studies at each trimester of pregnancy. Liquid Aged Garlic Extract has been successfully recommended for more than 50 years as an over-the-counter medicine for pregnant women in Japan.

Drug Interactions with Aged Garlic Extract


— Coumadin

Safety of intake of Aged Garlic Extract with coumadin has become a question since both show blood-thinning properties. A double-blind, placebo-controlled clinical trial by Rozenfeld et al. (1999) suggests that there is no toxic synergism between these substances and that Aged Garlic Extract is safe in conjunction with Coumadin®.


No evidence of increased hemorrhage was observed in a clinical study with deep vein thrombosis (DVT) patients on oral anticoagulation (Coumadin) therapy who were administered 5 ml of Kyolic® Aged Garlic Extract™ twice a day for 12 weeks. Aged Garlic Extract is relatively safe and poses no serious hemorrhagic risk for patients on oral anticoagulation (Coumadin) therapy with close monitoring.

Aged garlic extract (AGE) was administered at a dose of 5 mL twice a day for 12 weeks in a double-blind, randomized, placebo-controlled pilot study. There was no evidence of increased hemorrhage in either the placebo or the AGE group.

— Statins
A recent double-blind randomized placebo-controlled clinical study using liquid Aged Garlic Extract (AGE) on cardiovascular patients has shown no contraindications with statins (cholesterol lowering drugs) and aspirin.

— Acetaminophen


Except for a slight increase in sulfate conjugation, Gwilt et al. (1994) found that Aged Garlic Extract did not affect the metabolism or efficacy of acetaminophen.

— Saquinavir
Drug-supplement interaction has been paid attention by medical arena and public recently. Dehydrated garlic powder product has been reported to have a drug interaction, especially AIDS treatment drug, such as Saquinavir that is influenced by P450 metabolism enzymes. Aged Garlic Extract (AGE) may not be necessary to have such negative interaction with medicines, since it has a special preparation method to eliminate odorous oil-soluble sulfur compounds by aging extraction process. This note has clearly pointed out this difference and arguments with original authors of the paper.

Dosages in Studies


The majority of the original clinical studies conducted utilized from 2-4 ml of the liquid Aged Garlic Extract, roughly equivalent to 1,200 mg of the powder, thus establishing the suggested daily intake of 1,200 mg. In clinical studies, as little as 1,800 mg of Aged Garlic Extract powder has shown immunostimulatory activity, enhancing Natural Killer cell activity (Kandil et al., 1987) in normal subjects, whereas as much as 10,000 mg has been safely taken by AIDS patients and has shown similar enhancement of these cells over a six week period (Abdullah et al., 1989). In two double-blind clinical studies, 7,200 mg of Aged Garlic Extract powder was shown to effectively reduce cholesterol, blood pressure and platelet aggregation having been safely taken for five to six months (Steiner et al., 1996b; Yeh et al., 1997). Another double-blind, randomized, placebo-controlled study (Steiner 2001) showed lower dosages (2.4 g and 4.8 g) of Aged Garlic Extract are significantly effective at thinning the blood. A clinical study by Gwilt et al. (1994) utilized 10 ml per day of liquid Aged Garlic Extract for three months and found increased sulfate conjugation (detoxification) of the analgesic acetaminophen. Even at this high dosage (more than twice the suggested daily dosage), no body odor was noted from subjects. Dimitrov et al. (1997) also utilized 10 ml/day in his clinical study showing a decrease in the levels of the serum prostaglandins and it was “tolerated well by all participants.”

Side Effects

No severe side effects were noted in the more than 40 clinical studies using Aged Garlic Extract confirming the safety of such preparations. The preparations were generally well tolerated, even at high dosages. Minor side effects, noted in only a few clinical studies, were few in number and were noted in less than 10% of the subjects. Main complaints included stomach discomfort, nausea, flatulence, diarrhea, or odor. Interestingly, some of the subjects taking the placebo in Lau’s study complained of heartburn and headaches, suggesting a psychological factor possibly plays a role in side effects.
Aged Garlic Extract contains numerous compounds that have demonstrated beneficial effects and a synergy of these compounds is likely responsible for the benefits of Aged Garlic Extract. For the sake of quality control, Aged Garlic Extract is standardized with S-allyl cysteine (SAC), a stable, effective, and safe organosulfur compound derived from garlic, which significantly and naturally increases during the aging process. Aged Garlic Extract preparations are standardized to contain no less than 0.05% SAC by dry weight. The following studies suggest that SAC can provide protection against oxidation, free radicals, pollution, cancer, and cardiovascular diseases. The bioavailability of SAC has also been confirmed in several models:

### Cardiovascular Effects of SAC

#### Cholesterol Lowering Effect


Abuirmeileh et al. (1991) found that SAC lowered total serum cholesterol and LDL cholesterol in hypercholesterolemic models. Cholesterol was lowered by inhibiting the activity of the key enzymes in cholesterol synthesis, ß-hydroxy-ß-methylglutaryl CoA synthetase and reductase, in the liver.


Qureshi, N. (1990) and Qureshi, A. (1990) found that SAC lowered serum cholesterol, LDL cholesterol and triglycerides in both normolipemic and hypercholesterolemic models. Cholesterol was lowered by the inhibition of key enzymes in cholesterol synthesis, ß-hydroxy-ß-methylglutaryl CoA synthetase and reductase, in the liver.

Key enzymes of lipogenesis, acetyl CoA carboxylase and fatty acid synthetase, were also significantly inhibited by SAC.

Among garlic preparations tested, maximum inhibition of cholesterol-producing enzyme activities was observed in this order: inhibited by SAC.

Kyolic® > SAC > commercial garlic oil > garlic powder


Yeh et al. (1994) found that SAC inhibited the synthesis of cholesterol and fatty acids in cultured liver cells.


Yeh and Liu (1998) found that water-soluble organosulfur compounds, such as S-alk(en)yl cysteines (SAC, S-ethylcysteine, etc.) and γ-glutamyl-S-alk(en)yl cysteines (γ-glutamyl-S-allylcysteine, γ-glutamyl-S-methylcysteine, etc.), derived from garlic inhibited 20-60% of the cholesterol biosynthesis in primary cultured hepatocytes, apparently through metabolic alteration. Alliin, a major water-soluble organosulfur constituent in garlic cloves, had no inhibitory effect. Oil-soluble sulfur compounds, including diallyl disulfide and diallyl trisulfide, also inhibited synthesis (10-15%), however, inhibition by these compounds was accompanied by the release of intracellular lactate dehydrogenase, indicating the reduction in cholesterol synthesis could be due to cytotoxicity. Based on IC50 and maximal inhibition, SAC was found to be one of the major garlic constituents responsible for non-toxic cholesterol reduction.

### Blood-Thinning Effect

Qureshi,. et al. (1990b) and Yu et al. (1990) and Abuirmeileh et al. (1991) found that SAC lowered the levels of plasma thromboxane B2 and factor 4 (blood clotting factors) in hypercholesterolemic models up to 30%. SAC also decreased platelet aggregation, or blood clotting, induced by the potent clotting agents, collagen and adenosine diphosphate.


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$_2$O$_2$)-induced oxidant injury. Pretreatment of cells overnight with SAC (4 mg/mL) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase release (LDH)$^5$ and lipid peroxidation induced by H$_2$O$_2$. The authors suggesting that these compounds may be effective in hampering the aging process and for prevention of atherosclerosis.


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 restenosis (narrowing or constriction) of blood vessels subjected to angioplasty.$^6$

Sickle Cell Anemia

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.


Antioxidative Effects of SAC


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

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 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 10 mmol/L. SAC also demonstrated radical and hydrogen peroxide scavenging activities in vivo.

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).

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.

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.

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
compounds were found to decrease Fe\textsuperscript{2+}- and glucose-induced lipid oxidation in plasma, kidney and liver \((p<0.05)\). The cysteine-containing compounds were also found to decrease fibrinogen, triglyceride, and cholesterol levels in plasma and liver, while they increased levels of \(\alpha\)-tocopherol in the liver, plasma, and kidney \((p<0.05)\).

Mundo et al. investigated the effects of \(S\)-allylcysteine (SAC) on early behavioral alterations, striatal changes in superoxide dismutase activity, lipid peroxidation and mitochondrial dysfunction induced by the systemic infusion of 3-nitropropionic acid (3-NPA) to models. SAC, given to the models 30 minutes before 3-NPA, prevented the hyperkinetic pattern by the toxin. 3-NPA alone produced decreased activities of manganese and copper/zinc-dependent superoxide dismutase, increased lipid peroxidation and mitochondrial dysfunction in the striatum. Pre-treatment of 3-NPA-injected models with SAC resulted in a significant prevention of all these markers.

Cell membrane damage in myocardial infarction-induced models increased enzymatic leakage, lipid peroxidation, and free radical formation. Oral pretreatment with SAC (100 mg and 150 mg/kg) improved superoxide dismutase, catalase, glutathione reductase, and ascorbic acid enzymatic activities. End measures of lipid peroxidation, TBARS, were decreased with SAC oral pretreatment (100 mg and 150 mg/kg). Padmanabhan and Prince (2006) conclude improvements made in lipid peroxide markers (decrease) and anti-oxidant status (increase) are due to the anti-oxidant effect of SAC.

Padmanabhan and Prince concluded that in subjects who suffered myocardial infarction (heart attack) due to prolonged myocardial ischemia (deficient blood flow to the heart), showed improvement in mitochondrial enzyme activities when pretreated with SAC. Researchers suggest this is due to SAC antioxidant qualities.

Perez-De La Cruz et al. sought to determine the antioxidant properties of \(S\)-allylcysteine (SAC) on lipid peroxidation and mitochondrial dysfunction induced by 3-nitropropionic acid (3-NPA), a neurotoxin. Concentrations of 3-NPA at 0.75-2.5 mM produced enhanced levels of lipid peroxidation, while increasing concentrations of SAC (0.1-2 mM) decreased the peroxidative effects of 3-NPA. SAC at 0.75 mM also prevented the 3-NPA (1 mM)-induced mitochondrial dysfunction. Researchers determined that the protective actions of SAC on 3-NPA-induced lipid peroxidation and mitochondrial dysfunction are due to its antioxidant properties.

Inhibits Advanced Glycation Endproduct (AGEP) Formation in Diabetics

\(S\)-allylcysteine, the key component in aged garlic extract (AGE), is a potent antioxidant that can inhibit advanced glycation endproduct (AGEP) formation in vitro, which can help to prevent diabetic complications.

Attenuating Ischemic Brain Damage


Numagami et al. (1996) found that when SAC was administered 30 minutes prior to ischemic insult there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in this middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species generated due to ischemia.

Numagami et al. (1998, 2001) studied the efficacy of SAC as a free radical scavenger using brain ischemia models. In a middle cerebral artery occlusion model, pre-ischemic administration of SAC improved (i) motor performance and (ii) memory impairment, and reduced (iii) water contents and (iv) the infarct size. In a transient global ischemia model, (i) the production of free radicals (alkoxyl radicals) as studied by electron paramagnetic resonance spectroscopy (EPR) was biphasic; the first peak occurring at 5 min and the second peak at 20 min after reperfusion. SAC did not attenuate the first peak, but did the second peak. (ii) The lipid peroxidation as estimated by TBA-RS increased significantly at 20 min after reperfusion. SAC decreased TBA-RS to the levels found without ischemia. These results suggest that SAC would have beneficial effects in brain ischemia and that the major protective mechanism may be the inhibition of free radical-mediated lipid peroxidation.
The protective effect of SAC on the ischemic damage is examined. Researchers used the human neuroblastoma cell line, SK-N-SH, and incubated it with or without SAC for 48hr and then exposed them to simulated ischemia (hypoglycemia and hypoxia), followed by simulated reperfusion (reoxigenation). SAC showed a neuroprotective effect against ischemic neuronal damage both in vitro and in vivo. SAC decreased the size of infarction after transient or global ischemic insults. While it did not alter the N-methyl-D-aspartate excitotoxicity, SAC significantly scavenged the endogenously or exogenously produced ONOO- and reduced ONOO- cytotoxicity. Further, SAC inhibited the activity of extracellular signal-regulated kinase (ERK) increased in cultured neurons exposed to oxygen-glucose deprivation. The present results indicate that SAC exerts its neuroprotective effect by scavenging ONOO- and inhibiting the ERK signaling pathway activated during initial hypoxic/ischemic insults.

Inhibits the Activation of NF-kB which Mediates Inflammatory Reactions

Hydrogen peroxide and tumor necrosis factor (which produces the oxidants superoxide and hydrogen peroxide) have been shown to activate a compound (nuclear factor kappa B, NF-kB) in immune T-cells, which is involved in immune and inflammatory reactions. Lau and his team of researchers at Loma Linda University found that SAC, by acting as an antioxidant, inhibits NF-kB activation. At doses of 0.5- to 2.0 mg/ml used in this study SAC was not toxic to human T Cells. Consequently, NF-kB is also essential for the expression of genes controlled by the AIDS virus. The authors suggested further research to see if SAC may be effective at preventing NF-kB mediated disorders.

Anticancer and Cancer-Preventive Effects of SAC

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.

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.

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.

Ameliorates Anticancer Drug, Doxorubicin, Cardiotoxicity

Clinical uses of Doxorubicin, a potent anticancer drug effective against a wide range of human neoplasms, have been limited due to its serious cardiotoxic effects, which are likely the result of generation of free radicals and lipid peroxidation. SAC has been reported to have antioxidant and radical scavenging effects. Thus, Ohnishi et al. (2000) examined the effect of SAC on doxorubicin toxicity. Severe doxorubicin toxicity was induced by a single intraperitoneal injection. SAC (30 mg/kg) was injected intraperitoneally daily for 5 days, starting two days prior to the administration of doxorubicin. Doxorubicin injection induced a mortality rate of 58%, with SAC treatment reducing the doxorubicin-induced mortality rate to 30%. The severe body weight loss caused by doxorubicin (13%) was also significantly attenuated by SAC treatment (9%). Treatment with SAC significantly reduced the level of serum creatine phosphokinase. Histological analysis demonstrated that heart and liver damage was significantly less severe in SAC treated subjects, than in those receiving only doxorubicin. These results suggest that SAC research may ultimately lead to a resolution of the adverse effects of doxorubicin treatment in cancer chemotherapy.
Song et al (1999) found that providing either 0.105 micromol diallyl disulfide or S-allyl cysteine by gastric gavage thrice weekly for 2 wk was effective in retarding DMBA bioactivation. Isomolar alliin was not effective.


Amagase, H. and Milner, J. 1992. FASEB J. 6(4): 3229. SAC supplementation markedly depressed the occurrence of DMBA-DNA adducts in mammary cells by 70 or 80%, respectively, but did not alter food intake or weight gain.

Liu et al. (1995) found that SAC is an effective inhibitor of N-methylnitrosourea-induced mammary tumors. Final tumor incidence was 81% in control models and 38% in those fed SAC.

SAC is an effective inhibitor of chemically induced transformation in vitro and in vivo. SAC reduced the formation of revertants of Salmonella typhimurium TA100 following exposure to NMOR (nitrosomorpholine), a known liver carcinogen. MNU-induced and DMBA-induced mammary tumors were reduced by SAC.

SAC inhibited the initiation of DMBA mammary carcinogenesis. Selenium appeared to enhance the activity of SAC.

Schaffer et al. found that SAC and DADS are effective inhibitors of N-methyl-N-nitrosourea (MNU)-induced mammary carcinogenesis. Garlic powder, SAC and DADS supplementation significantly delayed the onset of mammary tumors compared to the control group. Tumor incidence 23 weeks after MNU treatment was reduced by 76, 41, and 53% in models fed garlic, SAC and DADS. Also the quantity of mammary DNA alkylation occurring 3 hours after MNU treatment was reduced; specifically, O6-methylguanine adducts were reduced by 27, 18, and 23% and N7-Methylguanine adducts decreased by 48, 22, and 21%.

Tiwari et al. (1993) found that SAC inhibited the growth and proliferation 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.

Li et al. (1995) also found that SAC inhibited the growth of transformed human breast cells and increased both glutathione-S-transferase and peroxidase levels in the non-transformed cells.

Takeyama et al. found that SAC inhibited the proliferation of nine human melanoma cell lines and one murine melanoma cell line in a dose dependent manner. SAC inhibited cellular growth and proliferation and modulated major cell differentiation marker of melanoma.

Welch et al noted time- and dose-dependent inhibition of cell growth of LA-N-5 human neuroblastoma cell cultures treated with SAC for two days.

Sumiyoshi et al. (1990) found that the incidence and frequency of colon tumors induced by the carcinogen dimethylhydrazine (DMH) were significantly inhibited by pretreatment with SAC. SAC also stimulated the activity of glutathione S-transferase, an enzyme known to assist in the detoxification of carcinogens in the liver and colon.
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.

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 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-15) 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 p34cdc2 protein expression. Suppression of p34cdc2 and cdc25C expression likely accounts for the ability of DADS to inhibit p34cdc2 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

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.

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

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

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.


Inhibited Both the Formation and Bioactivation of a Liver Carcinogen

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.

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 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.


Nakagawa et al. (1985) found that SAC completely suppressed the cytotoxicity (cell-killing power) of the potent liver toxin, carbon tetrachloride (CCl4), whereas four control drugs were found to be less effective at protecting the liver cells.


Billington et al. (1998) found that pretreatment with SAC for several days reduced BB toxicity in subsequently prepared liver slices. Up to 1 mM SAC was added to the culture medium of liver slices, suggesting this compound (or a metabolite) may be the GSH-sparing agent.

Hsu et al. (2006) found that acetaminophen-induced depletion of glutathione (GSH) content in blood and organs could be lessened by S-allylcysteine and S-propyl cysteine due to their antioxidant tendencies. Consequently, models demonstrated suppressed oxidation, inflammation, coagulation, with improved liver function.

Brain, Neurotrophic, Anti-aging, and Anti-depression Effects of SAC


Numagami et al. (1996) found that when S-allyl cysteine, a constituent in Aged Garlic Extract was administered 30 minutes prior to ischemic insult there was a significant decrease in ischemic damage. This was indicated by decreased water (swelling of the brain) in this middle cerebral artery occlusion model. In a global ischemia model, SAC decreased the amount of reactive oxygen species generated due to ischemia.


Nishiyama and other researchers at the University of Tokyo (1997) found that SAC enhanced neuronal survival and branching of hippocampal culture studies. The authors suggested that it may be helpful in the development of therapeutic and/or prophylactic drugs for neurodegenerative disorders. S-allyl cysteine (SAC), an organosulfur compound in Aged Garlic Extract with a thioallyl group (CH3=CH-CH2-SH) promoted the axonal branching of cultured neurons, according to Moriguchi et al. (1997). SAC also promoted neuronal survival.

An in vitro simulated ischemia model and an in vivo transient global ischemia model were used to study the protective effect of SAC, a compound found in AGE. It was reported that SAC showed neuroprotective effects against in vitro and in vivo ischemic neuronal damage.

Ito et al. (2003) reported that S-allyl-L-cysteine (SAC) protected neuronal cells against amyloid β-protein (Aβ)-induced cell death in a concentration-dependent manner and protected them against tunicamycin-induced neuronal death, which may be triggered by endoplasmic reticulum (ER) dysfunction in nerve growth factor (NGF)-differentiated PC12 cells.

S-Allyl-L-cysteine (SAC) was shown to protect the hippocampal neurons in the CA3 area and the dentate gyrus (DG) from the cell death (in an organotypic hippocampal slice culture (OHC) induced by Amyloid-β (Aβ) and ibotenic acid (IBO). These results suggest that SAC and L-glutamate protects cells from death in specific areas of the hippocampus.

In a study by Negi, et al., SAC was shown to aid in the protection of spinal neurons against glutamate neurotoxicity in organotypic spinal cultures (OSCs). This study also reported that SAC may aid in suppressing the loss of motor neurons induced by glutamate.
AGE and SAC were reported to decrease apoptosis by enhancing endogenous antioxidant defenses in a study determining their effects on Aβ-induced apoptosis and reactive oxygen species (ROS) generation in a pheochromocytoma (PC12) cell line.

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.

S-Alllyl-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.

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 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 granule 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.

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Amagase (2001, 2002) stressed the importance of choosing appropriate, bioavailable marker compounds in standardizing herbs to assure quality and utility of the final product. Such markers can also assure compliance in clinical studies. For example, allicin is often thought to be an active ingredient for standardizing garlic products, though this compound is transient, actually not found in any product, and not bioavailable. S-allylcysteine (SAC) is a more appropriate choice since it has shown beneficial effects in many studies and is bioavailable. Numerous recent clinical studies, utilizing 'allicin-standardized' products have shown a lack of efficacy, likely due to inappropriate standardization.


Physical, chemical, and biological properties of S-allylcysteine (SAC) were investigated. SAC showed stable properties under tested conditions, and its acute/subacute toxicity was very minor. The pharmacokinetics of SAC was investigated after oral administration of garlic supplement containing SAC to human volunteers. SAC from garlic consumption was rapidly absorbed from the gastrointestinal tract, however, the half-life and excretion time were more than 10 h and 30 h, respectively.


This review article clarifies the real active compounds in garlic and different preparations. While not all of the active compounds of garlic are known, and allicin-like transient compounds are not directly active. Ample research shows that an allicin-free garlic preparation, such as Aged Garlic Extract (AGE) that is standardized with a bioavailable component, S-allyl cysteine, is active and various effects of garlic may be attributed to it. Various other chemical constituents in garlic including non-sulfur compounds like saponins, contribute to the essential biological activities of garlic.

SAC Has Confirmed Safety


Imada, from the M.D. Anderson Cancer Center, found that the LD 50 for oral intake of SAC in models was 8890 mg/kg body weight in male models and 9390 in female models. (Consequently, the toxicity of allicin was almost thirty-fold that of SAC, 309 and 363, respectively.) In subacute toxicological studies of SAC, pathological effects were not seen until 500 mg/kg.


Geng et al. (1997) checked the toxicity of SAC in cultures of human T Cells and found that 0.5 to 2.0 mg/ml did not affect cell viability.

Pharmacokinetics

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.

Chemistry

Allicin is not active or a 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 this compound for standardization.
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:

**Antioxidative and Radioprotective Effects of SAMC**


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 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 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 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. 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.
Liver Protective Effects of SAMC Induced Liver Injury through Suppression of P450 2E1 Activity.

To further understand this mechanism, glutathione (GSH) contents in the liver and CYP2E1 activities were determined. No change was seen in GSH contents of the liver by SAMC, but SAMC improved the activity of cytochrome P450 2E1. ALT activity was increased by APAP in plasma, but was decreased by SAMC.

The effect of SAMC administration on plasma ALT activity, GSH contents, and cytochrome P450 2E1 activity were determined. Administration of APAP increased ALT activity in plasma 72-fold, and lipid peroxidation 2.9-fold after 6 hours. However, SAMC was able to return both ALT activity and lipid peroxidation back to normal levels. ALT activity, GSH contents, and cytochrome P450 2E1 activity, and the expression of stress protein HSP70i were determined. HSP70i is enhanced by a complex metabolite of APAP, (NAPQI) and APAP and HSP70i were determined. No change was seen in GSH contents of the liver by SAMC, but SAMC improved the activity of cytochrome P450 2E1.

The effect of SAMC or NAC on acetaminophen (APAP)-induced liver injury was evaluated using a model system. SAMC was given 24h before and 2h after APAP administration, and plasma ALT activity, GSH contents, and cytochrome P450 2E1 activity were determined. The data indicated that ALT activity was increased by APAP in plasma, but was decreased by SAMC.

The effect of SAMC on APAP-induced liver injury through suppression of P450 2E1 activity was confirmed. SAMC pretreatment also suppressed cytochrome P450 2E1 activity since SAMC suppressed an enzyme representative of P450 2E1 activity. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation (oxidation of liver tissue) and the decrease in liver COQ9H2 levels. SAMC was found to be more effective than the other chemicals used, suggesting an antioxidative effect.

In order to elucidate the mechanism by which SAMC confers liver protection; however, the mode of action may be different between these compounds.

S-allylmercaptocysteine (SAMC) or Acetyl cysteine (NAC) on acetaminophen (APAP)-induced liver injury was determined. SAMC and NAC both had an effect on the GSH contents of the liver, which indicates the potent liver toxin, carbon tetrachloride (CCl4), whereas four positive control drugs (vitamin E, α-tocopherol, CoQ10, and GSH) were found to be less effective at protecting the liver from free radicals generated by CCl4.

Protection from Carbon Tetrachloride and Paracetamol were effective than the other chemicals used.
Anticancer Effects of SAMC

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.

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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.

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 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 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

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. 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.

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 et al. had previously reported that S-allyl mercaptocysteine (SAMC) inhibited growth, arrested cells in G2/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 (JNK1) and capase-3 are then triggered, which signals pathways leading to apoptosis.
Models were provided a semi-purified, casein based diet with or without 57 or 570 µmole/kg of SAC, DADS or SAMC for 13 weeks prior to determination of aberrant crypt foci (ACF) and aberrant crypt number. All treatments, except 57 µmole/kg SAC, significantly lowered ACF compared to controls. ACF was significantly reduced by DADS and SAMC at both concentrations tested. This study revealed that all ally sulfur compounds are not equivalent in retarding early preneoplastic markers for colon cancer.

Inhibited the Growth of Prostate Cancer Cells


Steiner et al. (1997) from East Carolina University found that S-allylmercaptocysteine (SAMC) in Aged Garlic Extract could inhibit the growth of the hormone responsive prostate cancer cell line CRL-1740. Even at 0.05 mM, SAMC totally suppressed the growth of CRL-1740 cells compared with the solvent treated control cells.


Pinto et al. (1997, 2000) found SAMC may inhibit the growth of androgen responsive human prostate cancer cells (LNCaP). Testosterone, a specific androgen or male hormone, enhances the activity/growth of LNCaP. SAMC was found to enhance the catabolism or degradation of testosterone. Therefore, these researchers suggested that SAMC, by catabolizing testosterone, hampered the progression or activation of these cancer cells. Prostate specific antigen (PSA) levels were markedly reduced after treatment with SAMC. PSA is a marker for prostate cancer.

Inhibited Growth of Erythroleukemia Cell Lines


Steiner et al. (1997) from East Carolina University found that S-allylmercaptocysteine (SAMC) in Aged Garlic Extract could inhibit the growth of two erythroleukemia cell lines (HEL and OCIM-1). HEL cells showed complete suppression of growth at $\geq 0.25$ mM SAMC and even at 0.1 mM SAMC inhibited HEL cell growth by $>70\%$. OCIM cells exhibited a 55% reduction in growth at 0.1 mM SAMC.


Steiner et al. (1997) further confirmed that S-allylmercaptocysteine (SAMC) in Aged Garlic Extract could inhibit the growth of two erythroleukemia cell lines (HEL and OCIM-1). It induced a dose-dependent inhibition with a 50% lethal dose of 0.046 mM for OCIM-1 cells and 0.093 mM for HEL cells. The authors concluded from analyses of $[3H]$ thymidine incorporation and high molecular weight DNA fragmentation that SAMC is an effective antiproliferative agent against erythroleukemia cells that induces death by apoptosis. Allyl sulfur compounds found in Aged Garlic Extract (AGE) have been determined by Lea et al. (2002) to inhibit histone acetylation and cell growth. Garlic extracts with thiosulfinate inhibited DS19 model erythroleukemia proliferation. DS19 cells incubated with S-allylmercaptocysteine (SAMC) or allyl isothiocyanate produced similar degree of downregulation of both histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities.
OTHER CONSTITUENTS IN AGED GARLIC EXTRACT

Oil-soluble and Water-soluble Organosulfur Compounds


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

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, methin, 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 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.

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

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

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

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 AOSC’s ability to reduce CYP2E1 enzyme which activates carcinogens nitrosomethylbenzylamine and azoxymethane responsible for inducing esophageal and colon cancers.
Garlic compounds DAS and DASO2 have proven effective in inhibiting chemical toxicity and carcinogenesis by competing for CYP2E1 enzyme, protecting against hepatotoxicity of acetaminophen, and inhibiting the bioactivation of tobacco carcinogen (NNK). However, these beneficial effects have only been observed with higher concentrations of garlic. Studies still need to be conducted using concentrations equivalent to dietary consumption and supplementation.

Reduced the Incidence of Chemically-Induced Cancers

Garlic and its associated allyl sulfur compounds have been shown to reduce the incidence of chemically induced breast, colon, skin, uterine, esophagus, and lung cancers. Aqueous suspensions and high exposure to S-allyl cysteine have been shown to inhibit early stage colon cancer. All treatments significantly lowered colon cancer model compared to controls (p < 0.05). This study reveals that all allyl sulfur compounds are not equivalent in retarding early stage of colon cancer.

Organosulfur Compounds in Aged Garlic Extract


γ-glutamyl S-allyl cysteine

Alliin

Allyl methyl sulfide

Diallyl disulfide

Diallyl sulfide

Diallyl trisulfide

Ajoene

Vinyl-dithines

S-1-propenylcysteine

S-1-allyl cysteine

S-1-methyl cysteine

S-1-allyl mercaptocysteine

Cycloalliin

Maillard Reaction Products

N-Fructosyl Glutamate and Nα-fructosyl Arginine (Fru-Arg)


Ryu, K et al., Joint ACS-ICoFF Symposium on Food Factors for Health Promotion, San Diego (USA) April 1-5, 2001.

Two research teams lead by O'Brien (1998) and Ryu (1998), reported unique Maillard reaction products in AGE. These include N-fructosyl glutamate and Nα-fructosyl arginine (Fru-Arg), which have antioxidant activity. They were generated through a non-enzymatic reaction of amino acids and reducing sugars during the aging of garlic. Fru-Arg completely scavenging hydrogen peroxide, a potent oxidant, at a very low dosage (50 µM). Since Fru-Arg was detected in high levels in AGE, but not in raw or boiled garlic, its presence may partly explain the antioxidative effects of AGE not shown by other forms of garlic.


Ide, N. et al., 7th International Symposium on the Maillard Reaction (Kumamoto) 10/29/2001-11/01/2001

Ide and Lau (1999) found that Fru-Arg significantly inhibited the oxidizing effects of the copper ion (Cu2+) on LDL cholesterol when incubated together (as shown by a reduction in TBA-RS formation). Pretreatment of pulmonary artery endothelial cells (cells which line the blood vessels of the lungs) with Fru-Arg inhibited cell damage caused by oxidized LDL (as indicated by a reduction in lactate dehydrogenase release, an indicator of cell damage, and a reduction in TBA-RS formation.) Incubation of Fru-Arg with macrophage immune cells also dose-dependently reduced the ability of oxidized LDL to initiate peroxide release from the macrophages. In a cell-free test tube system, Fru-Arg was shown to scavenge the hydrogen peroxide free radical.
Ide et al. (2001) reported that they found four of 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acids (MTCCs) as antioxidants in AGE. Interestingly, the concentrations of these compounds in the extract were shown to increase in progression of the natural aging process. Antioxidant properties of these compounds were further studied using several in vitro assay systems. All of four MTCCs showed strong hydrogen peroxide activities. Particularly, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-beta-carboline-1,3-dicarboxylic acid [(1S, 3S)-MTCdiC] was the most potent hydrogen peroxide scavenger, and the activity was stronger than a common antioxidant, ascorbic acid. To elucidate the mechanism, we analyzed the metabolite of (1S, 3S)-MTCdiC in hydrogen peroxide/peroxidase system using HPLC-APCI-MS/MS. The data suggest that the compound may function as an electron donor and scavenge hydrogen peroxide in this system. MTCCs also inhibited the peroxidation of linoleic acid caused by incubating with 2,2'-azobis(2-amidinopropane) hydrochloride [AAPH] at 37°C. Macrophages were incubated with LPS at 37°C and 5% CO2 for 20h, and the release of nitric oxide (NO) metabolites were measured using a spectrophotometer. LPS significantly increased the release of NO metabolites from the macrophages. Among four MTCCs identified in AGE, only dicarboxylates, (1S, 3S) and (1R, 3S)–MTCdiC, significantly inhibited the release at low concentrations, suggesting that MTCCs, which are formed during the natural aging process, are potent antioxidants in AGE, and that AGE would be useful for prevention of disorders associated with oxidative stress.

Yoshida et al. (2001) have reported four new antioxidants identified in Aged Garlic Extract (AGE). These compounds, 1,2,3,4-tetrahydro-beta-carboline derivatives, show strong scavenging activities. Among these compounds, (1S, 3S)-1-methyl-1,2,3,4-tetrahydro-beta-carboline-1,3-dicarboxylic acid was found to be stronger than ascorbic acid, a common antioxidant. Chemical analytical data indicates that these four compounds were not detected in raw garlic, but their presence was increased during the natural aging process. These four new compounds may contribute to the antioxidant activities of AGE. In a study that used liquid chromatography mass spectrometry (LC-MS), four tetrahydro-β-carboline derivatives were found to have strong hydrogen peroxide scavenging activities. These compounds found in AGE were shown to increase during the aging process and were not detected in raw garlic. This study suggests that these compounds in AGE are potent antioxidants, and may play an important role in preventing disorders that are associated with oxidative stress.


Matsuura, (1998, 2001) showed that garlic cloves contain proto-eruboside B. When cloves are processed, a glucose molecule is removed from proto-eruboside B through an enzymatic hydrolysis releasing eruboside B. Eruboside B consists of four sugar moieties (one glucose and three galactose) and a unique steroid aglycone, named ß-chlorogenin.

Itakura et al. (1998, 2001) concluded, after analyzing various saponin profiles in the Allium species, that ß-chlorogenin could be the best candidate for identification and distinction of garlic from the other Allium vegetables.


Matsuura, H  et al.: Cholesterol lowering effects of saponins from garlic, 3rd International Congress on Phytomedicine (3rd ICP), Munich (Germany) October 11-13, 2000.


Matsuura et al. (1988, 1989, 1998) identified and isolated new steroidal glycosides from garlic, which belong to the saponin group and have anti-fungal activity.

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


Matsuura found that Aged Garlic Extract contained a small amount of oligofructanes not found in other forms of garlic. Fructanes have been shown to enhance the growth of the friendly bacteria Bifidobacterium bifidum.

Enhanced Immune Cell Activity/ Anti-tumor Effects


Hirao et al. (1987) found that the F-4 protein fraction in Aged Garlic Extract strongly stimulated peritoneal macrophage activity and exhibited cytostatic activity in vitro. F-4 also stimulated the proliferating activity (ability to reproduce) of spleen cells. In vivo, F-4 induced the stimulation of carbon clearance activity. The authors concluded that was effective for the suppression of tumor cell outgrowth through the stimulation of immunoresponder cells.


Lau et al. (1991), found that the F-4 protein fraction from Aged Garlic Extract stimulated the proliferation of T-lymphocytes (immune cells).


Morioka et al. (1993) found that a protein fraction is isolated from Aged Garlic Extract enhanced the ability of human peripheral blood lymphocytes (white blood cells) to destroy tumor cells. Moreover, the protein fraction significantly stimulated the lymphokine (interleukin-2)-activated killer activity. The protein fraction also enhanced the proliferation of lymphocytes induced by the immune-stimulating agents, interleukin-2 and concanavalin-A, suggesting a possible reduction of the dosage of interleukin-2 in cancer immunotherapy.
Enhanced Nerve Survival

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 (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

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 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 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

Allixin showed a dose-related inhibition of histidine+ revertants induced by AFB1. (AFB1 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 AFB1-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 (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-pyrones (DHP) was found to show similar activity with less toxicity. These two compounds may be useful as external neurotrophics.
Chemistry


Allicin is not active or a 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 this compound for standardization.


Allixin was induced by irradiating fresh garlic cloves with sunlight or UV light. The induced allixin was analyzed by HPLC, and the accumulated amounts of allixin were 3.1-6.3 µg/g under these conditions. The importance of this study presents the possibility of allixin induction by light irradiation.

Selenium


In a comparative study among various garlic products on the market in Germany, Kyolic Aged Garlic Extract was found to have the most selenium of the eight products tested, containing 25-50% the selenium of raw garlic.

Selenium compounds in garlic exhibited chemopreventive effects by inhibiting the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary adenocarcinomas and azoxymethane-induced colon cancer and benzo[a]pyrene-induced forestomach tumors.

Using HPLC inductively coupled plasma MS (HPLC-ICP-MS) analysis, Se-methlyselenocysteine (MeSeCys), $\gamma$-glutamyl-Se-methlyselenocysteine ($\gamma$-Glu-Se-Me-Cys), selenomethionine, and nonmetabolized selenate were identified in water extracts of garlic seedlings when hydroponic enrichment of selenium (Se) was implemented. High selenium (Se) garlic could be an ideal nutritional supplement of dietary Se for cancer prevention.

Peroxidase


Peroxidase, which decomposes the oxidizing compound hydrogen peroxide, which is produced by vital reactions, is found in many vegetables and plants, including garlic. The degree of peroxidase activity in garlic products varies substantially. A Kyolic ® Liquid Aged Garlic Extract preparation containing vitamin B1, vitamin B12 and liver extract and Leopin-5 ®, an Aged Garlic Extract preparation containing ginseng, B vitamins and other nutritional factors, were found to have peroxidase activity. Studies of five other garlic products on the market showed no such activity.

Others


New antioxidant compounds were found in the garlic skin. These compounds are also found in AGE. Six phenylpropanoids were identified. Determination and assay of these chemical compounds have been done by state-of-the-art chemical analysis, such as HPLC, LC-MS, etc.


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.

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, 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, 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.

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.

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.

Fructosyl-arginine (Fru-Arg) is a unique compound only found in AGE and no other garlic products have this kind of compounds in it. Fru-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.

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 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.
Although less bioavailable (<10%) than SAC in garlic, Ichikawa et al. (2006), suggest that cycloalliin is more suitable as a chemical marker for garlic due to its highly stable nature when stored or processed.

In a review on AGE, it was stated how garlic gains antioxidant activity during the aging process via different constituents found in garlic: SAC, Fru-Arg, and tetrahydro-β-carbolines. Liquid chromatography mass spectrometry (LC/MS) data showed all of the compounds were either not present or were in extremely lower concentrations in raw garlic, but were increased during the aging process.

The history of garlic use in folk medicine dates back to ancient times when it was supplied to the workers building the pyramids. Even the ancient Roman civilization used garlic as a tonic. Currently, garlic is one of the most studied plants with most research focusing on its unique chemistry; it contains more than 5% sulfur compounds, and its biological activities, such as cholesterol lowering effects, and anti-platelet aggregation. Thus, the focus of this paper is the history of garlic and research completed on its unique chemistry.
Review of the Benefits of AGE, SAC, and SAMC


In a review, Borek discussed the numerous benefits of AGE. With over 580 research studies, AGE has been shown to be beneficial in many areas including cardiovascular protection (i.e., lowering cholesterol, blood pressure, etc.), increase in circulation, enhance immunity, and preventing many forms of cancer and neurogenerative disease. AGE has also been shown to have anti-aging effects that may help with improving memory, endurance and learning.

Garlic, especially Aged Garlic Extract (AGE) has been mentioned and reviewed in this article as a part of Encyclopedia of Food Science. It introduces wide variety of benefits from garlic based upon the scientific literature of AGE.

In a study using HPLC and LC/MS, S-allyl-L-cysteine (SAC), fructosylarginine, and 1,2,3,4-tetrahydro-ß-carboline-3-carboxylic acids were shown to be principal bioactive components of aged garlic extract. These 3 compounds were present and were found to be more stable and bioactive than thiosulfinates. The levels of these components were also found to increase during the aging process. This suggests that additional health benefits may be provided by the aging process in garlic.

The sulfur-containing compounds in garlic may modify cancer-cell growth by targeting anti-oxidant pathway, which regulates cell growth or death. Among the various chemicals, S-allyl mercaptocysteine (SAMC), only found in Aged Garlic Extract (AGE) has significant effect on this pathway. Detailed cancer cell control by SAMC has been described.

The supplement discusses the health benefits of culinary herbs and spices. In particular, reviews of aged garlic extract studies indicate that it may prove beneficial in cardiovascular health. Clinical trials examine the effect of garlic on lipid levels and lipoproteins, as well as its anticlotting and blood pressure effects. Other studies find that herbs and spices, including garlic and its constituents, may have an inhibitory effect on carcinogens at all stages of the cancer process by interfering with Phase I and Phase II enzymes.

In a review on garlic, both water-and lipid soluble allyl sulfur compounds are effective in blocking a myriad of chemically induced tumors through different mechanisms and reactions that happen in the body. Also, that anticarcinogenic potential of garlic can be influenced by several dietary components including specific fatty acids, selenium and vitamin A.

The ancient tradition of utilizing garlic would qualify it as part of "folk medicine" or "alternative/complementary medicine". Scientists from around the world have identified a number of bioactive substances in garlic that are water-soluble (e.g., S-allylmethycysteine) found many in AGE, and fat-soluble (e.g., S-allylsulfide). Mechanisms of action are being elucidated by modern technology. The current state of knowledge will find a place as a "complement" to known methods of disease prevention and treatment. Our goal now should be to examine garlic together with other agents to evaluate possible efficacy and toxicity under conditions of actual use in humans.

This article is an overview of garlic product and regulatory situation in Europe. Many garlic products including Kyolic/Aged Garlic Extract have been marketed as herbal medicine or food supplements in Europe for some medicinal purposes.
tumor inoculation. It has been reported to potentiate and restore various kinds of immune reactions, and suppress the growth of allogenic or syngenic tumors even when administered prior to their development. PSK, a protein-bound polysaccharide derived from the mushroom, has demonstrated an array of benefits in various studies. Raw garlic contains only small amounts of SAC, which increases with aging and is standardized in the Aged Garlic Extract preparations. SAC (S-allyl cysteine) is a water-soluble, sulfur-containing amino acid found in processed garlic (except garlic oil). It is odorless and has a antibacterial activity.

Adducts develop in the initiation stage of cancer where a carcinogen binds to DNA and initiates a change. At this point, various enzymes act to repair the DNA. If the systems are adequate, the damaged DNA will be fixed and repaired. However, if such systems are inadequate, the damaged DNA will begin to reorder the DNA to its proper sequence. This process is called repair, and the DNA will be repaired. If the DNA is not reordered correctly, it will be changed and the cell will begin to die. This process is called apoptosis.

As seen in this paper, Milner et al. (1990) found a 96% correlation between adducts and final tumor development. Hence, the presence of adducts pretty well predicts future tumor development.

Apoptosis is the fragmentation of cells into tiny particles that are then engulfed by phagocytes or immune cells. It is a controlled cell death mechanism, and it is important to prevent the formation of adducts.

Bioactivation is the process by which chemical compounds are converted into reactive species, such as free radicals or oxidants. BP (Benzo-(a)-pyrene) is a potent procarcinogen causing a number of animal tumors. It is found in cigarette smoke, charcoal broiled meat and automobile exhaust. Foods that are processed or cooked at high temperatures, especially charbroiling of foods rich in fats or carbohydrates, typically results in the generation of BP.

Bioactivation takes place when nitrite, a preservative typically added to foods such as hot dogs and lunch meats, combines with endogenous nitric oxide or thiobarbituric acid reductive substances, are indicators of oxidation, where a high level indicates increased generation of free radicals or oxidants.

Lactate dehydrogenase (LDH) is an enzyme found inside of cells that leaks out into the culture medium when cell membranes are damaged. The amount of free radicals or oxidants produced in the body is an indicator of cell membrane damage. A higher level of LDH in the solution indicates a greater level of membrane damage.

Cut off from their blood supply, cells begin to die and produce free radicals. Also, when the obstruction is cleared and oxygen-carrying blood returns to the vessel, significant oxidation can take place.

Starting with the obstruction of a blood vessel, there is a disruption of the endothelial cells lining the vessel, causing a secondary narrowing or constriction of the vessel. This narrowing or constriction is called restenosis. SMC (smooth muscle cell) proliferation is the excessive growth and transformation of smooth muscle cells (SMC) that line blood vessels. It takes place once a scar or injury has damaged the lining of a vessel to sort of “patch” the scar. SMC can change into immune cells.

Vitamin C is well known for preventing the formation and damage caused by these compounds. Glutathione S-transferases are internal enzymes critical for detoxification of chemical compounds such as carcinogens. Glutathione is the excessive growth and transformation of smooth muscle cells (SMC) that line blood vessels. When cells are cut off from their blood supply, they begin to die and trillions of free radicals are produced. Also, when the obstruction is cleared and oxygen-carrying blood returns to the vessel, significant oxidation can take place.

Since high cholesterol levels are at least one factor strongly associated with an increased risk of developing heart disease, the NCEP was established to help reduce cholesterol levels. It has published specific guidelines which include a goal of reducing total cholesterol to <200 mg/dl, LDL cholesterol <160 mg/dl or <130 mg/dl if two or more risk factors for heart disease are present. Such risk factors include a family history of heart disease, smoking cigarettes, diabetes, obesity, hypertension, low HDL or being male. Dietary advice includes a low fat diet containing <30 percent of total calories coming from fat, less than 10% of fat calories coming from saturated fat, and less than 300 mg cholesterol per day. This is usually recommended for six months and if ineffective, saturated fat is further reduced to 7% of total calories and cholesterol intake is reduced to <200 mg per day. Medication is recommended if dietary approach is not successful.

Angioplasty is a procedure where a balloon catheter is inserted into an artery blocked by a plaque. The catheter is then inflated to flatten the plaque against the wall of the artery. Angioplasty is intended to make a wider opening for blood to flow. However, following angioplasty, endothelial cells lining the vessel are disrupted and “fatty streaks” (regions of accumulated cholesterol, especially oxidized cholesterol) begin to develop. Ultimately, these fatty streaks can cause a secondary narrowing or constriction of the vessel.

SAC (S-allyl cysteine) is a potent immomodulatory treatment widely used in human cancer therapy. It is a deficiency of blood to tissues, organ or cells due to constriction or actual obstruction of blood vessels. When cells are cut off from their blood supply, they begin to die and trillions of free radicals are produced. Also, when the obstruction is cleared and oxygen-carrying blood returns to the vessel, significant oxidation can take place.

LDH (lactate dehydrogenase) is an enzyme found inside of cells that leaks out into the culture medium when cell membranes are damaged. The amount of free radicals or oxidants produced in the body is an indicator of cell membrane damage. A higher level of LDH in the solution indicates a greater level of membrane damage.