Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

Pharmacologic Activities of Aged Garlic Extract in Comparison with Other Garlic Preparations[1]

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ABSTRACT We investigated the pharmacologic activities of four garlic preparations, raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (DGP) and aged garlic extract (AGE). The study used three animal models, i.e., testicular hypogonadism (hypospermatogensis and impotence) induced by warm water treatment, intoxication of acetaldehyde and growth of inoculated tumor cells. RGJ was found to be effective only in recovery of testicular function. The efficacy of HGJ was observed in three models; however, it did not improve impotence. DGP was effective in recovery of spermatogenesis and stimulated acetaldehyde detoxification. Significant beneficial effects of AGE were found in all three models. Although all four garlic preparations significantly enhanced natural killer (NK) and killer cell activities of the spleen cells of tumor-bearing mice, only AGE and HGJ inhibited the growth of inoculated tumor cells. These results suggest that different types of garlic preparations have different pharmacologic properties, and among the four garlic preparations studied, AGE could be the most useful garlic preparation. J. Nutr. 131: 1080S–1084S, 2001.

KEY WORDS: • *garlic* • *pharmacology* • *mice*

Many health foods and plant-derived substances touted to improve health are sold around the world. However, conclusive evidence for their benefits is frequently lacking. Garlic (*Allium sativum*) is believed by many people to be useful for disease prevention. In ancient Egypt and Rome, garlic was given to laborers and soldiers, possibly to mitigate fatigue or to prompt recovery from physical exhaustion (Essman 1984). After that period, garlic was used to treat hypertension, cardiac disease, inflammation, diabetes and cancer, according to folk medicine (Essman 1984). Research has recently focused on the preventative and curative effects of garlic on cardiovascular disease (Jacob et al. 1993, Randerson 1993) and cancer (Block 1994, Dorant 1993).

The different garlic products that are marketed may be divided into allicin potential products and nonallicin potential products. The former are made from raw garlic and the latter are made from so-called processed garlic. All may differ significantly in the substances they contain. Thus, questions arise concerning whether all garlic products are created equal and which forms are best for improving health.

The purpose of these investigations was to compare garlic products for improving health. We investigated the pharmacologic activities of four garlic preparations, raw garlic juice (RGJ),[3] heated garlic juice (HGJ), dehydrated garlic powder (DGP) and aged garlic extract (AGE), using three animal models, i.e., testicular hypogonadism (hypospermatogenesis and impotence), acetaldehyde intoxication and growth of tumor cells.

MATERIALS AND METHODS

Animals. Male ddY or ICR mice were purchased from Japan SLC (Hamamatsu, Japan). They were kept in an air-conditioned room (23 ± 3°C, 55 ± 10% humidity) and had free access to a commercial diet (CE-2, Kurea Japan, Tokyo, Japan) and water.

Garlic preparations. The garlic preparations used in this study were raw garlic juice (RGJ), heated garlic juice (HGJ), dehydrated garlic powder (DGP) and aged garlic extract (AGE).

RGJ was prepared from skinned raw garlic cloves that were crushed in a Waring blender for 1 min, together with an equal weight of water. The mixture was then allowed to stand for 30 min at 25°C. After filtration through cheesecloth, the raw garlic sample was obtained.

HGJ was prepared from skinned raw garlic cloves that were heated to 95°C for 30 min. The preparations for HGJ were identical to those used for RGJ. DGP, one of the garlic preparations used as material for food supplements and sold over the counter in Japan, was purchased commercially. It has been said to be composed mainly of the sulfur-

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[3] Abbreviations: AGE, aged garlic extract; DGP, dehydrated garlic powder; HGJ, heated garlic juice; NK, natural killer; RGJ, raw garlic juice.

containing glycoside "scordinine." AGE is manufactured by Wakunaga Pharmaceutical as follows: garlic cloves were sliced and soaked in a water/ethanol mixture and naturally extracted/aged for 10 mo at room temperature.

Each of these garlic preparations, except for DGP, was stored in the freezer at −80°C until they were adjusted to 15% extracts with distilled water before administration to the mice.

Contents of alliin and allicin in four garlic preparations. RGJ contained 0.162% allicin but no alliin. HGJ contained 0.266% alliin and 0.001% allicin. DGP contained 0.462% alliin but no allicin. AGE contained 0.003% alliin but no allicin.

Experiment 1

The effect on testicular hypogonadism. Induction of hypogonadism in mice was conducted using the modified method of Amendola et al. (1990). Male mice (ddY strain, 10 wk old, n = 10/group) were placed in cages (150 mm in length × 40 mm in diameter) and their pelvic regions were immersed in 42°C water for 30 min. After 1 d of warm water treatment, garlic preparations were administered orally by stomach tube at doses of 4 mL/(kg · d) for 13 d. Male sexual behavior was tested 13 d after warm water treatment. Each male mouse was mated with two estrous female mice that had been injected subcutaneously with both estradiol (1 mg/kg, every 3 d) and progesterone [10 μg/(kgd)] for 13 d. All mounting and intromission behaviors were recorded during a 30-min test period. The test was performed between 18:00 and 24:00. We considered suppression of sexual behavior as an impotence condition. One day after the evaluation of sexual behavior, mice testes were removed under deep diethyl ether anesthesia to assess the recovery of spermatogenesis. The organs were immediately placed into Bouin's fixative and fixed for 24 h. Fixed organs were subsequently embedded in paraffin wax. Sections (3 μm in thickness) were cut and stained using the periodic acid/Schiff procedure for classification according to the steps of spermatid growth. Seminiferous tubules were divided into three stages of development by classification of spermatids as follows: *1*) immature tubules (below step 4 spermatids); *2*) middle level of maturation (between step 5 and 8 spermatids); and 3) almost mature tubules (over step 9 spermatids). In this experiment, the effects of garlic preparations on recovery of spermatogenesis were calculated by the quantity of spermatids-bearing seminiferous tubules vs. the quantity of almost mature tubules. Testosterone (Sigma Chemical, St. Louis, MO) was used in this experiment as an active drug. It was injected subcutaneously at doses of 10 μg/kg every 3 d during the 13-d test period (n = 5 administrations).

Experiment 2

The effect on acetaldehyde intoxication. Male mice (ddY strain, 7 wk old) were injected with acetaldehyde (Wako Pure Chemical, Osaka, Japan) at doses of 500 mg/kg to induce acute acetaldehyde intoxication. Garlic preparations were administrated orally by stomach tube at doses of 30 mL/kg 1 h before the injection of acetaldehyde. The number of mice surviving 5 d after acetaldehyde administration was counted and the survival rate was calculated. In this assessment, if mice survived >5 d, their survival period was recorded as 5 d.

Experiment 3

Effect of garlic on the growth of inoculated tumor cells and immunoresponder cells. This experiment was conducted according to the method of Kyo et al. (1998). ICR strain male mice (7 wk old) were inoculated in the hypoderm of the back with 10^6 cells of Sarcoma-180 (American Type Culture Collection, Rockvillle, MD) in 100 μL of PBS. Twenty-four hours after carcinoma cell inoculation, garlic preparations were administrated orally by stomach tube at doses of 10 mL/kg every other day for 3 wk (n = 11 administrations). The size of the tumors was measured using a micrometer and the volume was calculated by the following formula:

$$\text{Tumor volume (mm}^3\text{)} = 0.75 \times (\text{shortest diameter})^2 \times (\text{longest diameter})^2$$

After measurement of the tumor size, natural killer (NK) and killer cell activities of spleen cells from tumor-bearing mice were measured. ^{51}Cr-labeled YAC-1 cells (American Type Culture Collection) were used for NK activity and ^{51}Cr-labeled Sarcoma-180 cells were used for killer cell activity. Spleen cells (7.5×10^5 cells) and ^{51}Cr-labeled YAC-1 cells or Sarcoma-180 cells (1.5×10^4 cells) were incubated for 24 h under 5% CO_2 /95% air at 37°C. After incubation, the medium was centrifuged at 15,000 *g* and the released ^{51}Cr in the supernatant was counted in a γ-counter. The cytotoxicity was calculated by the following formula:

$$\%\text{Cytotoxicity} = [(\text{experimental release} - \text{spontaneous release})/(\text{maximum release} - \text{spontaneous release})] \times 100$$

As an active drug, Krestin (Kureha Chemical, Japan) was used in this experiment, which is approved as an anticancer drug by the Ministry of Health and Welfare of Japan and widely applied to cancer patients, is a protein-bound polysaccharide derived from the mushroom, *Coriolus versicolor*. It was administered orally at 1 μg/kg in the same manner as administration of garlic preparations.

Statistical analyses. The data were analyzed with the SAS/STAT Software, release 6.12 (SAS Institute, Cary, NC). Data in tables are presented as means ± SEM.

RESULTS

Experiment 1

Effect on testicular hypogonadism. Tables 1 and 2 show the results of sexual behaviors. Appearance of mounting of the normal group, which was not treated with warm water, was 100%. That of the control, which was administered with water every day, was significantly reduced to 50% ($P < 0.05$). RGJ, HGJ, DHP, AGE and testosterone inhibited the reduction in appearance of mounting induced by warm water treatment; however, only AGE significantly restored the behavior ($P < 0.05$). Normal mice had 8.0 mountings within 30 min, whereas control mice showed only 4.6 mountings. RGJ, HGJ and DHP had no effect. AGE and testosterone inhibited the reduction in the number of actual mountings, but their effects were not significant. Appearance of intromission in the nor-

TABLE 1

Effect of garlic preparations on reduced mounting behaviors induced by warm water treatment[1,2]

Group	Appearance rate of mounting	Counts of mounting
	%	
Normal	100	8.0 ± 1.7
Control	50#	4.6 ± 2.1
RGJ	70	4.5 ± 1.5
HGJ	90	5.5 ± 1.5
DGP	70	4.7 ± 1.4
AGE	100*	7.0 ± 1.3
Testosterone	90	6.5 ± 1.4

1 Values are means ± SEM; # $P < 0.05$, compared with normal group (analyzed with Student's *t* test); * $P < 0.05$ compared with control group (analyzed with Fisher's exact test).

2 RGJ, raw garlic juice; HGJ, heated garlic juice; DGP, dehydrated garlic powder; AGE, aged garlic extract.

TABLE 2

Effect of garlic preparations on reduced intromission behavior induced by warm water treatment[1]

Group	Appearance rate of intromission	Counts of intromission
	%	
Normal	80	3.9 ± 1.3
Control	20#	1.2 ± 0.8
RGJ	50	1.7 ± 0.8
HGJ	40	1.4 ± 0.7
DGP	30	0.5 ± 0.3
AGE	70	3.2 ± 0.9
Testosterone	60	2.7 ± 0.8

[1] Values are means ± SEM; # $P < 0.05$, compared with normal group (analyzed with Student's *t* test). See Table 1 for abbreviations.

mal group was 80%. That of control mice was significantly reduced to 20% ($P < 0.05$). RGJ, DHP and HGJ slightly inhibited the reduction in appearance of intromission, but their effects were not significant. AGE and testosterone moderately inhibited the reduction in appearance of intromission but their effects also were not significant. Actual intromission in the control group was 3.9 within 30 min, whereas intromission in the control mice was reduced to 1.2.

Table 3 shows body and testis weights 14 d after warm water treatment. Body and testis weights were not different among garlic preparation and testosterone groups.

Table 4 shows the results of the recovery of spermatogenesis. Warm water treatment induced death of spermatocytes and spermatids in seminiferous tubules. In control mice, spermatid-bearing tubules comprised 2.95%. AGE and testosterone significantly enhanced spermatid-bearing tubules ($P < 0.05$ and $P < 0.01$, respectively), whereas RGJ only slightly enhanced them. HGJ and DGP showed no effects. Almost mature seminiferous tubules, bearing >step 9 spermatids, were 0.2% in the control group. RGJ, HGJ and DGP showed no effect on the appearance of almost mature tubules, whereas AGE and testosterone enhanced their recovery.

Experiment 2

Effects on acetaldehyde intoxication. **Table 5** shows survival rates of mice injected with acetaldehyde intraperitoneally. All of the control mice died within 5 d after acetaldehyde injection. RGJ could not protect against acetaldehyde-induced death. HGJ was slightly effective. DGP and AGE significantly protected against acetaldehyde intoxication ($P < 0.05$, 0.01, respectively). The survival period of the control group 5 d after acetaldehyde administration was 0.7 d. RGJ showed no effect. HGJ, DGP and AGE prolonged the survival period, and the effect of AGE was significant ($P < 0.01$) (Table 5).

TABLE 3

Effect of garlic preparations on weight of body and testis[1]

Group	Body weight	Testis weight
	g	
Normal	40.8 ± 0.6	0.283 ± 0.013
Control	39.6 ± 0.6	0.094 ± 0.004
RGJ	39.7 ± 0.5	0.089 ± 0.004
HGJ	40.5 ± 0.7	0.094 ± 0.004
DGP	40.4 ± 0.8	0.092 ± 0.004
AGE	40.7 ± 0.8	0.091 ± 0.003
Testosterone	40.4 ± 0.8	0.093 ± 0.007

[1] Values are means ± SEM. See Table 1 for abbreviations.

TABLE 4

Effect of garlic preparations on recovery of spermatogenesis[1]

Group	Appearance rate of spermatid-bearing tubules	Appearance rate of over step 9 spermatid-bearing tubules
	%	
Control	2.95 ± 0.89	0.20 ± 0.13
RGJ	6.00 ± 1.44	0.05 ± 0.05
HGJ	4.80 ± 0.84	0.05 ± 0.05
DGP	5.85 ± 1.54	0.25 ± 0.20
AGE	10.55 ± 3.12*	1.05 ± 0.42
Testosterone	12.70 ± 3.24**	1.55 ± 1.17

[1] Values are means ± SEM; * $P < 0.05$, ** $P < 0.01$, compared with control group (analyzed with Dunnet's multiple comparison test). See Table 1 for abbreviations.

Experiment 3

Effects on the growth of inoculated tumor cells and immunoresponder cells. Body weight of the mice did not differ among the groups during the 3-wk administration of garlic preparations with Krestin (data not shown). **Table 6** reveals the size of tumors resulting from growth of inoculated Sarcoma-180 cells. The tumor size of the control was 228 mm^3. RGJ and DGP treatment did not affect the growth of the tumor. Similarly, although HGJ and AGE reduced mean tumor size compared with controls, the effect was not significant. The effect of Krestin was significant ($P < 0.05$). To determine the relationship between inhibition of tumor cell growth and immune-enhancing properties, we determined NK and killer cell activities of spleen cells prepared from Sarcoma-180 cell–bearing mice. As shown in **Table 7**, NK cell activity against YAC-1 cells was moderately enhanced by all garlic preparations and Krestin. Furthermore, killer cell activity against Sarcoma-180 cells was significantly enhanced by all garlic preparations and the effects were similar to those of Krestin (Table 7).

TABLE 5

Effect of garlic preparations on acetaldehyde intoxication[1]

Group	Survival ratio	Survival period
	%	*d*
Control	0	0.7 ± 0.4
RGJ	0	0.9 ± 0.3
HGJ	40	2.2 ± 0.6
DGP	50*	2.4 ± 0.7
AGE	80**	3.2 ± 0.5**

[1] Values are means ± SEM; * $P < 0.05$, ** $P < 0.01$, compared with control group (survival ratio was analyzed with Fisher's exact test and survival period was analyzed with Dunnet's multiple comparison test). See Table 1 for abbreviations.

TABLE 6

Effect of garlic preparations on the growth of inoculated tumor cells[1]

Group	Size of tumor
	mm^3
Control	228 ± 55
RGJ	240 ± 58
HGJ	173 ± 51
DGP	252 ± 47
AGE	138 ± 23
Krestin	84 ± 15*

[1] Values are means ± SEM; * $P < 0.05$, compared with control group (analyzed with Dunnet's multiple comparison test). See Table 1 for abbreviations.

DISCUSSION

Pharmacologic activities of four garlic preparations (RGJ, HGJ, DHP and AGE) were investigated using three animal models in these studies. RGJ was effective only in the recovery of testicular function. The efficacy of HGJ was observed in three models; however, it did not improve impotence. DGP improved recovery of spermatogenesis and stimulated acetaldehyde detoxication. Beneficial effects of AGE were demonstrated in all three models.

Carlsen et al. (1992) first reported a decline in sperm density of human semen during the past 50 years. Similarly, Pajarinen et al. (1997) reported that the incidence of normal spermatogenesis decreased among middle-aged Finnish men between 1981 to 1991. They also reported the incidence of disorders of spermatogenesis and pathologic alterations in testes. It has been suggested that these alterations in male reproductive organs were induced by environmental toxins and chemicals such as alcohol, drugs and industrial solvents. Marked decrease of sperm density is related to male sterility, which is diagnosed as male sexual dysfunction. In urology, male sexual dysfunction includes both hypospermatogenesis and impotence. In oriental medicine, several herbs, including garlic, have been used to improve male sexual dysfunction since ancient times. Consequently, we examined garlic using the testicular hypogonadism model. Antispermatogenic chemicals, such as antitumor agents in the model of Gomes et al. (1973) or agents in the cryptorchidism model (Amatayakul et al. 1971), have been used to evaluate male antisterility therapies. These models suffer from the lengthy period required for evaluating a response. We used a simpler model, namely, the warm water model. Male sterility can be divided into hypospermatogenesis and impotence. Ideally antisterility substances should influence both symptoms. Interestingly, the warm water model influenced not only hypospermatogenesis but also the reduced behavior. Male sexual behaviors that were influenced included mounting and intromission with estrous females.

Our results showed that AGE significantly enhanced spermatogenesis after warm water treatment. RGJ, HGJ and DHP, on the other hand, were only slightly effective. Water-administered mice had decreased intromission counts compared with the normal group. AGE significantly improved impotence, whereas RGJ and HGJ were only slightly effective. DGP showed no effect. The effects of AGE were equivalent to those of testosterone. We recently demonstrated that AGE significantly improved peripheral circulation in a cooling rewarming test, which evaluated peripheral circulation. Again, the other three garlic preparations had no effect (Ushijima et al. 1997). The anti-impotence response to AGE may be due to its effects on peripheral circulation. Because oxygen and nutrients are obviously required in spermatogenesis, and it is well known that blood circulation to the testis is poor, improvement in circulation would likely result in improved nutriture and thus spermatogenesis. Furthermore, our previous studies (Ushijima et al. 1997) revealed that AGE significantly improved physical performance, prolonging both active swimming and treadmill running in rodents. Thus, it is possible that AGE might enhance the supply of oxygen to seminiferous tubule cells and stimulate energy metabolism in the testis.

Certain garlic products are known to be hepatoprotective against liver toxins (Nakagawa et al. 1989, Sumioka et al. 1998). Alcohol is one toxin known to cause liver failure in the case of chronic overconsumption. The toxicity of alcohol is due in part to acetaldehyde, which is metabolized from alcohol in the liver. Acetaldehyde is subsequently metabolized into acetic acid by mitochondial aldehyde dehydrogenase. Enhancement of aldehyde-dehydrogenase activity is one way in which to avoid acetaldehyde intoxication. In the present studies, all mice receiving only water died within 5 d after acetaldehyde treatment. AGE and DGP protected against acetaldehyde intoxication. HGJ was also slightly effective, although RGJ was not. It is unclear what might explain the protection provided by AGE and DHP, although enhanced aldehyde dehydrogenase is a possibility. Further studies are clearly required.

AGE inhibits the growth of transplanted tumor cells and enhanced NK and killer cell activities (Kyo et al. 1998). In the present studies, AGE inhibited the growth of sarcoma-180 cells transplanted in mice. Although HGJ was slightly effective, RGJ and DHP were not effective. All four garlic preparations were equivalent in their ability to promote NK cell activity, i.e., show nonspecific cytotoxicity of the immune system, and killer cell activity, i.e., show specific cytotoxicity. These results suggest that AGE and HGJ serve as potent biological response modifiers on NK and killer cells, and subsequently inhibit the growth of tumor cells. However, other factors appear to be involved in the antiproliferative effects of AGE.

From our experiments, AGE was the most effective compared with the other garlic preparations examined. In the experiment on antitumor activity, the four garlic preparations equally enhanced NK and killer cell activities, but RGJ did not inhibit the growth of inoculated tumor cells. The differ-

TABLE 7

Effect of garlic preparations on natural killer (NK) and killer cell activities of spleen cells[1]

Group	NK activity	Killer activity
	% cytotoxicity	
Control	6.6 ± 01.1	0.7 ± 0.7
RGJ	8.1 ± 1.2	4.1 ± 0.7*
HGJ	8.9 ± 0.7	4.4 ± 0.6*
DGP	11.1 ± 1.4*	5.9 ± 1.4**
AGE	8.8 ± 1.0	4.4 ± 0.8*
Krestin	8.8 ± 1.1	4.5 ± 1.3*

[1] Values are means ± SEM; * $P < 0.05$, ** $P < 0.01$, compared with control group (analyzed with Dunnet's multiple comparison test). See Table 1 for abbreviations.

ence between RGJ and other three garlic preparations is the presence of allicin, i.e., garlic has potential antitumor activity, but allicin appeared to suppress its potency. The reason why RGJ did not produce the same response might relate to its oxidative activity. Imai et al. (1994) investigated the antioxidant effects of RGJ, HGJ and AGE using the low level chemiluminescence method accompanied by lipid peroxidation. They found that RGJ and HGJ enhanced the emission, whereas AGE was inhibitory. The reduced effectiveness of RGJ might be due to one or several oxidative components.

These findings provide evidence that different garlic preparations have different pharmacologic properties. Among the four garlic preparations studied, AGE was most consistent in the models examined. Further investigations are required to clarify which compounds in AGE contribute to these protective effects.

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