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Effect of Garlic on the Hepatic Glutathione S-Transferase and Glutathione Peroxidase Activity in Rat

Garlic Effect on the Glutathione S-Transferase and Glutathione Peroxidase

Keun Huh, Jong-Min Park and Sang-Il Lee

Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 632, Korea

Address for correspondence: *Keun Huh, Department of Pharmacology, College of Pharmacy, Yeungnam University, Gyongsan 632, Korea*

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Abstract □ It was attempted to observe the effect of garlic on the hepatic glutathione s-transferase and glutathione peroxidase activity in this study. Glutathione s-transferase (EC 2.5.1.18) are thought to play a physiological role in initiating the detoxication of potential alkylating agents, inclnding pharmacologically active compounds. Glutathione peroxidase (EC 1.11.1.9) might play an important role in the protection of cellular structures against oxidative challenge. The activities of glutathione s-transferase and glutathione peroxidase in rat liver were increased by the treatment of garlic juice. Allicin fraction, heat-treated allicin fraction and garlic butanol fraction markedly inhibited glutathione s-transferase activity *in vitro*, whereas glutathione peroxidase activity was significantly increased in heat-treated allicin fraction and garlic butanol fraction.

Keywords □ Allicin, Glutathione s-transferase, Glutathione peroxidase

The widespread use of garlic(*Allium sativum* L.) as a flavoring agent in food is well known. It is also known that garlic has various pharmacological effect and medicinal purpose[1,2]. A scientific basis for the medicinal use of garlic extract was established by Lehmann[3].

Recent studies have shown that garlic components regulate the many metabolic diseases, such as atherosclerosis[4,5], diabetes[6,7] and gout[8].

Glutathione s-transferase, phase II enzyme, are regarded as detoxifying enzyme, which catalyzed the first step in mercapturic acid formation[9,10]. They conjugate glutathione with a large number of electrophilic compounds, many of which are potentially dangerous to the cell function[11,12,13].

By the way, it was reported that glutathione peroxidase protects the cell from hydrogen peroxide and organic hydroperoxides by reduction with concomitant oxidation of reduced glutathione[14,15,16].

These enzymes are widely distributed among eukaryotes and often presented in relatively high concentration intracellularly[17]. They are thought to play an important role in the biotransformation of many pharmacologically active compounds with glutathione intermediated biochemical reaction[18,19].

In the previous study, it was recognized that garlic components increased microsomal drug metabolizing enzyme activity, phase I enzyme which is concerned to first step metabolism of drugs, in liver[20].

Therefore, the present work was undertaken to study the effect of garlic juice, allicin fraction, heat-treated allicin fraction and garlic butanol fraction on the hepatic glutathione s-transferase, phase II enzyme, and glutathione peroxidase.

EXPERIMENTAL METHODS

Materials

Glutathione reductase, bovine serum albumin, NADPH were obtained from Sigma chemical Co. and 1-chloro 2, 4-dinitrobenzene from Junsei chemical Co. and reduced glutathione from Fluka A.G. The other reagents used were of reagent grade.

Isolation of allicin and garlic butanol fraction

Allicin fraction (1g) was obtained from 2kg of garlic bulbs according to the modified procedure of the method of Cavallito *et al*[21]. The procedure for the isolation of allicin fraction is shown in scheme 1.

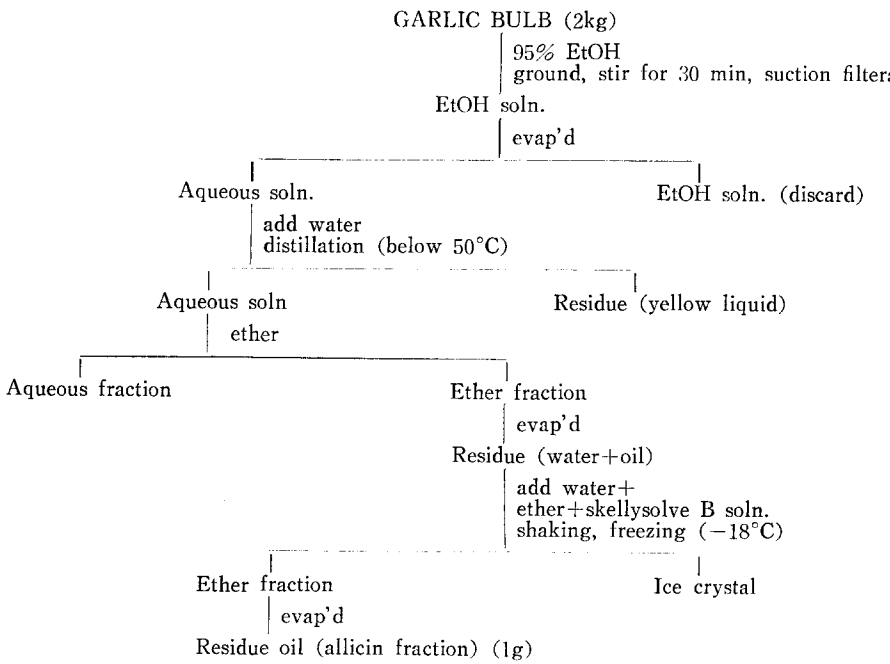
Garlic butanol fraction (7g) was obtained from 1kg of garlic bulbs according to the method of Namba *et al*[22].

Treatment of animals

60 male Sprague-Dawley rats weighing 150~160g were used for all studies. They were devided into 3 groups. One group, the control, received water and another group received 5% boiled garlic juice, a third group received 5% fresh garlic juice instead of water. This dietary regimen was continued for 25 days before sacrifice. All experimental rats were allowed free access to food and water but deprived of food for 16 hours prior to sacrifice.

Preparation of liver cytosolic and microsomal fraction

The animals were killed by exsanguination from the abdominal aorta. The liver was exhaustively perfused with cold 0.15 M NaCl soln. through the hepatic veins until it was uniformly pale and quickly removed. After mincing, the pieces of liver were homogenized in cold 0.25 M



Scheme 1. Isolation of allicin fraction from garlic bulb.

sucrose by three passes of a motor-driven teflon pastle in a glass homogenizing vessel. The homogenate (20% w/v) was sequentially centrifuged at 600×g, 10,000×g and 105,000×g. Then the 105,000×g supernatant was used as the cytosolic fraction and pellet as the microsomal fraction.

Enzyme assay

Glutathione s-transferase activity was assayed by monitoring the formation of the thioether between glutathione and 1-chloro-2, 4-dinitrobenzene according to the procedure of Habig *et al*[23]. Enzyme activity is defined as n mole per mg protein per minute at 25°C. Glutathione peroxidase activity was measured spectrophotometrically by a modification of the procedure described by the Paglia *et al*[24]. The standard assay mixture (3 ml) contained 0.1 M Tris-HCl, pH 7.2, 1 mM glutathione, 0.2 mM NADPH, 2 IU glutathione reductase, 0.25 mM hydrogen peroxide and enzyme soln. Protein was determined by the method of Lowry *et al*[25], and bovine serum albumin as standard.

Serum transaminase (alanine transaminase) activity was estimated according to the procedure described by Reitmann and Frankel[26]. A unit of transaminase is expressed as the Karmen unit[27] per ml of serum.

RESULTS and DISCUSSION

The effect of garlic juice on the body weight and serum transaminase activity Shown as Fig. 1, the body weight (A) and liver weight (B) were not changed significantly by the treatment of garlic juice comparing with control group. It was also observed that the activity of serum alanine transaminase was not changed comparing to the control group (Fig. 1. C).

These results indicated that garlic treatment to rats would not induce hepatocellular damage in this experimental condition.

The effect of garlic juice on the hepatic cytosolic glutathione s-transferase Table I shows the increment of liver cytosolic glutathione s-transferase activity following repeated-administration of garlic juice during 5, 10, 15, 25 days. Time dependent increase of glutathione s-transferase in garlic juice-fed rats were compared

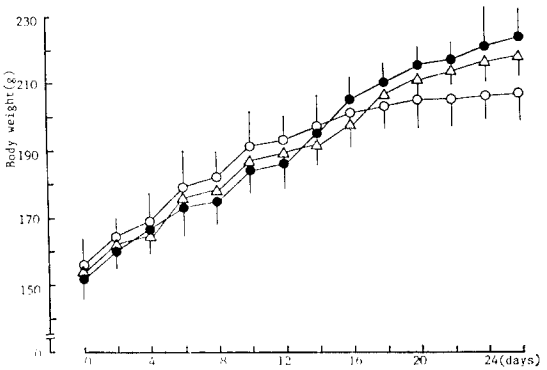


Fig. 1-A. Changes of body weight in male rats fed the garlic juice.
The garlic juice was offered until the time of sacrifice. Values are mean±S.E. of 5 rats in each group. -○-: Control group, -●-: 5% boiled garlic juice fed group, -△-: 5% fresh garlic juice-fed group.

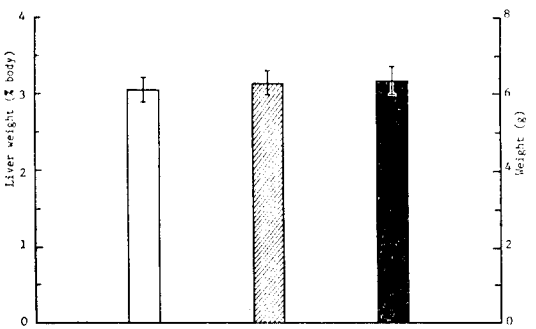


Fig. 1-B. Effect of garlic on the liver weight in male rats.
Garlic was fed instead of water for 25 days. Values are mean±S.E. of 5 animals in each group. □: Control group, ▨: 5% boiled garlic fed-group, ■: 5% fresh garlic fed-group

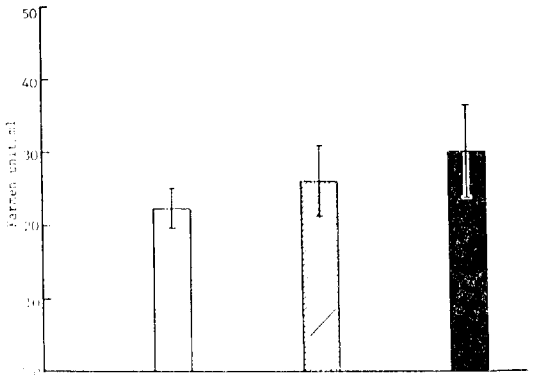


Fig. 1-C. Effect of garlic on the serum alanine transaminase activity in rats.
Garlic was fed instead of water for 25 days. The assay procedure was described in the text. Values are mean±S.E. of 5 animals in each group. □: Control group, ▨: 5% boiled garlic juice-fed group, ■: 5% fresh garlic juice fed-group

with that of control group. The increasing effect was greater in boiled garlic juice than fresh garlic juice-treated group. This result suggested that the heat stable components may play a role of the increasing enzyme activity.

The effect of garlic juice on the hepatic microsomal glutathione s-transferase The activity of liver microsomal glutathione s-transferase in rats were not significantly changed following feeding of 5% garlic juice (Table II). It suggested that garlic may susceptibly influenced enzyme activity in cytosolic fraction than microsomal fraction. The discrepant result in microsomal enzyme activity compared to cytosolic enzyme was not clearly understood.

The effect of garlic on the hepatic glutathione peroxidase activity Fig. 2 shows the effect of garlic treatment on the activity of the liver glutathione peroxidase. The activity of glutathione preoxidase was induced by garlic treatment. Furthermore, the enzyme activity was significantly enhanced by boiled garlic juice treatment. As mentioned above, it suggested that some heat stable substances of garlic might increase the hepatic glutathione peroxidase activity.

The effect of allicin fraction on enzymatic activity of cytosolic glutathione s-transferase in vitro

Table I. Changes of hepatic cytosolic glutathione s-transferase in male rats fed the garlic juice for 25 days

Treatment \ Days	0	5	10	15	25
		(n moles/mg protein/min)			
Control	366±23.3	354±18.7	369±19.5	363±20.1	370±20.6
5% boiled	366±23.3	385±16.5	405±13.6	441±13.6*	448±16.3*
5% fresh	366±23.3	379±18.7	392±17.9	409±12.5	418±15.4

Each values are mean±S.E. of 5 experiments. *; $p<0.05$

Table II. Changes of hepatic microsomal glutathione s-transferase in male rats fed the garlic juice for 25 days

Treatment \ Days	0	5	10	15	25
		(n moles/mg protein/min)			
Control	74±5.2	68.8±8.4	65.2±10.1	80.2±13.6	82.1±14.6
5% boiled	74±5.2	79.8±4.6	80.3± 6.5	82.5± 7.8	88.6±10.2
5% fresh	74±5.2	77±10.2	79.2±10.8	74.3±12.3	85.4±15.2

Each values are mean±S.E. of 5 experiments.

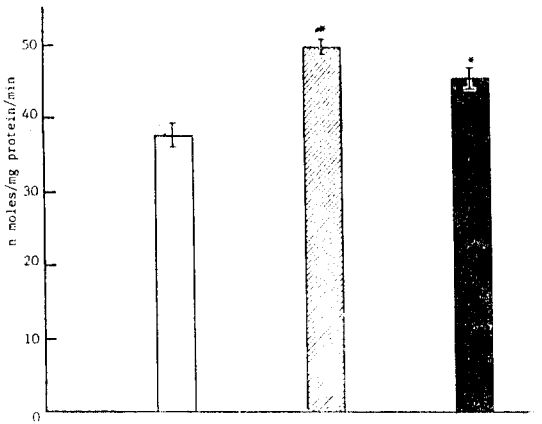


Fig. 2. Effect of garlic on the hepatic cytosolic glutathione peroxidase activity in rats.
Garlic was fed instead of water for 25 days. The assay procedure was described in the text. Values are mean±S.E. of 5 animals in each group. □ : Control group, ▨ : 5% boiled garlic-fed group, ■ : 5% fresh garlic-fed group, *; $p<0.05$

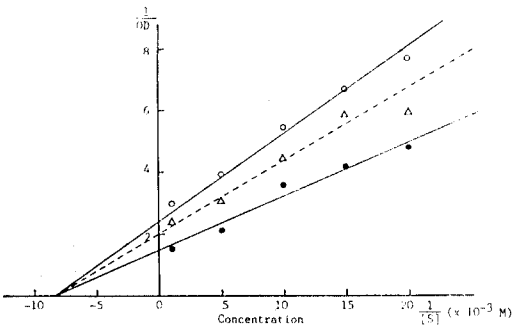


Fig. 3. Lineweaver-Burk plots of the hepatic glutathione s-transferase activity with 1-chloro-2, 4-dinitrobenzene substrate.
The reaction mixture contained 0.1M phosphate buffer, pH 6.5, various concentration of 1-chloro-2, 4-dinitrobenzene, 1 mM glutathione, allicin and heat-treated allicin fraction (5×10^{-6} g/m*l*) and enzyme soln. The values are mean of 3 experiments. -●- : 1-chloro-2, 4-dinitrobenzene, ···△··· : 1-chloro 2, 4-dinitrobenzene+allicin fraction, -○- : 1-chloro-2, 4-dinitrobenzene+heat-treated allicin fraction

The allicin fraction effect on the cytosolic glutathione s-transferase activity was demonstrated *in vitro* system and the results shown in Fig. 3. Both allicin and heat-treated allicin fraction (5×10^{-6} g/ml) decreased V_{max} values comparing with control, but not affected the K_m values. With 1-chloro-2, 4-dinitrobenzene as substrate, allicin and heat-treated allicin fraction, noncompetitive inhibition pattern were observed, respectively. It indicated that allicin and heat-treated allicin fraction might affect the other site including allosteric effect in the enzyme.

The effect of garlic butanol fraction on the enzymatic activity of cytosolic glutathione s-transferase in vitro

The activity of rat liver glutathione s-transferase in the presence of garlic butanol fraction (8×10^{-6} g/ml) increased the K_m value (1.4×10^{-4} M) comparing with control value (1.2×10^{-4} M). On the other hand, V_{max} was not changed. With various concentration of 1-chloro-2, 4-dinitrobenzene as substrate, garlic butanol fraction presence or absence, competitive inhibition pattern

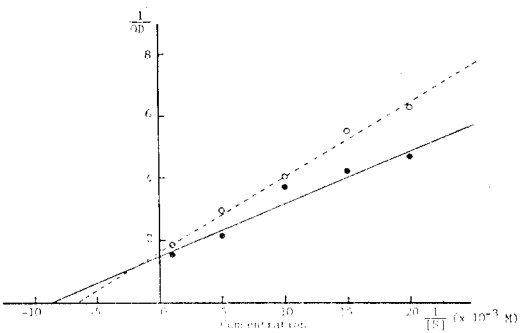


Fig. 4. Lineweaver-Burk plots of the hepatic glutathione s-transferase activity with 1-chloro-2, 4-dinitrobenzene substrate.
The reaction mixture contained 0.1M phosphate buffer, pH 6.5, various concentration of 1-chloro-2, 4-dinitrobenzene, 1mM glutathione, garlic butanol fraction (8×10^{-6} g/m*l*) and enzyme soln. The values are mean of 3 experiments. -●- : 1-chloro-2, 4-dinitrobenzene, ···○··· : 1-chloro-2, 4-dinitrobenzene+garlic butanol fraction

were obtained. This result suggested that garlic butanol fraction would regulate substrate binding site in enzyme (Fig. 4).

The effect of allicin fraction on the enzymatic activity in hepatic glutathione peroxidase

The glutathione peroxidase activity of liver was powerfully enhanced by heat-treated allicin fraction (1.3×10^{-5} g/ml), whereas the enzyme activity was not activated allicin fraction compareing with control value (Fig. 5).

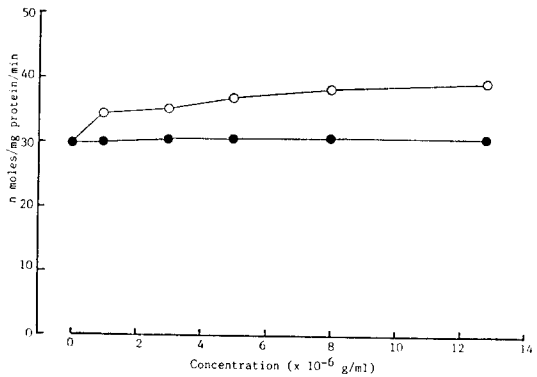


Fig. 5. Effect of allicin and heat-treated allicin fraction on the hepatic glutathione peroxidase activity *in vitro*.
The assay procedure was described in the text. Each value is a mean of 3 experiments. -●- : allicin fraction, -○- : heat treated allicin fraction

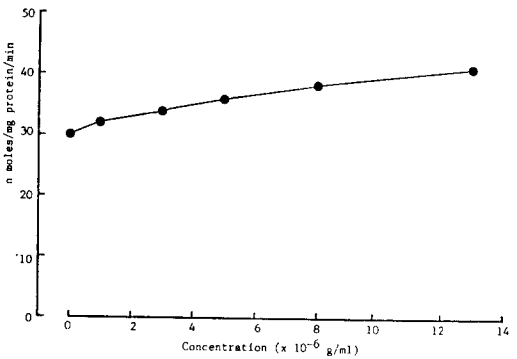


Fig. 6. Changes on the hepatic glutathione peroxidase activity in various concentration of garlic butanol fraction *in vitro*.
The assay procedure was described in the text. Each value is a mean of 3 experiments.

As mentioned above, the heat-treated allicin fraction may modulate the hepatic glutathione peroxidase activity

The effect of garlic butanol fraction on rat hepatic glutathione peroxidase The effect of garlic butanol fraction on rat hepatic glutathione peroxidase was observed that the butanol fraction increased the glutathione peroxidase activity significantly as shown in Fig. 6. Garlic butanol fraction (1.3×10^{-5} g/ml) in reaction mixture showed a 40% increment of enzyme activity.

These observations led us to conclude that the garlic components which are stable substances in heat may regulate the hepatic glutathione s-transferase and glutathione peroxidase activity. These results also indicated that, according to the chemical properties of allicin which is unstable in heat[28], other components than allicin in garlic may regulate the hepatic glutathione s-transferase and glutathione peroxidase which are detoxifying enzymes.

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