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A Study on the Effect of Garlic to the Heavy Metal Poisoning of Rat

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When garlic (Allium sativum) was administered to rat per os simultaneously with cadmium, methylmercury and phenylmercury to detect the protective effect against the heavy metal poisoning, accumulation of heavy metals in liver, kidneys, bone and testes were decreased, and histopathological damages and the inhibition of serum alkaline phosphatase activities by heavy metals were reduced. Such effect of garlic was not shown in the 1.7% garlic treated group and most remarkable in the 6.7% garlic treated group.

The protective effect of garlic was superior to those of 2,3 dimercapto-1-propanol (BAL) and D-penicillamine (PEN), and nearly similar to those of 2,3-dimercaptosuccinic acid (DMSA) and N-acetyl-DL-penicillamine (APEN), the current remedies, while garlic was not effective as a curative agent for heavy metal poisoning.

The excretion of cadmium was enhanced, more through feces than urine by garlic but the effect to the urinary excretion of cadmium was not significant comparing with DMSA or APEN when cadmium was ip injected in the first 3 days during the 12 days of oral administration of DMSA, APEN or garlic.

Key Words: *Garlic, Cadmium, Mercury, Heavy metal poisoning, Prevention of Heavy Metal Poisoning, Treatment of Heavy Metal Poisoning*

INTRODUCTION

Various kinds of heavy metals exist on the earth's crust, including some toxic metals such as antimony (Sb), arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), beryllium (Be) and nickel (Ni). Among these, cadmium and mercury have proved to be extremely toxic to mankind while their usage in various industries has increased rapidly in this century. The worldwide annual consumption of mercury has reached well over 10,000 tons and the cadmium consumption exceeds 20,000 tons (Guthrie and Perry 1980; Tsuchiya, 1983). These heavy metals pollute the environment more and more as a result of rapid industrialization. In Korea, the concentrations of mercury and cadmium in the atmosphere of Seoul were 0.007-0.014 $\mu g/m^3$ and 0.0111-0.024 $\mu g/m^3$, respectively (Kwon et al., 1980; Yum et al., 1980). In the Han river and its major tributaries 2-15 $\mu g/l$ of cadmium, and 0.63-2.7 $\mu g/l$ of mercury were detected (Ditri and Ditri, 1977), and some of the data indicate the potential of threat to health of residents. The toxicity of heavy metals varies with each other depending on target organs. Mercury destorys brain cell,

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inhibits the division of sex chromosomes, and induces genetic defects, while cadmium induces damage to liver and testes, in addition to inhibiting spermatogenesis. Numerous studies for heavy metal poisoning have reported by Parizek and Zahor (1956), Schroeder and Balassa (1961), Aberg and Ekmant (1969), Richardson and Murphy (1974), Kershaw and Dhahir (1980), Nagase et al. (1984) and others. Studies on the mutagenicity and carcinogenicity of metals were also performed (Nomiyama, 1982; Christley and Webster, 1983).

According to the mechanism of toxicity, heavy metals combine preferentially with -SH radicals of proteins and enzymes and inhibits their functions. Active research on the protective effect of chelating agents containing -SH radical is being carried out for prevention and treatment of heavy metal poisoning in the fields of biochemistry, pharmacology, medicine and public health (Imura et al., 1980; Yamane et al., 1981). Initially, -SH compounds, such as BAL (Longcope and Luetscher, 1949) and penicillamine (Aposhin, 1958), were developed as remedies for heavy metal poisoning. Later, this was followed by the development of compounds with less toxicity and side effects, such as 2.3-dimercaptosuccinic acid (Friedheim and Corvi, 1975), N-acetyl-DL-penicillamine (Gabard, 1976), and polythiol resine (Clarkson and Small, 1973). Considering the pharmacological effect of these -SH compounds (Shim, 1967; Cho and Lee, 1975), it is expected that garlic, which is used as one of essential spices in Korean cooking and contains an abundant quantity of -S-S-compounds such as diallyldisulfide, propylallydisulfide, and -SH compounds such as glutathione, thiolactic acid, cystine, cysteine, homocystine, and Vitamin B, will provide a protective effect in human body against heavy metal poisoning by reacting with the metals and producing sulfur compounds.

This study was carried out to investigate the protective effect of garlic against heavy metal poisoning. Doses of garlic with cadmium and mercury respectively, were administered to albino rats and the effect of garlic on the histopathological change and accumulation of heavy metals in the target organs, such as liver, kidneys, testes, and bone tissues were investigated. In addition, a comparative study on protective effect of garlic against cadmium poisoning was done with current remedies of 2,3-dimercaptosuccinic acid (DMSA), N-acetyl-DL-penicillamine (APEN), BAL and penicillamine (PEN), whose excellent protective effect on cadmium and mercury poisoning was already recognized. To understand the protective mechanism of garlic in heavy metal poisoning, cadmium and garlic were administered together and the effect of garlic on the excretion of the metal were observed, and compared with the effect of DMSA and APEN.

MATERIALS AND METHODS

1. Materials

Among male Sprague-Dawley rats bred 4 weeks under optimal condition, experimental animals were selected under the standards of body weight, motility, and of others in every experiment.

To detect the protective effect of garlic against cadmium and mercury poisoning, cadmium chloride ($CdCl_2.1/2\ H_2O$), methylmercuric chloride (CH_3HgCl) and phenylmercuric acetate ($C_2H_5HgC_2H_3O_2$) were mixed with drinking water in various concentrations. Raw garlic peeled and crushed was mixed with the feed, and was administered per os in various concentrations of 1.7% 3.35% and 6.7% which were considered to be 50, 100 and 200 ppm as allicin level.

To compare the protective effect of garlic against cadmium poisoning with those of current remedies which were developed before and have been used for treatment of the heavy metal poisoning as chelating agents, 2.3 dimercapto-1-propanol (BAL), D-penicillamine (PEN), 2,3-dimercaptosuccinic acid (DMSA) and N-acetyl-DL-penicillamine (APEN) were used. Cadmium chloride, 1 mg/kg, was injected intraperitoneally for 7 days. BAL (17.5 mg/kg), PEN (40 mg/kg), DMSA (60 mg/kg) and APEN (60 mg/kg), equivalent to 1/6 LD_{50}, were administered orally with drinking water. And raw garlic mixed with feed in 6.4%, equivalent to 1/6 LD_{50} of allicin considering intake amount of feed, was administered orally for 30 days after the cadmium injection.

To detect the effect of garlic to the excretion of cadmium, 0.5 mg/kg of cadmium chloride was injected intraperitoneally for 3 days. Garlic was mixed with the feed to make a concentration of 6.4% and was administered orally from 24 hours before the application of cadmium injections for 12 days. To make a comparative study of the effect of garlic, 30 mg/kg of diallyldisulfide, 75 mg/kg N-acetyl-DL-penicillamine 75 mg/kg were administered orally with drinking water for 12 days.

2. Methods

To detect the protective effect of garlic against cadmium and mercury poisoning, subject animals

were devided into four groups, such as (1) a control group that had not been exposed to heavy metals and had been bred with commercial feed only, (2) a garlic treated group (3) a heavy metal treated group, and (4) a garlic and heavy metal treated group. The heavy metal treated group was subdivided into a cadmium treatment group, a methylmercury treated group, and a phenylmercury treated group. The garlic and heavy metal treated group was subdivided into the 1.7% garlic and metal treated groups, the 3.35% garlic and metal treated groups and the 6.7% garlic and metal treated groups. Garlic and heavy metals were administered for 12 weeks and at the completion of this experimental period, blood samples were collected, and the alkaline phosphatase activity was measured by the Bowers and McComb method (Gilford Instrument Lab., 1982). Liver, kidneys, and testes were removed and the amount of cadmium in those organs were measured using the atomic absorption spectrophotometer (Shimadzu AA630-11). From samples of brain, liver and kidneys, the amount of total mercury was measured under the cold vapor technique using AAS. Thin slide preparations of liver, kidneys, brain and testes were stained with H-E stain method and examined histopathologically.

To compare the protective effect of garlic against cadmium poisoning with those of current remedies, cadmium was injected intraperitoneally for 7 days and current remedies of BAL, PEN, DMSA and APEN, and garlic were administered orally for 30 days from the beginning of cadmium injection. At the same time to compare the curative effect of garlic with current remedies to the cadmium poisoning, current remedies and garlic were administered orally for 23 days after the cesation of 7 days of cadmium injection. The experimental subject was divided into 6 groups of Cd-alone treated group, DMSA-Cd treated group, BAL-Cd treated group, APEN-Cd treated group, PEN-Cd treated group, and garlic-Cd treated group. Doses of cadmium were administered intraperitoneally for 7 days: doses of current remedies (DMSA, BAL, APEN, and PEN) were administered with drinking water from the first day of cadmium injection for 30 days, and the doses of garlic mixed into the feed were administered orally from cadmium injection for 30 days. In order to observe the curative effect of garlic in cadmium poisoning, poisoning was induced following the same procedure as above by cadmium injections intraperitoneally for 7 days and garlic and current remedies were administered for 23 days from 24 hours after cadmium injection. The dosage of garlic and the remedies was doubled in the last week to make the same dosage used in the experiment to compare the protective effect. A comparative study of protective and curative effect was performed mainly on the amount of cadmium accumulation in the liver. Kidneys, testes, and bone tissue and on the histopathological changes of the testes. The same method as above was employed for the determination of the amount of cadmium accumulation and histopathological changes.

To detect the effect of garlic to the excretion of cadmium from rat the experimental subject was divided into Cd-alone, garlic-Cd, diallyldisuifide-Cd, DMSA-Cd and APEN-Cd treated groups, and samples of urine and feces were collected from each metabolic cage in which 5 rats were bred. From the first day of experiment, i.e., 24 hours before the start of the cadmium injection, the oral administration of garlic, diallyldisulfide, DMSA, and APEN was conducted for 12 days, while the cadmium were administered intraperitoneally from the second day once a day for 3 days. The quantity of cadmium excretion was measured by collecting the urine and feces separately at 24 hour intervals from the beginning of cadmium administration on the second day. The method used to measure the amount of cadmium excretion was same as the above.

RESULTS

1. The protective effect of garlic against cadmium and mercury poisoning

1) The protective effect of garlic against cadmium poisoning

(1) Weight change

The weight of all the experimental albino rat on day 1 of the experiment were set to be 216 g. After 12 weeks, the control group weighed 348.0 g and the garlic (3.35%) treated group weighed 352.0 g which were a 61-63% increase while the slope of weight gain of the cadmium (100 ppm) treated group flattened after week 5 and weighed 318 g after 12 weeks with a 47% increase (Figure 1).

In groups treated with garlic and cadmium simultaneously, the weights were 321.3 g for the garlic (1.7%)-Cd treated group, 338.0 g for the garlic (3.35%)-Cd treated group, and 352.5 g for the garlic (6.7%)-Cd treated group, showing increases of 48%, 50%, and 63% respectively. The weight gain for the garlic (6.7%)-Cd treated group was approached that of the control group (Figure 1).

(2) The change of alkaline phosphatase activity in

Table 1. Serum alkaline phosphatase activities and cadmium levels in various tissues of rat treated with cadmium and/or garlic

M±S.D

Treatment		No. of	Alkaline Phosphatase	Cadmium (ug/g)		
Garlic (%)	Cd (ppm)	rat	(μ/l)	Liver	Kidney	Testis
0	100	10	72.78±13.74 (89.43)	33.86±3.93	33.93±2.65	0.77±0.28
1.70	100	9	70.89± 8.28 (87.16)	31.36±3.61	35.32±5.51	0.68±0.16
3.35	100	8	78.33±14.94 (96.31)	29.02±5.16*	29.55±3.15**	0.59±0.08
6.70	100	8	87.56±14.22 (107.17)*	25.28±4.05**	29.39±3.16**	0.54±0.09*
Control	0	6	81.33± 9.83 (100.00)	0.09±0.05	0.38±0.30	ND
Garlic (3.35%)	0	6	88.86±14.51 (109.25)	0.16±0.08	0.31±0.58	ND

* : p 0.05 compared with cadmium-alone treated group
**: p 0.01 compared with cadmium-alone treated group

Table 2. Mercury levels in various tissues of rat treated with mercury and/or garlic

Treatment+		No. of	Mercury (μg/g)		
Mercury (ppm)	Garlic (%)	rat	Brain	Liver	Kidney
0	0	10	0.09±0.04	0.11±0.05	0.18±0.11
0	3.35	10	0.06±0.04	0.12±0.07	0.16±0.10
4	0	10	0.31±0.18	3.01±0.97	5.19±1.68
MMC 4	3.35	10	0.27±0.11	2.82±1.03	4.02±1.15
4	6.70	10	0.21±0.08	2.34±0.82	3.68±0.95*
4	8.00	10	0.18±0.04*	1.82±0.83**	3.41±0.91**
6	0	8	0.10±0.04	2.91±1.34	13.86±2.75
6	1.70	10	0.09±0.07	2.81±0.09	14.06±4.62
PMA 6	3.35	10	0.09±0.04	1.96±1.01	12.92±1.50
6	6.70	10	0.09±0.05	1.68±0.08**	11.68±2.11

\+ MMC: Methylmercuric choride
PMA : Phenylmercuric acetate
* : $p<0.05$ compared with mercury-alone treated group
** : $p<0.01$ compared with mercury-alone treated group

Table 3. Cadmium levels in various tissues of rat simultaneously treated with cadmium and/or various thio compounds

Unit: μg/g (wet)

Treatment+	No. of	Organ			
	rat	Liver	Kidney	Testis	Bone
Cd-alone	20	40.77±6.42	15.28±2.13	2.77±0.84	6.03±0.74
Cd-garlic	20	27.91±5.17**	13.60±1.96*	1.37±0.26**	1.50±0.42**
Cd-DMSA	20	23.14±5.57**	13.50±1.93**	1.12±0.27**	0.51±0.21**
Cd-BAL	20	32.22±4.70**	18.50±3.41**	1.79±0.55**	2.50±0.74**
Cd-APEN	20	27.96±7.46**	13.36±3.51*	1.58±0.64**	1.79±0.75**
Cd-PEN	20	36.05±6.42*	18.44±2.78**	2.13±0.74*	1.89±0.54**

\+ DMSA: 2,3-dimercaptosuccinic acid
BAL : 2,3-dimercapto-a-propanol
APEN: N-acetyl-DL-penicillamine
PEN : D-penicillamine
* : $p<0.05$ compared with cadmium-alone treated group
** : $p<0.01$ compared with cadmium-alone treated group

Table 4. Cadmium levels in various tissues of rat treated with thio compounds after cadmium injection

Unit: ug/g (wet)

Treatment	No. of rat	Organ			
		Liver	Kidney	Testis	Bone
Cd-alone	20	40.77 ± 6.42	15.28 ± 2.61	2.77 ± 0.62	6.03 ± 0.74
Cd-garlic	20	32.96 ± 7.18**	14.72 ± 3.18	2.02 ± 0.53**	1.84 ± 0.23**
Cd-DMSA	20	32.56 ± 5.73**	14.89 ± 2.31	1.57 ± 0.62**	1.56 ± 0.96**
Cd-BAL	20	38.80 ± 9.00	15.70 ± 1.81	2.25 ± 0.59**	2.54 ± 0.79**
Cd-APEN	20	36.49 ± 5.69*	15.40 ± 4.67	1.98 ± 0.71**	2.69 ± 0.96**
Cd-PEN	20	40.17 ± 6.79	19.05 ± 2.87**	2.36 ± 0.73	2.59 ± 1.14**

(Abbreviation same as Table 3)

Table 5. The excretion of cadmium via urine in groups treated simultaneously with cadmium and thio compounds

Unit: ug/rat

Exp. Day	Exp. Group / Treatment	Cd-alone+		Cd + garlic		Cd + DMSA		Cd + APEN		Cd + diallyldisulfide	
		Cd-amount	(Cumm.%)	Cd-amount	(Cumm.%)	Cd-amount	(Cumm.%)	Cd-amount	(Cumm.%)	Cd-amount	(Cumm.%)
1st	Thiol comp.										
2nd	Thiol + Cd										
3rd	Thiol + Cd	56.43	(37.13)[a]	56.48	(37.16)[a]	56.65	(35.39)[a]	59.71	(41.18)[a]	56.35	(37.07)[a]
4th	Thiol + Cd	57.78	(36.72)[b]	111.75	(55.52)[b]	81.35	(43.25)[b]	104.61	(56.86)[b]	92.23	(48.56)[b]
5th	Thiol comp.	54.93	(36.53)	102.11	(59.42)	80.91	(45.65)	89.38	(57.86)	87.56	(41.67)
6th	Thiol comp.	5.84	(37.79)	12.16	(62.16)	13.86	(48.54)	13.19	(60.86)	12.04	(54.31)
7th	Thiol comp.	3.89	(38.63)	5.56	(63.39)	4.71	(49.52)	3.01	(61.55)	4.78	(55.35)
8th	Thiol comp.	5.26	(39.77)	5.56	(64.62)	4.39	(50.44)	4.50	(62.58)	4.28	(56.29)
9th	Thiol comp.	2.47	(40.30)	3.06	(65.29)	3.56	(51.18)	4.34	(63.57)	2.94	(56.93)
10th	Thiol comp.	2.05	(40.75)	2.95	(65.94)	2.67	(51.74)	3.00	(64.25)	2.65	(57.51)
11th	Thiol comp.	1.70	(41.11)	2.65	(66.52)	1.87	(52.13)	2.70	(64.87)	1.90	(57.93)
12th	Thiol comp.	1.70	(41.48)	1.91	(66.94)	1.76	(52.49)	1.82	(65.28)	1.72	(58.30)
Total amount of Cd injected (ug/rat)		463.0		455.0		480.0		439.0		457.0	

Cumm.% : Cummulative percentage of excretion amount based on total amount of cadmium injected
DMSA : 2,3-dimercaptosuccinic acid
APEN : N-acetyl-DL-penicillamine
\+ : Thiol compounds were not administered in Cd-alone group.
a : Percentage of excretion amount based on 1st injection amount of cadmium
b : Cummulative percentage of excretion amount based on sum of 1st and 2nd injection amounts

the blood.

Concerning the alkaline phosphatase activity in the blood, the garlic (3.35%)-only treated group showed a 9% increase compared with the control group, while the cadmium treated group showed an 11% decrease. In groups treated with garlic and cadmium simultaneously, the garlic (6.7%)-Cd treated group showed a significant increase compared with the Cd-alone treated group (Table 1).

(3) Cadmium accumulation in the tissues

Concerning the amount of cadmium accumulation in the liver tissue, the garlic (1.7%)-Cd treated group showed little change compared with the Cd-alone treated group, while the garlic (3.35%)-Cd treated group showed a 14% decrease, and the garlic (6.7%)-Cd treated group showed a 25% decrease.

The amount of cadmium accumulation in the kidney of the garlic (1.7%)-Cd treated group did not show any significant change compared with the Cd-alone treated group, while those of the garlic (3.35%)-Cd and garlic (6.7%)-Cd treated groups showed a 13% decrease.

Similarly, the amount of cadmium accumulation in testes of the garlic (3.35%)-Cd group also showed a significant decrease comparing with that of the cadmium treated group (Table 1).

Table 6. The excretion of cadmium via feces in groups treated with cadmium and thiol compounds

Unit: μg/rat

Exp. Group / Exp. Day / Treatment	Cd-alone+ Cd-amount (Cumm.%)	Cd + Garlic Cd-amount (Cumm.%)	Cd + DMSA Cd-amount (Cumm.%)	Cd + APEN Cd-amount (Cumm.%)	Cd + diallyldisulfide Cd-amount (Cumm.%)
1st Thiol comp.					
2nd Thiol + Cd					
3nd Thiol + Cd	1.21 (0.80)[a]	1.07 (0.71)[a]	1.41 (0.88)[a]	1.61 (1.11)[a]	1.23 (0.81)[a]
4th Thiol + Cd	1.93 (1.11)[b]	1.95 (1.00)[b]	2.15 (1.12)[b]	2.22 (1.33)[b]	1.91 (1.03)[b]
5th Thiol comp.	1.59 (1.02)	1.64 (1.03)	1.93 (1.15)	1.98 (1.32)	1.54 (0.87)
6th Thiol comp.	0.39 (1.11)	0.48 (1.13)	0.53 (1.26)	0.66 (1.48)	0.45 (0.97)
7th Thiol comp.	0.33 (1.18)	0.40 (1.22)	0.45 (1.35)	0.65 (1.62)	0.41 (1.06)
8th Thiol comp.	0.14 (1.21)	0.41 (1.31)	0.31 (1.42)	0.37 (1.71)	0.40 (1.15)
9th Thiol comp.	0.16 (1.24)	0.31 (1.38)	0.24 (1.47)	0.29 (1.77)	0.31 (1.21)
10th Thiol comp.	0.10 (1.26)	0.20 (1.42)	0.23 (1.51)	0.33 (1.85)	0.22 (1.26)
11th Thiol comp.	0.07 (1.28)	0.20 (1.47)	0.18 (1.56)	0.25 (1.91)	0.19 (1.30)
12th Thiol comp.	0.05 (1.29)	0.12 (1.49)	0.15 (1.58)	0.18 (1.95)	0.13 (1.33)
Total amount of Cd injected (ug/rat)	463.0	455.0	480.0	439.0	457.0

(Abbreviation same as Table 5)

(4) Histopathological change in liver, kidneys and testes

There was no significant difference of the histopathological change in liver, kidneys and testes between the garlic treated group and the control group. However Cd-alone treated group showed conspicuous liver cell necrosis at central zone of hepatic lobules, focal necrosis of proximal tubular epithelial cells, cloudy swelling in renal tubules, hydropic degeneration and necrosis with atrophy in germinal epithelium of seminiferous tubule.

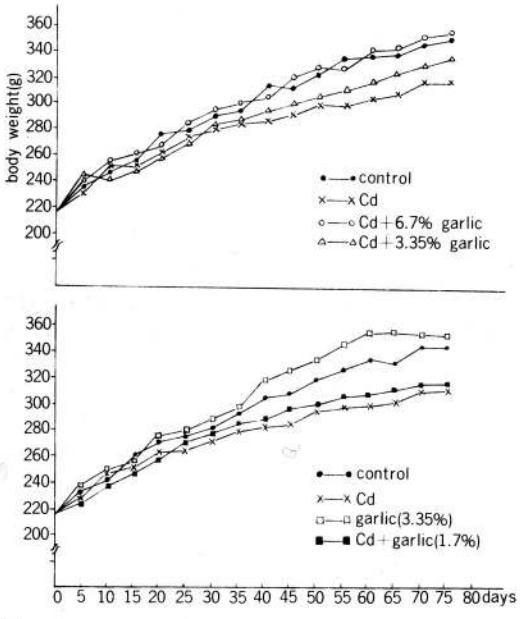

Fig. 1. Mean body weights of rat exposed to Cd or Cd - garlic

Table 7. Thio compounds and trace metals in garlic extract

Thio compounds (%) (Shim, 1967)		Metals (μg/g) (Cha, unpublished)	
Diallyldisulfide	23-39	Fe	1.90-2.80
Diallyltrisulfide	13-19.3	Cu	0.95-2.50
Propylallyldisulfide	3.7-4.5	Zn	0.13-0.45
Diallylsulfide	1	Cd	0.002-0.06
Glutathion	93.5 (mg%)	Pb	0.01-0.18
Vitamin C	5.3 (mg%)	Se	0.72-1.52
Thio amino acid	0.5-1.3 (mg%)		

When dosages of garlic were administered with cadmium the garlic (1.7%)-Cd treated group showed little difference in histopathological change compared with the Cd-alone treated group. A slight histopathological change appeared in the garlic (3.35%)-Cd treated group. There was a noticeable difference in the garlic (6.7%)-Cd treated group compared with the Cd-alone treated group in hepatic cell cords, sinusoids of liver and kidney in addition to a mild degenerative change in the germinal epitheiium of seminiferous tubule.

2) The protective effect of garlic against mercury

poisoning

(1) Change of mercury amount accumulated in the tissue.

The total amount of mercury accumulated in the brain tissue did not decrease significantly in the garlic (3.35%)-methylmercury treated group compared with the group treated with 4ppm methylmercury, while in the garlic (6.7%)-methylmercury and garlic (8.0%)-methylmercury treated groups the mercury accumulation decreased 32% and 40%, respectively. The amount of mercury accumulation in the brain tissue in the group treated with 6ppm phenylmercury was only 1/3 of that of the methylmercury treated group, and there was no significant difference in the garlic-phenylmercury treated group compared with the phenylmercury alone treated group.

The total amount of mercury accumulated in the liver was greater in the methylmercury treated group compared with the phenylmercury treated group. In groups treated with garlic together with mercury, the garlic (8.0%)-methylmercury treated group showed a decrease of 40% compared with the mercury treated group while the garlic (3.35%)-phenylmercury treated group began to show a decrease of 33% compared with the phenylmercury treated group.

The total mercury accumulated in kidney of the phenylmercury treated group was 3 times more than that of the methylmercury treated group and there was no decrease in the garlic (3.35%)-methylmercury treated group compared with the mercury-alone treated group (Table 2).

(2) Histopathological changes in brain and kidney tissues

The extent of damage of brain tissue in the phenylmercury treated group was not severe but in the methylmercury treated group the myelin sheath in the white matter was dehydrated and a large number of Purkinje cells showed degenerative and necrotic change. Among the groups treated with garlic and mercury simultaneously, the garlic (3.35%)-methylmercury treated group showed no difference in the degree of tissue damage, while the garlic (6.7%)-methylmercury treated group showed a mild degenerative change in the Purkinje cells. The degree of damage of the kidney due to mercury was similar in both phenylmercury and methylmercury treated groups. While severe degeneration and necrosis of the epithelium of proximal convoluted tubule was observed in the mercury-alone treated group, only mild damage was observed in garlic (6.7%)-mercury treated group, and maintained a normal architecture in the garlic (8.0%)-methylmercury treated group.

2. Comparison of the protective effect between garlic and current remedies against cadmium poisoning

(1) The protective effect of garlic and current remedies of BAL, PEN, DMSA and APEN

When an evaluation of the protective effect of garlic against cadmium poisoning is based on the cadmium accumulation in the liver, kidneys, testes and brain tissues, its degree of the protective effect was similar to the level of APEN and was superior to that of PEN or BAL. When compared with DMSA, the levels of the cadmium accumulation in the liver and kidneys were similar, but the accumulation levels in testes and brain tissue decreased significantly in the DMSA treated group. When PEN or BAL was administered together with cadmium, it increased the cadmium accumulation in kidney compared with Cd-alone treated group (Table 3).

When the histopathological change in testes due to cadmium alone treatment were compared with those due to simultaneous treatment with current remedies and cadmium, only a small degree of tissue damage was observable in the DMSA-Cd or APEN-Cd treated group, and no difference in the degree of tissue damage was observed in the BAL-Cd PEN-Cd treated group.

(2) The curative effect of garlic and current remedies

To compare the curative effect of garlic with current remedies they were administered from 24 hours after the 7 days' injection of cadmium. No significant difference between garlic and DMSA on the effect of reducing the concentration of the cadmium accumulated in the tissue was observed. In treatments with garlic or DMSA, the concentrations of cadmium in the testes and bone were clearly lower than those of the Cd-alone treatment, with only a small difference in the liver, but no difference in the kidneys. With the exception for the bone, BAL did not reduce the cadmium accumulation in the tissues.

Garlic did not decrease the cadmium accumulation in the other organs than the bone tissue below 80% of the levels of the Cd-alone treated group, and although the curative effect of garlic was superior to that of APEN in the liver, kidneys, and bone except for the testes, there was no statistical significance. The curative effect of PEN was inferior to that of garlic or APEN in all organs (Table 4).

Garlic and the current remedies did not recover histopathological changes of testes damaged by cadmium.

3. The effect of garlic on cadmium excretion from rats exposed to cadmium

The cadmium excretion was investigated in groups treated with cadmium and garlic, DMSA, or APEN. The effect of urinary excretion in the garlic-Cd treated group was clearly observed 2-3days later than the occurrence in the DMSA-Cd or APEN-Cd treated groups, and the cumulative excretion rate for 10 days in the garlic-Cd treated group did not reach the levels of APEN-Cd or DMSA-Cd treated group (Table 5).

But in the case of fecal excretion, the effect on the cadmium excretion was noticed from the second day and the cumulative excretion rate for 10 days in the garlic-Cd treated group exceeded that of APEN or DMSA, hence the enhancing effect of cadmium excretion of garlic is evident in feces rather than in urine excretion (Table 6).

In the case of diallyldisulfide-Cd treatment, the effect of cadmium excretion enhancing was conspicuous in the feces rather than in urine excretion, therefore it was similar to that of garlic. The effect of cadmium excretion enhancing through urine was weaker than that of the DMSA-Cd or APEN-Cd treatments. However comparing the effect of enhancing cadmium excretion between diallyldisulfide and garlic, diallyldisulfide was inferior to garlic.

DISCUSSION

Korean garlic (*Allium sativum*) consists of many sulfur compounds containing -SH and -S-S- radicals, and various kinds of metal. Therefore the sulfur compounds in garlic may form chelate compound with metals in body and are expected to have protective effect against heavy metal poisoning (Ishikuro, 1981). The garlic also contains much selenium, which antagonizes heavy metal poisoning including cadmium and enhances protective effect furthermore (Table 7). Korean has used garlic as a spice since acient time. In 1985, garlic production reached 256,000 M/T in Korea, with a consumption of 3.8 kg per capita, and it is estimated that the figures will continue to increase to 333,000 M/T in 1991 (Ministry of Agriculture & Fishes, 1986).

1. The protective mechanism of garlic against cadmium and mercury poisoning

It has been reported that alkaline phosphatase, a kind of enzyme in the cell membrane and a biochemical indicator of biological function, is distributed in target organs of cadmium poisoning, such as small intestinal epithelium, proximal convoluted tubule of kidney and osteoblast (Wada and Ono, 1977), and Kobayashi (1977) has reported that the activity of alkaline phosphotase in a rat with cadmium poisoning dropped to 50% over a three month period. Yoneyama and Sharma (1983) reported that methylmercury and phenylmercury inhibited the activity of potassium-dependent phosphatase, glutamine synthetase and alkaline phosphatase, which are enzymes in cell membrane.

In this study cadmium, methylmercury, and phenylmercury were administered respectively to rats simultaneously with garlic for 12 weeks and the results were compared with those of cadmium of mercury alone treated groups. The inhibition of alkaline phosphatase activity in the blood showed a trend of recovery in the garlic (3.35%)-metal treated group, and same values to that of the control group were indicated in the garlic (6.7%)-metal treated group.

In considering the cadmium accumulation and the histopathological changes in liver, kidneys, bone and testes which are target organs of cadmium poisoning, a significant decrease of cadmium accumulation was apparent in both garlic (3.35%)-Cd treated group and garlic (6.7%)-Cd treated group. Tissue damages, such as the edematous change of renal glomerulus and necrosis of liver cells, were at recovery in the garlic (3.35%)-Cd treated group and nearly recovered to show normal architecture in the garlic (6.7%)-Cd treated group. Parizek and Zahor (1956) have reported that a low concentration on cadmium (0.03 mg/kg) caused irreversible damage to the germinal epithelium of seminiferous tubule in testes and inhibits spermatogenesis due to necrosis of the reproductive tissues and interstitial tissues. In this study, cadmium accumulation in the testes in garlic (6.7%)-Cd treated group were reduced to 30% of that in Cd-alone treated group and only a small histopathological change in the germinal epithelium of seminiferous tubule were in indicated. The protective effect of garlic against testes damage due to cadmium, resembling the case of zinc and thiol compounds as reported by Gunn (1963) and Mason and Young (1964), was highly recognized. In the case of brain tissue which is a target organ of methylmercury poisoning, Daniel and Gage (1972) has reported that when there has been an long term exposure to phenylmercury, some of the mercury accumulates in the brain tissue after methylation, and the accumulation level is 5-10% of methylmercury. The accumulation level in kidneys is 10 times that of methylmercury. On the effect of-SH compounds to the mercury accumulation in the brain tissue Berlin

and Rylander (1964) have reported that BAL and penicillamine cannot reduce the amount of mercury accumulation in brain of the mouse, and also Kasuya (1972) has reported that L-cysteine. D-penicillamine and BAL cannot prevent the growth inhibitory action on the brain cell due to mercury poisoning.

With respect to mercury accumulation in brain this study showed a decrease of 32.2% in the garlic (6.7%)-methylmercury treated group compared with the methylmercury treated group, and as to changes in the brain tissue, only a small level of demyelinization was found in the white matter of cerebellum while no damage appeared in the Purkinje cells.

Garlic had no effect on the mercury accumulation in the brain tissue of the garlic (6.7%)-phenylmercury treated group, and the protective effect against the damage of brain could not be observed because there was little histopathological damages in the mercury treated group. It has been explained by Aaseth and Friedheim (1978) that in case of BAL and penicillamine, they cannot reduce the mercury accumulation in the brain cells, because their mercury compounds are reabsorbed and reaccumulated in the brain tissue. However the protective effect of garlic against mercury poisoning of the brain tissue can be interpreted as a result of smaller amount of mercury being absorbed into the brain tissue due to the enhanced excretion of mercury compounds from the body by the garlic rather than a relationship between garlic and brain tissue.

2. Comparison of the protective effect of garlic with the current remedies against cadmium poisoning

According to the chelating mechanism of the metal in living body, BAL and penicillamine form metal-chelates in the blood or outside cell and exerete the metal through urine. But they are considered inappropriate as remedies because the toxicity of the metal is increased when they are reabsorbed by the kidney or redistributed in the brain cell during the excretion process (Tepperman, 1947; Cherian, 1980).

2,3-dimercaptosuccinic acid (DMSA), a derivative of BAL, with 1/30 of the toxicity of BAL, discharges the metal through urine after forming metal-DMSA chelate compounds in the blood without reabsorption by the kindey. N-acetyl-DL-penicillamine, highly rated along with DMSA, stimulates the discharge of metals from the brain tissue and red blood cells by passing through the cell membrane. Since there is no information on garlic, the protective effect of garlic against heavy metal poisoning was investigated in target organs of cadmium poisoning, such as testes, liver, kidneys, and bone tissues and compared with results from other remedies. In the case of testes, the amounts of cadmium accumulation in the BAL-Cd treated group and PEN-Cd treated group were decreased by 26.5% and 23% of the Cd-alone treated group, in DMSA and APEN-Cd treated groups there were 45% and 44% decrease respectively and in the garlic-Cd treated group to 44%, thus the figure for garlic was close to those of the DMSA and APEN. For the cadmium accumulation in the liver, the effect of garlic were similar to that of DMSA-Cd treated and the APEN-Cd treated groups, and in the case of the BAL-Cd treated group there was a higher rate of cadmium discharge than that of the PEN-Cd treated group. The cadmium accumulation in kidneys showed an aproximately 20% increase of the Cd-alone treated group in the BAL-Cd treated and the PEN-Cd treated groups, respectively, due to the reabsorption in kidneys, as mentioned by Cantilena and Klaassen (1981), Yonaga and Morita (1981), and Muragami and Webb (1981). But in the instance of the garlic-Cd treated group, it shows a 10-20% decrease of the Cd treated group as in the case of the APEN-Cd treated group and DMSA-Cd treated group, demonstrating that there is no reabsorption with garlic like the instance of BAL. Based on the above results, the protective effect of garlic against heavy metal poisoning is superior to that of DMSA and APEN.

3. The effect of garlic on the excretion of cadmium

The accumulation and excretion of cadmium from the body varies according to the method of administration and the amount of excretion. Part of the absorbed cadmium in the liver is excreted in the bile as glutathione-Cd compound before the synthesis of metalothionein and secreted through the pancreas and intestinal mucous membrane. An extremely small amount of the secreted cadmium is re-absorbed in the small intestine and excreted via the urine while the majority is discharged in the feces through the intestine , thus it is commonly accepted that feces are the main route of excretion.

In this study, the amount of cadmium excretion measured in the feces and urine, collected 24 hours a day for 10 days by administering doses of garlic, DMSA, and APEN respectively with cadmium to albino rats bred in metabolic cages, was compared with the total administered amount of cadmium by the cumulative percentage. The urinary excretion of the cadmium in the garlic-Cd treated group did not

reach the level of DMSA or APEN treated group but the fecal excretion was higher than that of DMSA and APEN, and the total amount of cadmium excretion was highest in the garlic-Cd treated group, followed by APEN and then DMSA. Aaseth (1976) and Cantilena and Klaassen (1981, 1982) have reported that when albino rats were injected with cadmium (1 mg/kg) and DMSA (0.75 g/kg), mercury (1.3 mg/kg) and APEN (0.6 mg/kg) respectively, 3-5 times of the amount of cadmium in the metal alone treated groups had been excreted through the urine. But in this study the discharge of cadmium through urine was relatively low in the DMSA-Cd treated group and APEN-Cd treated group. It is assumed that the orally administered DMSA and APEN reacted with the cadmium in the intestine and were excreted in the feces while Aaseth (1976) and others administratered the chemicals by injection. It is also possible to interpret this as, unlike the polythiol resin which is not absorbed in the intestine during digestion and absorption, with garlic-Cd treatment, small amount of cadmium are excreted through the urine and the majority is excreted in the feces by enhancing the excretion process.

To compare the cadmium excretion effect of diallyldisulfide with that of DMSA, doses of diallyldisulfide, the main component of garlic, were administered together with cadmium. The urinary excretion effect did not reach that of DMSA but as in the garlic-Cd treated group, the fecal-excretion effect was higher compared to the DMSA group. Although the total fecal and urinary excretion amount in the diallyldisulfide group was higher than that of the DMSA group, it did not reach those of garlic.

As a summary, when the Korean garlic mixed with feed and heavy metals were simultaneously administered with garlic to albino rat, accumulation of heavy metals in liver, kidney, bone and testes decreased and histopathological damages were reduced. The garlic also decreased the inhibitory action of alkaline phosphatase activity in blood, which is occurred in heavy metal poisoning.

The effect of garlic was not appeared in the 1.7% garlic treated group; protective effect began to be appeared in the 3.35% group and the heavy metal accumulation decreased more than 40% in the 6.7% group comparing with the metal-alone treated group.

The protective effect of garlic against heavy metal poisoning is considered to be superior to current remedies, BAL and D-penicillamine, and similar to 2,3-dimercaptosuccinic acid and N-acetyl-DL-penicillamine. But the curative effect on heavy metal poisoning was not significant.

When garlic was administered with cadmium, the excretion of cadmium was enhanced, more through feces than urine.

Based on the above results, it is assumed that the protective effect of garlic containing -SH and -S-S- radicals is caused by forming sulfur compound with heavy metals in the body and promoting excretion of heavy metals through bile juice in feces.

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