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Influence of Garlic on the Disposition and Toxicity of Lead and Cadmium in the Rat

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Abstract

Garlic (*Allium sativum* L., Liliaceae) was investigated for its potential to prevent the accumulation of lead or cadmium, major environmental pollutants, and to reduce their toxic effects in rats. The oral feeding of minced fresh garlic during intraperitoneal injection of lead acetate or cadmium chloride, daily for 6 weeks significantly decreased the accumulation of these toxic metals and prevented the metal sensitive biochemical alterations in blood, liver and kidney. The ability of garlic to provide glutathione, biosynthesize metallothionein or similar protein, and its antioxidant properties appear to protect against potential oxidative damage to tissues by lead or cadmium. The regular intake of garlic may be beneficial in reducing the toxic effects of these heavy metals in the exposed population.

Introduction

Garlic (*Allium sativum* L.) has a wide range of beneficial effects (Forum, 1999). This is relevant in view of currently increasing public and scientific interest in herbal medicines. Garlic is known to stimulate the body's immune system and protect against heart disease and strokes by reducing cholesterol levels and hypertension (Rastogi & Mehrotra, 1991; Forum, 1999). Garlic has demonstrated anticancer effects owing to its potential to slow tumor growth, attributed to its chemical constituents mainly diallyl sulfide, diallyl disulfide and diallyl trisulfide (Sparnins et al., 1988). Garlic has also shown protection against damage from oxidation and free radicals (Lang & Chang, 1981; Forum, 1999). Investigations on the molecular mechanism of lead and cadmium toxicity have revealed the involvement of oxidative damage to the affected tissues. Changes in glutathione levels and in the activities of various antioxidant enzymes indicative of lipid peroxidation have been implicated in oxidative stress due to the toxicity of these metals (Muller, 1986; Jamall et al., 1989; Gurer et al., 1998; Adonaylo & Oteiza, 1999). The reactive oxygen species generated in lead or cadmium intoxication at different levels damage essential biomolecules such as proteins, lipids and DNA (Halliwal & Gutteridge, 1990; Hermes-Lima et al., 1991; Stohs & Bagchi, 1995). As glutathione (GSH) is an important antioxidant and garlic is a rich source of several organosulfur compounds capable of providing GSH, it can be anticipated that garlic may protect against oxidative damage due to lead and cadmium toxicity. The role of GSH and glutathione peroxidase in detoxification of heavy metals has been widely demonstrated (Kang & Enger, 1988; Suzuki & Cherian, 1989). In the present study, therefore, the influence of feeding minced garlic during daily administration of lead acetate or cadmium chloride for six weeks on their toxic effects was investigated in rats. Since metallothionein (MT), a low molecular weight cysteine rich protein is induced by various metals and has a role in metal detoxification, protects against oxidative stress and acts as free radical scavenger, it was also included in the investigation (Bremner, 1987; Sato & Bremner, 1993).

Materials and methods

Materials

Lead (II) acetate trihydrate (analytical reagent grade, BDH, Poole, UK) and cadmium (II) chloride monohydrate (LOBA Chemie, Indo-Australia, Bombay, India) were purchased. All other chemicals used were of analytical reagent grade. Fresh garlic was purchased from the vegetable market, minced and a homogeneous suspension prepared in distilled water a few minutes before administration.

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Experimental

Thirty-six male albino rats (150 ± 10 g) of the Industrial Toxicology Research Centre's colony, maintained on standard pellet diet (M/s Ashirvad Industries Ltd. Roper, Punjab, India, metal contents of diet, in ppm dry weight, Co 8.0, Cu 16.0, Mn 62.0, Zn 106.0, Fe 139.0) and drinking water *ad libitum* were divided equally into six groups, housed in stainless steel cages in an air conditioned room and treated five days a week for 6 weeks as follows:

Group I: No treatment (Control)
II: Garlic 40 mg/kg/4 ml of distilled water, through gastric intubation (orally), twice a day (10 AM and 4 PM)
III: Lead acetate 5 mg Pb/kg/4 ml, intraperitoneally once a day (11AM)
IV: Garlic as in group II + lead acetate as in group III
V: Cadmium chloride 0.5 mg Cd/kg/4 ml, intraperitoneally once a day (11 AM)
VI: Garlic as in group II + cadmium chloride as in group V

All animals were decapitated 24 hr after the last treatment, and blood, liver and kidney were collected.

Biochemical assays

Standard procedures were adopted to determine the activities of blood δ-aminolevulinic acid dehydratase (ALAD) (Berlin & Schaller, 1974), serum aspartate aminotransaminase (ASAT) and alanine aminotransaminase (ALAT) (Reitman & Frankel, 1957), and levels of hepatic and renal glutathione (GSH) (Jallow et al., 1974) and metallothionein (MT) (Onosaka & Cherian, 1982).

Metal analysis

The accurately measured samples of blood (0.5 ml), liver and kidney (0.5 g) were digested in conc. HNO_3 (2.0 ml) using Microwave Digestion System (MDS-2000, CEM Corporation NC, USA). The digested samples were brought-up to 3.0 ml with double distilled water and read on a Flame Atomic Absorption Spectrometer (Varian 250 plus) for lead (283.3 nm), cadmium (228.8 nm), zinc (213.9 nm) and copper (324.7 nm) against suitable standards processed identically.

Statistical analysis

One-way analysis of variance was used to analyse the mean difference between six treatment groups for each metal and biochemical parameter separately after ascertaining the homogeneity of variance between treatment groups. The individual treatment difference between two groups was assessed by computation of least significant difference by taking t-value for error df at 5% level of significance (Zar, 1984).

Results

The administration of lead or cadmium for 6 weeks increased blood, liver and kidney levels of each toxic metal in rats and the concentration of the latter was quantitatively more than the former. The co-administration of garlic significantly decreased the accumulation of these metals (Tables 1 and 2). The activity of blood δ-ALAD was decreased, those of serum ASAT and ALAT were increased, and hepatic and renal GSH levels were depleted in animals exposed to lead or cadmium alone. The changes in these biochemical indicators of metal intoxication were significantly less marked in garlic co-administered animals (Table 3). The feeding of garlic alone increased hepatic and renal MT or MT-like proteins. The increases in their levels were far more marked by the metals than by the garlic alone. Co-administration of garlic and cadmium enhanced the MT contents of liver and kidney; there was no influence of garlic co-administration on the lead-induced MT like proteins (Table 4). Garlic alone had no influence on zinc and copper levels of blood, liver and kidney, except a slight increase in renal zinc. The administration of lead increased their zinc levels while that of cadmium

Table 1. Effect of garlic and lead co-administration on the accumulation of lead in blood and tissues of rat.

Treatment	Blood (μg/ml)	Liver (μg/g, fresh tissue)	Kidney (μg/g, fresh tissue)
Control	0.09 ± 0.02	1.16 ± 0.19	N.D.
Garlic	0.10 ± 0.03	0.97 ± 0.71	N.D.
Lead-control	0.88 ± 0.07^{a}	18.56 ± 2.72^{a}	54.44 ± 8.14^{a}
Garlic + Lead	0.75 ± 0.07^{a,b}	12.69 ± 2.31^{a,b}	38.43 ± 8.07^{a,b}

N.D. Non-detectable; Mean ± S.D. (n = 6) $^{a}p < 0.001$ vs control; $^{b}p < 0.001$ vs lead-control at 5% level of significance (ANOVA).

Table 2. Effect of garlic and cadmium co-administration on the accumulation of cadmium in blood and tissues of rat.

Treatment	Blood (μg/ml)	Liver (μg/g, fresh tissue)	Kidney (μg/g, fresh tissue)
Control	N.D.	N.D.	N.D.
Garlic	N.D.	N.D.	N.D.
Cadmium-control	2.18 ± 0.21[a]	115.60 ± 8.90[a]	134.92 ± 22.99[a]
Garlic + Cadmium	1.21 ± 0.11[a,b]	89.00 ± 12.70[a,b]	111.53 ± 4.67[a,c]

N.D. Non-detectable; Mean ± S.D. (n = 6) [a]$p < 0.001$ vs control; [b]$p < 0.001$, [c]$p < 0.01$ vs cadmium-control at 5% level of significance (ANOVA).

Table 3. Influence of garlic and lead or garlic and cadmium co-administration on metal sensitive biochemical parameters in rat.

Treatment	Blood δ-ALAD (μmol ALA/min/l erythrocyte)	Serum ASAT (nmol hydrozone formed/min/mg protein)	Serum ALAT (nmol hydrozone formed/min/mg protein)	Hepatic GSH (μmol/g, fresh tissue)	Renal GSH (μmol/g, fresh tissue)
Control	6.43 ± 0.20	8.69 ± 0.25	10.17 ± 1.08	8.63 ± 0.83	4.93 ± 0.94
Garlic	5.63 ± 0.26[a]	8.56 ± 0.55	10.24 ± 1.18	7.41 ± 1.00	5.06 ± 0.88
Lead-control	3.10 ± 0.11[a]	17.69 ± 1.54[a]	27.07 ± 1.31[a]	4.51 ± 0.54[a]	3.14 ± 0.26[a]
Garlic + Lead	4.92 ± 0.13[a,c]	14.22 ± 1.58[a,c]	23.92 ± 0.84[a,c]	6.11 ± 1.02[a,d]	4.50 ± 0.46[d]
Cadmium-control	4.13 ± 0.31[a]	44.09 ± 2.01[a]	52.37 ± 2.45[a]	2.88 ± 0.69[a]	2.16 ± 0.26[a]
Garlic + Cadmium	4.53 ± 0.05[a,d]	40.70 ± 1.78[a,c]	50.92 ± 1.94[a]	4.02 ± 0.34[a,e]	3.46 ± 0.33[b,d]

Mean ± S.D. (n = 6) [a]$p < 0.001$, [b]$p < 0.01$ vs control; [c]$p < 0.001$, [d]$p < 0.01$, [e]$p < 0.05$ vs lead-control/cadmium-control at 5% level of significance (ANOVA).

Table 4. Influence of garlic and lead or garlic and cadmium co-administration on hepatic and renal metallothionein levels in rat.

Treatment	Hepatic MT (μg/g, fresh tissue)	Renal MT (μg/g, fresh tissue)
Control	28.06 ± 6.61	20.19 ± 5.17
Garlic	64.02 ± 8.17[b]	70.12 ± 9.93[a]
Lead-control	142.62 ± 32.93[a]	167.96 ± 5.05[a]
Garlic + Lead	135.92 ± 26.15[a]	145.95 ± 21.77[a,e]
Cadmium − control	283.66 ± 32.65[a]	147.74 ± 17.59[a]
Garlic + Cadmium	322.57 ± 17.23[a,d]	300.40 ± 21.47[a,c]

Mean ± S.D. (n = 6) [a]$p < 0.001$, [b]$p < 0.05$ vs control; [c]$p < 0.001$, [d]$p < 0.01$, [e]$p < 0.05$ vs lead-control/cadmium-control at 5% level of significance (ANOVA).

increased their zinc as well as copper levels. The co-administration of garlic and lead or garlic and cadmium considerably reversed these alterations (Tables 5 and 6). Lead had no influence on blood and tissue copper, and cadmium had no effect on blood copper levels (data are not shown).

Discussion

Garlic is a rich source of several organosulfur compounds. Within minutes of crushing fresh garlic, the enzyme alliinase starts producing allyl sulfur compounds, notably diallyl sulfide, diallyl disulfide and diallyl trisulfide, which possess anticancer and antioxidant properties (Lang & Chang, 1981; Forum, 1999). Besides these, allylmethyl disulfide, allylmethyl trisulfide (Lang & Chang, 1981) and E and Z isomers of ajoene, a potent antithrombotic organosulfur compound (Block et al., 1986), have been isolated from garlic bulbs. Intact garlic cloves have also been found to contain S-allyl cysteine-S-oxide (alliin) which is converted into allyl 2-propene thiosulfinate (allicin) by the action of the enzyme allinase (Block et al., 1986). The co-administration of garlic decreased the accumulation of lead and cadmium in tissues and consequently reversed the metal induced biochemical alterations which indicate complexation of lead and cadmium (soft lewis acid) with the free "lone pair" of electrons on the sulfur atom (soft lewis base) of various organo sulfides present in garlic and the subsequent excretion of such complexes. The possibility of an interaction between lead acetate or cadmium chloride and amino and carboxylic

Table 5. Influence of garlic and lead or garlic and cadmium co-administration on zinc levels in blood and tissues of rat.

Treatment	Blood (μg/ml)	Liver (μg/g, fresh tissue)	Kidney (μg/g, fresh tissue)
Control	5.11 ± 6.68	25.37 ± 1.58	18.77 ± 2.00
Garlic	5.30 ± 0.91	24.67 ± 3.07	21.43 ± 2.32[b]
Lead-control	11.75 ± 0.69[a]	32.85 ± 2.85[a]	22.72 ± 3.53[b]
Garlic + Lead	9.07 ± 1.04[a,c]	27.40 ± 1.91[d]	19.68 ± 2.29
Cadmium-control	7.97 ± 1.33[a]	32.03 ± 1.86[a]	26.55 ± 1.55[a]
Garlic + Cadmium	6.27 ± 0.22[b,d]	28.23 ± 1.19[b,d]	25.57 ± 0.64[a]

Mean ± S.D. (n = 6) [a]$p < 0.001$, [b]$p < 0.05$ vs control; [c]$p < 0.001$, [d]$p < 0.01$ vs lead-control/cadmium-control at 5% level of significance (ANOVA).

Table 6. Influence of garlic and cadmium co-administration on copper levels in tissues of rat.

Treatment	Liver (μg/g, fresh tissue)	Kidney (μg/g, fresh tissue)
Normal animal	5.63 ± 0.55	7.52 ± 0.70
Garlic	5.51 ± 0.35	7.51 ± 0.49
Cadmium-control	7.54 ± 0.61[a]	9.99 ± 0.82[a]
Garlic + Cadmium	5.63 ± 0.36[b]	8.21 ± 0.19[b]

Mean ± S.D. (n = 6) [a]$p < 0.001$ vs control; [b]$p < 0.001$ vs cadmium-control at 5% level of significance (ANOVA).

groups of S-allyl cysteine-S-oxide (alliin), a constitutent of garlic, to form their readily excretable complex can not be ruled out.

The antioxidant property of garlic and its ability to biosynthesize hepatic and renal MT or MT-like proteins also seems to be responsible for the protection against oxidative damage and tissue depletion of GSH by lead and cadmium. The binding and protective role of MT in heavy metal toxicity are well documented (Bremner, 1987; Sato & Bremner, 1993). Higher hepatic and renal MT and GSH levels in animals co-administered garlic and cadmium and their higher GSH levels in animals co-administered garlic and lead than in those given metal alone support this assumption. The increase in tissue GSH appears to be a protective response to metal intoxication as observed upon garlic co-administration. However, tissue MT levels were lower in animals co-administered garlic and lead than in those administered lead alone which suggests the possibility of a faster excretion of lead bound to MT like proteins in the former.

The increase in liver and kidney levels of zinc and copper in animals administered cadmium and in their zinc levels in those administered lead is probably associated with the induction of hepatic and renal MT or MT-like proteins by these metals. The induced MT may have enhanced the uptake of dietary zinc and copper and is capable of binding these essential trace elements. It appears that the lead induced MT like proteins have more affinity for zinc than for copper. However, the co-administration of garlic has considerably reduced the cadmium or the lead induced increase in tissue zinc and copper obviously due to the depletion of toxic metals and, hence, the decrease in their toxic effects brought about by garlic.

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