Investigation of effects of time of measurement and modes of administration on cadmium accumulation in rat liver under some medicinal plants food supplemented diet

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ABSTRACT

Context and Objectives: Cadmium (Cd) toxicity leads to cell and organ damage, we comparatively examined the protection ability of different medicinal plants on Cd liver accumulation following different treatment interventions and modes of administration. **Materials and Methods:** Rats were fed either 7% w/w *Zingiber officinale*, 7% w/w *Allium Sativum*, 10% w/w Lycopersicon esculentum, 5%, w/w Garcinia kola (all in rat chow), while Cd (200 ppm) was given in drinking water. Additives were administered together with (mode 1), a week after (mode 2) or a week before metal exposure (mode 3) for a period of six weeks. Cd liver was determined using AAS and compared using analysis of variance (ANOVA). **Results:** All additives significantly ($P < 0.5$) reduced the accumulation of Cd in the liver. After adjusting for time and mode of administration, mean %protection for week 4 was significantly lower by 14.1% ($P = 0.02$) from that for week 2 but the means did not differ with respect to additive used or mode of administration, no statistically significant interaction between modes of administration and either of additives used or time of administration in their respective relationships to percentage protection from Cd. **Conclusion:** Additives significantly reduced Cd accumulation through a reduction in absorption and enhancement of metal excretion.

Key words: Accumulation pattern, *Allium sativum*, Cadmium, *Garcinia kola,* liver, Lycopersicon esculentum, mode of administration, *Zingiber officinale*

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INTRODUCTION

Humans are exposed to cadmium through a variety of means such as occupational environments, food, air, water, industrial products.[1,2] This exposure leads to various dysfunctions, which are multi-organ and multi-systemic in nature as cadmium binds to tissues, macromolecules, and metallothionein. Its binding to biological components such as proteins and non-protein sulfhydryl groups causes derangements in enzymes and lipids as it increases the production of reactive radicals, oxidative tissue damage, and loss of membrane functions, and it can also have deleterious effects by deactivating DNA repair activity.[3,4]

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Tissue and cellular damage associated with cadmium exposures lead to severe complications and diseases like increased blood pressure, anemia, osteoporosis, myocardial dysfunctions, proteinuria, renal dysfunction, hepatic dysfunction, non-hypertrophic emphysema, pulmonary edema, and death.[5] In the management of metal poisoning, chelators are primarily used as these chelators can shield the metal from the biological targets by way of mobilization and excretion,[2,6] but they are not as useful in reversing the damage done by exposure to these toxicants.[7] Reports on the increasing role and usefulness of medicinal plant products in maintenance and optimization of health have lead to research on the efficacy of these medicinal plants/ nutrient products on the amelioration of the metal-induced toxicity.[8] These plant products are reported to affect the bioavailability, transport, and toxico-dynamics of metals in the tissues.[1,2,9-11]

Allium sativum (garlic) is claimed to have both prophylactic and curative effects[12-14] through its antioxidant properties. It contains many sulfur-active principles reported to contribute its metal-chelating properties.[2,15] *Zingiber officinale's* (ginger's) medicinal value is reported to be through its radical-scavenging properties and as an antioxidant.[9,16] *Garcinia kola* is considered as an antioxidant[17] and antidote to various poisons;[18] it contains oxalates and tannins, which have been reported to be chelators of divalent metal.[2,19,20] Tomato (*Lycopersicon esculentum*) is also a source of antioxidants.[21,22] It is among a group of plants reported to synthesize metal-chelating proteins, peptides, phytochelatins (PC), and other heavy metal-binding complexes analogous to metallothioneins when exposed to heavy metal ions.[23,24]

We have recently shown that *Garcinia kola,*[1] *Allium sativum,*[2] *Zingiber officinale,*[9] and *Lycopersicum esculentum*[10] reduced the hepatotoxic effect of cadmium through a hepato-protective role. The present study is to comparatively study the effects of these nutrients and medicinal plants on cadmium accumulation in the liver of rats following different treatment interventions. The study also aims to determine whether the modes of administration yielded different results and whether these differences varied with the additive used or with the time of measurement.

MATERIALS AND METHODS

Animals and experimental design

Seven-week-old Male Wistar rats weighing 150-180 g were obtained from the animal house of the faculty and used for the study. The animals were kept at constant room temperature with 12 h of light/dark cycles. All animals were fed with normal rat chow and had access to tap water *ad libitum* during the period of acclimatization. We sought and received ethical approval from the Federal University of Technology Faculty Ethical Committee for this study; "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable.

Preparation of nutrient substance and heavy metals

Fresh ginger rhizome, garlic, *Garcinia Kola,* and tomato were purchased from the market, ground and sieved (particulate size of 250 μm). Each of the medicinal plants was mixed with rat chow - 7% w/w of *Zingiber officinale* in rat chow, 7% w/w of *Garlic* in rat chow, 10% w/w of Lycopersicon esculentum in rat chow, 5%, w/w of *Garcinia kola* in rat chow - and fed to each of three groups of 15 rats – yielding 12 groups of rats. One group did not receive any of the medicinal plants so that, in total, there were 13 groups of animals and all were exposed to 200 ppm cadmium chloride in tap water accessed directly from the drinking water bottle. These concentrations were determined based on findings from our earlier reported studies.[1,2,9-11,25]

Experimental protocol

Treatment group one was fed with normal rat chow and cadmium (Cd = 200 ppm) only. Treatment group two was fed with rat chow and one of the nutritional medicinal plants mixed with rat chow (7% w/w of *Zingiber officinale* in rat chow, 7% w/w of *Garlic* in rat chow, 10% w/w of Lycopersicon esculentum in rat chow, 5% w/w of *Garcinia kola* in rat chow); the exposure and feeding started same time. Treatment group three was fed with normal rat chow and water mixed with Cd = 200 ppm for the first week and then with rat chow mixed with one of the medicinal plants as per the previously stated concentrations and tap water without cadmium from the second to the sixth week. Group four was fed with rat chow mixed with one of the medicinal plants as per the previously stated concentrations for one week, and after that, fed with normal rat chow and Cd (200 ppm) in drinking water for the remaining five weeks. Thus, there were three modes of administration of the additive with cadmium. For mode one the cadmium and the additive were given at the same time; for mode two, cadmium was given only during the first week and administration of the additive started from the second week and continued for the duration of the study; and for mode three, the additive was given only during the first week and administration of cadmium started from the second week and continued for the duration of the study. Fortnightly, up to six weeks, five of the animals from the different treatment groups were sacrificed, and percentage protection provided by the additives was estimated.

The grouping and feeding patterns are summarized in Table 1. All administrations were through the oral route.

Tissue preparation and analysis

At the end of the experimental period, the rats were sacrificed under chloroform anesthesia. Liver (1 g) was

Table 1: Summary of specimen grouping and six weeks feeding pattern

Week	Group 1	Group 2	Group 3	Group 4
1	F+WCd	FGa+WCd	F+WCd	FGa+W
2	F+WCd	FGa+WCd	FGa+W	F+WCd
3	F+WCd	FGa+WCd	FGa+W	F+WCd
4	F+WCd	FGa+WCd	FGa+W	F+WCd
5	F+WCd	FGa+WCd	FGa+W	F+WCd
6	F+WCd	FGa+WCd	FGa+W	F+WCd

F=Feed (rat chow); W=Eater; FGa=Feed-nutrient concentrate; WCd=Cadmium in water (Cd=200ppm) excised and transferred to polypropylene vials for analysis. Before acid digestion, a porcelain mortar was employed to grind and homogenize the tissue samples in 5 mls of normal saline. After digestion of all samples, the concentrations of Cd were analyzed using flame atomic absorption spectrophotometer (Perkin Elmer A.A. 3030) with D2 background correction device.

Statistical analysis

This study aimed to determine whether the percentage protection provided by different additives differed significantly. The additives used were garlic, ginger, *Garcinia kola*, and tomato. Each of these additives was given in the presence of cadmium. The study also aimed to determine whether the modes of administration yielded different results and whether these differences varied with the additive used or with the time of measurement.

The percentage protection provided by the presence of each additive was obtained using the formula:

$$\frac{Cd_{1j} - Cd_{ij}}{Cd_{1j}} \times 100 \qquad (1)$$

where Cd_{1j} is the concentration of accumulated cadmium in the liver sample when exposed to cadmium without the food additive (treatment group 1) at time *j*, (*j* = *2, 4, 6, weeks*). Cd_{1j} is the mean value for accumulated cadmium concentration in the liver obtained from the sample of five rats sacrificed at each time point.

Cd_{ij} is the concentration of accumulated lead in the liver sample when exposed to cadmium in the treatment group *i*, (*i* = *2, 3, 4*) at time *j*, (*j* = *2, 4, 6, weeks*). Cd_{ij} is the mean value for accumulated cadmium concentration in the liver obtained from the sample of five rats fed a given additive using a particular mode of administration at time points 2, 4, and 6, respectively.

Descriptive data analysis yielded means and standard error of means for the percentage protection for the explanatory variables - modes of administration, additives, and times of administration. The analysis of variance (ANOVA) method was used to determine whether the means differed with respect to any of the levels of the explanatory variables, and models included the interaction terms to allow for assessment of whether or not the differences due to mode of administration differed with respect to additive or time of administration. Where necessary, Bonferroni corrections for multiple comparisons were applied.

Data analysis was done using Stata version 12.0, and Origin™ 5.0 (Microcal Software Inc., Northampton, USA) created graphical displays of the data. Estimates were considered statistically significant of the associated *P* value less than 0.05.

RESULTS

Effect of various additives and cadmium co-administration on accumulation in the liver

As illustrated in Figures 1-3, cadmium concentration in the liver increased over time in those rats given the metal in water with rat chow without the nutrient products. Figure 1 shows that, using administration mode 1, the

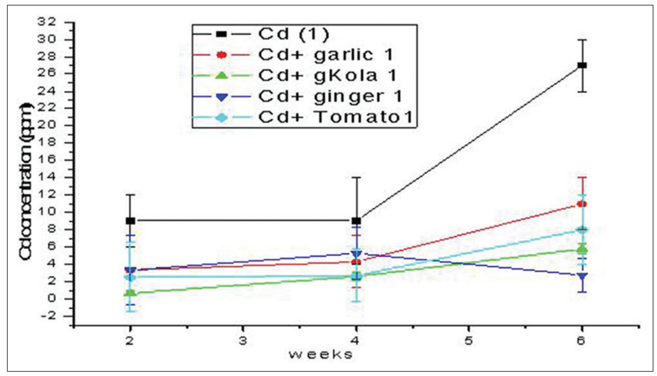

Figure 1: Effects of the various nutrient substances on cadmium accumulation in the liver of rats when animals were exposed to cadmium and nutrients at same time.

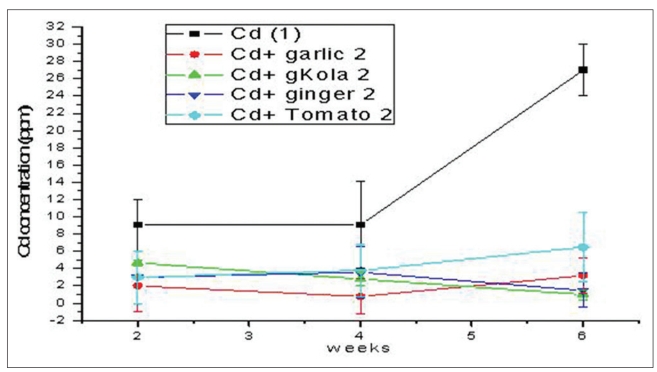

Figure 2: Effects of the various nutrient substances on cadmium accumulation in the liver of rats when nutrients were used after a week's exposure to cadmium.

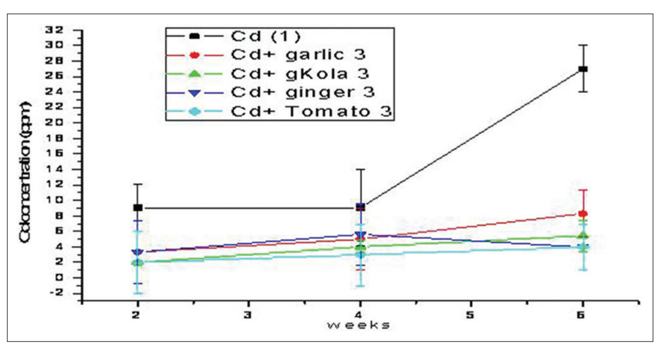

Figure 3: Effects of the various nutrient substances on cadmium accumulation in the liver of rats when animals were exposed to cadmium after a week's exposure to the nutrients.

cadmium concentration in the liver increased over time when given alone or in the presence of garlic, tomato, and *Garcinia kola* but falls over time in the presence of ginger. The additives used all significantly ($P < 0.05$) reduced the accumulation of cadmium in the tissue across the six weeks of study. When administration mode two was used, cadmium concentration increased in the presence of tomato and garlic as additives but not in the presence of *Garcinia kola* and ginger [Figure 2]. The additives used all significantly ($P < 0.05$) reduced the accumulation of cadmium in the tissue across the six weeks of study. When administration mode three was used, cadmium concentration decreased over time in the presence of ginger but not in the presence of the other additives [Figure 3]. The additives used all significantly ($P < 0.05$) reduced the accumulation of cadmium in the tissue across the six weeks of study.

Table 2 gives the percentage protection calculated using equation (1) for each sample of five rats sacrificed within the different treatment groups at the different times for the different modes of administration. For mode one, percentage protection appeared for fall over time in the presence of garlic, tomato, and *Garcinia kola* but not in the presence of ginger. For all four additives, percentage protection generally increased from week two to week six when administration mode two was used. When administration mode three was used, percentage protection tended to increase from weeks two to six in the presence of garlic, ginger, and tomato but decreased in the presence of *Garcinia kola*.

Table 2: Summary of percentage protection by the nutritional medicinal plants on cadmium accumulation in the liver

	Cadmium			
	Garlic	Ginger	Tomato	Garcina kola
Mode 1				
Week 2	63.3	63.3	72.4	90
Week 4	52.6	41.8	70.2	41
Week 6	59.3	89.8	70.3	56
Mode 2				
Week 2	77.9	77.9	67	37.5
Week 4	91.2	60.4	58.1	46.9
Week 6	88.1	94.5	76	94
Mode 3				
Week 2	63.3	63.3	78	72.4
Week 4	44.9	37.9	67	44.8
Week 6	69.3	85.3	85.2	62.2

Data shows percentage protection in each treatment group

Summary statistics in Table 3 showed that the mean percentage protection was highest for mode two (72.4%, 95% CI = 61.4-83.4%) and lowest for mode one (64.2, 95% CI = 54.9-73.4%), but the differences between means were not statistically significant. Results further showed that mean percentage protection was lowest for Garcinia kola as an additive (60.5%, 95% CI = 46.4-74.7%) and highest for tomato (71.5%, 95% CI = 66.3-76.8%). Again, the differences between means were not statistically significant. Table 3 also shows that the mean protection at week four was lowest (54.7%, 95% CI = 45.6-63.8%) but highest at week six (77.5%, 95% CI = 69.4-85.6%). One-way analysis of variance revealed that mean percent protection for week six was significantly higher by 22.8% ($P = 0.001$) from that for week four. However, the means for weeks four and six, respectively, did not differ from those for week two. When we adjusted for the type of additive used and the mode of administration, multi-way ANOVA revealed that the mean percentage protection at week four was significantly lower than that for week two (difference = -14.1%, 95% CI = -25.8 − -2.4, $P = 0.02$). Multi-way analysis of variance revealed that the relationship between the modes of administration and percentage protection was the same at all times of administration and for all additives. That is, there was no statistically significant interaction between time of administration and mode of administration or between additive and mode of administration in their relationship to percentage protection in the presence of cadmium.

Table 3: Means and 95% confidence intervals (in brackets) for percentage protection for the different modes, additives and times

Factor	Mode 1		Mode 2		Mode 3		Total	
	n	Mean (95%CI)	*n*	Mean (95%CI)	*n*	Mean (95%CI)	*n*	Mean (95%CI)
Additive								
Tomato	3	57.6 (43.4-71.7)	3	49.7 (38.2-61.1)	3	48.1 (41.1-55.1)	9	71.5 (66.3-76.8)
Ginger	3	38.4 (10.4-66.4)	3	53.4 (44.2-62.6)	3	43.0 (30.8-55.3)	9	68.2 (54.6-81.9)
Garlic	3	37.2 (19.5-54.9)	3	64.4 (37.6-91.2)	3	57.7 (31.3-84.2)	9	67.8 (57.3-78.3)
Garcinia kola	3	55.0 (41.8-68.1)	3	47.9 (10.8-85.1)	3	51.9 (37.7-66.1)	9	60.5 (46.4-74.7)
Time								
2 weeks	4	37 (25.5-48.5)	4	34.7 (18.5-51.0)	4	36.9 (30.7-43.0)	12	68.8 (61.3-76.4)
4 weeks[d]	4	41.9 (21.1-62.7)	4	60.6 (52.5-68.6)	4	55.6 (43.6-67.7)	12	54.7 (45.6-63.8)***
6 weeks	4	62.2 (55.9-68.5)	4	66.3 (51.5-81.1)	4	58.1 (47.4-68.8)	12	77.5 (69.4-85.6)***
Total	12	64.2 (54.9-73.4)	12	72.4 (61.4-83.4)	12	64.5 (55.4-73.5)		

*=P<0.05, **=P<0.01, ***=P<0.001, [d]-Week 4 mean significantly different from that for week 6

DISCUSSIONS

Some medicinal plants are reported to produce metal-chelating peptides from phytochelatin synthase (PCS), a protease-like enzyme from glutathione.[24,26] These proteins such as metallothioneins and phytochelatins (PC) can play essential roles in the binding, transport, and accumulation of metals (toxico-kinetics) in the body tissues. Other properties that can affect the toxico-kinetics of metals include organo-sulfur compounds found in garlic, which are reported to inhibited superoxide production by xanthine-xanthine oxidase and also posses' metal-chelating capability.[27,2] Oxalates and tannins are reported to be chelators of divalent metal.[1,19,20] All the medicinal plants used in this study are regarded as sources of antioxidants and as such, used both prophylactically and curatively. The confidence intervals for the mean percentage protection for the respective modes of administration at each time point all excluded zero giving evidence that at a given time of administration, each of the modes could be expected to give a level of protection significantly different from zero. The antioxidant and anti-inflammatory properties (of ascorbate, glutathione, and α-tocopherol), phenolic, and vitamin-rich mixtures, which can give a synergistic effect incorporating both the radical scavenger[28] and the transition-metal ion chelation properties,[29,30] can account for the protective ability of the additives used in the study.

The modes of administration used in the study aim to aid our understanding of the best use these medicinal products in the management of cadmium accumulation and toxicity. Interactions in mode one (metal and additives given simultaneously) will provide information about the absorption in the presence of the additives, while mode two will throw more light on whether the additives enhanced the metal excretion and mode three on whether the additives prevented or reduced the absorption of the metal in question.[31] It was observed that all the three modes employed showed significant protective interactions, further confirming that the additives used affected the toxico-kinetics of metal. But, mode two yielded the highest mean percentage protection and mode one the lowest; this type of variation was observed at week four but not at weeks two and six. This type of variation was observed in the presence of ginger and garlic only. Our results revealed that there was no statistically significant interaction between modes of administration and additive used, in their relationships to percentage protection, but all the additives used significantly reduced the accumulation of this metal in the liver not minding the mode of administration. Neither was there statistically significant interaction between time of administration and mode of administration in their relationship to percentage protection in the presence of cadmium.

The absence of statistically significant difference between the different modes highlights that these additives can effectively reduce the accumulation of cadmium either by enhancing secretion, preventing accumulation and absorption, chelating or making the cadmium unavailable up to the same degree. It also showed that the additives were effective up to the same degree as there was no significant interaction between modes of administration and additive used, in their relationships to percentage protection. In general, our results have demonstrated the need to use diets with natural compositions for dietary toxicity studies. These additives have protective effect against Cd-induced liver damage by reducing Cd accumulation. However, the details of the mechanism of these additives on liver toxicity remains to be clarified by further studies to explain the interactions and mechanisms of actions of these nutrients and medicinal products in order to expand our knowledge and understanding of the interactions and how they can affect the toxico-kinetics of these injurious substances we tend to accumulate within our environment.

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