

A comparative study of the effect of some nutritional medicinal plants effect on lead accumulation in the liver following different modes of administration

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ABSTRACT

Context and Objectives: Lead (Pb) toxicity leads to cell damage in many organs of the body. Using different treatment interventions and modes of administration we comparatively examined the protective ability of some medicinal plants on liver Pb accumulation. **Materials and Methods:** Rats were fed on 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 Pb (100 ppm) was given in drinking water. The additives were administered together with (mode 1), a week after exposure to (mode 2) or a week before metal exposure to (mode 3) the metal for a period of 6 weeks. The metal accumulations in the liver were determined using atomic absorption spectrometry and compared using analysis of variance. **Results:** Some additives significantly ($P < 0.05$) reduced, while others enhanced Pb accumulation. Mode 2 yielded the highest mean % protection and mode 3 the lowest, no significant interaction between modes of administration and time of measurement in their relationships to percentage protection, but there was statistically significant ($P < 0.05$) interaction between modes of administration and additive used in their relationships to percentage protection. **Conclusion:** Protective effects of medicinal plants are varied and depend on the nature of lead exposure.

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

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INTRODUCTION

Lead (Pb) has been shown to have a multisystem effect seriously affecting the nervous, circulatory, skeletal, renal, hematopoietic, and endocrine systems.^[1] Long-term exposure to Pb may also result in a nephropathy or renal adenocarcinoma, neuromuscular weakness,^[2,3] behavioral, and neurochemical alterations.^[3-5] These can occur through a derangement of the antioxidant enzymes and neurotransmitter balance.^[4,5] Pb toxicity is reported to occur through its affinity for proteins and its capacity to simulate calcium and iron (Fe) channels.^[6] The increase in intracellular calcium together with the increase in reactive oxygen species, which is reported to occur with increase in lead exposure,

can trigger apoptosis through the cytochrome C release and a fall in the mitochondrial potential.^[6,7]

The use of chelators are usually the primary cause of management with metal poisoning and these chelators can shield the metal from the biological targets by way of mobilization and excretion,^[8] but they are not always useful in reversing the damage done by exposures to these toxicants.^[6] Reports on the increasing role and usefulness of medicinal plant products in maintenance and optimization of health have led to research on the efficacy of these medicinal plants/nutrient products' effects on the amelioration of the metal-induced toxicity.^[9-13] These plant products are reported to affect the bioavailability, transport, and toxico-dynamics of this metal in the tissues.

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Allium sativum's (garlic) metal chelating properties,^[9,14] *Zingiber officinale*'s (ginger) radical scavenging properties.^[11,15] *Garcinia kola* characteristics to act as chelators of divalent

metal,^[9,16,17] and Tomato's, (*Lycopersicon esculentum*) abilities to synthesize metal chelating proteins, metallothioneins, peptides, phytochelatins^[12,18,19] have all been reported and documented. These medicinal plant products are also reported for their prophylactic and curative uses, which is related to their antioxidant properties.^[9,20,21]

We have recently shown that *G. kola*,^[9] *A. sativum*,^[10] *Z. officinale*^[11] and *L. esculentum*^[12] reduced hepatotoxic effect of lead through a hepato-protective role. This study is to comparatively study the effects of these nutrients and medicinal plants on lead accumulation in the liver of rats following different treatment interventions. 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.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar rats of about 7 weeks old 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 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; and "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, *G. kola* and Tomato were purchased from the market at Okija, Nigeria, from January to April (mainly during the dry season). Professor C. Ufearo of the Nnamdi Azikiwe University Nigeria did the authentication. Materials were ground with a kitchen blender and sieved using a very fine sieve (particulate size of 250 µm). Each additive –7% w/w of *Z. officinale*, 7% w/w of *A. sativum*, 10% w/w of *L. esculentum*, 5% w/w of *G. kola* – was mixed with rat chow and fed to different groups of animals. Each group received directly from the drinking water bottle tap water that contained 100 ppm lead acetate. The lead was prepared from Lead acetate. Ten millilitre of 1000 ppm lead was further diluted with 90 ml of distilled water. Lead estimation was calculated from the calibration curves. These concentrations were arrived at following our earlier reported studies^[9-12] with a calibration curve, which was prepared for the estimation of the sample and control materials.

Experimental protocol

Group 1 was fed with normal rat chow and lead (Pb = 100 ppm,) only. Group 2 was fed with rat chow and one of the nutritional medicinal plants (7% w/w of *Z. officinale*, 7% w/w of Garlic, 10% w/w of *L. esculentum*, 5%, w/w of *G. kola*) mixed with rat chow along with the lead acetate water, the exposure to lead and feeding starting same time. Group 3 was fed with normal rat chow and water mixed with Pb (100 ppm), for the 1st week and then with rat chow mixed with one of the additives and tap water without lead from the 2nd to the 6th week. Group 4 was fed with rat chow mixed with one of the additives for 1 week, and after that they were fed with normal rat chow and Pb (100 ppm) in drinking water for the remaining 5 weeks. The grouping and feeding patterns are summarized in Table 1. All administrations were through the oral route.

Tissue preparation

At the end of the experimental period, the rats were sacrificed under chloroform anesthesia. Liver (1 g) was excised and transferred in polypropylene vials for analysis. Before acid digestion, a porcelain mortar was employed to grind and homogenize the dry tissue samples in 5 ml of normal saline. After digestion of all samples the concentrations of Pb was analyzed using flame atomic absorption spectrophotometer (AAS) (Perkin Elmer A.A. 3030 Waltham, MA USA) with D2 background correction device. Cold vapor technique was used for the analysis of Pb (Kingston and Jessie, 1988; Medham, 2000). Lead was estimated using the AAS at 283 nm wavelength.

Statistical analysis

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

$$\frac{Pb_{1j} - Pb_{ij}}{Pb_{1j}} \times 100 \quad (1)$$

Where,

Pb_{1j} is the concentration of accumulated lead in the liver sample when exposed to lead alone (treatment Group 1)

Table 1: Summary of specimen grouping and 6 weeks feeding pattern

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

F=Feed (rat chow); W=Water; FGa=Feed-nutrient concentrate; WPb=Lead in water (Pb=100 ppm)

at time j , ($j = 2, 4, 6$, weeks). Pb_{ij} is the mean value for accumulated lead concentration in the liver obtained from the sample of five rats sacrificed at each time point.

Pb_j is the concentration of accumulated lead in the liver sample when exposed to lead in the treatment group i , ($i = 2, 3, 4$) at time j , ($j = 2, 4, 6$, weeks). Pb_{ij} is the mean value for accumulated lead 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.

Means and standard errors for the percentage protection were obtained for the various additives, modes of treatment and times of administration. Analysis of variance (ANOVA) with Bonferonni's posttest analysis was performed to determine whether the means differed with respect to each of the aforementioned sources of variation - additives, modes of treatment and times of administration. In addition the ANOVA was used to examine whether the relationship of each of additive and time of measurement with percentage protection differed with respect to mode of treatment. The non-parametric test for trend was also used to examine whether the percentage protection increased or decreased with time of administration. Stata version 12.0 was used to carry out data analysis and Origin™ 5.0 (Microcal Software Inc., Northampton, USA) created graphical displays of the data. $P < 0.05$ was considered as statistically significant.

RESULTS

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

Figures 1-3, each show that the concentration of lead accumulated in the liver tissue over time for each of the additives and treatment groups (modes of administration). Excepting for tomato given using mode 2, the concentration of lead generally fell over time after the 2nd week. The additives used all significantly ($P < 0.05$) reduced the accumulation of lead in the first 2 weeks of the study for all modes of administration except for garlic in mode 3. This pattern was observed at weeks 4 and 6, but not at week 2. In addition, confidence intervals (CIs) for mode 3 at weeks 4 and 6 and mode 1 at week 4 indicated that the means for these modes at these times did not differ significantly from 0, due to the fact that, in the presence of lead some of the additives gave little or no protection. As one of the study objectives was to determine whether there was interaction between mode of administration and time, ANOVA was also used to examine whether the nature of the variation in means for mode of administration was the same at each of the three measurement times -2, 4 and 6 weeks.

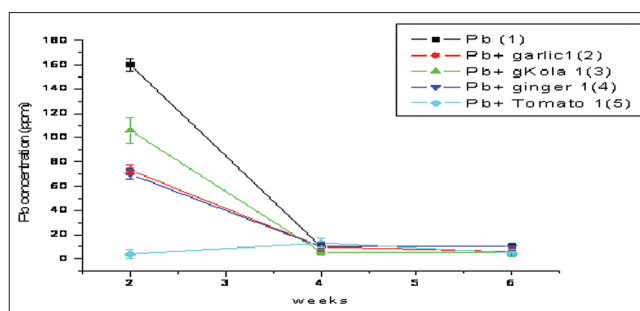


Figure 1: Effects of the various nutrient substances on lead accumulation in the liver of rats when animals were exposed to lead and nutrients at same time

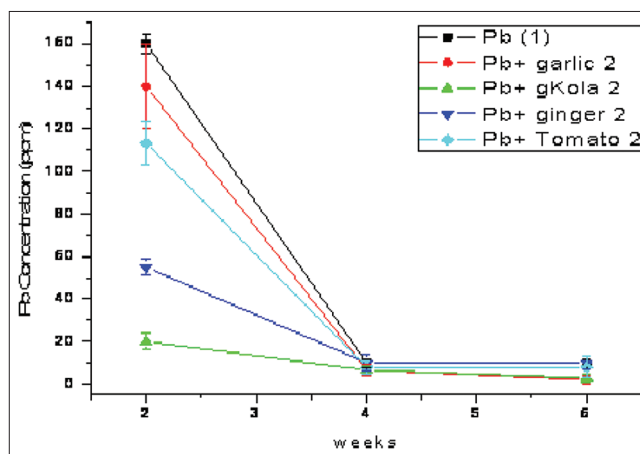


Figure 2: Effects of the various nutrient substances on lead accumulation in the liver of rats when nutrients were used after a week's exposure to lead

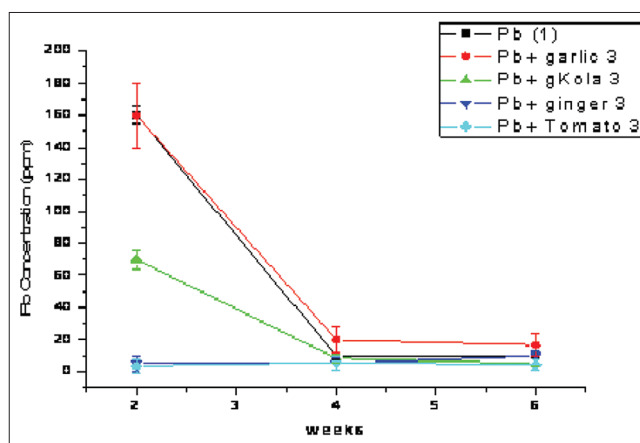


Figure 3: Effects of the various nutrient substances on lead accumulation in the liver of rats when animals were exposed to lead after a week's exposure to the nutrients

Comparative analysis of percentage protection to lead

Table 2a gives the mean percentage accumulation for the samples of rats sacrificed at the different time periods within groups defined by additive and mode of administration. For ginger, the level of protection tended to fall to zero as time passed and for tomato, an actual increase

in the lead accumulation, leading to negative percentage protection was observed.

Analysis of variance was used to determine whether the nature of the variation in means for mode of administration was the same for each additive. There was statistically significant interaction between modes of administration and additive used, in their relationships to percentage protection. This means that the differences between the modes were not the same for all additives. The adjusted mean values, produced by the ANOVA, for the percentage protection for groups defined by additive and mode combinations revealed that the percentage protection offered by mode 3 in the presence of garlic was actually negative – indicative of increasing lead levels relative to the amounts when lead is given alone - and significantly different from the percentage protection obtained for the other modes in the presence of garlic [Tables 1 and 2]. Results in Table 2b further showed

that the differences between the modes for the other additives were not significantly different.

DISCUSSION

Increasing drug toxicity due to metabolic activation and other unwanted side-effects of synthetic drugs has led to the increasing global demands and use of herbal remedies in the management and treatment of various ailments and to promote health.^[9-12] Our results showed that the additives used affected the accumulation levels of lead in the liver. Some of them significantly reduced the accumulation, while others had no effect or, interestingly, increased its accumulation. The additives used all significantly reduced the accumulation of lead in the first 2 weeks of the study for all modes of administration except for garlic in mode 3. Though nutrients and nutritional status^[9,22] affect the rate of accumulation and or excretion of metals, the observed decrease in the lead concentrations seen across all modes is not due to any analytical error. Jezierska and Witeska,^[23] had reported that accumulation is a function of uptake and elimination, the unexpected patterns of metal accumulation seen here has been reported by others. We also observed Pb accumulation increased with initial exposure but decreased with further exposure in our studies, this is not due to any experimental or analytical error as such patterns have been reported by others.^[24,25,29]

Many reports exist in literature about the efficacy of medicinal plant products in ameliorating or reducing the toxicity and accumulation of lead in tissues^[9,14] due to their metal chelating, antioxidant and scavenging properties,^[18,19] and have also highlighted that these properties are not

Table 2a: Summary statistics (sample means) of percentage protection by the nutritional medicinal plants, time and mode of administration

	Garlic	Ginger	Tomato	Garcina kola
Group 2				
Week 2	54.1625	56.25	97.5	33.75
Week 4	0	0	-33.3	44
Week 6	44.1	0	48.9	47.9
Group 3				
Week 2	12.4938	65.625	29.1875	87.5
Week 4	33.3	0	20	33.3
Week 6	80	0	20	72
Group 4				
Week 2	0	96.875	97.9188	56.25
Week 4	80	50	43.2	0
Week 6	33.9	0	66.1	0

Table 2b: Means and 95% CIs (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								
Garlic								
Unadjusted	3	32.8 (-1.0-66.5)	3	41.9 (1.4-82.5)	3	-55.4 (-115.0-4.2)	9	6.4 (-32.5-45.4)
Adjusted		32.8 (3.3-62.2)		41.9 (12.5-71.4)		-55.4 (-84.8-25.9)**a		
Ginger								
Unadjusted	3	18.8 (-19.3-56.8)	3	21.9 (-22.5-66.3)	3	49.0 (-7.8-105.7)	9	29.9 (4.4-55.3)
Adjusted		18.8 (-10.7-48.2)		21.9 (-7.6-51.3)		49.0 (19.5-78.4)		
Garcinia Kola								
Unadjusted	3	41.9 (33.3-50.4)	3	64.3 (31.5-97.0)	3	18.8 (-19.3-56.9)	9	41.6 (21.8-61.4)
Adjusted		41.9 (12.4-71.4)		64.3 (34.8-93.7)		18.7 (-10.7-48.2)		
Tomato								
Unadjusted	3	37.7 (-39.8-115.2)	3	23.1 (16.8-29.3)	3	69.1 (36.9-101.3)	9	43.3 (15.4-71.2)
Adjusted		37.7 (8.2-67.2)		23.1 (-6.4-52.5)		69.1 (39.6-98.5)		
Time								
2 weeks	4	60.4 (33.3-87.5)	4	48.7 (14.1-83.3)	4	62.8 (16.0-109.6)	12	57.3 (37.6-77.0)
4 weeks	4	2.7 (-29.5-34.9)	4	21.7 (5.7-37.6)	4	-1.7 (-71.9-68.5)	12	7.5 (-17.0-32.1)*b
6 weeks	4	35.2 (11.3-59.2)	4	43.0 (3.3-82.7)	4	0.0 (-54.8-54.8)	12	26.1 (1.6-50.6)
Total	12	32.8 (12.2-53.3)	12	37.8 (19.7-55.8)	12	20.4 (-15.1-55.8)		

*P<0.05, **P<0.01, ***P<0.001, ^aSignificant difference between mode 3 and mode 1 for garlic. ^bSignificant difference between week 4 and week 2, means. CI=Confidence interval

same for all nutrient substances.^[26,27] Enhancement of the antioxidant capacity of the liver, reduction of hepatocyte injury and lipid peroxidation, improvement of barrier functions and antioxidant activity, decrease oxidative DNA damage in the liver and increased hepatic detoxification and bile production are some of the ways by which nutrient substances can offer a hepatoprotective ability. Our result shows that these products gave protection in varying degrees. It was observed that ginger gave less protection as the weeks progressed in all modes of treatment, while tomato was associated with an increase in accumulation at week 4 using mode 2 when compared with the control values.

The mode of administration of the additive substances and metals was designed to give an understanding as to how these plant products/additives affect the bioavailability of lead.^[30] Administration of the lead and the additive at the same time, as per mode 1, would provide indication of whether the additives prevented the absorption of lead. Administration of lead during the 1st week only, as per mode 2, followed by administration of the additive starting from the 2nd week until the end of the study would indicate whether the additive enhanced excretion of the accumulated metal, while administration of the additive in the 1st week only, as per mode 3, followed by administration of lead from week 2 onwards would indicate whether the additives prevented the absorption and accumulation of the metal. The summary statistics shown indicated that mode 2 yielded the highest mean percentage protection and mode 3 the lowest; this showed that the additives used in the study were effective mainly through reducing the absorption of Pb in the liver. Results revealed there was no statistically significant interaction between modes of administration and time of measurement in their relationships to percentage protection. The frequency and mode of administration is reported to affect efficacy of treatment.^[28]

The trend of increased mean percentage protection for modes 1 and 2 relative to mode 3 was observed in the presence of garlic and *G. kola* only. In the presence of lead some of the combinations of modes and additives can be expected to give little or no protection as the CIs produced from these data were relatively wide and included zero for some of these estimates. Our study showed there was statistically significant interaction between modes of administration and additive used with percentage protection being negative for garlic used according to mode 3 and significantly different from the percentage protection obtained when garlic is used based on mode 1. This difference between modes was not observed for the other additives.

Medicinal plant products affect the toxicodynamics of metals in tissues at different degrees, some by decreasing

accumulation and uptake of these metals and also enhancing their elimination from the tissues. Medicinal plant products are not always useful in the reduction of metal accumulation and toxicity; their use can also be harmful, and the identification of the roles played by these substances becomes more important if they are to be used in this regard. More studies are needed to understand these interactions and efficacy of these substances together with the best modes of administration that will significantly impact on their usefulness in the management of metal exposures and toxicity.

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