

Comparative study on the hepatoprotection to heavy metals of *Zingiber officinale*

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ABSTRACT

Context: *Zingiber officinale* (Zingiberaceae) is a herb used for culinary and therapeutic purposes due to its anti-inflammatory and antioxidant potentials. **Objectives:** We examined its protective ability against mercury (Hg), lead (Pb) and cadmium (Cd) accumulation in the liver. **Materials & Methods:** Ground *Zingiber officinale* (7%, w/w of feed) was administered to rats either at the same time with the exposure of heavy metals (group 2), a week after exposure to heavy metals (group 3) or given a week before heavy metal exposure (group 4) for six weeks. Animals were exposed to either of Hg (10 ppm), Cd (200 ppm) and Pb (100 ppm) in drinking water. The heavy metal accumulations in the liver were determined using AAS. **Results:** Weight losses induced by these metals were not reversed by *Zingiber officinale* administration. There was a significant ($P < 0.01$) increase in protection to Pb (97%) and Cd (63%) accumulation when compared to Hg (32%) at week 2. The protective ability was significantly ($P < 0.01$) decreased at week 4 when compared to week 2 for Cd and Pb but not to Hg in groups 3 (50%) and 4 (52%). At week 6, hepatoprotection to Hg (44%) and Cd (85%) was significantly ($P < 0.01$) different but not to Pb which was only significant ($P < 0.05$) in week 2 of treatment for all groups. **Discussion and Conclusion:** *Zingiber officinale* affected the bioavailability, elimination and uptake of these metals in a time-dependent way with highest beneficial reducing effect to Cd followed by Hg and least protection to Pb in the liver.

Key words: Accumulation, cadmium, lead, liver, mercury, protection, *Zingiber officinale*

INTRODUCTION

Oxidative stress, altered physiological and biochemical characteristics^[1,2] leading to organ damage^[3-6] occur with heavy metal exposures. Heavy metals impart their toxicological effects mainly through molecular interactions with sulfhydryl groups on various molecules,^[7-16] generation of reactive oxygen species (ROS) and weakening the antioxidant defense system of cells and altering calcium and Fe²⁺ channels transport.^[17,18]

Zingiber officinale (ginger), an aromatic but pungent food spice, is used for its medicinal value.^[19-28] Its antioxidant properties^[21] contribute to its radical scavenging

properties, a characteristic suited for metal chelation and hepatoprotection.^[22,28] Antioxidant supplementation is reported to be beneficial in metal toxicity.^[28] This study is performed to comparatively analyze the hepatoprotection of *Zingiber officinale* to lead (Pb), cadmium (Cd) and mercury (Hg) accumulation in the liver of rats.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar rats of about seven-week-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 of light/dark cycles. The individual animal body weight was recorded weekly throughout the experiment. 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 Faculty Ethical Committee for this study.

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Preparation of nutrient substance and heavy metals

Fresh ginger rhizome was purchased from the main market at Nnewi Nigeria. Ground and sieved (particulate size of 250 μm) *Zingiber officinale* were mixed with rat chow (7% w/w of *Zingiber officinale* in rat chow) and fed to the animals. Pb-exposed groups received tap water that contained 100 ppm lead acetate. Cadmium-exposed groups received tap water that contained 200 ppm cadmium chloride while mercury-exposed groups received tap water that contained 10 ppm mercury chloride water directly from the drinking water bottle.

Experimental protocol

Group 1 was fed with normal rat chow and either one of the heavy metals in drinking water (Cd = 200 ppm, Hg = 10 ppm, Pb = 100 ppm). Group 2 was fed with rat chow mixed with 7% *Zingiber officinale* and either one of the heavy metals in drinking water (Cd = 200 ppm, Hg = 10 ppm, Pb = 100 ppm). The exposure and feeding started at the same time. Group 3 was fed with normal rat chow and either of the heavy metal (Cd = 200 ppm, Hg = 10 ppm, Pb = 100 ppm) in drinking water for the first week and then with rat chow mixed with *Zingiber officinale* and tap water from the second to the sixth week. Group 4 was fed with rat chow mixed with *Zingiber officinale* for one week. After that they were fed with normal rat chow and either one of the heavy metal (Cd = 200 ppm, Hg = 10 ppm, 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 and analysis

At the end of the experimental period, the rats were sacrificed under chloroform anesthesia. Liver (1g) 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, in all samples, the concentrations of Pb and Cd were analyzed using flame atomic absorption spectrophotometer (Perkin Elmer A.A. 3030) with D2 background correction device. Cold vapor technique was used for the analysis of Hg.^[30,31]

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

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

F = Feed (rat chow); W = Water; FGa = Feed-ginger concentrate; WHm = Heavy metal in water (Cd=200 ppm, Hg= 10 ppm, Pb= 100 ppm).

Statistical analysis

The results are expressed as mean \pm SEM. Student's *t*-test and two-way analysis of variance (ANOVA) with Bonferonni's post-test was performed, where applicable, using Graph Pad Prism version 5.0 for Windows (GraphPad Software, San Diego, Ca, USA). A *P* value of 0.05 was considered statistically significant.

RESULTS

Body weight changes

It was observed that the treatment of the animals with both nutrients and heavy metals significantly ($P < 0.05$) reduced the body weight when compared with animals not exposed to any of the nutrients or heavy metals [Figure 1]. *Zingiber officinale* administration also significantly ($P < 0.05$) reduced the body weight. *Zingiber officinale* did not significantly alter the weight loss induced by Hg and Cd exposures, but caused a significant ($P < 0.05$) revision of the weight loss induced by Pb. Such gain in weight was significantly less than those observed in control animals during the course of study.

Effect of *Zingiber officinale* administration and heavy metal together on accumulation in the liver

The results of administration of *Zingiber officinale*, Hg, Cd and Pb concurrently on the accumulation of heavy metal in liver are presented in Figures 2-4. The percentage protection offered differed when *Zingiber officinale* was ingested at the same time with the metals (group 2) as presented in Table 2. There was a significantly ($P < 0.01$) higher percentage protection to Cd accumulation when compared to Hg and/or Pb at week two. However, the percentage protection to Cd accumulation was significantly ($P < 0.05$) increased when compared with the other heavy metals at week 2. *Zingiber officinale* provided a significantly ($P < 0.05$) highest protection to Cd

Table 2: Summary of percentage protection by ginger on heavy metal accumulation in the liver

Group 2	Mercury	Cadmium	Lead
Week 2	31.8	63.3	56.3
Week 4	18.5	41.8 [†]	0
Week 6	64.9	89.8 [†]	0* [†]
Group 3			
Week 2	47.9	77.9	65.6
Week 4	50.0	60.4 [†]	0
Week 6	62.4 [†]	94.5 [†]	0*
Group 4			
Week 2	31.8	63.3	96.9
Week 4	52.5	37.9 [†]	50
Week 6	44.8	85.3 [†]	0* [†]

Data shows percentage protection in each treatment group. * = $P < 0.01$ vs Hg and cadmium in week 6 of corresponding group; [†] = $P < 0.01$ vs week 4 for each treatment group

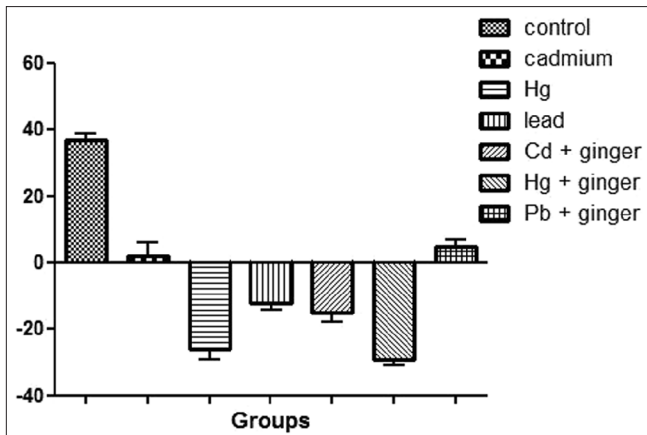


Figure 1: Effects of heavy metal on body weight gain in rats treated with ginger

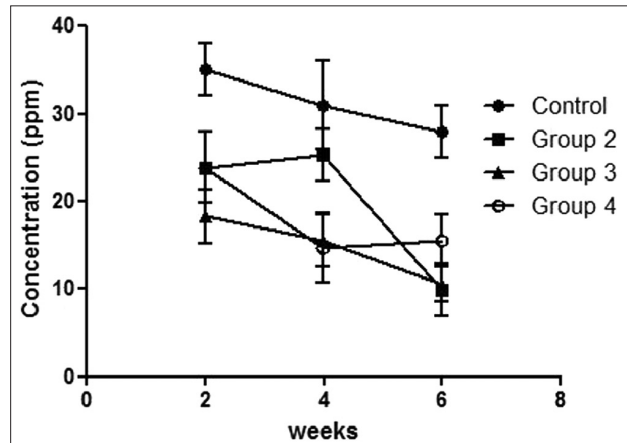


Figure 2: Effect of ginger on accumulation of mercury in liver of rats

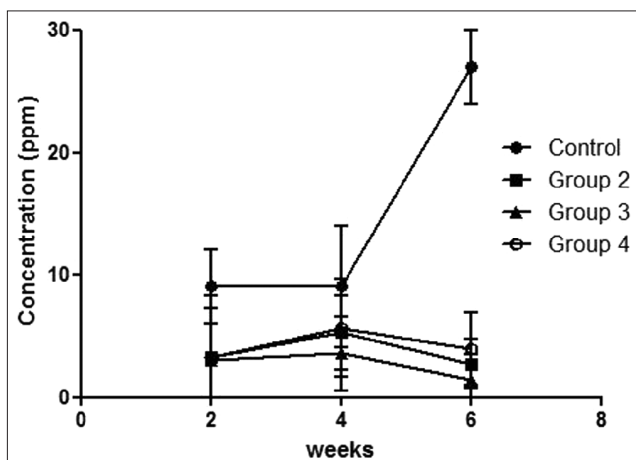


Figure 3: Effect of ginger on accumulation of cadmium in liver of rats

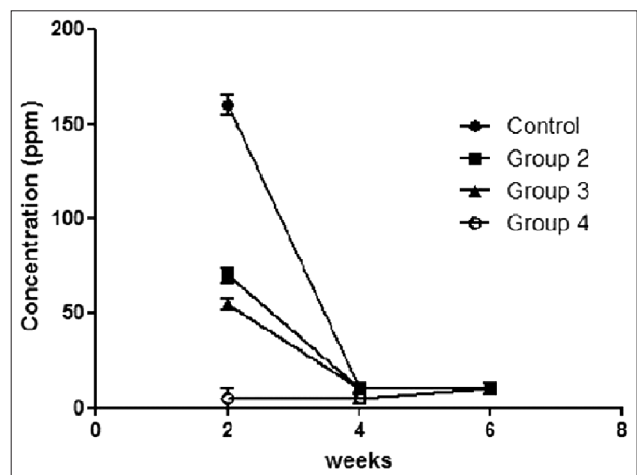


Figure 4: Effect of ginger on accumulation of lead in liver of rats

accumulation at week 4 when compared to the protection to Hg and Pb metal. However, there was no level of protection to Pb observed at week 4. At week 6, the percentage protection to Hg and Cd accumulation in the liver was significantly ($P < 0.01$) greater than protection to Pb. The percentage protection of *Zingiber officinale* at this period was significantly different in the three heavy metal groups. The summary of percentage protection offered by ginger to heavy metal accumulation in the liver is as follows: cadmium > mercury > lead.

Effect of one week administration of heavy metal before ginger on accumulation in the liver

The results of the effect of administration of heavy metal for one week before ginger supplementation on liver content of heavy metal are presented in Figures 2–4. This is represented as group 3. There was a significant ($P < 0.01$) increase in levels of protection by *Zingiber officinale* for cadmium when compared with lead and mercury [Table 2]. *Zingiber officinale* administration offered little or no protection to lead accumulation in the liver at week four whereas it reduces significantly ($P < 0.05$) Hg and Cd accumulation by

50 and 60% respectively. However, the protection at week 6 offered by *Zingiber officinale* was significantly ($P < 0.01$) higher in Cd-treated group (95%) when compared to Hg-treated (62%) and Pb-treated groups (0%). For this treatment protocol, *Zingiber officinale* treatment offered a highest protection to Cd accumulation in the liver when compared to Hg and Pb metals. There was no protection of *Zingiber officinale* to lead accumulation in the liver. The results show that *Zingiber officinale* offered the highest protection to Cd followed by Hg and least protection to Pb.

Effect of prior administration of ginger on heavy metal accumulation in liver

In the last protocol in which *Zingiber officinale* was given first to experimental animals before administration of the heavy metals (Table 2, group 4), there was a significant ($P < 0.01$) increase in protection to Pb (97%) and Cd (63%) accumulation when compared to Hg (32%) at week 2. The percentage protection offered by *Zingiber officinale* to accumulation of Pb and Cd in the liver was however significantly ($P < 0.01$) decreased at week 4 when compared to week 2, though the value was significantly

($P < 0.05$) increased in Hg-treated group. The percentage protection offered by *Zingiber officinale* at week 4 to Hg and Pb was comparable. At week 6, the percentage protection by *Zingiber officinale* to both Hg (44%) and Cd (85%) was significantly ($P < 0.01$) different to each other while there was no protection to Pb (0%) at this period. On the whole, ginger offered the greatest protection to cadmium accumulation followed by Hg and least protection to Pb accumulation.

DISCUSSION

The bioavailability and toxicity of heavy metals on animals is strongly influenced by the biology of the organism. This is because many organisms have developed some elimination methods that help to excrete even the assimilated quantities of the heavy metals. The kinetics of elimination and uptake of these chemicals/ metals from the body can display some unexpected patterns.^[32,33] We would expect that under continuous exposure, there is a tendency for total internal body concentrations to increase as long as the animal is exposed to the metal, bearing in mind that these metals bioaccumulate and are not degradable. However, some researchers have reported an initial dramatic increase in accumulated heavy metals which then decreased even though animals were fed / exposed constantly to the metals.^[34,35] Such reports had earlier been thought to be experimental or analytical errors but are now seen to have a biological basis.^[32,34-36] Our results also presented with such overshoots and non-conformity with expected patterns which can be attributed to the kinetics of the metal assimilation, uptake and excretion. According to Laskowski et al.^[33] an animal may show a lag in physiological response that results in a delay in the onset of efficient elimination and/or decrease in metal assimilation following an exposure to high doses of these metals.

Chelation of these metals is an effective method of treatment of the metal-induced toxicity since it enhances the mobilization and excretion of metallic cations.^[15,28,37] Nevertheless, there is increasing evidence suggesting that diet and/or metabolic differences may influence heavy metal uptake and/or excretion.^[38] Nutrients are reported to affect bioavailability, toxicodynamics, and transport to target organs, and influence the immunologic, biochemical, or cytologic functional responses to heavy metals.^[28,29,39]

Zingiber officinale has been reported to act as an antitoxic agent^[19,20] because of its rich antioxidant properties^[21] which contribute to its free radical scavenging properties, a characteristic suited for metal chelation.^[22] In our studies, we observed that *Zingiber officinale* offered some hepatic protection to heavy metal accumulations in the liver,

such percentage protections varied with the metal and methodology of exposures.

Grzanna et al.^[40] reported that *Zingiber officinale* modulates biochemical pathways activated in chronic inflammation, which may include its gastric protection,^[23,24] chemopreventive and antioxidant potentials as well as a modulating effect on phase II detoxification enzyme and reduced glutathione.^[41] All this may contribute to the kinetics of elimination of these metals from the system. Our observations were that *Zingiber officinale* had less percentage protection over Pb exposures. Protection offered to this metal accumulation was immediate but did not last with increase in durations of exposures. Protection offered to Hg and Cd exposures also showed a duration-dependent effect with initial and late increases at the second and sixth weeks but more to Cd than to Hg.

Also *Zingiber officinale* offered more protective ability through the elimination of Cd and Hg, but when given before exposure to the heavy metals, it significantly reduced the accumulation of Pb in the liver. After two weeks of treatment, *Zingiber officinale* did not offer any further protection to Pb exposures in all treatment groups. The results of the present study highlight the benefit of *Zingiber officinale* to be used as a nutrient supplement in heavy metal toxicity probably due to its antioxidant property.

Our studies focused on the accumulation, elimination and bioavailability of heavy metals following exposures and treatment with *Zingiber officinale*, a nutrient substance famed for its antioxidant and anti-inflammatory properties. We had earlier reported its hepatoprotection to cadmium-induced toxicity through amelioration of some liver enzymes.^[27] In conclusion, *Zingiber officinale* affected the bioavailability, kinetics of the metal assimilation, uptake and excretion in a time-dependant manner, with more hepatic protection to Cd and less to Hg and Pb, as such it is beneficial for the reduction of heavy metal burden. Our results highlight the hepatoprotective effect of this nutrient substance against heavy metals, these hepatoprotective effects against other chemicals that induce hepatic stress and hepatotoxicity have been reported by other researchers^[22,27,28,42-44] and our studies further confirm it. Our limitations include the non-observance of the toxicokinetics of these metals due to treatment with the nutrient substances and the bioaccumulation of these metals in other tissues. There is a therefore need for further studies to investigate the effect of *Zingiber officinale* on heavy metal accumulation in other organs and its protective abilities through antioxidant assays.

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