Comparative analysis on the effect of palm oil (*Elaeis guineensis*) in reducing cadmium and lead accumulation in liver of Wistar rats

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ABSTRACT

Context: Palm oil from *Elaeis guineensis* is an edible nutrient substance with anti-inflammatory and antioxidant properties. We examined its protective effect against lead (Pb) and cadmium (Cd) accumulation in the liver. **Materials and Methods**: 12% w/w of palm oil (PO) in rat chow concentrate was fed to rats exposed to Cd (200ppm) and Pb (100ppm) in drinking water at different feeding regimens. PO was administered either at the same time with the metals (group 2), post-treatment after exposure (group 3) or pre-treatment before exposure (group 4) for six weeks. The heavy metal accumulations in the liver were determined using AAS. **Results**: Weight losses induced by these metals were significantly ($P<0.05$) reversed by PO administration. Analysis among the groups showed that post-treatment group had a significant ($P<0.05$) higher percentage protection to Cd, but same time treatment for Pb ($P<0.05$) when compared with other groups. The protective ability to PO was only significantly ($P<0.05$) increased for Pb at week 2, but showed a time-dependent significant ($P<0.05$) increase for Cd across all treatment regimens. **Conclusion**: PO is beneficial in reducing metal accumulation in the liver and has a higher hepatoprotective effect to Cd compared to Pb at the selected doses by possibly affecting the processes of uptake, assimilation and elimination of these metals.

Key words: Accumulation, cadmium, heavy metals, lead, liver, protection

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INTRODUCTION

Palm oil is a vegetable oil with high content of tocopherols, tocotrienols and carotenoids which act as potent antioxidants.[1] It is processed from the mesocarp of the fruits of the oil palm tree (*Elaeis guineensis*), and has a rich source of natural antioxidants with anti-inflammatory properties. The constituents include beta carotene (precursors of vitamin A), vitamin E, sterols, phospholipids, glycolipids and squalene.[2,3]

These antioxidants present in palm oil has been reported to aid in maintenance of tissues, prevention of oxidative deterioration of cellular membranes through their radical scavenger properties[4] and promotes growth induction of some hepatic drug-metabolizing enzymes. These include glutathione-S-transferase, aminopyrine N-demethylase and ethoxyresorufin-O-deethylase,[5-7] as such it has been used as a traditional antidote to toxicity,[8] tumorigenesis[9] and hepatocarcinogenesis.[10-12]

Inhibition of the liver enzymatic function, through an increase in lipid peroxidation, production of reactive radicals, oxidative tissue damage, loss of membrane functions and hepatic congestion[13-15] have been reported as mechanisms of heavy metal induced damage. Cadmium (Cd) and lead (Pb) are major environmental pollutants, toxic at even low concentrations and are able to generate reactive radicals leading to cellular damages[16,17] and hepatotoxicity in humans and animals.[18,19]

Since antioxidant supplementation has been found to be beneficial in metal toxicity[20-22] and palm oil has been reported to have anti toxic and hepatocarcinogenic effects. The present study was designed to investigate and comparatively analyze the hepatoprotective effects

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of this nutrient substance on Cd and Pb (heavy metal) accumulation in the liver of rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing about 150-180 g, obtained from the animal colony of the NIMR Lagos were used for this study. They were randomly assigned to cages to acclimatize before the commencement of the study. Ethical approval for this study was sought and received from the Faculty of Science Ethical Committee and was in conformity with the guidelines for the "Care and Use of Animals". The animals were maintained at room temperature (25°C) with 12 h of light/dark cycles and had access to normal rat chow and tap water *ad libitum* during the acclimatization period. Body weight of these animals was recorded during the course of the study. The groupings for the study are as shown in Table 1. Each group had a population size of $n = 15$. From these groups, five (5) animals are randomly sacrificed every two weeks for tissue collection and analysis.

Preparation of heavy metals and diet

This diet preparation was done as reported earlier.[22,23] In brief, palm oil was mixed with the rat chow to form a palm oil concentrate (12% w/w of red palm oil in rat chow) at 88:12 w/w ratios and kept in a dessicator. Lead-exposed groups were given Pb acetate in water at a concentration of 100 ppm Pb. The groups exposed to Cd were given cadmium chloride in the drinking water as 200 ppm Cd.

Experimental design

Group 1 received normal rat chow and either Cd=200 ppm or Pb= 100ppm in drinking water. Group 2 received 12% w/w of red palm oil in rat chow and either Cd=200 ppm or Pb= 100 ppm in drinking water with exposures beginning same time. Group 3 received normal rat chow and either Cd=200ppm or Pb= 100 ppm in drinking water for the first week (post treatment group) then 12% w/w of red palm oil in rat chow from the second to the sixth week. Group 4 received 12% w/w of red palm oil in rat chow for one week (pre treatment group) then either Cd=200 ppm or Pb= 100 ppm in drinking water for the remaining 5 weeks. Group 5 received normal food and tap water, while group 6 was fed with the palm oil rat chow concentrate and water alone. Table 1 summarizes the grouping and feeding patterns, while the oral route was the method of exposures. The concentrations of the chosen metals were obtained through preliminary studies and review of literature.[23,24] Care was taken to choose a concentration that will assure the survival of the animals throughout the period of study.

Tissue analysis of heavy metals

After six (6) weeks of exposure and treatment, the animals were sacrificed under chloroform anesthesia. One (1g) liver sample was excised and homogenized in 5mls of normal saline. Acid digestion was done using 10 ml of concentrated HNO_3 at room temperature.[25] After digestion, the concentrations of cadmium and lead were analyzed using (AAS) atomic absorption spectrophotometer (Perkin Elmer A.A. 3030).[26] All analysis for the heavy metal concentrations in the liver samples was done at weeks 2, 4, and 6. The percentage protections of the nutrient substances were determined from compassion of the 2, 3 and 4 with group 1 as shown in Table 1.

Statistical analysis

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

RESULTS

Effect of palm oil on liver metal burden

The results of the liver heavy metal burden for the various treatment regimens are shown in Figures 1 and 2. We observed an initial dramatic increase in accumulated Pb which then decreased, even though animals were fed / exposed constantly to the metals, but the accumulation increased throughout the period of study for Cd. Treatment with PO reduced the accumulation patterns for both metals.

Treatment with PO showed different percentage protection

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

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

F = Feed (rat chow); W = Water; FPO = Feed-palm oil concentrate; WHm = Heavy metal in water (Cd=200ppm, Pb= 100 ppm)

for the different treatment regimens (concurrent, pre and post treatments), the summary of these percentage protections are presented in Table 2. The summary of these protection ability shows that palm oil caused a significantly (P<0.05) higher protection to lead when compared with its protection to cadmium at week 2 when it was administered concurrently with the heavy metal, but at weeks 4 and 6, no significant protection to lead accumulation was observed with PO. The protective ability of palm oil to Cd was significantly (P<0.05) highest at week 2 when compared to the protection at week 4 and 6. On the whole PO administration offered a significant (P<0.05) greater protection to Cd when compared with Pb in this treatment protocol.

In the post treatment group, there was a significant (P<0.05) increase in protection by PO for Cd when compared with lead, this was observed to be time-dependent. The percentage maximum protection was significantly (P<0.05) higher at week 6 in Cd-treated group (93%) when compared to Pb-treated (0%). After week 2, palm oil offered protection to Cd and least hepatoprotection for Pb was observed.

When palm oil was first given to the experimental animals before exposure to either of Cd or Pb (pre treatment group), the protection was significantly (P<0.05) higher to Pb (81.3%) accumulation compared to Cd (66.9%) at two weeks, but this was significantly (P<0.05) decreased by the fourth week. By the sixth week, the protection to Cd liver burden (59.3%) was now significantly (P<0.05) higher when compared to Pb liver burden. Palm oil offered no protection to Pb beyond the second week of administration.

Body weight changes

The body weight of rats treated with heavy metals and PO is presented in Figure 3. Exposure to heavy metals and treatment with nutrients affected the body weight and presented significant (P<0.01) changes when compared to the controls not exposed to any of the heavy metals or PO. Our observations were that PO significantly (P<0.05) altered the Cd and Pb induced weight loss in the exposed animal groups.

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

Group 2	Cadmium	Lead
Week 2	77.9	93.8#
Week 4	44.9*	0*#
Week 6	66.7*†	0#*
Group 3		
Week 2	39.4	31.3#
Week 4	66.9*	0*#
Week 6	92.6*†	0*#
Group 4		
Week 2	66.9†	81.3#
Week 4	39.4	0#
Week 6	59.3†	0#

Data shows percentage protection in each treatment group. * = P<0.01 vs week 2; † = P<0.01 vs week 4 for each treatment group; # = P<0.01 vs each metal in each corresponding week

DISCUSSIONS

Our findings were that the treatment with palm oil caused

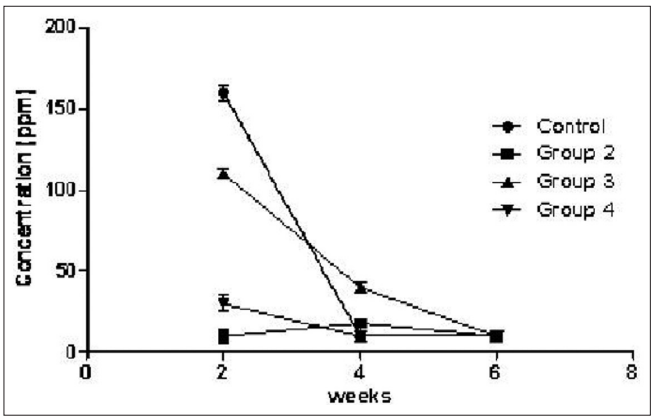

Figure 1: The concentration of lead in the liver of rats at weeks 2, 4 and 6

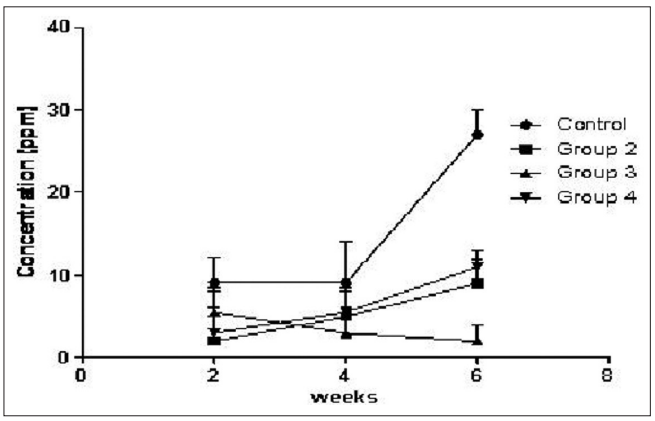

Figure 2: The concentration of cadmium in the liver of rats at weeks 2, 4 and 6

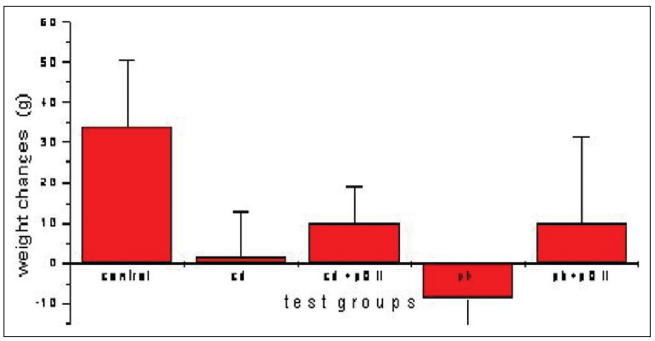

Figure 3: Effect of palm oil, cadmium and lead on weight changes in rats

a reduction in the liver heavy metal burden in animals exposed to these metals as it affected the dynamics of uptake, elimination and accumulation of Cd and Pb within the liver. We observed an initial dramatic increase in accumulated Pb which then decreased even though animals were fed / exposed constantly to the metals, but the accumulation increased throughout the period of study for Cd. Treatment with PO reduced the accumulation patterns for both metals. The percentage protection offered differed when *palm oil rat chow concentrate* was administered at the same time with Pb and Cd (group 2), or given a week before or after the exposure to these metals (group 3 and 4).

On the changes in weight, we observed that PO positively altered the Cd and Pb induced weight loss in the study animals, this is not in accordance with reports by Edem[13] who used 30% w: w palm oil in their study, our study used 12 % w: w of palm oil in diet[27] which can reduce the oxidative stress related to increased oil contents in diet.

The liver is not necessarily the only organ in terms of accumulation of heavy metals after chronic dosing. Other organs such as the kidneys, bone, red blood cells, accumulate lead and cadmium much more than the liver. An explanation for the reduction of heavy metal burden in the liver may be that the metals are redistributed from liver to other organs by palm oil. Studies were not performed to check if palm oil increases the excretion of these metals (in urine, bile or feces). We had earlier reported that oil from palm significantly reduced the GOT and GPT increase in the liver induced by exposures to heavy metal,[8,28] the study then suggests that the oil from palm has a hepatoprotective role as has been highlighted by some researchers.[8,29]

These indicate that the management and treatment patterns with this nutrient substance on exposure to toxicity/accumulation could present with different results. Our observations were that PO offered more protective ability to Cd than to Pb in all treatment regimens as it significantly reduced the accumulation of these metals in the liver in a time dependant manner. The unexpected patterns observed in the kinetics of elimination and uptake of these chemicals/metals have been reported even with continuous exposures and is not due to experimental or analytical errors.[22-24] Nutrient substances like PO are reported to provide a great deal of antioxidants which can affect the toxico-dynamics and bioavailability of heavy metals following exposure.[23,30] PO is able to reduce the bioavailability of these metals by possibly complexing and enhancing its elimination/excretion from the tissues like some other nutrient substances.[17,23,31]

The increase in weight gain in the animals exposed to the metals could be attributed to an increase in food intake as fat enhances satiety and consumption through its palatability properties,[32] the caloric content of the diet may also contribute to this increase in weight gain.

The results of the present study highlight the benefit of *palm oil* being used as a food and nutrient supplement. It protects the liver against heavy metal induced oxidative damage in rats by reducing its liver accumulation. The hepatoprotective effect might be correlated with its antioxidant, free radical scavenger effects also hepatic drug-metabolizing enzymes induction and probably chelating properties. Need for further studies to investigate the effect of palm oil on heavy metal accumulation in other organs and its protective abilities through antioxidant assays, also its hepatic phases metabolizing enzyme activities.

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