JMBR: A Peer-review Journal of Biomedical Sciences
December 2013, Vol. 12 No.2 pp 166-172

EVALUATION OF SERUM LEVELS OF CADMIUM AND LEAD IN OCCUPATIONALLY EXPOSED PAINTERS WITH ADMINISTRATION OF PROBIOTIC (Lactobacillus pentosus KCA 1) SUPPLEMENTED YOGURT: A PILOT STUDY

OSADOLOR, H. B., IGHARO, O. G., OKUO, R. O. AND ANUKAM, K. C.

ABSTRACT

Cadmium and Lead are extremely toxic metals found in industrial workplaces. They are also found in some industrial paints and may represent hazards when sprayed. Exposure to Cadmium fumes may cause flu-like symptoms including chills, fever, and muscle ache sometimes referred to as "the cadmium blues." Occupational exposure is a common cause of cadmium and lead poisoning in adults. Lactobacillius pentosus KCA1 used in this study is the recently discovered and sequenced lactobacilli of probiotic strain with a potential for detoxification of heavy metals. Probiotic has been defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements. The study was aimed at evaluating the detoxification potential of probiotic Lactobacillius pentosus KCA1 in painters occupationally exposed to cadmium and lead. Serum levels of Lead and Cadmium were determined in thirty professional painters and ten non painters as control using Atomic absorption spectrophotometer (AAS Model SOLAAR 969 UNICAM series). Probiotic supplemented yogurt was prepared and administered to the occupationally exposed painters (as exposed subjects) and its effect on serum levels on Cadmium and Lead were also determined. The results obtained show that mean ± standard error of mean (S.E.M.) of serum levels of Cadmium (0.012 ± 0.01ppm/L) and Lead (0.025 ± 0.003ppm/L) were higher in painters and significantly different compared with values obtained in non painters (controls); (0.005 ± 0.002 ppm/L) and (0.02 ± 0.01 ppm/L) for Cadmium and Lead respectively; ($p < 0.05$). Mean serum level of Lead in the painters after administration of probiotic supplemented yogurt (0.008 ± 0.002 ppm/L) was significantly reduced compared with levels before administration of the yogurt (0.025 ± 0.003ppm/l); ($p < 0.001$). From this study, serum level of Cadmium and Lead were reduced in occupationally exposed painters by probiotics (Lactobacillus pentosus KCA1) which was prepared in form of yogurt. Comparatively, serum Lead levels were more significantly reduced than Cadmium levels in the occupationally exposed painters.

INTRODUCTION

Due to natural sources or human activities, heavy metal ions are found in surface water, wastewater, waste and soils. Cadmium and Lead are heavy metals that are toxic to the body, they pose occupational, environmental and lifestyle hazard. Cadmium and Lead are chemical elements heavy/ transition metals. They are soft, malleable, ductile, bluish white and divalent metals, (Holleman et al., 1985;Akinola and Ekiyoyo, 2006)).

Cadmium and Lead have no useful biological role. They are found in some industrial paints and may represent hazard when sprayed. An operation involving removal of cadmium paints by scraping or blasting may pose significant hazard. Cadmium is also present in the manufacturing of some types of batteries, (Besellt, 2008).

Lead adversely affects numerous part of the body system and causes forms of health impairment and diseases that arises after a period of exposure as short as days (acute exposure) or as long as several years (chronic exposure).Common symptoms of acute lead poisoning are loss of appetite, nausea, vomiting, stomach cramps, constipation, difficulty in sleeping, fatigue, moodiness, headache, joint or muscle aches, anemia and decreased sexual drive (Bruton et al.,2007).

KEYWORD: *Cadmium, Lead, Lactobacillus pentosus KCA1, Probiotics.*

Osadolor, H. B., Igharo O. G., Okuo, R. O. and Anukam, K. C.
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.

Correspondence:
Dr H.B.Osadolor
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.
E –mail:humphrey.osadolor@uniben.edu

Acute lead poisoning from uncontrolled occupational exposures has resulted in fatalities. Long term (chronic) overexposure to lead may result in a severe damage to the blood forming, nervous, urinary, and reproductive system (Grant, 2009).
Probiotics organisms are living organisms that are thought to be beneficial to their host organism. According to the currently adopted definition by FAO/WHO, Probiotics are: Live microorganisms which when administered in adequate amounts confer a health benefit on the host. Lactic acid bacteria (LAB) and Bifidobacteria are most common types of microbes used as probiotics; but certain yeast and bacilli may be used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures such as in yogurt, soy yogurt,or as dietary supplements. Probiotics are also delivered in fecal transplants, in which stool from a healthy donor is delivered like a suppository to an infected patient, (WHO, 2001).

The motivation for the study was based on the fact that casual painters work with little or no protective devices such as face mask, hence the need to study how long and short term exposures could constitutes any occupational hazard. Lactobacillus pentosus KCA1 is a newly isolated and newly sequenced lactobacillus of a probiotic strain (Anukam et al., 2004). The organism was first isolated from the vagina of a healthy Nigerian woman and the genome sequence has shown that it has potential for detoxification of heavy metals, lead and cadmium particularly, (Anukam et al., 2004).

Therefore, the study was aimed at primarily investigating the level of Cadmium and Lead toxicities in the occupationally exposed painters and to determine the detoxifying potential of probiotic (Lactobacillus pentosus KCA1) supplemented yogurt on Cadmium

and Lead poisoning in the painters recruited for the study.

MATERIALS AND METHOD

Study Area and Population

This study was carried out in Benin City, a metropolitan city and the capital of Edo State, South- South of Nigeria.

Thirty (30) male professional painters between Twenty six and Fifty five years of age served as test subjects, while fifteen non-painters who were apparently healthy males aged between twenty five and thirty five years were recruited as controls. The painters have been in painting work for duration ranging between three and twenty six years.

Informed consent was sought and obtained from all the test and control subjects prior to sample collection. All samples were obtained using standard phlebotomy techniques.

Sample Size and Sampling Method

The study was designed to be an experimental pilot study. This accounted for the use of group sampling technique and a sample size of forty five (thirty test subjects and fifteen control subjects). The test subjects recruited for the study were from a group of painters using same types of household paints in a construction site in Benin City.

Inclusion Criteria

Professional painters who have been on the vocation for durations ranging between three to twenty six years were recruited as test subjects. The structured questionnaires administered showed that they were apparently healthy based on their medical history; and there was none who had used any recommended safety mask to prevent or reduce inhalation of toxic metals from paints.

The control subjects were apparently healthy adults (average age of 32 years) who had no history of exposure to toxic metals either by occupation or lifestyle.

Exclusion Criteria

Painters who had less than three years duration in painting vocation were excluded from the study. Also, painters who had on-going health challenges and history of recent hospitalization were not recruited as part of the test subjects.

Sample Collection

Pre- and Post-treatment serum samples were collected from the subjects. Five millitres (5mL) of venous blood samples was collected from each of the subjects with sterile disposable needles and syringes, this was dispensed into a clean dry specimen container while avoiding haemolysis. Sample was left on the bench to stand at least one hour before centrifugation. The serum was then transferred into vials and stored at -20^0 C.

Preparation of Probiotic Supplemented Yogurt

Culture of Lactobacillus pentosus KCA1

MRS (de Man, Rogosa and Sharpe) broth was used to culture the organism; it was incubated for 24hours anaerobically. After incubation, it was centrifuge and supernatant was discarded. The organism was suspended in phosphate buffered saline was added to the yogurt in this form.

Preparation of yogurt:

Five (5) litres of yoghurt was prepared following the steps below:

- 3.43g of milk (commercially acquired) was weighed and added to 20ml of distilled water and mixed properly.

- 200µl of the starter culture (Lactobacillius pentosus KCA1) was added and mixed properly.
- It was incubated anaerobically for 24hours.
- 171.6g of MRS broth was weighed to 1 litre of distilled water in a pot.
- It was mixed properly and pasteurized at 80ºC for 20 minutes and stirred at intervals aseptically.
- It was allowed to cool to 40ºC,
- 20mls of the starter culture prepared was added to it, and mixed properly.
- 200µl of Lactobacillus pentosus suspended in buffer was added to it and mixed properly.
- It was transferred to a plastic container with a fitted cap aseptically.
- The yoghurt was incubated anaerobically for 24hours.
- 20g of sugar, 20mls of vanilla flavor were added to it and was mixed properly.
- It was dispensed aseptically in a 50ml plastic container.

Storage and Administration of Yogurt to the Subjects

One hundred (100) plastic containers (50ml each) of yogurt were taken from 5 litres of yogurt; they were refrigerated and dispensed to the subjects daily for ten (10) days. This approach was discretionary.

Post treatment blood samples were collected from the subjects after ten days of administration of the yogurt. The samples were analyzed for Cadmium and Lead using Atomic Absorption Spectrophotometer.

Sample Analysis

Cadmium and Lead were estimated in Mcgill Analytical laboratory, Benin City, using atomic absorption spectrophotometer (AAS Model-Solaar 969 Unicam Series). Standard Quality Assurance Practices were strictly adhered to.

Statistical Analysis

The single factor ANOVA both parametric and non –parametric was used to determine significant differences in the Cadmium and Lead levels between the test and the control groups.

Where a significant difference (P- value $\leq$ 0.05) was detected, the Duncan Multiple Range Test was used to locate the source of the difference.

All statistical analyses including the descriptive statistics were computed using Statistical Package for Social Sciences (SPSS) version 16.0 computer package.

RESULTS

Table 1.0 shows the mean ± standard error of the mean of serum Cadmium and Lead levels in painters (test subjects) and non-painters (control subjects).As indicated, mean serum levels of Cadmium in painters (0.012±0.01ppm/L) were higher and statistically different from values obtained in non-painters (0.005±0.002ppm/L), (P<0.05). Though Lead levels were slightly higher in painters (0.025±0.003ppm/L), when compared to non-painters (0.020±0.010ppm/L), the difference was not statistically significant, (P>0.05).

TABLE 1.0: Serum levels of Cadmium and Lead in Painters (Test Subjects) before Treatment with Yogurt and in Non-Painters (Control Subjects)

	Painters		Non-painters		P-Value	Significance Level
Parameters	N	Mean±S.E.M	N	Mean±S.E.M		
Cadmium (ppm/L)	30	0.012±0.010	15	0.005±0.002	0.018	P<0.05
Lead (ppm/L)	30	0.025±0.003	15	0.020±0.010	0.461	P>0.05

Table 2.0: Comparison of Pre- and Post -Treatment levels of Cadmium and Lead in Painters (Test Subjects)

Parameters	n	Mean ±S.E.M (Pre-treatment)	Mean±S.E.M (Post-treatment)	P-Value	Significance Level
Cadmium(ppm/L)	30	0.012±0.010	0.032±0.008	0.029	P<0.05
Lead (ppm/L)	30	0.025±0.003	0.008±0.002	0.000	P<0.001

Table 2.0 and Figure 1.0 show mean values of Cadmium and Lead levels in painters before and after treatment with probiotics supplemented yogurt. Serum Lead level(0.008±0.002) after treatment was lower and statistically different from value obtained before

treatment(0.025±0.003); P<0.001.However, serum Cadmium level was unexpectedly higher after treatment (0.032±0.008) than values obtained before treatment (0.012±0.010). This difference was significant (P< 0.05).

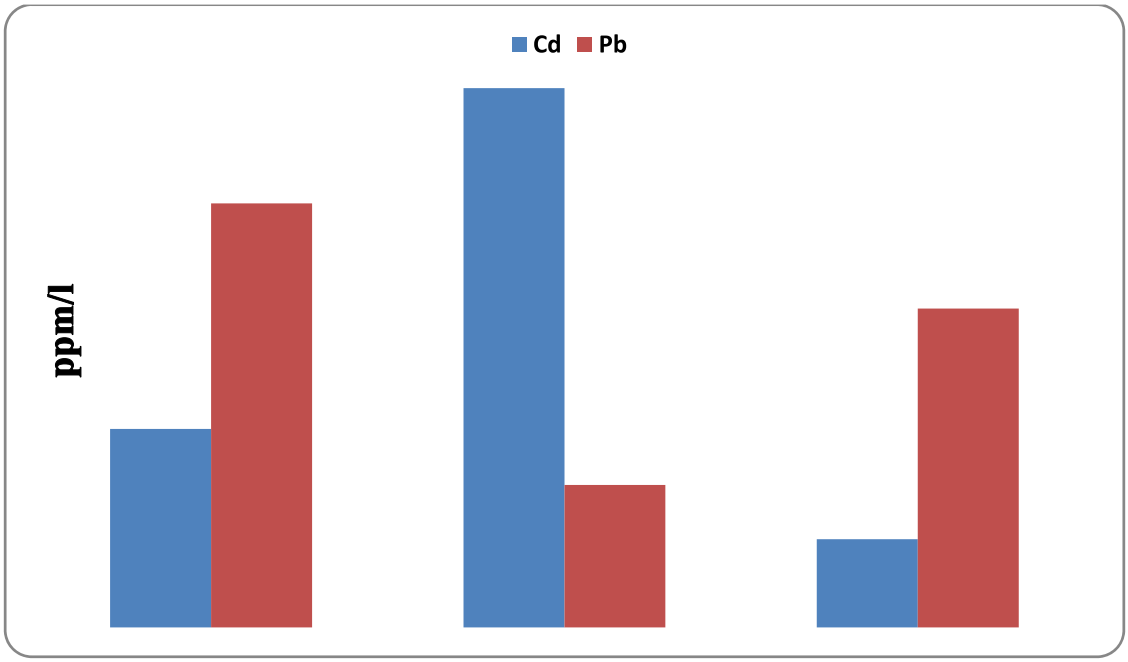

Fig.1.0: Chart showing the serum levels of Cadmium and Lead in painters before and after treatment with probiotics supplemented yogurt and in non-painters.

DISCUSSION

Cadmium and Lead are common pollutants and they pose occupational hazards to different individuals which include painters, (Jarup,1998; Jarup et al.,1998). Occupational exposure of human to heavy metals may have long term deleterious effect on the liver and renal function (Orisakwe et al., 2007).

In the present study, serum levels of Cadmium and Lead of thirty (30) painters and fifteen (15) non-painters were estimated before and after administration of probiotics supplemented yogurt.

As shown above, serum levels of Cadmium and Lead in painters were higher than values obtained in non-painters. The difference in serum cadmium in painters and non-painters was statistically significant, ($P < 0.05$). However the difference between serum Lead levels of painters compared with non-painters was not statistically significant, ($P > 0.05$).This may be attributed to the fact that most paints in common use are now de-leaded.

In a similar study, Orisakwe et al., 2007 observed that occupational exposure of humans to paints increased the blood Lead and Cadmium levels when compared with non-paint factory workers.

The organism, Lactobacillus pentosus KCA1 used in this study has been shown to have genetic potentials to detoxify heavy metals and the appropriate use of this

organism seems to be a new hope for detoxicification of heavy metals. It was reported from the genomic sequence of Lactobacillus pentosus KCA1 that there are about 5934 proteins in the organism and three of these proteins were reported to have potentials to bind Cadmium and Lead, (Anukam et al.,2006).

Conclusion

From this pilot study, it was observed that only the level of Cadmium was significantly elevated in painters. Though lead levels were raised in painters, the difference was not significant. These elevated levels were significantly reduced in occupationally exposed painters by probiotic (Lactobacillus pentosus KCA1) which was prepared in form of yogurt. However, there was more reduction in serum Lead levels than Cadmium. This significant reduction may have been accounted for by the recently sequenced probiotic Lactobacillus pentosus KCA1 prepared in form of yogurt in this study. Based on the reported potential of probiotic Lactobacillus pentosus KCA1 to detoxify some heavy metal, ease of administration of the yogurt, low or absence of side effects, it is therefore recommended that probiotic-rich products like Lactobacillus pentosus KCA1-enriched yogurt could be utilized as alternative or supplement in treatment some of heavy metal toxicities. It is however recommended that a more detailed work should be carried out along this line of research to further establish the observations of this pilot study.

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