



Effects of Bacterial Cellulose Nanofiber on Lead Concentration in Kidney and Liver Tissues of Wistar Rats

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ABSTRACT

Background and objectives: Lead (Pb) is among the most toxic pollutants that affect health of both humans and animals. Finding a way to prevent Pb accumulation in animals' bodies seems necessary. Bacterial cellulose nanofiber (BCNF) can remove heavy metals from aqueous solutions. This study investigates effects of oral consumption of BCNF, as a chelator, on Pb concentration in the kidney and liver tissues of rats.

Methods: Sixteen Wistar rats (aged 6-8 weeks) were divided into four groups: 1. control, 2. fed with Pb, 3. fed with Pb (50 µg/g) and BCNF (16 µg/g) simultaneously, and 4. fed with Pb and BCNF with 4 hours interval. The rats were euthanized, and the kidney and liver tissues were separated. After acidic digestion of the tissue samples, Pb concentration was measured by atomic absorption spectrometry.

Results: The mean concentration of Pb in the kidney and liver tissues of rats fed with Pb and BCNF were significantly lower than that of rats fed only with Pb. In addition, the mean Pb concentration in rats of group 3 was lower than that of group 4.

Conclusion: The results of this study showed the favorable effects of BCNF on prevention of Pb accumulation in the kidney and liver tissues of rats. Moreover, removal of Pb may be related to binding of BCNF with Pb in the gut or blood. More studies are necessary to determine the exact mechanisms through which BCNF can reduce Pb accumulation.

Keywords: [Rats Wistar](#), [Lead](#), [Tissue](#).

INTRODUCTION

Heavy metals are among the most toxic pollutants in the environment, and lead (Pb) has been reported as the most toxic heavy metal (2). Mining Pb or other metals and using Pb compounds such as paint, ceramics, gasoline, and acidic batteries lead to the introduction of Pb to the environment (1). Lead can enter animals' bodies by inhaling polluted air and drinking or eating Pb-contaminated water and foods (5). Even at very low concentrations, Pb exerts toxic effects on the nervous system, circulatory system, kidneys, liver, and endocrine glands, especially in young children (7, 14). Various studies have reported Pb contamination in animals and humans (1,3).

In this regard, finding ways to prevent Pb contamination and remove Pb from contaminated tissues of animals seems essential. The use of nanotechnology has been proven effective in removing heavy metals from water sources (4,12). Cellulose is present in high levels in plant cells and in lower levels in algae, fungi, and bacteria (9). Cellulose nanofibers (CNF) are consisted of cellulose fibers with dimensions smaller than 100 nm from different sources (9). Bacterial cellulose can be obtained from different species of bacteria, especially *Gluconacetobacter xylinus* (17).

Bacterial CNF (BCNF) has a high specific surface area, strong strength, low weight, and high biodegradability (15, 22). Given the ability of BCNF to remove heavy metals from liquids, it can be useful in medical engineering and water treatment (11,20, 22). Currently, there is no effective strategy for reducing Pb contamination in living organisms, and current treatments for Pb poisoning in animals are limited to supportive therapies, such as serum therapy. Considering the potential of BCNF in removal of heavy metals from aqueous solutions (6, 22), the purpose of this study was to investigate effects of BCNF on Pb's tissue uptake in Wistar rats.

MATERIALS AND METHODS

Wistar rats aged 6-8 weeks were purchased from the Pasteur Institute of Iran (Amol

branch) and kept in plexiglas cages in the animals house of Golestan University of Medical science under controlled temperature (22–23 °C), humidity (60%), and a 12:12 light–dark cycle. The experimental procedure was performed according to the Guide for the Care and Use of Laboratory Animals proofed by CCAC (2009). The study was also approved by the research committee of Gorgan University of Agricultural Sciences and Natural Resources (ethical approval code: 8/21692). The animals received BCNF 1% (1.6 µg/g, Nano Novin Polymer Co., Sari, Iran) and lead acetate (50 µg/g) (Chem lab Co., , Belgium daily. Table 1 summarizes the characteristics of the BCNF, which was used in the present study (18,19). The rats were weighed every morning. Lead acetate and BCNF were prepared daily. The rats were treated after 7 days of familiarity with the laboratory environment. The treated rats were divided into a control group and three experimental groups (13). Animals in group 1 (control group, n=4) were fed with standard water and food. Animal in group 2 were fed with water-soluble lead acetate by gavage for 6 weeks (n=4). Animals in group 3 were fed with lead acetate and BCNF simultaneously by gavage for 6 weeks (n=4). Animals in group 4 were fed with lead acetate and BCNF with 4 hours intervals by gavage for 6 weeks (n=4). One day after the last administration, the rats were euthanized with ketamine-xylazine (50 mg/kg). The kidneys and liver were removed and stored at -20 °C. After acidic digestion of sampled tissues, Pb concentration was measured by atomic absorption spectrometry (Trance 1800, Aurora Biomed, Germany). Obtained results were entered into SPSS₁₉ software and analyzed with one-way analysis of variance. Mean values were compared using the Duncan's multiple range test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean Pb concentrations (µg/g) in the kidney and liver tissues of rats in each group are shown in table 2.

Table1- Characteristic of the BCNF gel

Characteristic	BCNF
Formula	(C ₅ H ₁₀ O ₅) _n
Nanofiber diameter	40 nm
Purity	≤% 99

Table 2- Mean Pb concentration in the kidney and liver tissues of rats in each study group

Tissue Group	Liver	Kidney
1 (control)	0	0
2	138 µg/g	48 µg/g
3	120 µg/g	17.5 µg/g
4	123 µg/g	22 µg/g

Based on the results, Pb concentration was significantly higher in the liver tissues than in the kidney tissues. The mean concentration of Pb in the kidney (48 µg/g) and liver (138 µg/g) tissues of rats that were only fed with Pb were higher than all BCNF-treated groups (Table 2). The mean concentration of Pb in the tissues of rats fed simultaneously with BCNF and Pb were lower than that in rats fed with BCNF and Pb with 4-hour intervals ($p>0.05$).

DISCUSSION

The concentration of Pb in the control group was zero, indicating the absence of Pb in the food and water of the treated rats. The mean Pb concentration in the kidney and liver tissues of rats simultaneously fed with Pb and BCNF decreased by 13% and 63.5%, respectively, compared with Pb-treated rats. Moreover, the mean Pb concentration in rats fed with Pb and BCNF with 4-hour intervals decreased by 11% and 54%, respectively, compared with those treated with Pb for 6 weeks. This indicates Pb removal from the body of the BCNF-treated rats. It is argued that BCNF absorbs heavy metals in aqueous media due to the presence of hydroxyl and amine groups. It seems that BCNF can maintain this property in animals' bodies (6, 8, 11, 16). Lead may bind to BCNF in the gut and become unabsorbable. In addition, the binding of Pb with BCNF in the blood and its excretion by the kidneys may reduce the uptake of Pb through the liver and kidneys (6, 11, 16). To justify this hypothesis, Pb should be measured in urine and feces. In this regard, transmission electron microscopy of urine and fecal samples can be helpful.

The mean concentration of Pb in the tissues of rats simultaneously fed with Pb and BCNF was lower than that in tissue of those fed with Pb and BCNF with 4-hour intervals. Lower Pb concentration in the rats simultaneously fed with Pb and BCNF can be explained by the binding of Pb to BCNF in the gut and possible elimination of Pb from the gastrointestinal tract before absorption by blood vessels (6, 8, 16). The mean Pb concentration in the liver

was significantly higher than that in the kidney. This might be related to the high level of blood circulation in the liver and the fact that the liver is the main organ responsible for cleansing the blood (14).

To date, no study has examined the biological effects of BCNF on animals. Hence, the obtained results were compared with results of studies on the effect of other Pb chelators on laboratory animals. Khazaeipour et al. (2010) reported that nanochitin, as a Pb chelator, can lower Pb concentration in the kidney and liver tissues of Wistar rats, which may be related to binding of Pb with amino and hydroxyl groups of nanochitin (13).

Pal et al. (2015) also reported a decrease in Pb tissue concentration of mammals by using curcumin–nanocurcumin, which is the main ingredient of turmeric (21). Marianti et al. (2017) demonstrated that chitosan protects the kidneys and liver from the toxic effects of Pb by chelating and removing Pb from the tissues (18). Another study reported that flaxseed oil could cause a 50% reduction in Pb concentration in the kidney of male laboratory rats (19). Johari et al. (2013) reported a similar effect for garlic extract in rats (10). According to the authors, the presence of free carboxyl and amino groups in the garlic extract is responsible for this activity. Similarly, a study reported that pomegranate extract could reduce Pb concentration in tissues of rats.

CONCLUSION

Based on the results, oral consumption of BCNF can be used for prevention of Pb accumulation in the body. In addition, BCNF may be used as a suitable supplementary drug in Pb-poisoned animals. Since BNFC may also reduce concentration of essential metals such as iron and calcium in the blood of both wild and domestic mammals, the use of this substance in industry and medicine needs further investigations.

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DECLARATIONS

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Ethics approvals and consent to participate

The experimental procedure was performed according to the Guide for the Care and Use of Laboratory Animals proofed by CCAC (2009) and was approved by the research committee of Gorgan University of Agricultural Sciences and Natural Resources (ethical approval code: 8/21692).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.

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