Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Original Article

Impact of Nanochitin on Serum Concentration of Iron and Calcium in Wistar Rats

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Received: 2021/10/18
Revised: 2021/11/02
Accepted: 2021/12/08

DOI: 10.29252/mlj.16.5.26

ABSTRACT

Background and objectives: Considering the increasing use of nanochitin for the removal of heavy metals from aqueous solutions, examining the biological effects of this substance on the level of essential metals for humans and animals is crucial. Therefore, this study investigated impact of oral administration of nanochitin on serum levels of iron (Fe) and calcium (Ca) in Wistar rats.

Methods: Twenty male Wistar rats were randomly divided into four treatment groups and one control group. Two groups were fed with nanochitin at doses of 1.6 and 2.6 µg/g for 6 weeks, and the other two groups received the mentioned doses for 10 weeks. Serum concentrations of Fe and Ca were measured using atomic absorption spectroscopy.

Results: Oral administration of 2.6 µg/g nanochitin for 10 weeks caused a significant decrease in serum Ca and Fe concentrations (p<0.05). Oral administration of 1.6 and 2.6 µg/g nanochitin for 6 weeks caused a non-significant reduction in serum Fe and Ca concentrations (p>0.05). However, nanochitin consumption for 10 weeks resulted in a significant decrease in serum Fe concentration but not Ca.

Conclusion: The limited reduction of serum Fe and Ca concentrations after oral consumption of nanochitin at a low dose and for a limited duration indicates that the controlled use of nanochitin could be safe for animals. However, complementary studies are needed to determine the exact effects of nanochitin on the animals' bodies. On the other hand, it is recommended to use Fe and Ca supplements after consuming high doses of nanochitin for longer periods.

Keywords: Iron, Calcium, Rats Wistar.

INTRODUCTION

Chitin is a polymeric substance derived from glucose, which is abundantly found in the skin of insects, marine animals, and fungi. It is a biopolymer with a definite structure of N-acetyl-B-D-glucosamine. Chitin is naturally combined with carbonates and proteins, and due to presence of active functional groups such as hydroxyl and amine, it has a high capability to absorb heavy metals (1).

Nanoparticles are particles of matter with diameter of 1 and 100 nm that have unique physical, chemical, mechanical, electrical, and magnetic characteristics. For example, they easily enter the cell and interfere with its normal and vital processes (2). Conversion of chitin to nanochitin further increases its beneficial properties (3). As a low-cost biopolymer, nanochitin is widely used in the chemical, pharmaceutical, food, textile, cosmetic, biotechnology, paper, and agricultural industries as well as in refining wastewater from heavy metal ions and pigmented substances (4). However, despite the various applications of this substance and its increasing use, the biological impacts of this chemical compound have not yet been carefully identified. As a promising bio-absorbent, nanochitin is used to absorb anionic and cationic contaminants, such as heavy metals (5). In a study by Khazaeipour et al. (2020), lead concentrations reduced in the liver of Wistar rats fed with nanochitin compared with a control group. In addition to showing the positive effects of nanochitin on toxic heavy metals such as lead, the mentioned study also pointed to the possible reduction of essential metals such as iron (Fe) and calcium (Ca).

As an important mineral element, the presence of Fe, in small amounts, is vital for normal functioning as well as efficient metabolism in mammals (6). This element exists in important biomolecules such as hemoglobin, myoglobin, cytochrome, and enzymes. It also contributes to the production of hemoglobin (the oxygen-carrying chemical in red blood cells) and myoglobin (a protein in muscle cells) (7). On the other hand, Ca is usually involved in bone formation and metabolism. The presence of Ca in the circulatory system, extracellular fluid, muscles, and other tissues is vital for vasoconstriction and dilation, muscle function, neurotransmission, intracellular signaling, and hormone secretion. This element contributes to formation of bones and teeth, activates enzymes in the body, and helps regulate blood pressure and blood clotting (8, 9)

Due to the ability of nanochitin to bind to heavy metals in aqueous media, entry of nanochitin in the bloodstream might lead to excretion of essential elements, such as Ca and Fe through urine. Considering the vital role of essential metals such as Fe and Ca in animals and humans and the expanding use of nanocomposites such as nanochitin in the pharmaceutical industry, it is essential to investigate the possible impact of nanochitin on the concentration of essential metals and their biological activity (10). In this regard, the present study was conducted to investigate effects of oral consumption of nanochitin on serum Fe and Ca concentrations in Wistar rats.

MATERIALS AND METHODS

This study was conducted on 20 male Wistar rats aged 8 to 10 weeks with an average weight of 150 to 180 g. The animals were purchased from the Amol Pasteur institute of Iran and kept in the Animal Center of Golestan University of Medical Sciences. The animals were kept at 20 ºC, 60% humidity, and 12 h:12 h light-dark cycles. The rats were fed with ready-made food and urban water (11). To maintain hygiene in animal cages, wood shavings of the cages' floor were changed weekly, and the floor was completely disinfected with 5% phenol.

The 5% nanochitin gel (Nanonovin Polymer Co., Iran) was used in this research. After being numbered, the rats were weighted and randomly assigned to four treatment groups (N=4) and one control group (N=4) as follows (12,13).

Control group: Rats in the control group had free access to water and food. Serum Fe and Ca concentrations were measured at baseline and 6 and 10 weeks after the intervention.

Treatment group 1: Treatment group 2: Rats in this group received 1.6 µg/g water dissolved nanochitin by gavage, every morning, for 6 weeks.

Treatment group 2: Rats in this group received 1.6 µg/g water dissolved nanochitin by gavage, every morning, for 10 weeks.

Treatment group 3: Rats in this group received 2.6 µg/g water dissolved nanochitin by gavage, every morning, for 6 weeks.

Treatment group 4: Rats in this group received

2.6 µg/g water dissolved nanochitin by gavage, every morning, for 10 weeks.

At the end of the study period, the rats were anesthetized, and blood samples (about 3 ml) were collected from the tail vein. Serum was obtained by centrifugation and then immediately transferred to the laboratory. The concentrations of Fe and Ca were measured using an atomic absorption spectroscopy instrument (Agilent 240Z AA with AC modulation, Agilent Technologies, USA). All experimental procedures were done in accordance with the guide for care and use of laboratory animals. The study was approved by the ethics committee of Gorgan University of Agricultural Sciences and Natural Resources (approval code: 8/25422) (14).

Data were expressed as mean and standard deviation. Statistical analysis of data was carried out in SPSS software (version 20).

One-way analysis of variance (ANOVA) and t-test were used to compare effect of the duration of nanochitin administration and effect of nanochitin concentration on serum Fe and Ca concentrations, respectively. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Normality of the obtained data was confirmed by the Kolmogorov-Smirnov test. Compared with the control group, oral administration of 1.6 µg/g nanochitin for 6 weeks and 10 weeks caused a slight decrease in the serum concentration of Ca and Fe ($p \geq 0.05$). Consumption of 2.6 µg/g nanochitin for 6 weeks did not significantly reduce the serum Ca concentration compared with the control group. However, consumption of 2.6 µg/g nanochitin for 10 weeks significantly reduced serum Ca compared with the other groups ($p < 0.05$) (Table 1).

Oral administration of nanochitin at both doses for 10 weeks significantly decreased serum Fe concentration compared with the control group ($p < 0.05$).

Table 1- Concentrations of Fe and Ca in the control and treatment groups

	Control group	Treatment group 1 (1.6 µg/g for six weeks)	Treatment group 2 µg/g for 10 (1.6 weeks)	Treatment group 3 (2.6 µg/g for six weeks)	Treatn (2.6 µ
	287.4	228.4	212	220	
	11.48	10.53	10.50	10.11	

DISCUSSION

This study revealed that the consumption of nanochitin at the doses of 1.6 and 2.6 µg/g for 10 weeks reduced the serum concentrations of Fe and Ca in Wistar rats. Of course, the decreasing trend of serum Fe and Ca differs due to different mechanisms of absorption, excretion, and the normal level of these two elements in the animal's body (15, 16). The concentration of serum Fe reflects the amount of Fe absorption from the gastrointestinal tract and also the level of Fe production in the liver (17). The nanochitin-associated decrease of Fe may be related to the binding of nanochitin to Fe in the gastrointestinal tract and fecal and urinary excretion of Fe (15-18). Therefore, determining the exact mechanisms through which nanochitin affects serum Fe concentrations seems essential. In this regard, transmission electron microscopy of urine and feces samples may be beneficial. Given the importance of serum Fe levels and the role of this element in maintaining animals' health, studies have investigated the impact of Nano zinc oxide has been widely used in agricultural and pharmaceutical industries in recent years. However, the effects of its long-term use, like that of nanochitin, on animals are not known. In this regard, Wang et al. (2015) demonstrated that nano zinc oxide consumption (500 µg/g) does not alter serum Fe level in rats (18-23).

The uptake of Ca occurs in the intestines, and several factors are involved in the Ca absorption in the gastrointestinal tract. For example, vitamin D increases the absorption of Ca in the gastrointestinal tract, while oxalate reduces intestinal Ca absorption. On the other hand, serum Ca level is affected by the level of hormones secreted by the thyroid and parathyroid glands (15). Despite the possible binding of nanochitin to Ca in the gastrointestinal tract and serum, oral administration of 1.6 µg/g nanochitin did not significantly affect serum Ca concentrations in Wistar rats. It seems that the hormonal system

controls the level of blood Ca in short periods (e.g. 6 weeks). Even if Ca absorption from the intestines decreases or Ca secretion by the kidneys increases, the body may be still able to regulate the serum Ca levels (15). In line with our findings, a previous study reported that oral administration of starch and inulin for 21 days did not change blood serum Ca levels in Wistar rats (24). Shockravi et al. (2011) indicated that the addition of oral phytase enzyme to the diet of rats did not change serum Ca levels in rats (25).

In the present study, the oral administration of 2.6 µg/g nanochitin for 10 weeks significantly decreased serum Ca level. This might be related to the binding of nanochitin to Ca and its subsequent excretion through the gastrointestinal tract or the kidneys (26). Our results showed that oral consumption of nanochitin at high doses and for longer periods can significantly reduce serum Ca concentration.

CONCLUSION

Based on the results, oral administration of nanochitin at low doses and for short periods has little effects on the serum concentrations of Ca and Fe. Thus, the limited and controlled use of this substance seems to be safe. However, long-term administration of nanochitin at the doses of 1.6 and 2.6 µg/g can disrupt the normal concentration of Fe and Ca, which might lead to complications such as anemia, lethargy, and muscle weakness. Therefore, nanochitin should be administered with caution. It is also recommended to use Fe and Ca supplements after consuming high doses of nanochitin for long periods.

ACKNOWLEDGMENTS

The present paper has been extracted from a master's degree thesis by Negin Valizadeh Keshmesha Tapeh, approved by the Gorgan University of Agricultural Sciences and Natural Resources. We are very grateful to Dr. Roghayeh Safari and Mohammad Mazandarani for their contributions to the statistical analysis of data.

DECLARATIONS

Funding

This project was funded by the Gorgan University of Agricultural Sciences and Natural Resources, Iran.

Ethics approvals and consent to participate

All experimental procedures were done in accordance with the guide for care and use of laboratory animals. The study was approved by the ethics committee of Gorgan University of Agricultural Sciences and Natural Resources (approval code: 8/25422).

CONFLICT OF INTEREST

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

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How to Cite:
Valizadeh KeshmeshTapeh N, Namroodi S, Taziki SH [Impact of Nanochitin on Serum Concentration of Iron and Calcium in Wistar Rats]. mljgoums. 2022; 16(5): 26-30 DOI: 10.29252/mlj.16.5.26