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

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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.

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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

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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

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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.

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DECLARATIONS

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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.

REFERENCES

1. Pinto PX, Al-Abed SR, Reisman DJ. Biosorption of heavy metals from mining influenced water onto chitin products. Chemical Engineering Journal. 2011 February1009-1002(3)166ł. [View at Publisher] [DOI:10.1016/j.cej.2010.11.091.] [Google Scholar]

2. Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol Lett. 2008;179(3):130-9. [View at Publisher] [DOI:10.1016/j.toxlet.2008.04.015.] [PubMed] [Google Scholar]

3. Musgrave C.B, Perry J.K, Merkle R.C, & Goddard W.A. Theoretical studies of a hydrogen abstraction tool for nanotechnology. Nanotechnology. 1991; 2(4): 187. [DOI:10.1088/0957-4484/2/4/004] [Google Scholar]

4. Tan TS, Chin HY, Tsai ML, Liu CL. Structural alterations, pore generation, and deacetylation of α- and β-chitin submitted to steam explosion. Carbohydr Polym. 2015;122: 321-8. [View at Publisher] [DOI:10.1016/j.carbpol.2015.01.016.] [PubMed] [Google Scholar]

5. Ghourbanpour J, Sabzi M, Shafagh N. Effective dye adsorption behavior of poly(vinyl alcohol)/chitin nanofiber/Fe(III) complex. Int J Biol Macromol. 2019; 137: 296-306. https://doi.org/10.1016/j.ijbiomac.2019.06.213 [View at Publisher] [DOI:10.1016/j.ijbiomac.2019.06.213.] [PubMed] [Google Scholar]

6. Latunde-Dada G.O, McKie A.T, & Simpson R. J. Animal models with enhanced erythropoiesis and iron absorption. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2006 Aprilł423. https://doi.org/10.1016/j.bbadis.2005.12.007 [View at Publisher] [DOI:10.1016/j.bbadis.2005.12.007.] [PubMed] [Google Scholar]

7. Schumann K. Safety aspects of iron in food. Annals of Nutrition and Metabolism. 2001; 45: 91–101. [View at Publisher] [DOI:10.1159/000046713.] [Google Scholar]

8. Weaver CM, Heaney RP. Calcium, Modern Nutrition in Health and Disease. 10. Advances in Nutrition. Lippincott Williams & Wilkins. 2006; 2(30): 290-292. [View at Publisher]

[Downloaded from mlj.goums.ac.ir on 2023-12-10]

[DOI: 10.52547/mlj.16.5.26]

9. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary Reference Intakes for Calcium and Vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Washington (DC): National Academies Press (US); 2011. PMID: 21796828. [View at Publisher] [PubMed] [Google Scholar]

10. Buerge T. and Weiss T. The laboratory Mice. Copy right Elsevier ISBEN.0-1233-6425-6.2004.

11. Al-Attar AM. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J Biol Sci. 2011; 18(1): 63-72. [View at Publisher] [DOI:10.1016/j.sjbs.2010.10.004] [PubMed] [Google Scholar]

12. Khazaeipour A, Nimrodi S, and Taziki Sh. The effect of nanokitin on the tissue uptake of lead acetate in the liver of rats. Scientific Journal of Gorgan University of Medical Sciences. 2019; 22 (74): 39-34. [Persian] [View at Publisher]

13. Zarei K, Najafpour Gh, Sharifzadeh M. Synthesis and Application of Nano-chitosan in Removal of Heavy Metals. Thesis in Chemical. Faculty of Chemical Engineering. Babol Noshirvani University of Technology (BUT). 2012. [Persian]

14. CCAC- Canadian Council On Animal Care . The care and use of farm animals in research, teaching and testing. Ottawa. 2009;12-5. [View at Publisher]

15. Yoo S, Chio KS, Ryu MH. A Study on the Effect of Chitin, Chitosan and Dithiocarbamate Chitosan on the Nickel Toxicity in Rat liver. Korean journal of environmental health. 2008 July ؛2008.91-285(4)34.; https://doi.org/10.5668/JEHS.2008.34.4.285 [View at Publisher] [DOI:10.5668/JEHS.2008.34.4.285.] [Google Scholar]

16. Allen LH. Calcium bioavailability and absorption: a review. Am J Clin Nutr. 1982; 35(4): 783-808. [DOI:10.1093/ajcn/35.4.783.] [PubMed] [Google Scholar]

17. Guthrie H.A. Basics of nutrition. Translated by Dr. Minoo Forouzani. Entesharat sherkate sahami Chehr Tehran. [Persian]

18. Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, et al. Acute toxicological impact of nano-and submi- cro-scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticle Research. 2008;10: 263-276. [View at Publisher] [DOI:10.1007/s11051-007-9245-3]

19. Ounjaijean S, Thephinlap C, Khansuwan U, Phisalapong C, Fucharoen S, Porter JB, Srichairatanakool S. Effect of green tea on iron status and oxidative stress in iron-loaded rats. Med Chem. 2008; 4(4): 365-70. [DOI:10.2174/157340608784872316] [PubMed] [Google Scholar]

20. Mehri Pirayvatlu A, Ali Panah Mogadam R, Mazani M, Manafi f, Malekzadeh V, Nemati A, Nagizadeh Bagi A. The effect of andrographolide extract on blood glucose and lipid profile in rats with secondary iron overload. Journal of Ardabil University of Medical Sciences. 2016; 16(4): 399-408. [View at Publisher]

21. Turgut G, Kaptanoğlu B, Turgut S, Enli Y, Genç O. Effects of chronic aluminum administration on blood and liver iron-related parameters in mice. Yonsei Med J. 2004; 45(1): 135-9. [DOI:10.3349/ymj.2004.45.1.135] [PubMed] [Google Scholar]

22. Moshtagi M, Moshtagi A, Mahdavi J, Pourmoghadas H. The effect of fluoride on the concentration of parameters related to iron metabolism in rats. Proceedings of the 3rd Congress of Rare Elements of Iran. Kashan University of Medical Sciences. 2012; 16(7): 715-716. [Persian] [View at Publisher]

23. Wang C, Lu J, Zhou L, Li J, Xu J, Li W. Effects of Long-Term Exposure to Zinc Oxide Nanoparticles on Development, Zinc Metabolism and Biodistribution of Minerals (Zn, Fe, Cu, Mn) in Mice. PLoS ONE. 2016; 11(10): e0164434. [View at Publisher] [DOI:10.1371/journal.pone.0164434] [PubMed]

24. Tavaria FK, Jorge MP, Ruiz LT, Ana Lúcia TG, Pintado ME, Carvalho JE. Anti-proliferative, anti-inflammatory, anti-ulcerogenic and wound healing properties of chitosan. Curr Bioact Comp. 2016; 12(2): 122-114. [DOI:10.2174/1573407212666160330204522] [Google Scholar]

25. Younes H, Coudray C, Bellanger J, Demigné C, Rayssiguier Y, Rémésy C. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. Br J Nutr. 2001; 86(4): 479-85. [View at Publisher] [DOI:10.1079/BJN2001430.] [PubMed]

26. Shokravi S, Mohamad shirazi M, Abadi A, Komeyli fonud R, Kimiyagar R. The effect of phytase supplementation on the status of zinc, iron and calcium in rats fed a diet containing Iranian perfitate bread (Sangak). Iranian Journal of Endocrinology and Metabolism. Shahid Beheshti University of Medical Sciences and Health Services. 2012; 13(5) :514-523. [Persian] [View at Publisher]

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