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**THE EFFECT OF ACUTE PRESCRIPTION OF ZINC OXIDE NANOPARTICLES ON
THYROID HORMONE IN ADULT MALE RATS**

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ABSTRACT

The widespread use of Nanoparticles has caused these compounds enter human life system rapidly. The purpose of this study was to investigate the effects of Zinc oxide Nanoparticles on thyroid hormones in adult male rats. In this study, 42 male Wistar rats (200±20 gr) were divided into 6 7-member groups, including group 1 saline, groups 2, 3, 4 2.5 mg/kg Nano Zinc oxide, and groups 5 and 6 that received 1.25 and 5 mg/kg Nano Zinc oxide intraperitoneally (ip). The blood samples of groups 2, 3 and 4 were taken respectively on the first, third and fourth days to determine the appropriate day for taking blood, and The blood samples of groups 5 and 6 were taken the third day after receiving drug to measure the amount of T3 and T4 hormones. 1.25 mg/kg of NanoZinc oxide significantly reduced the amount of T3 on the third day. NanoZinc oxide of 2.5 mg/kg significantly reduced the levels of T4 and T3 on the third day and T4 on the fourteenth day. NanoZinc oxide of 5mg/kg significantly reduced the level of T4 and T3 in the third day. Acute injection of NanoZinc oxide reduces the amount of thyroid hormone T3 and T4. The effect occurs in different doses, but is more significant in the medium and long term, and its mechanism of action is needed to be carefully analyzed.

Keywords: Nano Zinc Oxide, Thyroid Hormone, Rat

INTRODUCTION

The thyroid gland is one of the largest endocrine and its hormones, thyroxine and triiodothyronine are important for human growth, development and metabolism.

Hypothyroidism is one of the most important endocrine hormone diseases in human and animal caused by deficiency of hormones produced in thyroid gland [1]. Each of the elements are somehow related to the synthesis, metabolism, and actions of thyroid hormones, and any disturbance in their normal value may underlie disorders in the effects of thyroid hormones and cause symptoms associated with these disorders [2]. There is evidence suggesting the role of Zinc in the function and formation of thyroid hormones [3]. According to the evidence, the triiodothyronine receptor needs Zinc for its correct biological activity [4]. The role of some elements, such as Zinc, on the thyroid gland is less known. However, more or less than normal absorption of this element can have a variety of effects on the metabolism of thyroid hormones [1].

Nanoparticles as a new type of material are greatly used in various fields due to their specific properties. The properties of Nano materials can be different from their bulk mode, and in some cases, unexpected physical and chemical properties are shown [5]. Some studies have shown that Nanoparticles are more toxic than the same combination at micro-size, and Nanoparticles of different sizes induce different levels of cytotoxicity and DNA damage [6]. Oxidative

stress represents a general mechanism of cellular damage caused by Nanoparticles, and the mechanism has been confirmed in many studies on the toxicity of Nanoparticles. Large amounts of ROS can be produced even at the presence of a small amount of Nano Zinc oxide in cells [7]. Among large number of Nanoparticles, Nano Zinc oxide has gradually attracted lots of attention in animal sciences. Strong proprietary energy and its associated effects that lead to higher conductivity and electron mobility than the bulk materials and other metallic oxides have provided more opportunities for the use of this Nanomaterial [8]. However, there are few studies on the beneficial effects of Zinc oxide Nanoparticles in physiological processes. Some studies have shown that in mammalian cells, Zinc oxide Nanoparticles cause higher levels of oxidative stress, resulting in inflammation and cell toxicity [9]. Brunner et al. have shown that following the exposure to concentrations of Zinc oxide Nanoparticles higher than 15 ppm, almost all human and rodent cells will die [10]. However, recent studies, in contrary with those findings, have shown that exposure to acute doses of Zinc oxide Nanoparticles causes antioxidant effects in hippocampal brain tissue of rats [11]. However, research on the potential

health risks of exposure to Nanoparticles has lagged behind the rapid development of Nanotechnologies [12].

With regard to novelty of research on Nanoparticles and their limited, contradictory and controversial effects on body systems, identification of positive or negative effects of these substances on the endocrine function of the thyroid gland is particularly necessary.

MATERIALS AND METHODS

In this study, Wistar male rats weighing 200 ± 20 gr prepared from the center of proliferation of laboratory animals at Ahvaz University of Medical Sciences were used. Animals were kept in the animal room under standard conditions at 22 ± 2 ° C. Four animals were kept in each cage, and 12 hours of light and 12 hours dark cycle, with food and water was considered for them. In order to getting accustomed with laboratory environment, the rats were kept in separate cages for a period of one week before each test. Then, animals were randomly divided into 6 groups as follows:

- 1) The group receiving saline (injection control) (n=7), that received 10ml/kg buffered saline (0.9%), and then their blood was taken to measure the levels of T3, T4.
- 2) Three groups receiving 2.5 mg/kg Zinc oxide Nanoparticles (NanoZnO) (n=7) that were bled on days 1, 3, and 14 after

receiving the drug to measure the levels of T3, T4. With the findings of these groups, the bleeding day for other groups was identified.

- 3) Two groups receiving 1.25 and 5 mg/kg Zinc oxide Nanoparticles (NanoZnO) (n=7) and then their blood was taken on the third day after receiving the drug to measure the levels of T3, T4.

The Nano Zinc oxide was prepared from Lolitec Company Germany with size of 50-80 nm, and the needed amount was ultrasonic bath in saline 0.9% for 15 minutes before the test, and was dispersed again by Shaker for 1 minute before each injection [13]. Drugs were injected into receiving groups intraperitoneally as an acute dose, and to measure the amount of T3, T4 hormones, sera were separated and were delivered to the lab under standard laboratory procedure.

Biochemical analysis: Hormones were measured using Electro chemiluminescence luminescence method (ECL) and Elecsys kit (Code 17121802 to 17551102 Code for T4 and T3).

Statistical methods: Results are expressed as mean \pm SEM. Data was analyzed using SPSS (Version 21). To evaluate the results of tests in different groups ANOVA, one-way and Post hoc, Tukey was used. In all cases, there is a difference between the groups at $P < 0.05$ is considered significant.

RESULTS

Comparing the effect of 2.5 mg/kg of Nano Zinc oxide: Figure 1 shows the level of T4 thyroid hormone in different groups of adult male rats that received 2.5 mg/kg of body weight of Nano Zinc oxide, and were bled on the first, third and fourteenth days. Statistical analysis shows the level of T4 in the group receiving 2.5 mg/kg that were bled on the first day was not significantly different than the saline group, while shows a significant decrease in groups that were bled on the third day (**P<0.01) and fourteenth day (*P<0.05).

Figure 2 shows the level of T3 thyroid hormone in different groups of adult male rats that received 2.5 mg/kg of body weight of Nano Zinc oxide, and were bled on the first, third and fourteenth days. Statistical analysis shows that the level of T3 in the group receiving 2.5 mg/kg on the first and fourteenth days was not significantly different than the saline group, while shows a significant decrease in groups that were bled on the third day (*P<0.05).

Comparing the effect of different doses of Nano Zinc oxide: Figure 3 shows the level of T4 thyroid hormone in different groups of adult male rats that received 1.25, 2.5, and 5 mg/kg of body weight of Nano Zinc oxide, and were bled on the third day. Statistical analysis shows that the level of T4 in the group receiving 1.25 mg/kg on the third days was not significantly different than the saline group, while shows a significant decrease in groups that received 2.5, and 5 mg/kg of Nano Zinc oxide, (**P<0.01) and (*P<0.05). respectively.

Figure 4 shows the level of T3 thyroid hormone in different groups of adult male rats that received 1.25, 2.5, and 5 mg/kg of body weight of Nano Zinc oxide, and were bled on the third day. Statistical analysis shows that the level of T43 in the group receiving 1.25, 2.5, and 5 mg/kg on the third days was significantly decreased compared to the saline group, (**P<0.01) and (*P<0.05), and (**P<0.01) respectively.

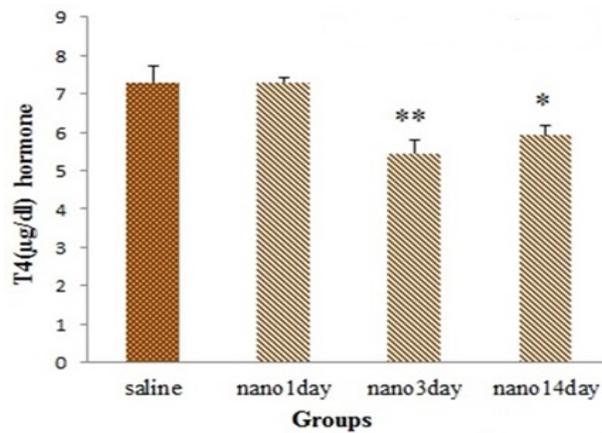


Figure 1. T4 thyroid hormone on the first, third and fourth days in the different groups of adult male rats receiving 2.5 mg/kg of Nano Zinc oxide compared to saline (symbol*). nano=NanoZnO (mg/kg).

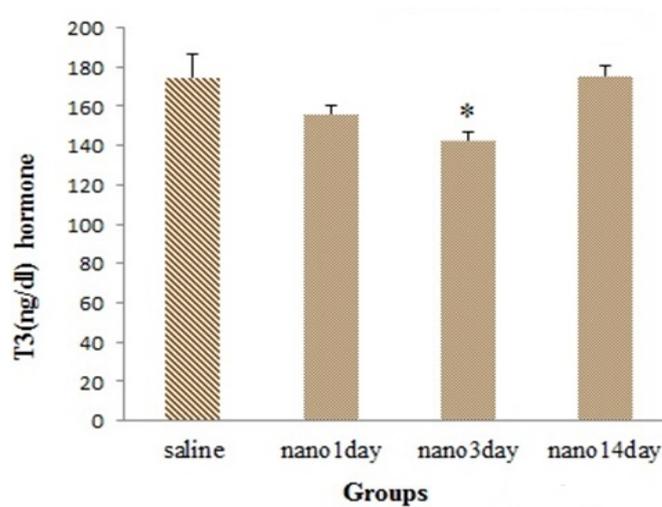


Figure 2. T3 thyroid hormone on the first, third and fourth days in the different groups of adult male rats receiving 2.5 mg/kg of Nano Zinc oxide compared to saline (symbol*). nano=Nano ZnO (mg/kg).

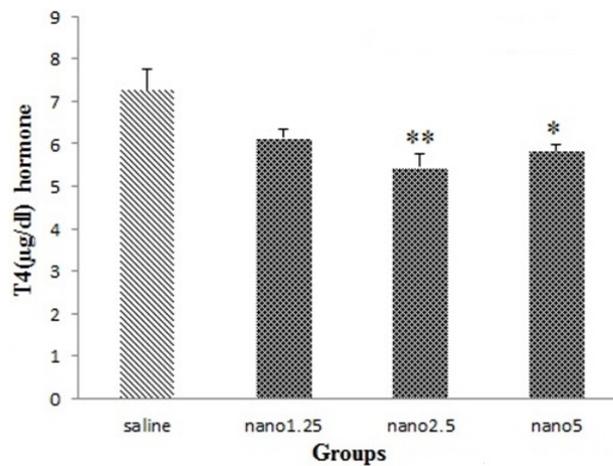


Figure 3. T4 thyroid hormone on the third day in the different groups of adult male rats receiving 1.25, 2.5, and 5 mg/kg of Nano Zinc oxide compared to saline (symbol*). nano=NanoZnO (mg/kg).

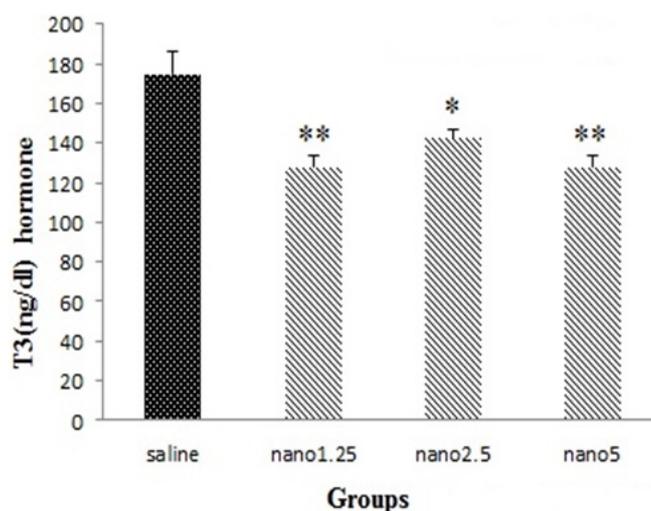


Figure 4. T3 thyroid hormone on the third day in the different groups of adult male rats receiving 1.25, 2.5, and 5 mg/kg of Nano Zinc oxide compared to saline (symbol*). nano=NanoZnO (mg/kg).

DISCUSSION

The findings of this study have shown that once intraperitoneally prescription of NanoZinc oxide can significantly reduce the level of thyroid hormones (T3 and T4) in male rats on the third day after injection. The measurement of these hormones in the first and fourteenth day after injection of 2.5 mg/kg Nano Zinc oxide was also performed in male rats. In these experiments, the levels of T4 and T3 did not significantly change on the first day after injection, and on the fourteenth day, only the level of T4 was significantly reduced compared to the saline group. Studies have shown that long-term prescription of Zinc supplements in small ruminators significantly reduces total concentrations of Thyroid hormones. However the free concentration of these hormones were reduced, but not in a

statistically significant amount[14]. Since the thyroid hormones system plays an important role in the metabolic performance, any malfunction of the endocrine system may cause irreparable damage to tissues. Research has shown that in mammalian cells, Zinc oxide Nanoparticles cause higher levels of oxidative stress, resulting in inflammation and cell toxicity [9]. This can reduce levels of these hormones through the effect on the thyroid gland or on release of thyroid stimulating hormone (TSH). Yet, more research is required in this regard. Studies have shown high levels of Zinc (Zn) in the diet, due to hypothyroidism may lead to a decrease in basal metabolism, hypercholesterolemia, copper and magnesium deficiency in the tissues, and increased risk of coronary artery disease [15]. However, other studies have shown that

exposure to Zinc oxide Nanoparticles do not have a significant effect on the activity of thyroid hormone beta receptors [16].

On the other hand, a study conducted on rats reported that the application of Zinc chloride reduces T3 and the ratio of T4/T3. This inhibition was attributed of the microsomal capacity to convert T4 to T3. [17]. It was also reported that increased Zinc in broiler chickens decreases levels of thyroxine (T4) and (T3) [18]. In another study on broiler chicken, it was found that T3 and T4 levels of plasma are influenced by dietary supplements containing Zinc, so that the values of 100 and 200 mg/kg of Zinc reduces T4 level of plasma in comparison with the control group, while T3 level of plasma reduced using 100 mg/kg of Zinc [19].

These data may suggest that a high intake of Zinc changes production or secretion of the thyroid hormone. In addition, it has been claimed that the reduced effects of thyroid may be due to the reduced regulatory effect of pituitary gland on thyroid gland, and decreased circulating thyroid hormones may be indicative of hypothyroidism due to Zinc toxicity [18]. These results are consistent with results of this study, and it seems that Zinc oxide Nanoparticles have significant effects in reducing the acute prescription of thyroid

hormones due to special properties resulted from its size.

Recent studies have shown that the central nervous system (CNS) is the major target organ for Nanoparticles. Thus, the effect of Nanoparticles on the CNS has attracted further attention. Nanoparticles can pass through the most powerful biological barriers like the blood-brain barrier through the olfactory nerves, and enter CNS and accumulate in various parts of the brain [20]. For this reason, it can be said that these Nanoparticles affect Hypothalamic-pituitary-thyroid axis and thus affect the level of thyroid hormones that requires further study.

CONCLUSION

Due to the changes and the results of this experimental study, it can be concluded that Zinc oxide Nanoparticles, even in small quantities, can have negative effects on the activity of the thyroid gland and disrupt production of its hormones.

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Conflict of interest: There is not conflict of interest in this study.

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