



Effect of Molybdenum Trioxide Nanoparticles (MoO₃ NPs) on Thyroid Hormones in Female Rats

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ABSTRACT

Background: In recent years, nanoparticles have been increasingly used in industries. However, there is a serious lack of information concerning their impact on human health and the environment. This study was investigated the effect of MoO₃ NPs on thyroid hormones in female rats.

Methods: In this study, 21 female Wistar rats were selected and divided into three groups of untreated control, sham and treatment group with seven rats in each group. During 28 days the sham group received normal saline and treatment group received 5 mg/kg (MoO₃ NPs) in normal saline. After the final injection, blood samples were collected and serum levels of total thyroxine (T₄), triiodothyronine (T₃), and thyroid-stimulating hormone (TSH) were measured and groups were compared together.

Results: The results showed that the serum levels of T₄ decreased significantly in group of 5 mg (MoO₃ NPs) in comparison with the control group (p<0.05). However, there was insignificant difference observed in T₃ levels when compared with the control group (p>0.05). The serum levels of TSH increased significantly in group of 5 mg/kg (MoO₃ NPs) when compared with the both groups of sham and control (p<0.05).

Conclusion: The results showed that MoO₃ NPs exposure induces the risk of thyroid dysfunction.

1. Introduction

In recent years, with development of nanotechnology, nanomaterials are recognized to have broad range of applications in the industry and biomedicine because of their large specific surface area, ultrahigh reactive surface sites, and quantum effects [1].

However, there is a serious lack of information on the effect of NPs on human health and environment. It is believed that the particle size, chemical nature, morphology, and surface chemistry of NPs are the main parameters that influence their levels of toxicity.

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Therefore, nanotoxicology still requires the necessary data and elucidations to provide true risk assessments of NPs [2]. In recent years, some researchers have reported that exposure to ultrafine particles (<100 nm) could cause tissue inflammation and pulmonary damages, which could lead to lung tumors and fibrosis [1, 3]. The mechanisms of NPs toxicity are not well recognized. However, it is believed that free oxygen radical production is often used to explain the toxicity of NPs. The free oxygen radicals induce a wide diversity of physiological and cellular events such as cellular stress, inflammation, DNA damage, and apoptosis [4]. Many studies reported about NPs toxic potential and oxidative damage in the cellular proteins, lipids, and DNA [4 -6]. Molybdenum (Mo) as a transition metal in periodic table is an essential trace element. The Mo functions as the 'molybdenum cofactor' in molybdoenzymes, including xanthine oxidase, aldehyde oxidase, and sulfite oxidase which are important to humans.

However, it is capable of producing toxic effects in animals or humans when ingested acutely or chronically, in excess [7-9].

Molybdenum trioxide (MoO₃) nano structure has become an attractive material due to its multifunctional uses such as photo catalysis, oxidative catalyst, gas sensors, photochromic coatings, lubricants, secondary batteries and as additives in paint [10]. Industrial effluents, which contain an excess amount of molybdenum, could contaminate environment and cause unfavorable effects in animals and humans [8,11]. Therefore, it is necessary to know about the potential risks of MoO₃ NPs. However, there is little information in literatures on the toxic and non-toxic effects of Mo NPs. According to recent publications, Mo NPs could induce cytotoxicity, genotoxicity, and oxidative stress in mouse skin fibroblast cells and show cytoprotective effects in MCF-7 and HT-1080 cells [11, 12]. Also in our previous work we found that the Mo NPs induced a reduction in the serum levels of testosterone in male rats, and histopathology of testis and liver showed a decrease in number of Leydig cells and an increase in chronic inflammatory cells respectively [13]. The aim of this study was to investigate the effect of MoO₃ NPs exposure on serum levels of thyroid hormones in female rats. Thyroid hormones have an important role in the

metabolism in all organs and systems, and it is reported that 10% of population suffers some level of thyroid dysfunction, representing a major public health problem [14]. Therefore, there is increasing attention to find the effect of environmental exposures on such changes. In this work we studied the effect of MoO₃ NPs on T₃, T₄, and TSH levels in female Wistar rats as an animal model.

2. Materials and Methods

2.1. Animals

Female Wistar rats were purchased from the School of Pharmacy at Zanjan University of Medical Sciences (ZUMS), Zanjan, Iran. The MoO₃ nanoparticles (APS 13-80 nm) were obtained from NANOSANY Corporation TM (Mashhad, Iran). Female rats with average initial body weight (200-250 g) were selected as the animal model for the study of MoO₃ NPs effects.

All animals (21 rats) were housed with 12 hr light/dark cycle at room temperature with relative humidity of 60%. Food and water were provided ad libitum. All animal experiments were performed in compliance with the ZUMS Ethics Committee.

2.2. Experimental design and treatment

In this experimental study, 21 female rats were selected and randomly divided into three groups of untreated control, sham and treatment group with seven female rats in each group. During 28 days the sham group received intraperitoneal injection of normal saline alone and treatment group received 5 mg/kg BW of MoO₃ NPs in normal saline every other day. One day after the final injection, the rats were anesthetized with ether before getting sacrificed, and blood samples were taken from the left heart atrium. In addition, body weights of rats measured before and end of treatment for all animals.

2.3. Hormone assays

Blood samples were centrifuged at 3,000 rpm for 10 min. Following centrifugation, serum from each sample was collected and frozen at -20 °C

until analysis. Serum levels of T₃, T₄, and TSH were determined using ELISA technique (Stat fax 3200, USA) and kits (Autobio Co., Ltd, China) according to the manufacturer's instructions. Serum levels of T₃, T₄, and TSH were determined using ELISA technique (Stat fax 3200, USA) and kits (Autobio Co., Ltd, China) according to the manufacturer's instructions.

2.4. Statistical analysis

For the statistical analysis, data were analyzed by SPSS 22.0. ANOVA. At first, the normal distribution was checked to verify the validity of the parametric ANOVA test for the present sample. Also, Tukey tests were used to compare the difference between the sham, control and treatment groups. The statistical significance for all tests was set at P-value<0.05. The results are presented as means ± standard deviation (SD).

3. Results and Discussion

In this study, we found that the serum levels of T₄ decreased significantly in group of receiving 5

mg (MoO₃ NPs)/kg BW in comparison with the control group with P-value of 0.012. However, there was insignificant difference observed in T₃ levels when compared with the sham and control groups with P-values of 0.542 and 0.584 respectively (p>0.05). The serum levels of TSH increased significantly in group of 5 mg/kg BW (MoO₃ NPs) when compared with the both groups of sham and control (p=0.00). The hormone assay results are presented in Figure 1 and Table 1.

Thyroid gland activity is controlled by a negative feedback mechanism. Thyrotropin releasing hormone (TRH) is released into hypothalamic-hypophyseal portal blood by hypothalamic neurons and binds to its receptors in the adenohypophysis. TRH stimulates secretion of TSH, which in turn triggers the thyroid gland to produce and release thyroid hormones. Deficiency of iodine molecules in thyroid gland leads to promote the conversion of T₄ into T₃. Furthermore, T₃ synthesis and degradation occurs through T₄ deiodination [14,15,16].

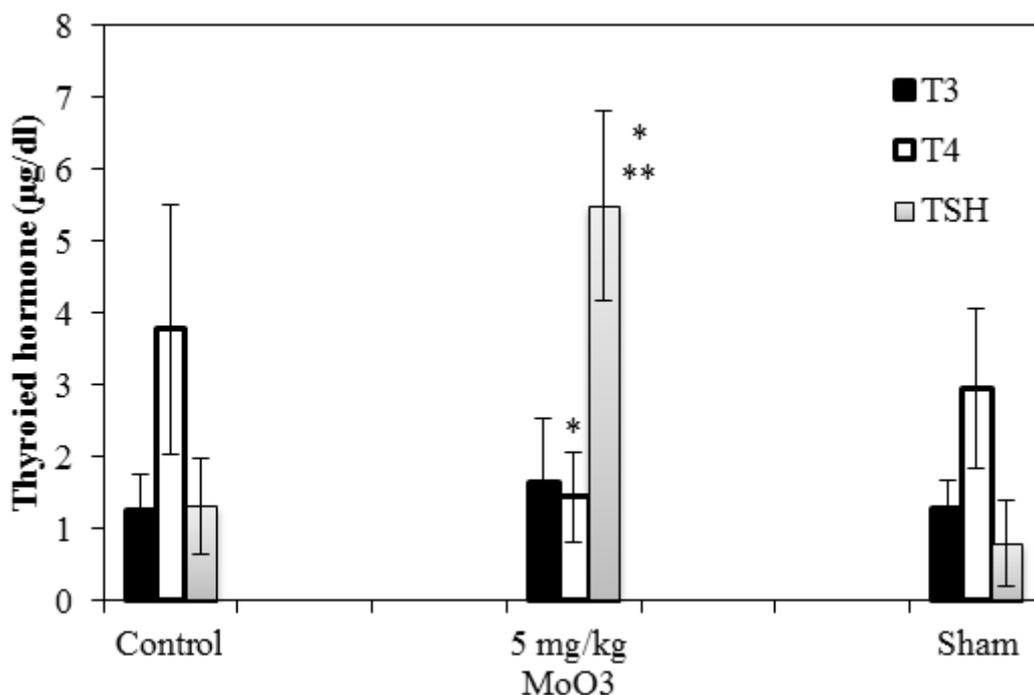


Fig. 1: Effect of MoO₃ NPs on T₃, T₄, and TSH Hormones (mean values ± SD, n=7).

* Significant difference between treatment group and control group (p<0.05)

** Significant difference between treatment group and sham group (p<0.05)

Table 1: Effect of MoO₃ NPs on T₃, T₄, and TSH hormones (mean values \pm SD, n=7).

	T ₃	T ₄	TSH
Control	1.25 \pm 0.506	3.77 \pm 1.734	1.32 \pm 0.665
5 mg/kg MoO ₃ NPs	1.66 \pm 0.875	1.44 \pm 0.629 *	5.50 \pm 1.326 *, **
Sham	1.28 \pm 0.380	2.96 \pm 1.113	0.81 \pm 0.598

* Significant difference between treatment group and control group (p<0.05).

** Significant difference between treatment group and sham group (p<0.05).

To date, in both in vitro and in vivo models, concerning the impact of NPs on thyroid function are conflicting and very limited. Hinthert et al. [17] showed that exposure to 5–10 nM of Ag NPs and to 0.1 nM of QDs alone induced a reduction in levels of transcripts encoding the TH-induced receptor β (TR β) and TH-repressed Rana larval keratin type I (RLKI), whereas treatment with ZnO NPs had no effect on TR β and RLKI transcript levels. In contrast, the administration of Cr NPs to heat-stressed Sprague-Dawley rats did not bring about significant modifications in TSH, T₃ and T₄ serum levels, indicating that these NPs

do not affect the metabolism of thyroid hormone [18]. Additionally, effect of MoO₃ NPs on body weight of animals before and end of treatment measured and the results showed that body weight of animals which received normal saline alone (sham group), increased significantly (p=0.045) in the end of treatment when compared with initial body weight in before treatment (Fig. 2 and Table 2). However, body weight reduced in those rats received MoO₃ NPs compared with both groups of sham and control. The MoO₃ NPs was effective in inhibiting weight gain at short term treatment.

Table 2: Effect of MoO₃ NPs on weight of rat before and after treatment (mean values \pm SD, n=7).

	Weight Before Treatment	Weight After Treatment
Control	218.75 \pm 9.465	231.25 \pm 10.308
5 mg/kg MoO ₃ NPs	212.14 \pm 21.381	206.43 \pm 25.119
Sham	203.57 \pm 12.817	224.29 \pm 20.902 *

* Significant (p<0.05)

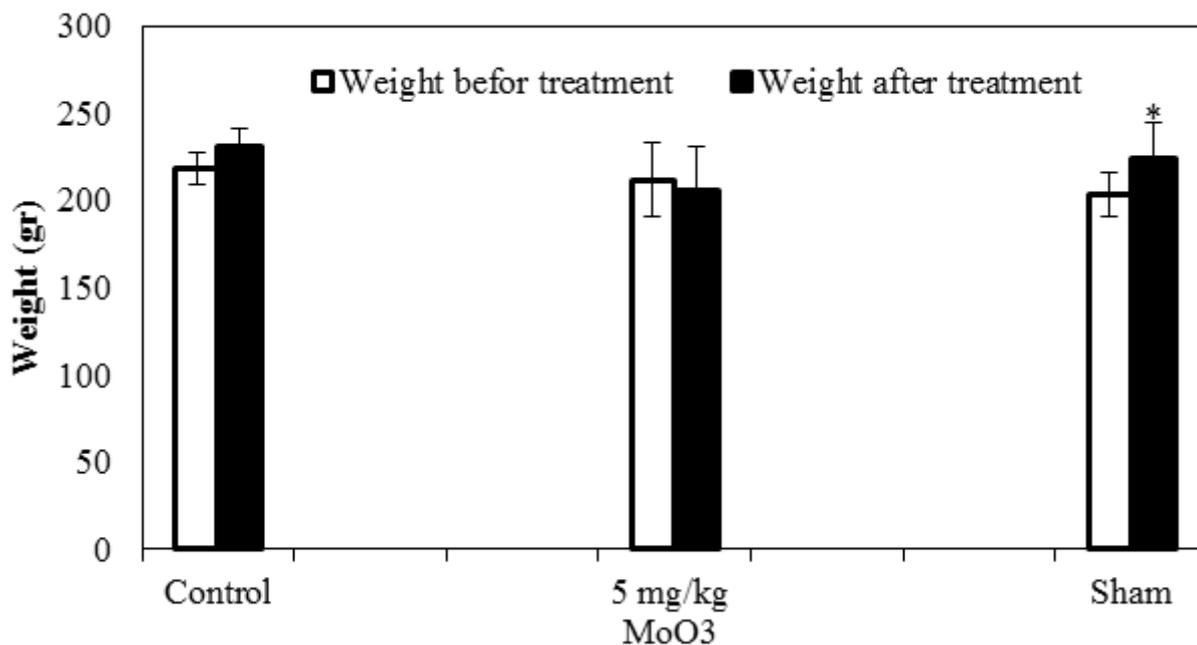


Fig. 2: Effect of MoO₃ NPs on Weight of Rat before and after Treatment (mean values \pm SD, n=7, * p<0.05).

Several studies are available in literature which investigated effect of in vitro and in vivo exposure to molybdenum nanoparticles. A recent study has illustrated Mo NPs to be non-toxic in HT-1080 and MCF-7 cell lines. Furthermore, it has been reported that Mo NPs significantly protect these cells from death induced by H₂O₂ and ZnO NPs [12]. By contrast, another study showed that Mo NPs induced oxidative stress, cytotoxicity, and genotoxicity in mouse skin fibroblast cells (L929) [11]. Other researchers have also reported about low toxicity of Mo- based nanoparticles. Braydich-Stolle et al. (2005) reported that molybdenum trioxide (MoO₃) induced low toxicity in rare cell mouse spermatogonia-A type (C18-4) [19] and according to Wu et al. study (2011), molybdenum disulfide (MoS₂) NPs to be nontoxic up to 3 mg/mL in human lung adenocarcinoma (A549) and human leukemic (K562) cells when exposed for 48 hr [20]. By contrast, more recently, Thao et al. (2014) reported that exposure to MoO₃ NPs induces apoptosis and generates reactive oxygen species (ROS) in breast cancer iMCF-7 cells [21]. In recent study, MoO₃ NPs exhibited a significant cytotoxic influence on lung and breast cancer cell lines and also showed high antibacterial activity

against Gram negative and positive bacteria [22].

In our previous study, our in vivo results, suggest that Mo NPs are toxic effect on serum levels of testosterone, AST, and LDH. However, there seemed to be no significant impact on the serum LH levels and hematological parameters [13]. Nano structures can move from body's entry portals into the circulatory and lymphatic systems, and finally into tissues and organs because of their very small sizes. Moreover, irreversible damages to cells occurs by various NPs through oxidative stress and/or organelle injury due to several factors, including size, shape, composition, and surface chemistry [1,23,24]. The possibility of chemicals and various NPs entering biological systems is of main concern to the public with regard to potential toxicity to biological system and development [25].

4. Conclusion

In conclusion, experimental findings demonstrated that intraperitoneal injection of MoO₃ NPs can have adverse effects on thyroid hormones, and it is important to control MoO₃

NPs contamination in environment.

Conflict of interest

The authors declare that they have no conflict of interest.

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