Effect of Molybdenum Trioxide Nanoparticles on Ovary Function in Female Rats

Fardin Asadi 1, Mansour Sadeghzadeh 1, Ahmad Jalilvand 2, Keivan Nedaei 3, Yasamin Asadi 4, Azam Heidari 5

1. Dept. of Pediatrics, Mousavi Hospital, Zanjan University of Medical Sciences, Zanjan, Iran
2. Dept. of Pathology, Mousavi Hospital, Zanjan University of Medical Sciences, Zanjan, Iran
3. Dept. of Medical Biotechnology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
4. National Nutrition of Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Dept. of Biochemistry, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

ABSTRACT

Background & Objective: Molybdenum trioxide nanoparticles (MoO₃ NPs) have widespread applications in industries. However, the toxic and non-toxic effects of MoO₃ NPs have not been fully investigated yet. In this study, effects of MoO₃ NPs on ovary function of female rats were studied.

Materials & Methods: In this study, 21 female rats were randomly divided into three groups (n=7): the first group did not receive any treatment, the second one received normal saline, and the third group received 5 mg/kg MoO₃ NPs in normal saline via intraperitoneal injection during a period of 28 days. Serum concentrations of estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone were measured. Also, changes of ovaries, uterine weights, uterine, and length of uterine horns were studied.

Results: The serum level of estrogen in the MoO₃ NPs exposed group was significantly attenuated; those of luteinizing hormones and follicle stimulating hormone were elevated while progesterone level change was insignificant. The weights of the right ovary and the uterine body decreased significantly in the exposed group.

Conclusion: Our data showed that MoO₃ nanoparticle exposure could cause an imbalance of sex hormones and decrease in body and ovarian weights in the female rats.

Keywords: Nanoparticles, Female rats, Reproduction

Introduction

With the widespread application of molybdenum trioxide nanoparticles (MoO₃ NPs) as photo catalysis, oxidative catalyst, gas sensors, photochromic coatings, lubricants, secondary batteries, and additives in paints (1), few studies are available on the toxic and non-toxic effects of MoO₃ NPs (2-6). Mo is considered as an essential trace element because it is part of molybdenum cofactor that is needed for the mammalian enzymes such as aldehyde oxidase, xanthine oxidase, and sulfite oxidase. Also, Mo exists in +4, +5 and +6 oxidation states which mean that when it enters the cell, it can readily contribute in red-ox reactions (7, 8). With respect to these facts, industrial wastes which contain an additional amount of MoO₃ NPs could cause unfavourable impacts in both humans and animals health (9, 5). It is believed that the toxicity of NPs is influenced by chemical nature, morphology, particle size, and surface chemistry of NPs in the environment. Therefore, it is vital to know the necessary information and clarifications in order to provide true risk assessments of NPs (10). According to literatures, exposure to ultrafine particles (<100 nm) could induce tissue inflammation and pulmonary damages, lung tumors and fibrosis (10, 11).

The mechanisms underlying NPs toxicity in biological systems are not clear. However, production of reactive oxygen species (ROS) is often involved in the toxicity of a wide range of NPs. The ROSs accumulation prompts a wide variety of physiological and cellular events including inflammation, cellular stress, DNA damage, and apoptosis (11, 12).
Although there is little information available about adverse effects of exposure to MoO₃ NPs, several in vitro and in vivo studies have reported these effects. According to earlier studies, Mo NPs could induce cytotoxicity, oxidative stress, and genotoxicity in mouse skin fibroblast cells and also show cytoprotective effects in HT-1080 and MCF-7 cells (3,5,13). Also, in our previous research we found that the Mo NPs induced alterations in the serum levels of sex hormones, and histopathology of testis and liver showed a decrease in number of Leydig cells and an increase in chronic inflammatory cells, respectively (14). In addition, other findings of our previous study demonstrated that Mo NPs exposure in female rats could induce the risk of thyroid dysfunction (15).

The aim of this study was to investigate the effects of MoO₃ NPs on female reproductive system. Recently, several in vitro and in vivo studies have investigated the adverse effects of environmental chemicals and NPs exposure on the female reproductive function (14-24). The findings of some existing literature have illustrated that many NPs disrupt the function of reproductive system by inducing cytotoxic effects on ovarian cells, damaging oogenesis and follicle maturation, and altering sex hormone levels (16). However, we found a number of conflicting results in the published studies because of some factors including type, size, doses of exposure, length of treatment, and route of administration of NPs (17-24).

There is increasing interest in ascertaining the effect of environmental exposures on dysfunction of reproductive system because of its significant role in female reproduction for the perpetuation. In this study, we investigated the effect of MoO₃ NPs exposure on ovarian dysfunction and serum levels of estrogen, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) in female rats. Also, the changes of ovaries and uterine weights, and uterine horn length were studied.

**Materials and Methods**

**Molybdenum Oxide Nanoparticles**

Nanoparticles of MoO₃ (99.94% purity, 13-80 nm) were purchased from NANOSANY Corporation™ (Mashhad, Iran). The size and structure of the MoO₃ NPs were analysed by transmission electron microscopy (TEM) (LEO 912AB).

**Animals and Treatment**

Adult female Wistar rats, aged 7-8 weeks, with average initial body weight (200-250 g) were purchased from the animal houses of the School of Pharmacy at Zanjan University of Medical Sciences (ZUMS), Zanjan, Iran. All rats were housed at room temperature with relative humidity of 60% with 12:12-hour light: dark cycles. Food and water were provided ad libitum. All experiments of animals were performed with compliance of the ZUMS Ethics Committee (ZUMS.REC.1393.37).

For experimental study, 21 female rats were randomly divided into three groups (n=7 per group): the first group did not receive any treatment (control group), the second one received normal saline (sham group), and the third group received 5 mg/kg BW MoO₃ NPs in normal saline (MoO₃NPs group) via intraperitoneal injection every other day for 28 days (total intakes: 14). In addition, body weights of rats were recorded at the beginning and end of the experimental period.

At day 29 (one day after the end of administration), the rats were sacrificed, and blood samples were obtained from the left heart atrium. The blood samples were centrifuged at 3,000 rpm for 10 minutes and serum obtained from each sample was collected and frozen at -20°C until analysis. Also, the ovaries of all animals were removed for more examination.

**Hormone Assays**

In order to perform hormone assays, serum levels of estrogen, progesterone, LH, and FSH were determined using ELISA technique (Stat fax 3200, USA) and commercial kits (Monobind Inc., USA) according to the manufacturer’s instructions.

**Ovaries and Uterine Indices**

The ovaries and uterus of all animals were weighed using an electronic weighing balance and the length of uterus and right and left uterus horns were recorded.

**Statistical Analysis**

Data were statistically analysed by SPSS 22.0. (SPSS Inc., Chicago, Ill., USA) ANOVA and Tukey tests were used to compare the difference between the treated groups and the control. The statistical significance for all tests was considered to P-value <0.05. The results are presented as means ± standard deviation (SD).

**Results**

**Molybdenum Oxide Nanoparticles Analysis**

The transmission electron microscopy (TEM) image of MoO₃ NPs is shown in Figure 1. The MoO₃ NPs have orthorhombic crystal structure and average diameters range of 13-80 nm.

![Figure 1. Transmission Electron Microscopy (TEM) image of MoO₃ NPs](image-url)
Sex Hormone Levels

The results showed that the serum levels of estrogen decreased significantly in group of MoO3 NPs in comparison with the control group with P-value of 0.022. However, insignificant difference was observed in progesterone levels when compared with the control groups (P>0.05). The serum levels of FSH and LH increased significantly in group of MoO3 NPs in comparison with the control group (P=0.00). The hormone assay results are presented in Table 1.

Table 1. Sex hormone levels of serum after MoO3 NPs exposure (mean values ± SD, n=7, * P<0.05)

| Parameters   | Control  | Normal saline | MoO3 NPs |
|--------------|----------|---------------|----------|
| Progesterone (µg/L) | 41 ± 19  | 47 ± 17       | 50 ± 19  |
| Estrogen (pmol/L)    | 210 ± 130| 125 ± 73      | 63 ± 21* |
| LH (IU/L)          | 1.0 ± 0.6| 1.4 ± 0.5     | 15.5 ± 5.7* |
| FSH (IU/L)         | 0.58 ± 0.39| 10 ± 8       | 43 ± 8*   |

* Significant difference between MoO3 NPs group and control group (P<0.05)

Body Weight Changes

According to the results presented in Figure 2, the body weight of rats increased significantly at the end of treatment in sham group in comparison with initial body weight before treatment (P=0.045).

Weight of Ovary and Uterine Changes

As shown in Figure 3, the weight of right ovary and uterine body decreased significantly in the MoO3 NPs group in comparison with the control group (P=0.001, 0.035 respectively). Also, there was insignificant change in left ovary weight when compared to the control group.

Length of Ovary and Uterine Changes

Moreover, insignificant change was observed in the lengths of uterine body and uterine horns in comparison with the control group as shown in Figure 4.

Figure 2. Weight changes of rats before and after MoO3 NPs exposure (mean values ± SD, n=7, * P<0.05).

Figure 3. Organ weight changes after MoO3 NPs exposure (mean values ± SD, n=7, * P<0.05).
Discussion

In this study, the effects of exposure to MoO₃ NPs on reproductive system in female rats were investigated and results demonstrated that intraperitoneal injection of MoO₃ NPs induced dysfunction in reproductive system and disrupted the balances of hormonal levels in female rats. The female reproductive system plays important role in fetal development and preservation of the species. However, the reproductive system of female is significantly more fragile than other systems. This is because of: 1) limitation of female gametes production in comparison with the reproductive male gametes; 2) periodic growth and regression of reproductive organs and their sensitivity to foreign bodies and stress influenced and regulated by hormones; and 3) the disturbance of female reproduction inevitably leading to abnormal fetal development (25).

Our findings of previous study showed that exposure to MoO₃ NPs led to a significant decrease in estrogen concentration and also increased the FSH, and LH concentrations (Table 1). Clearly, the estrogen hormone plays a main role in facilitating the differentiation of granulosa cells, as well as the induction of receptor systems for FSH and LH levels. However, it was obvious that increase in FSH leads to the promotion of estrogen levels, but a significant decrease of estrogen was shown in the MoO₃ NPs treated group in this study. Additionally, the results of our previous study showed that serum levels of FSH and LH in MoO₃ NPs treated group increased significantly and also estrogen level decreased in a dose-dependent manner (26). In general, MoO₃ NPs exhibited the greatest influence on the hypothalamus and increased GnRH secretion, which was responsible for increasing the FSH level and promoting estrogen production. However, this conflicting result found in our study regarding estrogen level may be due to: 1) the direct impact of nanoparticles on the ovaries, which further suppressed follicular growth and reduced estrogen; or 2) interference with the normal functioning of follicles. Therefore, the number of estrogen secreting follicles decreased, resulting in reduced estrogen secretion (15,26). In addition, insignificant increase in the progesterone level was observed in MoO₃ NPs group when compared with the control group. It is reported that progesterone is the main component in ovulation, implantation, and maintenance of pregnancy in female reproduction (27). Unfortunately, the stage of the estrous cycle in the animals was not determined prior to treatment in this study, which should be considered in future studies.

Also, the body weight of animals of sham group which received normal saline increased significantly in the end of treatment compared with their initial body weight before treatment (P=0.045). However, the body weight reduced in the rats which received MoO₃ NPs compared with both groups of sham and control. Moreover, exposure to MoO₃ NPs leads to body weight loss of about 3% lower than the weight of animals in the beginning of the experimental period. The MoO₃ NPs was effective in inhibiting weight gain at short term treatment (15). It is clear that body weight is a sensitive indicator which is affected by toxic chemicals. Also, gastrointestinal disorders, loss of appetite, or delayed absorption of foods are some of the most important factors that lead to reduction in body weight (28,29). In addition, we found a significant decrease in right ovary weight and uterine weight in animals which received MoO₃ in compared to the control group. This might be related to the development of the uterus by affecting the release of sex hormones, such as GnRH, LH, and FSH by NPs. Meanwhile, it should also be noted that dynamic activity and rigorous control of hormones increase the sensitivity of this system to external bodies and physiological stress compared with other physiological processes (25,30,31).

There are few studies about MoO₃ NPs effects on female reproductive system. But some similar results were found in the published literature on the effect of different NPs. For example, exposure to TiO₂ NPs...
showed a toxic effect on Chinese hamster ovary-K1 (CHO-K1) cell (17). Also, in another study, a decrease in serum levels of progesterone, LH, FSH, and testosterone along with an increase in estrogen concentration were shown in female mice treated with TiO2 NPs (18). By contrast, in a different study, it was reported that exposure to nickel nanoparticles (Ni NPs) induces an increase in FSH and LH, and decrease in estradiol serum levels and also injured ovaries and uterus tissues in female rats (19). Moreover, the viability of Chinese hamster ovary cells (CHO) decreased when exposed to graphene oxide NPs (20). Additionally, treatment of Chinese hamster ovary (CHO-K1) cells by aluminium oxide NPs (Al2O3 NPs) showed that penetration of Al2O3 NPs to cytoplasm of CHO-K1 cells induced concentration-dependent cytotoxic damage (21). In this context, Liu et al. demonstrated that zinc oxide NPs (ZnO NPs) did not change body weight and levels of the estrogen and progesterone hormones. But a reduction in the ovary organ index was observed in the domestic hens by ZnO NPs (22). Furthermore, a significant increase in uterine weight and no significant change in ovaries were observed in the female rats treated with ZnO NPs (23). Conversely, administration of ZnO NPs to female rats did not affect serum levels of FSH, LH, and estradiol (24). Furthermore, the exposure of silicon carbide nanowires to CHO cell viability caused a reduction of reproduction rate (32). Similarly, examination of Cadmium Tellurium/Zinc Tellurium Quantum Dots (CdTe/ZnTe QD) on follicle development and oocyte maturation in female mice was reported to be associated with a decrease in the rate of antrum cavity formation (33). Our findings are consistent with the results of previous studies. In brief, we observed the MoO3 nanoparticle exposure could cause an imbalance of sex hormones and decrease in body and ovarian weights in the female rats.

Conclusion

Taking what was mentioned into account, we might conclude that exposure to MoO3NPs can have adverse effects on sexual hormones, uterine, and ovaries which lead to negative impact on reproductive health. These results will provide a basis for future studies of MoO3NPs exposure for human health.

Acknowledgements

The authors are grateful to the Zanjan University of Medical Sciences, Zanjan, Iran for all financial and other supports.

Conflict of Interest

Authors declared no conflict of interests.

References

1. Cheng L, Shao M, Wang X, Hu H. Single-crystalline molybdenum trioxide nanoribbons: photocatalytic, photoconductive, and electrochemical properties. Chem Eur J. 2009; 15(10): 2310-16. [DOI:10.1002/chem.200802182] [PMID]

2. Krishnamoorthy K, Veerapandian M, Yun K, Kim SJ. New function of molybdenum trioxide nanoplates: Toxicity towards pathogenic bacteria through membrane stress. Colloids Surf B Biointerfaces, 2013; 112: 521-4. [DOI:10.1016/j.colsurfb.2013.08.026] [PMID]

3. Akhtar MJ, Ahamed M, Alhaadq Alh, Alshamsan A, Khan MA, Alrookayen SA. Antioxidative and cytoprotective response elicited by molybdenum nanoparticles in human cells. J Colloid Interface Sci. 2015; 457: 370-7. [DOI:10.1016/j.jcis.2015.07.034] [PMID]

4. Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol. 2010; 30(3): 343-2. [DOI:10.1016/j.reprotox.2010.06.002] [PMID]

5. Siddiqui MA, Saqib Q, Akhmed M, et al. Molybdenum nanoparticles-induced cytotoxicity, oxidative stress, G2/M arrest, and DNA damage in mouse skin fibroblast cells (L929). Colloids Surf B Biointerfaces. 2015; 125: 73-1. [DOI:10.1016/j.colsurfb.2014.11.014] [PMID]

6. Fakhri A, Nejad PA. Antimicrobial, antioxidant and cytotoxic effect of Molybdenum trioxide nanoparticles and application of this for degradation of ketamine under different light illumination. J Photochem Photobiol B. 2016; 159: 211-7. [DOI:10.1016/j.jphotobiol.2016.04.002] [PMID]

7. Schwarz G, Mendel RR, Ribbe MW. Molybdenum cofactors, enzymes and pathways. Nature. 2009; 460(7257): 839-7. [DOI:10.1038/nature08302] [PMID]

8. Ikuchi K, Hamano S, Mochizuki H, Ichida K, Ida H. Molybdenum cofactor deficiency mimics cerebral palsy: differentiating factors for diagnosis. Pediatr Neurol. 2012; 47(2): 147-9. [DOI:10.1016/j.pediatrneurol.2012.04.013] [PMID]

9. Shrivas K, Agrawal K, Harmukh N. Trace level determination of molybdenum in environmental and biological samples using surfactant-mediated liquid-liquid extraction. J Hazard Mater. 2009; 161(1): 325-9. [DOI:10.1016/j.jhazmat.2008.03.092] [PMID]

10. Thakur M, Gupta H, Singh D, et al. Histopathological and ultra structural effects of nanoparticles on rat testis following 90 days (chronic study) of repeated oral administration. J Nanobiotechnology. 2014; 12: 42-4. [DOI:10.1186/s12951-014-0042-8] [PMID] [PMCID]

11. Oberdörster G, Maynard A, Donaldson K, et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol. 2005; 2: 8. 1-35. [DOI:10.1186/1743-8977-2-8] [PMID] [PMCID]

12. Capasso L, Camatini M, Guaitieri M. Nickel oxide nanoparticles induce inflammation and genotoxic effect in lung epithelial cells. Toxicol Lett. 2014; 226(1): 28-4. [DOI:10.1016/j.toxlet.2014.01.040] [PMID]

13. Braydich-Stolle L, Hussain S, Schlagler JJ, Hofmann MC. In vitro cytotoxicity of nanoparticles in mammalian germine stem cells. Toxicol Sci. 2005; 88(2): 412-9. [DOI:10.1093/toxsci/kfi256] [PMID] [PMCID]

14. Asadi F, Mohtaseb M, Dadashi N K, Haj-Soleymani F, Jalilvand A, Heidari A. Effect of Molybdenum Nanoparticles on Blood Cells, Liver Enzymes, and Sexual Hormones in Male

Volume 27, March & April 2019

Journal of Advances in Medical and Biomedical Research
Rats. Biol Trace Elem Res. 2017; 175(1): 50-56. [DOI:10.1007/s12118-016-0765-5] [PMID]

15. Asadi F, Amirmoghaddami H, Shamsheddin M, Nedaei K, Heidari A. Effect of Molybdenum Trioxide Nanoparticles on Thyroid Hormones in Female Rats. J Hum Environ Health Promot. 2016; 4(4): 189-95. [DOI:10.29252/hjehp.1.4.189]

16. Ivacicoli I, Fontana L, Leso V, Bergamaschi A. The Effects of Nanomaterials as Endocrine Disruptors. Int J Mol Sci. 2013; 14(8): 16732-1. [DOI:10.3390/ijms140816732] [PMID] [PMCID]

17. Lu PJ, Ho IC, Lee TC. Induction of sister chromatid exchanges and micronuclei by titanium dioxide in Chinese hamster ovary-K1 cells. Mutat Res. 1998; 414(1-3): 15-20. [DOI:10.1016/S1383-5718(98)00034-5]

18. Zhao X, Ze Y, Gao G, et al. Nanosized TiO2-Induced Reproductive System Dysfunction and Its Mechanism in Female Mice. Plos one. 2013; 8(4): 59378. [DOI:10.1371/journal.pone.0059378] [PMID] [PMCID]

19. Kong L, Tang M, Zhang T, et al. Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. Int J Mol Sci. 2014; 15(11): 21253-9. [DOI:10.3390/ijms151121253] [PMID] [PMCID]

20. Batraskaita D, Grinceviciute N, Snitka V. Impact of graphene oxide on viability of Chinese hamster ovary and mouse hepatoma MH-22A cells. Toxicol in Vitro. 2015; 29(5): 1195-200. [DOI:10.1016/j.tiv.2015.05.004] [PMID]

21. Di-Virgilio AL, Reigosa M, Amal PM, Lorenzo de Melea MF. Comparative study of the cytotoxic and genotoxic effects of titanium oxide andlanium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells. J Hazard Mater. 2010; 177(1-3): 711-8. [DOI:10.1016/j.jhazmat.2009.12.089] [PMID]

22. Liu XQ, Zhang HF, Zhang WD, et al. Regulation of neuroendocrine cells and neuron factors in the ovary by zinc oxide nanoparticles. Toxicol Lett. 2016; 256: 19-22. [DOI:10.1016/j.toxlet.2016.05.007] [PMID]

23. Jo E, Seo G, Kwon JT, et al. Exposure to zinc oxide nanoparticles affects reproductive development and biodistribution in offspring rats. J Toxicol Sci. 2013; 38(4): 525-30. [DOI:10.2131/jts.38.525] [PMID]

24. Esmaeillou S, Moharamnejad M, Hsankhani R, Tehrani AA, Asadi F. Hostile of ZnO nanoparticles in healthy adult mice. Environ Toxicol Pharmacol. 2013; 35(1): 67-71. [DOI:10.1016/j.etap.2012.11.003] [PMID]

25. Sun J, Zhang Q, Wang Z, Yan B. Effects of Nanotoxicity on Female Reproductivity and Fetal Development in Animal Models. Int J Mol Sci. 2013; 14(5): 9319-37. [DOI:10.3390/ijms13059319] [PMID] [PMCID]

26. Asadi F, Fazelipour S, Hooshmand Abbasi R, et al. Assessment of Ovarian Follicles and Serum Reproductive Hormones in Molybdenum Trioxide Nanoparticles Treated Rats. Int J Morphol. 2017; 35(4): 1473-81. [DOI:10.4067/S0717-95022017000401473]

27. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocr Rev. 1997; 18(4): 502-19. https://doi.org/10.1210/edrv.18.4.0308 [DOI:10.1210/er.18.4.502] [PMID]

28. Kim HY, Lee SB, Lim KT, Kim MK, Kim JC. Subchronic inhalation toxicity study of 1,3-dichloro-2-propanol in rats. Ann Occup Hyg. 2007; 51(7): 633-43.

29. Chatterjee-Chakrabarty S, Miller BT, Collins TJ, Nagarmami M. Adverse effects of methylphenidate on the reproductive axis of adolescent female rats. Fertil Steril. 2005; 2: 1131-8. [DOI:10.1016/j.fertnstert.2005.03.071] [PMID]

30. Weihua Z, Saji S, Makinen S, et al. Estrogen receptor (ER) beta, a modulator of ERalpha in the uterus. PNAS. 2000; 97(11): 5936-41. [DOI:10.1073/pnas.97.11.5936] [PMID] [PMCID]

31. Alwasel S. Effect of Maternal Food Restriction on the Uterus of Female Rats from the First and Second Generation. Advances in Reproductive Sciences. 2016; 4: 23-30. [DOI:10.4236/arsci.2016.42004]

32. Jiang J, Wang J, Zhang X, et al. Activation of mitogen-activated protein kinases cellular signal transduction pathway in mammalian cells induced by silicon carbide nanowires. Biomaterials. 2010; 31(31): 7856-62. [DOI:10.1016/j.biomaterials.2010.07.023] [PMID]

33. Xu G, Lin S, Law WC, et al. The invasion and reproductive toxicity of QDs-transferrin bioconjugates on preantral follicle in vitro. Theranostics. 2012; 2(7): 734-45. [DOI:10.7150/thno.4290] [PMID] [PMCID]