RESEARCH ARTICLE

Synthesis of silver-cobalt nanoparticles by chemical reduction method and its effects on serum levels of thyroid hormones in adult male rats

Zohreh Parang [1]*, Davood Moghadamnia [2]

[1] *Department of Physics, Shiraz Branch, Islamic Azad University, Shiraz, Iran*

[2] *Young Researchers and Elite Club, Shiraz Branch, Islamic Azad University, Shiraz, Iran*

ARTICLE INFO

Article History:
Received 30 May 2018
Accepted 13 July 2018
Published 15 August 2018

Keywords:
Adult Male Rats
Silver-Cobalt Nanoparticles
Thyroid Hormones

ABSTRACT

Objective(s): Silver-cobalt nanoparticles have anti-fungal properties and are used in medicine. In this research, the effect of silver-cobalt nanoparticles on serum levels of T3 and T4 hormones was investigated. Silver-cobalt nanoparticles were synthesized by chemical reduction.

Methods: In this experimental study, 28 male adult Wistar rats (approximately 180-220 gr) were used. Animals were divided into 4 groups of 7. The control group were not affected by any treatment. Experimental groups 1 and 2 received 25 and 100 mg / kg of silver-cobalt nanoparticles, in which silver nanoparticles were synthesized during 75 seconds intraperitoneally for 14 days, respectively. The experimental group 3 received 25 mg / kg of silver-cobalt nanoparticles, in which silver nanoparticles were synthesized during 300 seconds intraperitoneally for 14 days. At the end of the trial, blood sampling was performed to measure hormones. The mean serum levels of T3 and T4 hormones were analyzed by appropriate statistical tests including ANOVA and Duncan test.

Results: Serum levels of T4 in experimental groups 2 and 3 showed a significant decrease compared to control group. Serum T3 level did not change significantly in all experimental groups compared to control group ($p < 0.05$).

Conclusions: Silver-cobalt nanoparticles reduced the serum T4 level.

How to cite this article
Parang Z, Moghadamnia D. Synthesis of silver-cobalt nanoparticles by chemical reduction method and its effects on serum levels of thyroid hormones in adult male rats. Nanomed Res J, 2018; 3(4): 236-244. DOI: 10.22034/nmrj.2018.04.008

INTRODUCTION

Today, nanotechnology is developing and expanding and is used in many areas including health, nutrition, environmental health and agriculture [1]. Silver nanoparticle is an antibacterial and antifungal agent and is used in medicine. Evidence suggests that germ cells and embryonic fibroblasts in rats are toxic with silver nanoparticles [2]. The mechanism of the side effects of silver nanoparticles on cells is to increase the expression of P53, decrease vital capacity of the cell, changes in glutathione production, changes in gene expression plans, especially inflammatory genes and planned cell death [2].

In a study by Shaheen et al in 2016, gold-silver nanoparticles improve diabetes-related disorders by limiting inflammation and reducing oxidative stress and enhancing the antioxidant defense system in diabetic rats with streptozotocin [3]. Kwan et al., in 2014, also showed that the silver nanoparticles change the proteoglycan expression in promoting the repair of the tendon [4]. In addition, in a study by Park et al. In 2010, it has been found that repeated feeding induction of silver nanoparticles may lead to organ toxicity and inflammatory responses in mice [5].

In a study by Crisan et al in 2018, the silver and gold nanoparticle complex with blueberries reduce inflammation in psoriatic plaques (a type

* Corresponding Author Email: *zohreh.parang@gmail.com*

of autoimmune skin disease) by inhibiting NF-KB activity [6]. Also, in a study by Liu et al in 2017, silver nanoparticles coated sutures had prolonged anti-inflammatory effects in intestinal anastomosis in mice [7]. In addition, in the study of Sivakumar et al in 2017, silver nanoparticles have anti-cancer therapeutic effects and provide healing effects of ulcers [8]. In a study by Van de Brule et al in 2016, silver nanoparticles could disturb intestinal bacterial activity in mice [9].

Colognato et al., in 2008, the effects of cobalt nanoparticles on peripheral leukocytes were investigated. The study found that cobalt nanoparticle causes cell poisoning [10]. Various doses of cobalt nanoparticles increase the fragmentation of DNA strand, leading to a large production of superoxide and hydroxyl radicals, and increases the percentage of cell toxicity and leads to an impairment in the repair of DNA-linked proteins [10].

In a study by Park and colleagues in 2015, cobalt ferrite nanoparticles were found to have photodynamic anticancer activity in different cancer cells [11]. Also, Vinardell et al., in 2015, showed that the cobalt oxide nanoparticle has anti-tumor activity and its mechanism of action is to produce reactive oxygen species or cell death and necrosis [12].

In a study by Yan et al. In 2018, cobalt and cobalt nanoparticles showed an increase in inflammatory cytokines, including tumor necrosis factor alpha (TNF-a), interleukin 1B (IL-1B), and interleukin-6 (IL-6), and induced ROS production, thereby causing cellular poisoning and inflammation [13]. In addition, in the study of Magaya et al. (2012) showed that nanoparticles based on copper, nickel and cobalt cause genetic toxicity and genetic cancer. Epigenetic factors, such as planned cell death and stimulating oxidative stress and pro-inflammatory effects, appear to play a decisive role in the effects of these nanoparticles [14]. A study by Cho and colleagues in 2012 found that cobalt oxide and nickel oxide nanoparticles can trigger chronic progressive pulmonary immune responses [15].

The thyroid gland secretes T4 and T3 hormones that regulate the metabolism of the body. So the function of the thyroid gland is very important. Key factors have regulatory effects on the hypothalamic-pituitary hormonal axis to control the production and secretion of thyroid gland hormones. Thyrotropin releasing hormone (TRH) is released from the paraventricular nucleus of the hypothalamus, which affects the anterior pituitary gland and results in TSH secretion [16 and 17]. T4 and T3 hormones are released due to the release of TSH [18].

Considering the increasing use of nanoparticles in the treatment of disorders such as cancer and their side effects, and also given the crucial role that thyroid hormones play in regulating various body functions, it is necessary to study the effects of silver-cobalt nanoparticles on thyroid hormones levels. The results can be used by therapeutic centers and endocrinology, and predictions need to be made on optimal use of this nanoparticle.

MATERIALS AND METHODS

Synthesis of silver-cobalt nanoparticles

The silver nanoparticles synthesized by the electrochemical method were used for preparation silver-cobalt core-shell nanoparticles. The chemical reduction method was used to produce silver-cobalt nanoparticles. For preparation of silver-cobalt core-shell nanoparticles, solution which containing 0.001 M of $COSO_4$ (Cobalt sulfate) and 0.01 M of CTAB (Cetrimonium bromide or Cetyl trimethylammonium bromide) was added to an equal volume of a solution which containing silver nanoparticles, stirred vigorously by a magnetic stirrer for 10 minutes. Then, 0.1 mL of another solution, including 0.8 M of $NaBH_4$ (Sodium borohydride) and 0.01 M of CTAB (Cetrimonium bromide or Cetyl trimethylammonium bromide), was added dropwise to the stirring solution. CTAB (Cetrimonium bromide or Cetyl trimethylammonium bromide) was added as the stabilizer. Then the color of the solution changed to dark brown, which indicating the formation of bimetal silver-cobalt nanoparticles. This experiment was performed at room temperature [19].

In fact, if we want to state the process of generating silver-cobalt nanoparticles in general, this process consists of three stages as follows:

A. Production of silver nanoparticles by electrochemical method

B: Add a solution containing cobalt salt

C: Add a reducing agent

In Fig. 1, the change in the color of the solution was seen after the addition of the reducing agent to the solution.

In this study, the synthesized silver-cobalt nanoparticles were centrifuged for 15 minutes with 14000 rounds. Then, in order to remove additional chemicals in the final product, the nanotube was washed three times washed three time with distilled water.

Animals

In this experimental study, 28 adult male Wistar rats weighing 180-220 gr were taken from the Animal Breeding Center of Shiraz Azad University. Animals were kept in separate 7 cages at temperatures of 22-24 ° C, 12 hours of darkness and 12 hours of light. Drinking water was provided to the animals from urban tap water and special food for the rat. In order to adapt the animals to the experimental environment after a few days, the animals were deployed.

Animal treatment

The animals were divided into 4 groups of 7, which included: control group: that were not affected by any treatment. Experimental groups 1 and 2 received 25 mg/kg and 100 mg/kg of silver-cobalt nanoparticles (in which silver nanoparticles were synthesized during 75 seconds) intraperitoneally for 14 days. Experimental group 3 received 100 mg/kg of silver-cobalt nanoparticles (in which silver nanoparticles were synthesized during 300 seconds) intraperitoneally for 14 days. Dose and type of injection and duration of injection were selected using previous studies [20-22].

Fig. 1: Silver-cobalt nanoparticles synthesized by using of silver nanoparticles which prepared during 75 seconds, (a) before adding a reducing agent, (b) after adding a reducing agent

After the end of the trial period, animals were affected by anesthesia with ether. Blood collection from the left ventricle of the heart. The blood samples were kept in laboratory for 20 minutes and centrifuged for 15 minutes at 5000 rpm. The serum was carefully isolated from the pasteurized pipette and poured into a normal test tube. Serums were stored in the refrigerator at -20 °C. The levels of T3 and T4 hormones were measured using radioimmunoassay (RIA) method.

Statistic analysis

The results of this study were analyzed by SPSS software version 22. The results of the experimental and control groups were expressed as mean ± standard error and analyzed by ANOVA and Duncan test. The statistical inference margin was considered as $p < 0.05$.

RESULTS AND DISCUSSION

Since in the method used to synthesize silver-cobalt nanoparticles, one of the parameters that influences the production of nanoparticles is the synthesis time, according to Fig. 2, it was observed that the absorption of silver-cobalt nanoparticles from silver nanoparticles produced in the time interval of 300 seconds is more silver-cobalt nanoparticles produced from synthesized silver nanoparticles in the 75-seconds period.

To investigation the effect of silver-cobalt nanoparticles on the thyroid, the prepared silver-cobalt nanoparticles in which silver nanoparticles

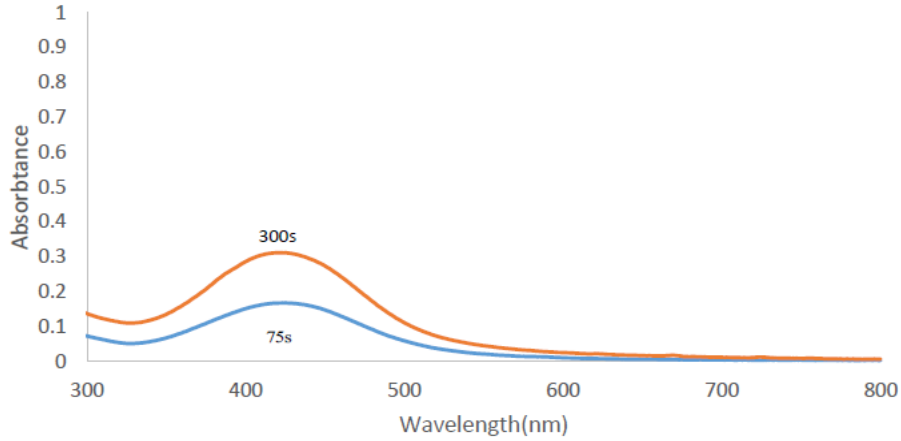

Fig. 2: Absorption spectra of silver-cobalt nanoparticles prepared from silver nanoparticles synthesized at intervals of 75 seconds and 300 seconds (The electrical current adjusted at 1amp and rotational speed was 3000 rpm).

were synthesized during 75 seconds and 300 seconds, were used. Because of the need for silver-cobalt nanoparticles to be injected for 14 days, it is necessary to review the durability of these nanoparticles over time. Therefore, the changes in the solutions color and absorption spectra were studied after a time. It was observed that the color of the solutions containing silver-cobalt nanoparticles in which silver nanoparticles was prepared during 75 seconds, slightly changed and the nanoparticles were deposited (Fig. 3).

As shown in Fig. 4, the absorption spectrum of silver-cobalt nanoparticles prepared from silver nanoparticles synthesized at a time interval of 75 seconds, after a few days, relatively small broadening with low displacement to lower wavelengths observed it turned out. The solution color of these nanoparticles is slightly altered and the solution is deposited, the absorption spectrum of these nanoparticles is about 425 nm.

As shown in Fig. 5, after several days, the color of the solutions of silver-cobalt nanoparticles in which silver nanoparticles was synthesized during 300 seconds, changed slightly and the nanoparticles were deposited.

As shown in Fig. 6, the absorption spectra of silver-cobalt nanoparticles prepared from silver nanoparticles synthesized during 300 seconds decreases over time. The absorption spectrum of these nanoparticles was detected at 415 nm.

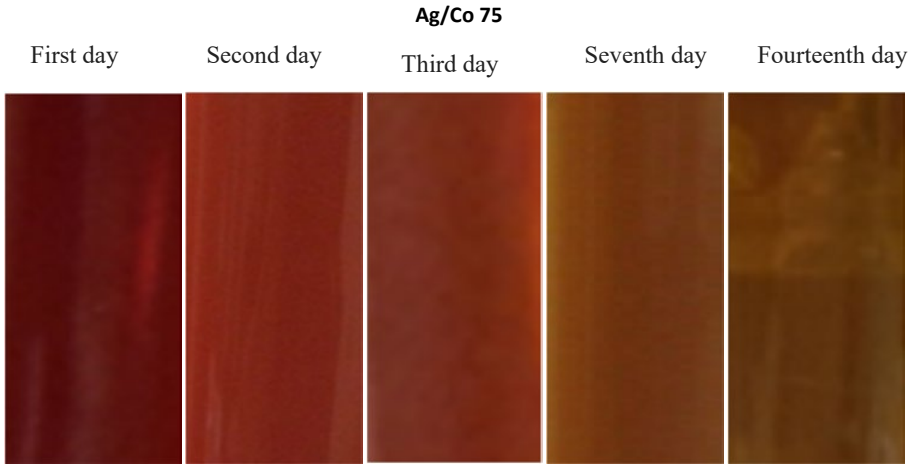

Fig. 3: Silver-cobalt nanoparticles produced from silver nanoparticles at different times of 75 seconds on the first, second, third, seventh and fourteenth days (respectively, from left to right).

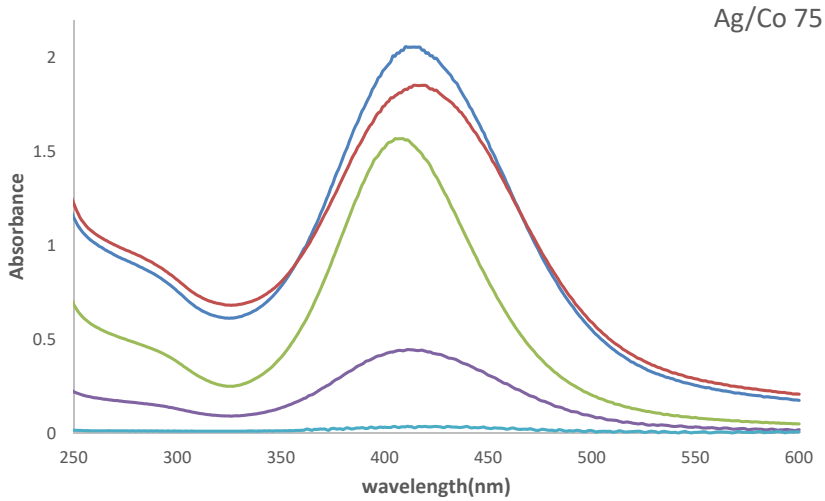

Fig. 4: Absorption spectra of silver-cobalt nanoparticles produced from silver nanoparticles at a different time interval of 75 seconds on the first, second, third, seventh, and fourteenth days (The electrical current adjusted at 1amp and rotational speed was 3000 rpm).

According to Fig. 7, the synthesized bimetallic nanoparticles have a core-shell structure. The transmission electron microscopy (TEM) was used to study the size and shape of silver-cobalt nanoparticles. In order to prepare the specimen, the diameters of silver-cobalt nanoparticles synthesized on a carbon-coated copper network were placed. Fig. 7 (a) and (b) shows the TEM image of silver-cobalt nanoparticles which obtained from silver nanoparticles synthesized during 75 and 300 seconds respectively. According to Fig. 7, the average diameter of silver-cobalt nanoparticles that prepared from silver nanoparticles synthesized during 75 and 300 seconds, is 27.45 nm and 13.5 nm, respectively. Therefore, increasing the synthetic time of silver nanoparticles reduces the size of silver-cobalt nanoparticles made from silver nanoparticles. Because nanoparticle nanotubes that were produced at a longer intervals were coated with less polyvinylpyrrolidone (PVP) compared to nanoparticles synthesized over a shorter period of time.

The mean serum concentration of T3 hormone did not change significantly in all experimental groups receiving silver-cobalt nanoparticles compared to the control group (Table 1). The mean serum concentration of T4 hormone in experimental groups 2 and 3 receiving silver-cobalt nanoparticles showed a significant decrease relative to the control group (Table 2) ($p < 0.05$).

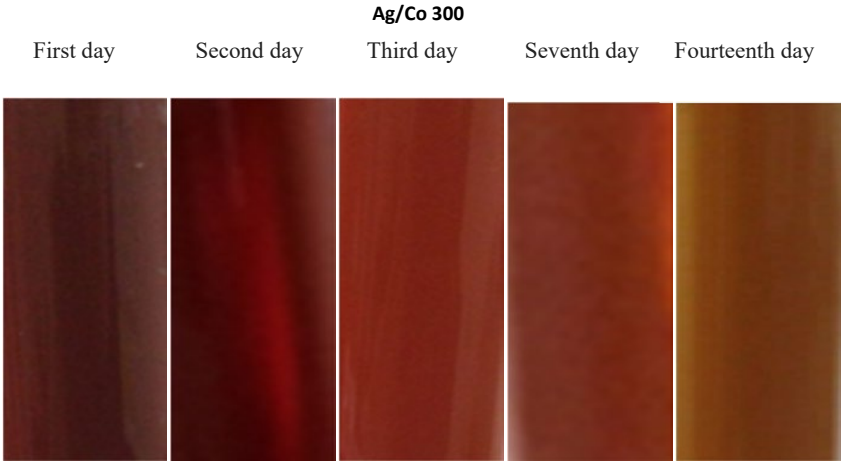

Fig. 5: Silver-cobalt nanoparticles produced from silver nanoparticles at intervals of 300 seconds on the first, second, third, seventh, and fourteenth days (respectively, from left to right)

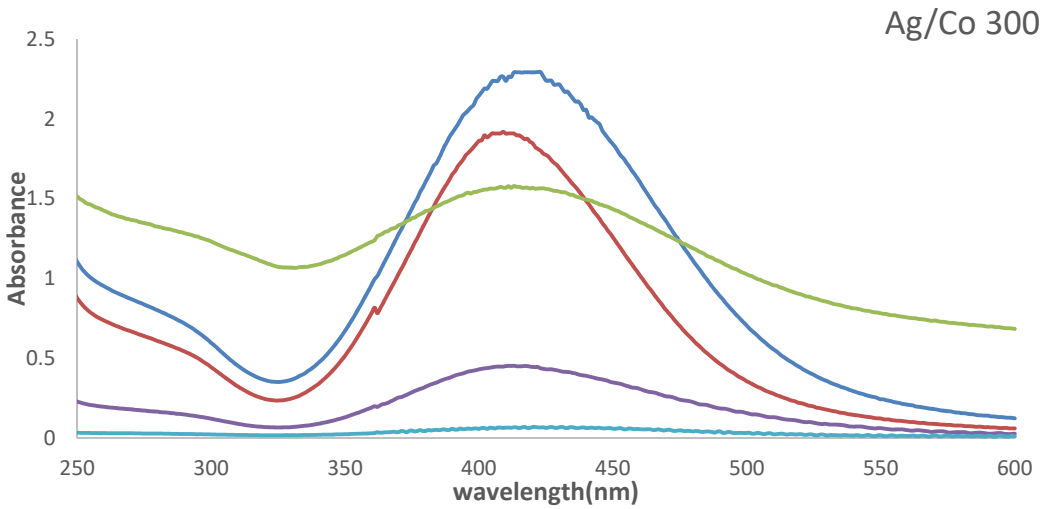

Fig. 6: Absorption spectra of silver-cobalt nanoparticles produced from silver nanoparticles at a different time interval of 300 seconds on the first, second, third, seventh, and fourteenth days (The electrical current adjusted at 1amp and rotational speed was 3000 rpm).

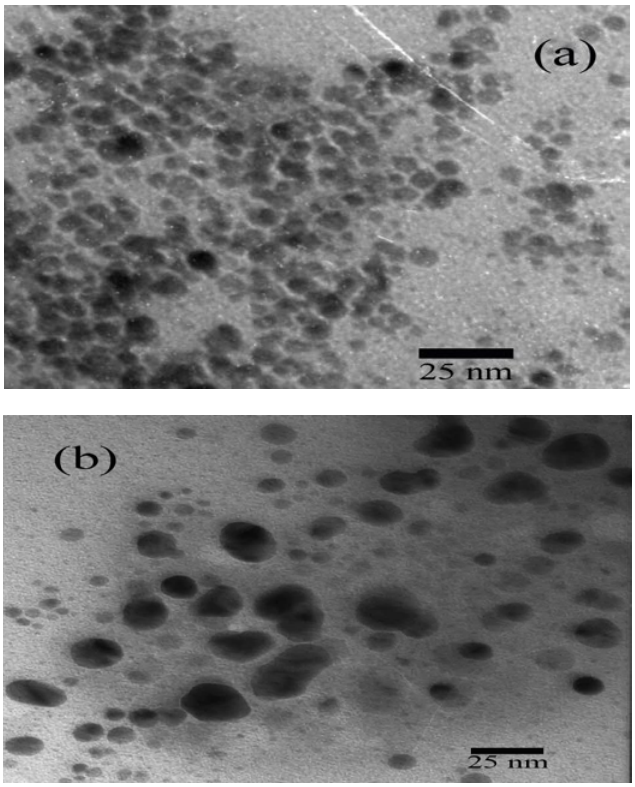

Fig. 7: TEM image of silver-cobalt nanoparticles produced (a) of silver nanoparticles synthesized during 75 seconds, (b) of silver nanoparticles synthesized in a period of 300 seconds (The electrical current adjusted at 1amp and rotational speed was 3000 rpm).

Table 1. Comparison of different amounts of silver-cobalt nanoparticles on mean serum concentration of T3 hormone in the tested groups.

Groups	Number of samples	T3(ng/ml)
Control	7	1.20±0.07
Experimental 1	7	1.40±0.14
Experimental 2	7	1.30±0.05
Experimental 3	7	1.37±0.07

Table 2. Comparison of different amounts of silver-cobalt nanoparticles on mean serum concentration of T4 hormone in the tested groups.

Groups	Number of samples	T4(ng/ml)
Control	7	4.43±0.36
Experimental 1	7	3.63±0.17
Experimental 2	7	3.31±0.25*
Experimental 3	7	2.94±0.37*

The * sign indicates a significant difference at the level of p <0.05 with the control group.

In the present study, the effect of silver-cobalt nanoparticles on the concentration of T3 and T4 hormones was investigated. The results showed that the T3 level did not change significantly in all experimental groups receiving silver-cobalt nanoparticles compared to the control group. The levels of T4 in the experimental groups 2 and 3 receiving silver-cobalt nanoparticles showed a significant decrease compared to the control group.

The core of the silver-cobalt nanoparticle is composed of silver nanoparticles. In a study, silver nanoparticles with doses of 10, 50 and 150 µg / kg/bw were used as gavage. In this study, silver nanoparticles with a dose of 150 µg / kg/bw

resulted in a significant increment in thyroxine. The concentration of TSH hormone in the group receiving doses of 50 and 150 µg / kg/bw relative to the control group showed a significant reduction [23].

Another study also showed that silver nanoparticles with a constant concentration of 16ppm were used for gavage for 30 days. In this study, there was a significant increase in T4 level in the experimental groups relative to the control group, but T3, TSH levels did not show a significant change in comparsion with the control group. In pathological studies, there was no trace of lesions caused by exposure to silver nanoparticles. Increased T4 levels in experimental groups compared to control group are probably due to impairment of T4 iodination [24].

It has been determined that nanoparticles cause impairment in thyroid function. Thyroid hormones are involved in regulating cardiac function, bone formation, and mental states. In addition, thyroid hormones play a important role in embryonic development,especially brain development. Hence, changing their physiological level decreases neuronal growth and differentiation in the cerebellum, hippocampus, and cortex [25, 26].

In vivo and in vitro studies have reported that nanoparticles affect thyroid hormones pathways. Exposure to silver nanoparticles and zinc oxide nanoparticles were investigated on the transmission of thyroid hormone in the frog tissue. The results showed that exposure to silver nanoparticles with 5-10 nm alone led to a decrement in the level of beta-receptor copies of thyroid hormones (TRBs). In addition, the silver nanoparticle is able to affect the function of thyroid hormones on versions of RLK1, TRB, which indicate endocrine disruptions [27].The results of the research showed that silver nanoparticles at a given dose of animals resulted in hyperthyroidism and then, with increasing dose of tissue, they were necrotized, and hormones also undergone significant changes [28].

The reduction of TSH hormone has been attributed to hypothalamic-pituitary pathology and liver damage. The action of removing toxins in the body is done by liver tissue and the tissue is exposed to the greatest damage by nanoparticles [29]. Studies have shown that exposure to various concentrations of silver nanoparticles can cause severe damage to the liver of the mouse and increase the activity of serum enzymes [30]. In addition, silver nanoparticles with a concentration above 50 ppm increase the amount of lesions in the liver tissue. Damage to the liver leads to the evolution of the cytoplasm of the liver cells along with the degeneration and necrosis of some cells and the accumulation of focal inflammatory cells in the liver associated with hyperemia [31]. Histologic findings have shown that exposure to cobalt nanoparticles can lead to more severe liver damage than cadmium chloride, however, serum symptoms show vague changes. Cobalt accumulates in the liver, kidneys, pancreas and heart. Cobalt salts and minerals induce oxidative damage to DNA through reactive oxygen species and inhibit DNA repair [32]. The silver-cobalt nanoparticle appears to cause damage to the thyroid hypothalamus-pituitary gland and decrease the level of the T4 hormone through liver damage.

In the study of Dziendzikowska et al in 2016, silver nanoparticles significantly reduced intra-testis and plasma levels of testosterone in 7 and 28 days after treatment in male rats [33]. In a study by Ahmed et al in 2016, silver nanoparticles decreased serum levels of testosterone and reduced the expression of spermatogenesis genes in comparison to the control group in albino rats. [34]. Due to the direct effect of testosterone on the production and proliferation of cells and the growth of the thyroid gland, on the one hand, and also because part of the effect of the hypothalamic TRH hormone on TSH is mediated through testosterone, it is likely that silver-cobalt nanoparticles, by reducing the secretion of testosterone, trigger The hypothalamic-pituitary-thyroid hormone axis function decreases, resulting in a significant reduction in T4 levels.

The silver-cobalt nanoparticle coating is made up of cobalt nanoparticles. In the study of Yu in 2017, cobalt has been shown to cause thyroid poisoning and leads to hypothyroidism. This study proved that cobalt poisoning on the thyroid may precipitate thyroid dysfunction and damage to thyroid hormone synthesis [35]. Kriss et al. Showed that treating patients with cobalt leads to hypothyroidism and thyroid hyperplasia [36]. The findings are consistent with the results of this study, which shows that silver-cobalt nanoparticles reduce the levels of T4 hormone. It seems that the reduction in T4 level caused by the silver-cobalt nanoparticle is due to its coating, which consists of a cobalt nanoparticle.

It seems that silver-cobalt nanoparticles can induce oxidative stress, generate reactive oxygen

species (ROS), reduce cellular antioxidants such as glutathione, and increase cellular involvement in immune processes by damaging the mitochondria of the cell as well as damage to DNA [3 , 10] and thereby disrupt the thyroid function and subsequently reduce the level of the T4 hormone.

According to the results of this research and similar studies, it is suggested that an additional study on the morphological changes of the thyroid follicles should be made using an electron microscope. The results of this research and other studies suggest that further investigations on the morphological changes in thyroid follicles taken using an electron microscope. It is advisable for physicians to inform patients of the possibility of developing thyroid disorders before starting treatment with silver-cobalt nanoparticles.

CONCLUSION

The silver-cobalt nanoparticle is likely to lower the level of the T4 hormone, although further studies are needed. The results observed in this study are similar to secondary hypothyroidism in clinical studies. In this case, despite the reduction of the T4 hormone, the normal T3 hormone is normal because in the anterior pituitary gland, the T3 hormone is more intrinsic to the free circulation of T4 hormone.

ACKNOWLEDGEMENT

Hence, we sincerely appreciate the close cooperation of the Vice-Chancellor of Research at Shiraz Azad University.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Wijnhoven S, Peijneburg W, Herberts C. Nano-silver-a review of available data and knowledge gaps in human and environmental risk assessment. J Nanotoxicology. 2009;3(2):109-138.
2. Ahamed M, AlSalhi MS, Siddiqui MKJ. Silver nanoparticle applications and human health. Clinica Chimica Acta. 2010;411(23-24):1841-8.
3. Shaheen TI, El-Naggar ME, Hussein JS, El-Bana M, Emara E, El-Khayat Z, et al. Antidiabetic assessment; in vivo study of gold and core-shell silver-gold nanoparticles on streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy. 2016;83:865-75.
4. Kwan KHL, Yeung KWK, Liu X, Wong KKY, Shum HC, Lam YW, et al. Silver nanoparticles alter proteoglycan expression in the promotion of tendon repair. Nanomedicine: Nanotechnology, Biology and Medicine. 2014;10(7):1375-83.
5. Park E-J, Bae E, Yi J, Kim Y, Choi K, Lee SH, et al. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environmental Toxicology and Pharmacology. 2010;30(2):162-8.
6. Crisan D, Scharffetter-Kochanek K, Crisan M, Schatz S, Hainzl A, Olenic L, et al. Topical silver and gold nanoparticles complexed with Cornus mas suppress inflammation in human psoriasis plaques by inhibiting NF-κB activity. Experimental Dermatology. 2018;27(10):1166-9.
7. Liu X, Gao P, Du J, Zhao X, Wong KKY. Long-term anti-inflammatory efficacy in intestinal anastomosis in mice using silver nanoparticle-coated suture. Journal of Pediatric Surgery. 2017;52(12):2083-7.
8. Sivakumar AS, Krishnaraj C, Sheet S, Rampa DR, Kang DR, Belal SA, et al. Interaction of silver and gold nanoparticles in mammalian cancer: as real topical bullet for wound healing— A comparative study. In Vitro Cellular & Developmental Biology - Animal. 2017;53(7):632-45.
9. van den Brule S, Ambroise J, Lecloux H, Levard C, Soulas R, De Temmerman P-J, et al. Dietary silver nanoparticles can disturb the gut microbiota in mice. Particle and Fibre Toxicology. 2015;13(1).
10. Colognato R, Bonelli A, Ponti J, Farina M, Bergamaschi E, Sabbioni E, et al. Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro. Mutagenesis. 2008;23(5):377-82.
11. Park BJ, Choi K-H, Nam KC, Ali A, Min JE, Son H, et al. Photodynamic Anticancer Activities of Multifunctional Cobalt Ferrite Nanoparticles in Various Cancer Cells. Journal of Biomedical Nanotechnology. 2015;11(2):226-35.
12. Vinardell M, Mitjans M. Antitumor Activities of Metal Oxide Nanoparticles. Nanomaterials. 2015;5(2):1004-21.
13. Yan X, Liu Y, Xie T, Liu F. α-Tocopherol protected against cobalt nanoparticles and cocl2 induced cytotoxicity and inflammation in Balb/3T3 cells. Immunopharmacology and Immunotoxicology. 2018;40(2):179-85.
14. Magaye R, Zhao J, Bowman L, Ding MIN. Genotoxicity and carcinogenicity of cobalt-, nickel- and copper-based nanoparticles. Experimental and Therapeutic Medicine. 2012;4(4):551-61.
15. Cho WS, Duffin R, Bradley M, Megson IL, MacNee W, Howie SEM, et al. NiO and Co3O4 nanoparticles induce lung DTH-like responses and alveolar lipoproteinosis. European Respiratory Journal. 2011;39(3):546-57.
16. Melmed S, Polonsky KS, P. Larsen R. Williams textbook of endocrinology. 12th ed. Sunders. Elsevir; 2011: 341-346.
17. Hall JE, Guyton A, Guyton and Hall Physiology Review.11th ed. Philadelphia: Saunders Press; 2005: 235-259.
18. Cooper DS, Klibanski A, Ridgway EC. DOPAMINERGIC MODULATION OF TSH AND ITS SUBUNITS: IN VIVO AND IN VITRO STUDIES. Clinical Endocrinology. 1983;18(3):265-75.
19. Christy AJ, Umadevi M. Synthesis and characterization of monodispersed silver nanoparticles. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2012;3(3):035013.
20. Garcia T, Lafuente D, Blanco J, Sánchez DJ, Sirvent JJ, Domingo JL, et al. Oral subchronic exposure to silver nanoparticles in rats. Food and Chemical Toxicology. 2016;92:177-87.
21. Hussein R, Sarhan O. Effects of intraperitoneally injected silver nanoparticles on histological structures and blood

parameters in the albino rat. International Journal of Nanomedicine. 2014:1505.
22. Li TZ, Gong F, Zhang BY, Sun JD, Zhang T, Kong L, et al. Acute toxicity and bio-distribution of silver nitrate and nano-silver with different particle diameters in rats. Zhonghu Shao Shang Za Zhi. 2016;32(10):606-612.
23. Saedi Marghmalki V, Agha-Taheri M. Effect of silver oxide nanoparticles on liver enzymes, thyroid hormone and thyroid-stimulating hormone concentrations in rats. 24th Iranian Congress of physiology and pharmacology. 2015; 2-12.
24. Rejali L, Moshtaghian SJ, Mahzouni P, Davood A. The effect of chronic consumption of silver nanoparticles on thyroid gland and pregnancy in rats. Qom Univ Med Sci J. 2015;9(7):20-28.
25. Lavado-Autric R, Ausó E, García-Velasco JV, del Carmen Arufe M, Escobar del Rey F, Berbel P, et al. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. Journal of Clinical Investigation. 2003;111(7):1073-82.
26. Ausó E, Lavado-Autric R, Cuevas E, del Rey FE, Morreale de Escobar G, Berbel P. A Moderate and Transient Deficiency of Maternal Thyroid Function at the Beginning of Fetal Neocorticogenesis Alters Neuronal Migration. Endocrinology. 2004;145(9):4037-47.
27. Hinther A, Vawda S, Skirrow RC, Veldhoen N, Collins P, Cullen JT, et al. Nanometals Induce Stress and Alter Thyroid Hormone Action in Amphibia at or below North American Water Quality Guidelines. Environmental Science & Technology. 2010;44(21):8314-21.
28. Sharifi AS,Naseri S,Rezaei Zarchi S, Rezaei R. Effect of silver nanoparticles on thyroid hormones and tissue in male rat.1st National Conference on nano science and Technology. 2010;1-340.
29. Afkhami-Ardakani M, Shirband A, Golzade J, Asadi-Samani M, Latifi E, Kheylapour M, et al. The effect of iron oxide nanoparticles on liver enzymes (ALT, AST and ALP), thyroid hormones (T3 and T4) and TSH in rats. J Shahrekord Univ Med Sci. 2013; 14(6): 82-8.
30. Seyedalipour B, Arefifar A, Khanbabaee R, Hoseini S M. Toxicity of silver nanoparticles on ALT, AST, ALP and histopathological changes in NMRI mice. J Mazandaran Univ Med Sci. 2015; 25(124): 183-193.
31. Jafarzadeh Samani R, Heydarnejad MS, Kabiri Samani M. A survey of acute histopathological effects of silver nanoparticles on liver, kidney with blood cells during oral administration in male mice (Mus musculus). J Shahrekord Univ Med Sci. 2015; 17(4): 97-107.
32. Simonsen LO, Harbak H, Bennekou P. Cobalt metabolism and toxicology—A brief update. Science of The Total Environment. 2012;432:210-5.
33. Dziendzikowska K, Krawczyńska A, Oczkowski M, Królikowski T, Brzóska K, Lankoff A, et al. Progressive effects of silver nanoparticles on hormonal regulation of reproduction in male rats. Toxicology and Applied Pharmacology. 2016;313:35-46.
34. Ahmed SM, Abdelrahman SA, Shalaby SM. Evaluating the effect of silver nanoparticles on testes of adult albino rats (histological, immunohistochemical and biochemical study). Journal of Molecular Histology. 2016;48(1):9-27.
35. Yu R. Cobalt Toxicity, An overlooked Cause of Hypothyroidism. Journal of Endocrinology and Thyroid Research. 2017;1(3).
36. Kriss JP, Carnes WH,Gross RT. Hypothyroidism and thyroid hyperplasia in patients treated with cobalt. J Am Med Assoc. 1955;157(2):117-21.