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Effectiveness of Magnesium oxide Nanoparticles in the Management of Thyroid Hormone Level

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Abstract

Hypothyroidism is a clinical dysfunction resulting from deficiency of thyroid hormones or, more rarely, from their impaired and it poses a threat to human health. Nanotechnology is being applied for the inorganic biomedical agents development due to the perfect biological activities of the nanoparticles. Nano inorganic metal oxides control the biological contaminants. The most widely utilized oxide in different fields is magnesium oxide nanoparticles. This work presents an investigation of the main synthesis method, characterization by several techniques, including Fourier transform infrared spectroscopy, Atomic Force Microscope, scanning electron microscopy and X-Ray Diffraction and the effect of magnesium oxide nanoparticles on serum levels of T4 hormone was investigated. Serum levels of T4 in experimental showed a significant decrease at 100 ppm compared to control group. Other concentrations did not change significantly compared to control group. Magnesium oxide nanoparticles (MgONPs) at concentration of 100 ppm reduced the serum T4 level.

Keywords: Synthesis, Nanoparticles; Magnesium Oxide; Thyroide.

Introduction

Thyroid hormones (T3 and T4) are dividing from tyrosine amino acid. About 95% of thyroid secreting hormones are T4 (thyroxine) whereas T3 plays the main role. The main part of T3 is obtained from converting T4 to T3 in peripheral tissues like liver, kidney and placenta. Some tissues like the brain and hypophysiscan also convert T4 to T3 but obtained hormone cannot be entered to blood and remains there. On the whole, 80% of blood's T3 is made in liver and 20% in thyroid. Secreting thyroid stimulating hormone (TSH) controls releasing thyroid hormones. The amount of TSH secreting is also adjusted by level of thyroid hormones in blood. Thyrotropin releasing hormone (TRH) secreted from hypothalamus adjusts TSH releasing from hypophysis somehow [1].

Graves and Hashimoto diseases are types of autoimmune diseases that damage the thyroid gland and affect its hormone production levels .They are also the main reason why the thyroid gland produces high amounts of hormones in the bloodstream by cause of hyperthyroidism [1].

The unique properties of green nanoscience made it possible to grow quickly by chemist and scientist. A nanotechnology deal with a small size of is 1-100 nanometers, so the particles called as nanoparticles (NPs) [2]. Different shapes and sizes of synthesized NPs of different chemicals can be obtained by changing the synthesis experimental method. In the recent years, the researchers turned to synthesize the NPs biologically instead of physical and chemical technique. Such biological production techniques are safe of toxicity, low cost [3,4] and eco-friendly [5]. In principle, the environmentally friendly properties helped in the success of the attempts made by researchers to synthesize nanomaterials from extracts of leaves and flowers, for example [6-8].

The aim of this work is to synthesize magnesium oxide nanoparticles (MgONPs) by Green synthesis methodology, in which Mg acetate is the initial precursors and orange peels. Orange peels can act as reducing agents for the purpose of metals oxides such as ZnO, TiO_2 and so on as it. The oxidizing and reducing agent is acted by plant extracts [9, 10]. Naturally, orange peels have a light yellow color. These peels provide complete protection for the selected fruit inner parts from the surrounding

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environment. Also, water is utilized in this process to get rid of traditional harmful chemicals [11, 12]. The inorganic MgO material have a wide band gap [13], as well, it is used remediation of toxic wastes, catalyst applications, material adsorption, reflecting and anti-reflecting coating types, ionic batteries and so on [14, 15]. In medical applications, MgO is employed to control the heartburn, regeneration of bones and sore stomach [16, 17]. MgONPs considered as a promising nanomaterial for tumor treatment [18]. It is shown from this survey that there is a lack of information regarding study the effect of MgO nanoparticles on hypothyroidism. Therefore, in this research, the synthesis method, characterization and biological effect of MgO nanoparticles on thyroid hormone level are discussed.

Materials and Methods

Preparation of Orange Peel Extract

Extracts of orange peels was prepared according to [19]. Fresh orange fruit was collected from the trees planted in the gardens of Diyala. Orange peels were washed with distilled water to remove any dust particles, and then left in our laboratory for 15 days to dry for the removal the moisture residue and finally grinding into fine powder for further treatment. The quantity of dried peels powder, exactly 10 gm was refluxed with 250 ml of deionized water for one hour. Then, Whatman filter paper 0.1µm was used for filtration process. The filtrate was kept in the refrigerator for the experimental study.

Synthesis of Magnesium Oxide Nanoparticles

For the synthesis of MgONPs, 2 grams of magnesium nitrate dissolved in 100 ml deionized water in a glass beaker. After that, 10 ml of the prepared aqueous peels extract was added drop by drop, where the magnetic stirrer was used for 6 hours to get a homogenous mixture. The next step was reducing the solution of magnesium nitrate into MgO. A change in colour is an indication that the reduction process has occurred. 1 N Sodium carbonate solution was added by drop-wise method to maintain the solution pH at 12. After that, the solution heated to 80 ºC for half an hour, in the meantime the nanoparticles are formed and settled in the bottle bottom and the solution was expelled at 5,000 rpm/min for 5 min. Finally, MgONPs passed in filtering and air drying processed overnight.

Biological Studies

Nintey people were selected in this work, sixty of them have autoimmune disease and thirty are healthy. Their ages ranged between (9-60) years and the specimens were collected during the period from 1st Nov 2019 until the end of Feb 2020 from Al-Kindy hospital. Venous blood samples (4-5 ml) have been collected from each individual of healthy control between 8AM and 11AM. The serum collected by putting the blood in a clean and dry gel tube and left to clot at 37 ºC for 20 min, then put it in the centrifuge device at 6000 rpm for 10 min, Serum are separated in a simple plastic tube and kept in the freezer till used for the assay. 10 microns of serum were added to 10 micro litters (µL) of colloidal nanoparticles in concentrations of 0, 25, 50, 100, 150, 175 and 200 ppm. The free T4 assay is a two-step, first, immunoassay anti- free T4 coated micro particles are combined with the free T4 present in the sample. Second step, the T4 acridinium-labeled conjugate is added to create a reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by Abbott device. The principle of Abbott was based on paramagnetic micro particle chemiluminescent technology. The reaction principle is as follows: nanoparticle is coated with three recombinant antigens (Ag) to band antibodies specific for free T4 hormone. After incubating the nanoparticles with serum (10 µL), the chemiluminescent reaction was measured.

Results and Discussion

The white coloured MgONPs were obtained by the green synthesis methodology. Magnesium nitrates ($Mg(NO_3)_2.6H_2O$, orange peels aqueous extract and sodium carbonate were utilized for nanoparticles synthesis procedure. The resulted nanoparticles were characterized by using AFM, X-ray diffraction, FTIR and SEM techniques.

Atomic Force Microscope of MgONPs

Atomic force microscopic examination allows identifying the plot topographies representing the surface elevation and the structure of the surface. This technique refers to digital images that allow quantitative measurements of surface features, such as root mean square roughness (Rq) and average roughness (Ra). Figure 2 illustrates the two dimensional AFM image of the synthesized MgONPs. The average grain size for the MgO NPs was 50.04 nm, as show in, and it can be seen that the nanoparticles were approximately sphere.

Avg. Diameter: 50.04 nm

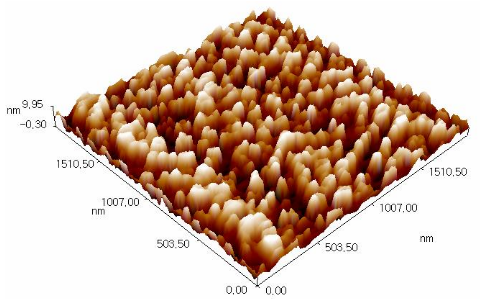

Fig. 1. AFM image to illustrate the size distribution of MgONPs.

X-ray Diffraction patterns (XRD)

X-ray or XRD was a useful technique to confirm the crystalline structure represented by crystallite size and crystal phase of MgO nanoparticles. XRD analysis was achieved at wavelength radiation of 1.5418 Å of Cu crystal at a scanning speed of 8.0000 deg/min for the driving axis (Theta-2Theta) and scanning range10.0000-120.0000 deg. The diffraction peaks were 42.9242, 62.3073, 39.7352) as shown in figure 2. The peaks in the XRD pattern of magnesium oxide nanoparticles were in accordance with JCDS 75–1525 which expressed the cubic structure of synthesized nanoparticles [20]. The existence of sharp peaks in the XRD spectrum of magnesium oxide sample confirmed the formation of nanoparticles, and increase in the peak width represented a decrease in the size of nanoparticles. Also, the absence of extra peaks in the synthesized nanoparticles confirmed their high purity.

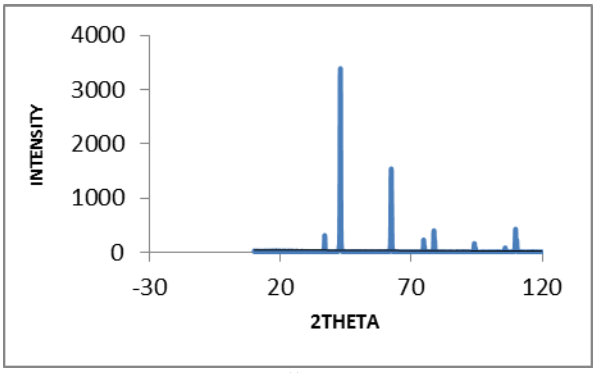

Fig. 2. X-ray diffraction MgONPs

Fourier Transforms Infrared Spectroscopy of MgONPs

Figure 3 performs the FTIR spectrum of synthesized magnesium oxide nanoparticles that showed strong peaks at 3415, 1633, 1500 and 434 cm^{-1}. the bands observed at the wavenumber of 3415 cm^{-1} indicated the adsorption of the O-H group which aid on the converting Mg acetate to Mg nanoparticles. While vibration stretches of aromatic C=C groups are represented by the two bonds 1633 and 1500 cm^{-1}. The peak observed at 434 cm^{-1} confirmed the formation of MgO nanoparticles.

Electron Microscope Images of MgONPs

The size and morphology of the synthesized magnesium oxide nanoparticles was studied through SEM micrograph as shown in figure 4. The scanning electron micrograph demonstrated agglomeration of some magnesium oxide nanoparticles. It is also clear that the synthesized nanoparticles have a spherical shape. The scanning electron microscope image showed that the average size of the synthesized nanoparticles was about 61 nm.

The Effect of MgONPs on FT4 Hormone

The result of free thyroxin hormone (free T4) of the present study shows a highly significant increase ($p \leq 0.05$) of its level in serum of thyroid patient in comparison to that level in the control group.

Data listed in table 1 and Figure 5 showed highly significant ($p \leq 0.05$) in free T4 level in sera of Hashimoto and Graves each patient to be compared with that levels in the controlling group. The results obtained indicate that the significant increase in free thyroxin hormone (FREE T4) in Graves' disease is due to the presence of immune bodies that increase the stimulation of the thyroid gland to produce this hormone. Highly significant in thyroxin hormone for Hashimoto disease comes from the inhibition to thyroid peroxidase enzyme by anti-thyroid peroxidase [18]. The results in Figure 6 indicate that the effect of nanoparticles on free thyroxin is little due to the lack of bind of the hormone with nanoparticles.

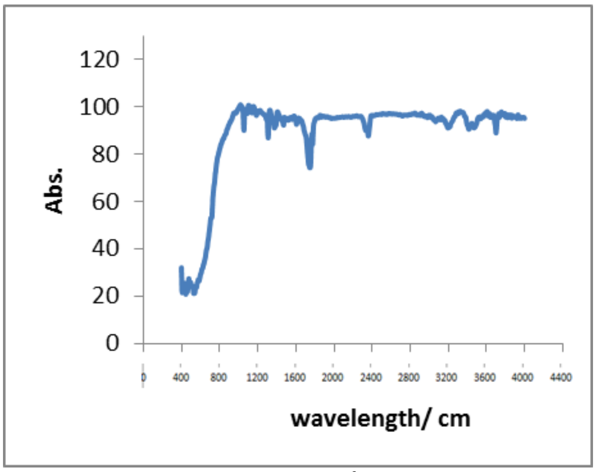

Fig. 3. FTIR spectra of MgONPs

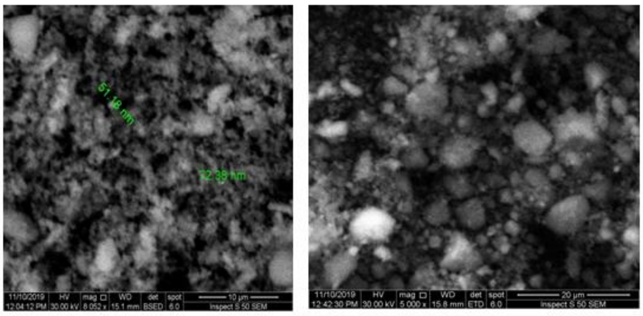

Fig. 4. SEM images of MgONPs

Table 1. Mean, SD and P-VALUE levels of FT4 in the sera of Graves and Hashimoto Patient and Control groups

	Mean ±SD		P-Value
Hashimoto	control	12.416±2.077	0.0001
	patient	6.470±2.078	
Graves	control	12.416±2.048	0.0001
	patient	24.486±5.040	

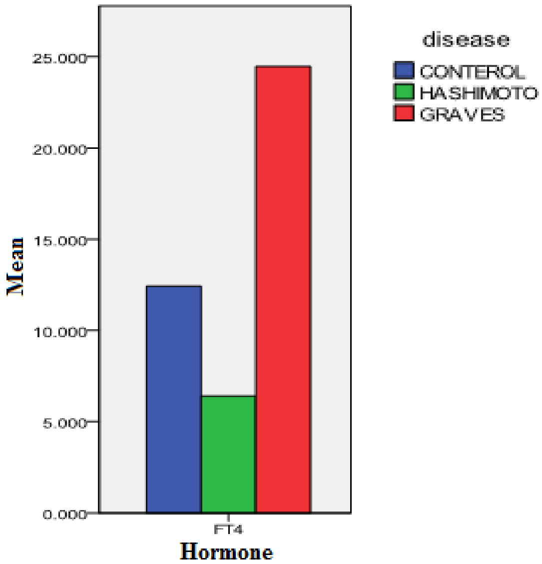

Fig. 5. Differences between mean for Graves and Hashimoto patient and control groups

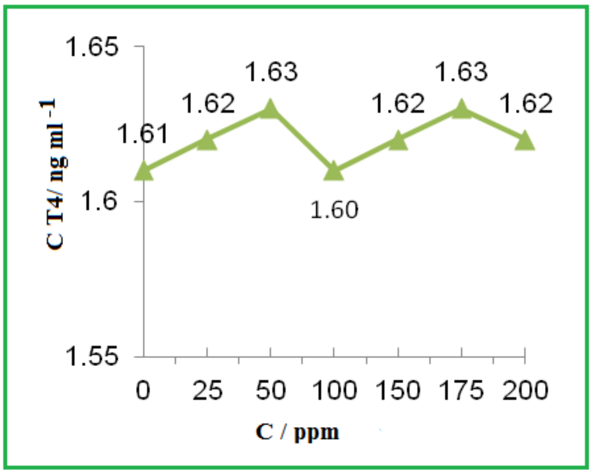

Fig. 6. Effect of MgONPs on free T4 hormone

Conclusion

Biocompatible and rapid synthesis of magnesium oxide nanoparticles using the orange peel extract is demonstrated with possible role of different phytochemicals as reducing and stabilizing agent. The prepared magnesium oxide nanoparticles were cubic in shape and characterized using FTIR, XRD, SEM and AFM techniques. The SEM image showed that most of the nanoparticles are spherical in shape. X-ray diffraction confirms the formation of crystalline structure. The present investigation provides an eco-friendly possibility for synthesis of magnesium oxide and biochemical effect on thyroxin hormone using natural product. MgONPs are likely to simulate the level of the free T4 hormone at all studied concentrations, except at 100 ppm, MgONPs lower the level of hormone. The results observed in this study are similar to secondary hypothyroidism in clinical studies.

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فعالية الجسيمات النانوية لأوكسيد المغنيسيوم في تنظيم مستوى هرمون الغدة الدرقية

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الخلاصة

ان قصور الغدة الدرقية هو خلل سريري ناتج عن نقص هرمونات الغدة الدرقية أو في حالات نادرة بسبب ضعفها ويشكل خطراً على صحة الإنسان. يتم تطبيق تقنية النانو لتطوير العوامل الطبية الحيوية غير العضوية بسبب الأنشطة البيولوجية المثالية للجسيمات النانوية، حيث تتحكم أكاسيد المعادن النانوية في الفعالية البيولوجية. يعد أكسيد المغنيسيوم النانوي من المركبات الأكثر استخدامًا في المجالات المختلفة. يمثل هذا العمل دراسة اكثرطرق التحضير الصديقة للبيئة ، والتشخيص من خلال عدة تقنيات مختلفة، بما في ذلك التحليل الطيفي بالأشعة تحت الحمراء، ومجهر القوة الذرية، والفحص المجهري الإلكتروني وانحراف الأشعة السينية وتأثير جزيئات أكسيد المغنيسيوم النانوية على مستويات مصل هرمون T4. أظهرت مستويات هرمون T4 في المصل انخفاضًا معنويًا عند تركيز 100 جزء لكل مليون لجسيمات اكسيد المغنيسيوم النانوية المحضرة مقارنة بالمجموعة السليمة. لم تتغير التركيزات الأخرى بشكل كبير مقارنة بالمجموعة السليمة. خفضت جزيئات أكسيد المغنيسيوم النانوية (MgONPs) بتركيز 100 جزء لكل مليون مستوى هرمون T4 في الدم.