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` Study the Effect of Nano-Indian Costus Extract on Thyroid Gland Injury

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Abstract:
The thyroid is an endocrine gland that produces and secretes triiodothyronine (T3), and thyroxine (T4) hormones. These hormones are essential for maintaining body homeostasis. Indian costus is a medicinal plant used in the treatment of thyroid disorders. The aim of the study is to use Indian costus in its particles and nanoparticles to treat the thyroid, which was infected with an overdose of melatonin. Nano Indian costus was prepared using microwave plasma and pulsed laser deposition (PLD). To determine the most effective technique for creating the Indian costus nanoparticles, the material was examined using an atomic force microscope (AFM) in its bulk and nanoparticle forms. It was found that the microwave plasma method gave better results than PLD, where the average nanoparticle diameter was 21.11nm for the microwave plasma method and 40.02nm for the PLD. Thyroid hormones regained their normal levels of T3 and T4 after receiving Indian costus nanoparticles prepared by microwave plasma, which proved more effective than Indian costus nanoparticles prepared by PLD.

Keywords: Indian costus, Atomic force microscope(AFM), microwave plasma, pulsed laser deposition (PLD), thyroid test T3 T4 TSH.

دراسة تأثير مستخلص القسط الهندي النانوي على إصابة الغدة الدرقية

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الخلاصة:
لغدة الدرقية هي غدة صماء تنتج وتفرز هرموني: ثلاثي يودوثيرونين (T3)، وثيروكسين (T4). هذه الهرمونات ضرورية للحفاظ على توازن الجسم. القسط الهندي هو نبات طبي يستخدم في علاج اضطرابات الغدة الدرقية. أهداف البحث هي استخدام القسط الهندي النانوي في جزيئاته الطبيعية وجزيئاته النانوية في علاج اختلال الغدة الدرقية ، التي أصيبت باستخدام جرعة زائدة من الميلاتونين. تم الحصول على حجم النانو باستخدام بلازما الميكروويف وترسيب الليزر النبضي (PLD) ، وفحص مجهر القوة الذرية (AFM) المستخدم لعينة القسط الهندي في كل من الجسيمات العادية والنانوية لتحديد التقنية الأكثر فعالية لإنشاء جسيمات القسط الهندي النانوية، فقد أظهر أن بلازما الميكروويف تعطي نتيجة أكثر تأثيرًا من الليزر حيث يبلغ متوسط القطر 21.11 نانومتر باستخدام البلازما الميكروويف و 40.02 نانومتر بطريقةPLD. كما استعادت هرمونات الغدة الدرقية مستوياتها الطبيعية بعد تناول جرعات من جسيم القسط الهندي المحضر ببلازما الميكروويف وهو أكثر فاعلية من جسيمات القسط الهندي المحضرة بالليزر .

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الكلمات المفتاحية: القسط الهندي ، مجهر القوة الذرية (AFM) ، بلازما الميكروويف ، ترسيب الليزر النبضي (PLD) ، اختبار الغدة الدرقية TSH T4 T3.

1. Introduction

The thyroid is an endocrine gland that produces and secrets thyroid stimulating hormone triiodothyronine (T3) and thyroxine (T4), reverse triiodothyronine(RT3), and calcitonin hormones. They regulate lipid and glucose metabolism, facilitate metabolic adaptations throughout reactions for variations in energy intake, regulate basal metabolism, thermogenesis, and oxidative metabolism. Thyroid dysfunction could affect any tissue in the body because many tissues express thyroid hormone receptors and rely on their activities to correct cell function. As a result, sufficient hormones are required for normal target tissue function. The thyroid gland's primary hormones are T3 and T4; For normal tissue function, T3 and T4, the main hormones produced by the thyroid gland, have a higher affinity for thyroid hormone nuclear receptors, which makes them more metabolically active. The intracellular deiodinase enzymes catalyse the conversion of T4 to T3, which functions to eliminate the iodine molecules of T4. This conversion is the main modulator of thyroid hormone content in both tissue and plasma [1,2].Thyroid conditions and natural therapies for endocrine problems, especially thyroid abnormalities, are becoming more common all over the world. Women are more prone than males to suffer thyroid dysfunction problems.

Thyroid problems are broadly classed as hyperthyroidism (high levels of thyroid hormones) or hypothyroidism (low levels of thyroid hormones). Hypothyroidism is a common endocrine condition characterized by low levels of the thyroid hormones (T3 and T4). Autoimmune thyroiditis is the most prevalent cause of hypothyroidism [3,4].

Indian costus has been used as a treatment plant to treat thyroid disorders. Indian costus is a medicinal plant that grows without cultivation in moist soil in the Himalayas and many parts of India. It is used in the treatment of many diseases and the treatment of thyroid disorders [5,6]. The aim of the work is to use the Indian costus plant extract to treat the infected thyroid, which was infected by an overdose of melatonin, causing damage to the thyroid tissues.

2. Material and Method:

Preparation of Indian Costus Aqueous Solution.

Indian costus (purchased from local herbal markets in Baghdad) was ground well with a special mill till it became a powder form. A 40 gm of this powder was mixed with 200ml of distilled water. The solution was stirred with a magnetic stirrer for 30 minutes at room temperature. The solution was converted into nanoparticles by two methods:

1- The first method uses microwave plasma operated by the electromagnetic wave at a frequency greater than 30 MHz. Indian costus was converted into nanoparticles by the heat produced by the plasma. It is a non-equilibrium (non-thermal) plasma with a continued wave of energy, and it has operation pressure ranging (10-5 torr up to atmospheric pressure); it is used in many medical and biological applications [8]. The microwave plasma employed in this investigation has a voltage of 175 volts, a gas flow of 2 liters per minute, and a frequency of 2.45 GHz [9].

2- The second method uses pulse laser deposition (PLD) employing the Neodymium-Doped Yttrium Aluminium Garnet (Nd:YAG) laser. Many characteristics make this kind of laser particularly useful, such as high laser light amplification, and the choice for energy storage in the active medium. It works at the wavelength (1,064 nm) [10,11].

3. Results and Discussion

The Indian costus properties were characterized by three types of analysis.

X-ray Fluorescence (XRF)

X-ray fluorescence (XRF) spectroscopy is a well-known, well-established, and non-destructive analysis method. It is often used to identify the main elemental compositions of materials. It is known for its high accuracy and precision. XRF laboratory analyses continue to be the standard method for providing high-quality geochemical data analyses in the investigation of elemental composition [12].

The chemical analysis of the Indian costus was obtained using (XRF) analysis. Table 1 shows the chemical composition of Indian costus powder. The table shows only the elements with high concentrations. The elements in the Indian coctus are the important elements that affect the function of the thyroid. Potassium helps in regulating the function of the thyroid. Reduced potassium causes the thyroid hormones to rise, which increases the thyroid gland activity and damages the kidneys. Calcium regulates the function of the thyroid gland by releasing calcium from the bones and increasing the quantity absorbed by the small intestine. It also raises calcium levels via raising parathyroid gland hormones. Low magnesium level leads to a decrease in the thyroid hormones, causing a reduction in the parathyroid hormones and making skeletal muscle receptors less sensitive to the thyroid hormone. Iron concentration is essential in the work of the thyroid; a low iron level leads to a low level of hormones, which contributes to hair loss [13].

Table 1: elements concentration for Indian costus powder

Symbol	Elements	Concentration
MgO	Magnesium	0.3336%
Al_2O_3	Aluminum	2.644%
SiO2	Silicon	4.119%
K_2O	Potassium	2.211%
CaO	Calcium	1.242%
Fe_2O_3	Iron	0.261%

The UV-Vis spectrometer

The ability to detect any molecule using ultraviolet-visible (UV-Vis) spectroscopy makes it one of the most popular analytical methods. UV-Vis spectroscopy measures the transmittance of UV-Vis light through a sample to determine the amount of light absorbed by a material (of thickness T) [14,15]; the absorbance (A) can be represented by:

$$A= -\log T \qquad \text{………} \quad (1)$$

The absorbance equation was derived to display the absorbance of a material at various wavelengths [16,17].

The UV-Vis analysis for the Indian costus and nanoparticles Indian costus prepared by plasma and laser at energy (200 mJ) are shown in Figure 1. The three absorbance peaks of the absorbance spectra of the bulk, nano Indian costus prepared by microwave plasma and the pulsed laser deposition method coincided; it is in the range between 200-250 nm. It is important to mention that the energy used was 200mJ not to break the bonds between the molecules of the substance, considering that it is a food substance, and high energy can break the bonds of molecules.

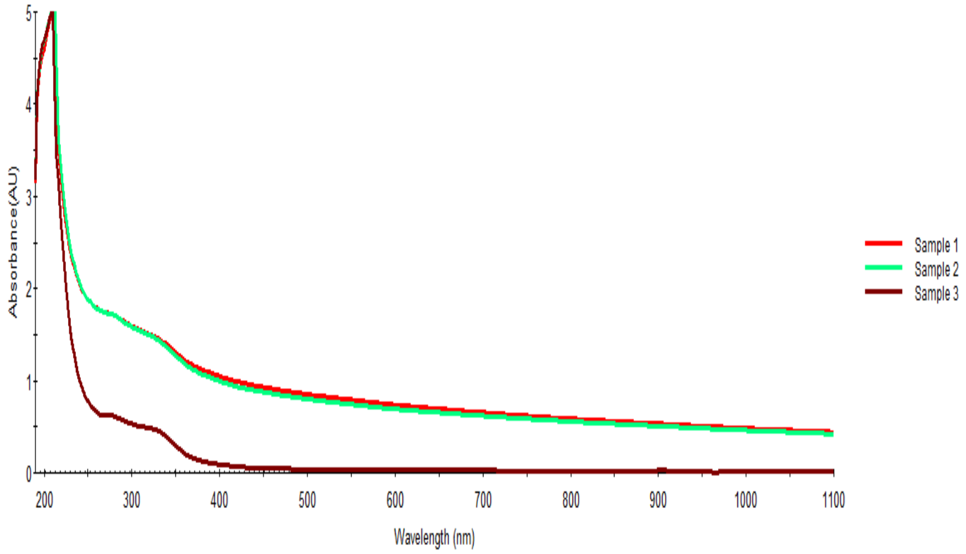

Figure 1: The UV-Vis Spectra for three nano Indian costus samples, sample1: the Indian Costus, sample2: the Nano Indian Costus(prepared Plasma) and Sample3 the Nano Indian Costus (prepared by PLD)

Atomic Force Microscope (AFM)

The AFM results of the aqueous solution of the Indian costus and nano Indian costus forms (produced by the two methods: the microwave plasma method and the pulsed laser deposition method) are shown in Tables 2, 3, 4 and 5. Figures 2, 3, and 4 show AFM images showing the distribution and homogeneity structure of Indian costus for the Indian costus and nano Indian costus. Table 2 shows that the average diameter for the nanoparticles prepared by microwave plasma was 21.1nm with maximum height of 76.78nm and arithmetic mean height of 10.87nm, which is better than the nanoparticles prepared by the pulsed laser deposition method. Table 3 displays the statistical features showing that the highest values of average value, maximum, median, R_a and RMS were for those prepared by plasma; the Skew and Kurtosis (the Atomic Force Microscope features) are with highest value in the normal Indian costus particles. Table 4 represents the height parameters; the lowest values were for the nanoparticles prepared by microwave plasma. In Table 5, the smallest values of the functional parameters were for those prepared by the pulsed laser deposition method.

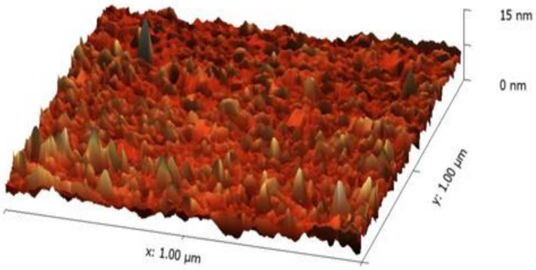

Figure 2: Atomic force microscope image of normal Indian costus.

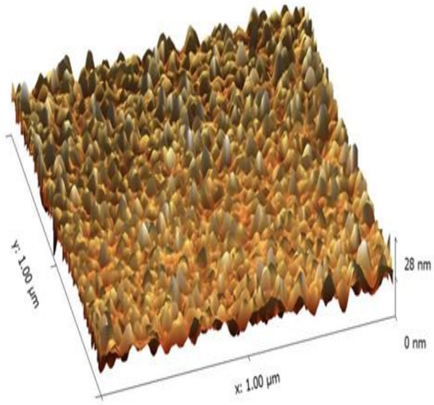

Figure 3: Atomic force microscope image for nano Indian costus (prepared by micowave plasma).

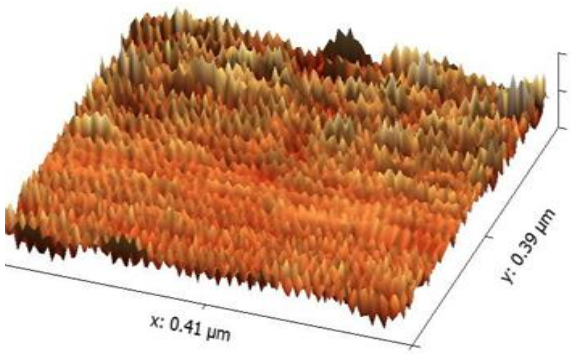

Figure 4: The atomic force microscope image for nano Indian costus (prepared by PLD).

Table 2: The atomic force microscope features.

Parameters	Average diameter (nm)	Root-mean-square height (nm)	Maximum height (nm)	Arithmetic mean height(nm)
Normal sample	108.4	41.50	176.1	34.74
Nano (plasma)	21.11	13.18	76.78	10.87
Nano (laser)	40.02	9.772	62.01	8.239

Table 3: The statistical features for Indian costus and nanoparticles Indian costus

Features	Normal	Laser	Plasma
Average value	4.90 nm	6.16 nm	14.57 nm
Minimum	0 nm	0 nm	0 nm
Maximum	15.23 nm	13.17 nm	27.98 nm
Median	4.83 nm	6.13 nm	14.57 nm
Ra	0.85 nm	0.91 nm	2.33 nm
Rms	1.13 nm	1.18 nm	2.99 nm
Skew	0.671	0.223	0.0163
Kurtosis	2.9	1.02	0.531

Table 4: The height parameters

Height parameters	Normal(nm)	Laser(nm)	plasma
Sa	16.66	8.211	3.922
Sq	21.21	10.50	5.134
Ssk	-0.8037	1.553	-0.8432
Sku	3.633	3.716	4.991
Sv	69.32	44.77	31.91
Sz	112.4	66.13	46.99

Table 5: The hybrid parameters, functional parameters and spatial parameters

Hybrid parameters	Normal(nm)	Laser(nm)	plasma
Sdq	0.7054 %		
Sdr	19.50 %		
Functional parameters	Normal	Laser	Plasma
Smrk2	16.36 %	7.098 %	8.199 %
Smrk2	90.34 %	76.03 %	87.59 %
Sk	45.45 nm	16.64 %	11.92 nm
Spatial parameters	Normal(µm)	Laser(µm)	Plasma(µm)
Sal	0.1260	0.135	0.1562
Std	0.7445	180.0	176.2
Ssw	0.9994	0.007	0.007

Experimental Groups

Five groups of rats of age between three and four months and of 350 gm weight were used in this study. The five groups (three rats in each group) were classified as follows: -

G1 = normal group without thyroid injury.

G2 = group with thyroid injury (damage in thyroid tissue).

G3=infected group treated with orally administered aqueous solution of Indian costus.

G4= infected group treated with Indian costus nano particles prepared by microwave plasma.

G5= infected group treated with Indian costus nano particles prepared by pulsed laser deposition (PLD).

The first method of preparing the Indian costus nanoparticles was by exposing the Indian costus solution to microwave plasma for five minutes; this time was decided so as not to destroy the chemical bonds between the molecules of Indian costus since it is a food substance and long-time duration destroys these bonds. The second method of preparing the Indian costus was by pulsed laser deposition (PLD) using a 4 Hz wavelength laser with 200 shots.

Thyroid dysfunction was stimulated in G2, G3, G4 and G5 rats by orally administering an overdose of melatonin with a concentration of 20 mg for each 1kg of body weight for seven consecutive days. This overdose of melatonin destroys thyroid tissue. The infection of the thyroid gland of the rats was confirmed by a blood serum test examining the thyroid hormones T3, T4 and TSH. 20 ml of the aqueous solution of Indian costus for each kg was orally administered to G3 rats for (10) days, G4 rats received the nano solution of Indian costus prepared by exposure to microwave plasma, and G5 rats received the solution of Indian costus prepared by PLD. After (10) days, blood serum was collected from the different groups of rats, and thyroid hormones (T3, T4, and TSH) were measured to determine the effect of the Indian costus on thyroid function. The TSH hormone regulates the thyroid gland's activity. When T3 and T4 are low, as in hypothyroidism, TSH levels are high to stimulate the thyroid to generate more T3 and T4. When T3 and T4 levels are high, as in hyperthyroidism, TSH will be low to stimulate the thyroid to decrease its activity. Figure 5 shows histograms for the **t**hyroid hormones levels of the five groups**.** The T3, T4, and TSH levels of G1 group represent the normal levels of these hormones. T3 and T4 hormones of G2 group were higher than the normal levels of G1; this indicates an overactive thyroid gland (hyperthyroidism). In G3, treated with Indian costus solution, there was a slight decrease in the value of T3, T4. The value of TSH in this group should be lower than the normal value since T3 and T4 are high (hyperthyroidism) compared with G2 (the infected groups) but are still higher than the normal levels of G1. For the G4 treated with the Indian costus solution exposed to the microwave plasma, T3 and T4 hormones were within the normal values of thyroid hormones. The results for the G5 treated with the Indian costus solution prepared by PLD showed an increase in T3, T4 and TSH hormones, indicating hyperthyroidism

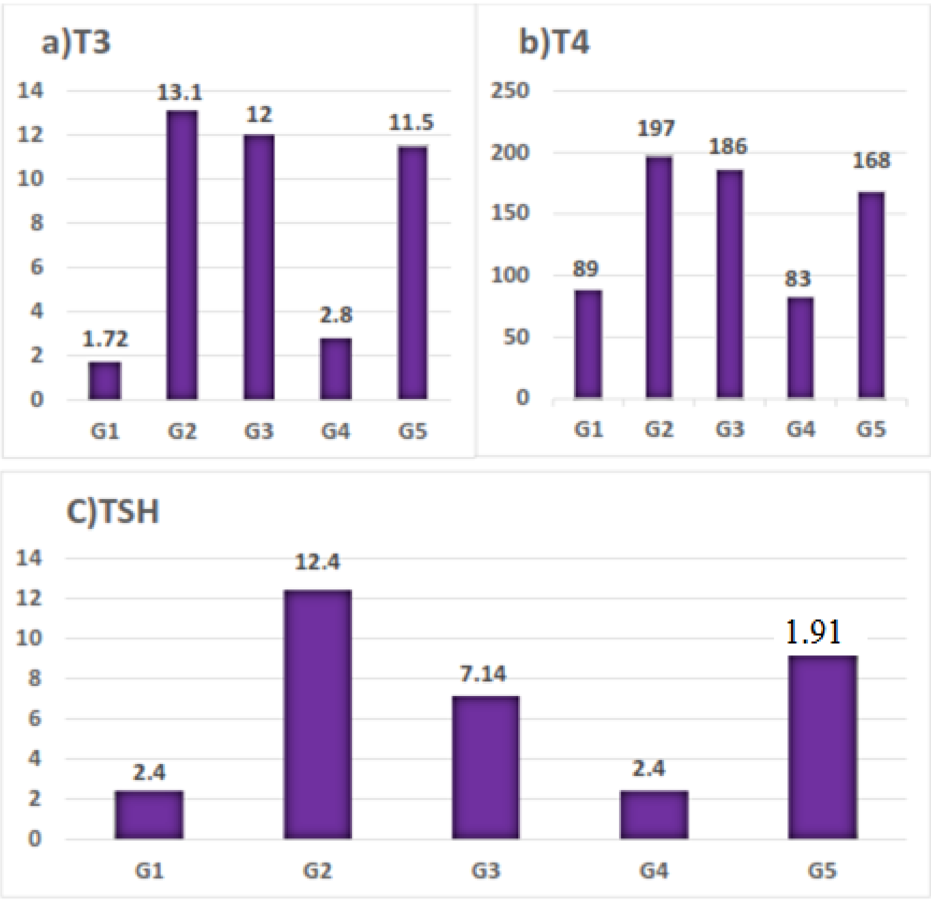

Figure 5: Histograms of thyroid hormones levels of the five rat groups (a) T3, (b) T4, (c) TSH.

4. Conclusion

Indian costus is employed as a nano particle in the medical industry, particularly for treating thyroid conditions. Two approaches were used in this work to prepare the Indian costus nanoparticles: the microwave plasma and the pulsed laser deposition methods. The results indicated that the plasma method is more successful in producing nano particles. The average nanoparticle diameter of the plasma method was 21.11 nm, while the average sample diameter of the PLD method was 40.02 nm. The results also showed that the surfaces of the nano particles are uniform and exhibited less contrast, where the three peaks representing normal and nano Indian nanomaterials appear to have the same value between 200 and 250 nm.

It was concluded that the nano Indian costus prepared using plasma produced results more effective than that of the nano Indian costus prepared by the 200mJ laser, because the arithmetic mean height for the nano material and the maximum height with the plasma prepared have higher values than that prepared by the laser approach. The prepared nano Indian costus was to treat the infected thyroid of rats, since it contains many minerals that aid in restoring the thyroid to its normal state; this was confirmed by measuring the T3, T4, and TSH levels. The treatment with nano Indian costus prepared by microwave plasma gave good results, and the thyroid hormones levels approached the normal levels.

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