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THE EFFECT OF COPPER OXIDE AND SELENIUM NANOPARTICLES ON VITAMIN D LEVELS IN WOMEN WITH INFERTILITY

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ABSTRACT

The nanoparticles of both copper oxide (CuO NPs) and selenium (Se NPs) were synthesized by a laser ablation method that is fast and safe in biological applications. It was Characterization by atomic force microscopy (AFM), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM), which showed that the size of the nanoparticles was 20-23 nm for Se NPs and 19-23 nm for CuO NPs. The effectiveness of these nanoparticles was examined on vitamin D levels in the group of women with primary infertility (PP) and the group of women with secondary infertility (SP). The results showed a highly significant activation effect of the copper oxide nanoparticles, with an activation rate equal to 13% for the primary sterility group (P = 0.003) and 23% for the secondary sterility group (P = 0.00)

Keywords: Nanoparticles; FESEM; Infertility; Vitamin D; CuO NPs; Se NPs

Introduction

Nanoparticles are commonly classified as particulate matter with zero dimension which is less than 100 nm(Christian *et al*. 2008). Copper is a physiologically essential mineral with multiple functions in the human body. CuO nanoparticles (CuO NPs) are strongly targeted towards biological applications due to their distinctive anti-bacterial and anti-fungal behaviour (Stevanović *et al*. 2019). also, selenium nanoparticle has several applications In the pharmacological fields where it works as antioxidants, anticancer agents, and others, where its apparent effect on the biological molecules of the body of the organism is not hidden(Adams and Barbante 2013; Ahmed, Elhissi, and Subramani 2012; Baalousha *et al*. 2014; Capek 2019; Clifford *et al*. 2019; Hornyak and Rao 2016; Leon, Chung, and Rinaldi 2020; Outline 2015; Ray and Salehiyan 2020).

Infertility is a medical disorder or scientific concept for people who do not have the potential to achieve conception from unsafe sexual intercourse for a span of 12 months(Stentz *et al*. 2019), there are two main forms of infertility (1) Primary infertility: It is infertility, in which no pregnancy happens indefinitely. (2) Secondary infertility: It is the form in which a condition of non-pregnancy occurs after a previous pregnancy or childbirth has occurred(Keskin *et al*. 2011).

Vitamin D is a steroidal fat-soluble substance that the body needs to absorb calcium, bone growth, muscular nervous function, control cell development, decrease inflammatory and healthy immune activity. Vitamin D is unique in humans because it could be obtained on the two types of cholecalciferol (Vitamin D3) or Ergocalciferol (Vitamin D2)(Belorusova and Rochel 2016; Boulevard *et al*. 2016; Charoenngam, Shirvani, and Holick 2019; Jin *et al*. 2019; Nagar *et al*. 2019; White 2011).

CuO NPs have a huge array of applications. The copper oxide nanoparticles demonstrate superior catalytic activity and selectivity as compared with ordinary copper oxide powder. Copper oxide (CuO Nps) nanoparticles are of great importance for biomedical and technical applications. They were used in treating biosensors, photodetectors, nanofluids, and wastewater (Sreeju, Rufus, and Philip 2017). Also though CuO nanoparticles have demonstrated their utility in biomedical applications; their potentially toxic effects have the main drawback for their usage in the medical field. NPS copper oxides can be harmful to mammalian cells and to vertebrates and invertebrates (Kung *et al*. 2015).

The Se NPs has properties that make it unique to other metals and metalloids. Researchers chose Se NPs for several reasons due to its use in various industries such as medicinal, chemical, electrical and electronics, ceramics, metallurgy, and glassmaking (Menon *et al*., 2019). The biological functions of Se are carried out primarily by selenoproteins, in which Se is introduced as selenocysteine (Sec) (Al-Quraishy, Adkhil, and Moneim 2015; Hosnedlova *et al*. 2018). Se most known role is to preserve redox homeostasis via a collection of antioxidant selenoenzymes or selenoproteins throughout the body.Se has a small therapeutic window and also the toxicity margins are very fragile while Se (SeNPs) nanoparticles have relatively reduced toxicity (Tran and Webster, 2012).

Polycystic ovary syndrome (PCOS) is a condition that affects a woman's hormone levels. Women with PCOS produce higher-than-normal amounts of male hormones. This

hormone imbalance causes them to skip menstrual periods and makes it harder for them to get pregnant.

The purpose of this study is to find out the effect of both copper oxide and selenium nanoparticle vitamin D levels in sera of patients with infertility and the possibility of having a therapeutic role in the future to raise vitamin D levels.

Materials and Methods

Synthesis of nanoparticles

The nanoparticles of selenium and copper oxide were prepared using a pulsed laser ablation method. The selenium tablet was prepared by pressing selenium powder by manual hydraulic pressure of 5 tons and then left in the mold for 15 minutes and then removed and washed with ethanol. Then a selenium disk with a diameter of 1 cm 2 was placed in a glass baker containing 5 ml of deionized water, which is 1 cm above the target, where the colloidal solution was prepared by irradiation of the target by Nd: YAG laser (type HUAFEI) of the wavelength of 1064 nm, 1 Hz frequency for 10 minutes. The laser energy used for eradication was 500 mJ / pulse, then the solution would begin to switch from colorless to red (Gangadoo *et al*., 2017). As for copper oxide (CuO NPs), a pure piece of copper was used under the same conditions, except that the energy exposure to the laser was 200 MJ and at a frequency of 6 Hz for 15 minutes(Gondal *et al*. 2013; Khashan, Jabir, and Abdulameer 2018). Figure 1 shows a method for preparing these nanoparticles.

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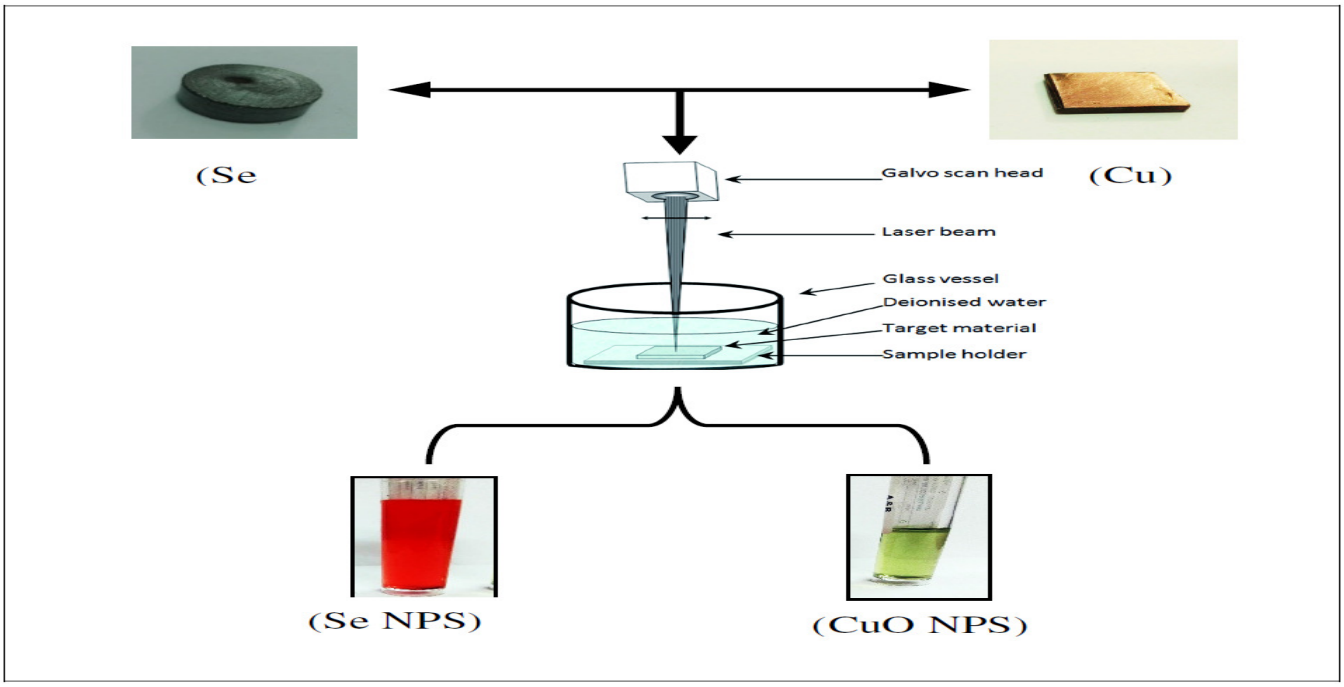

Fig. 1 : Synthesis of selenium and copper oxide nanoparticles by pulsed laser ablation

Characterization

CuO and Se nanoparticles were characterized by some devices. Size and morphology of nanoparticles were measured by field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). As for the elements or compounds that make up these particles (structure), they have been examined with X-ray diffraction (XRD) and UV–visible spectra analysis to see the absorbs of the Plasmon region.

Patients

In this study, samples were collected from ninety Iraqi women specifically from Anbar Governorate: 60 women have infertility (P) in addition to 30 fertile women (C). They were divided into three groups as follows: 30 women with primary infertility (PP), 30 women with secondary infertility (SP) and 30 fertile women (C).

Serum 25-OH vitamin D measurement

Vitamin D levels were measured in both patients and the control by using Monobind Inc. ELISA Kit (USA). This was done in two stages, as the first stage took place without the presence of nanoparticles, while the second stage was measured for the same groups in the presence of nanoparticles, where the measurement was once with the presence of selenium nanoparticles and once with the presence of copper oxide nanoparticles, as the incubation period for the nanoparticles with serum was 15 minutes Whereas, the volume of these particles was one-fifth of the serum added in the measurement.

Results and Discussion

UV–visible spectra analysis

Figure 2 shows the absorption spectrum of CuO and Se nanoparticles in highly purified water, where we can observe a high intensity absorption peak of around 220 nm for copper oxide nanoparticles and 270 nm for selenium nanoparticles, in addition to a peak around 650 nm of low intensity They return to CuO NPs and others about 535 nm refer to Se NPs called plasmon regions (SPR)

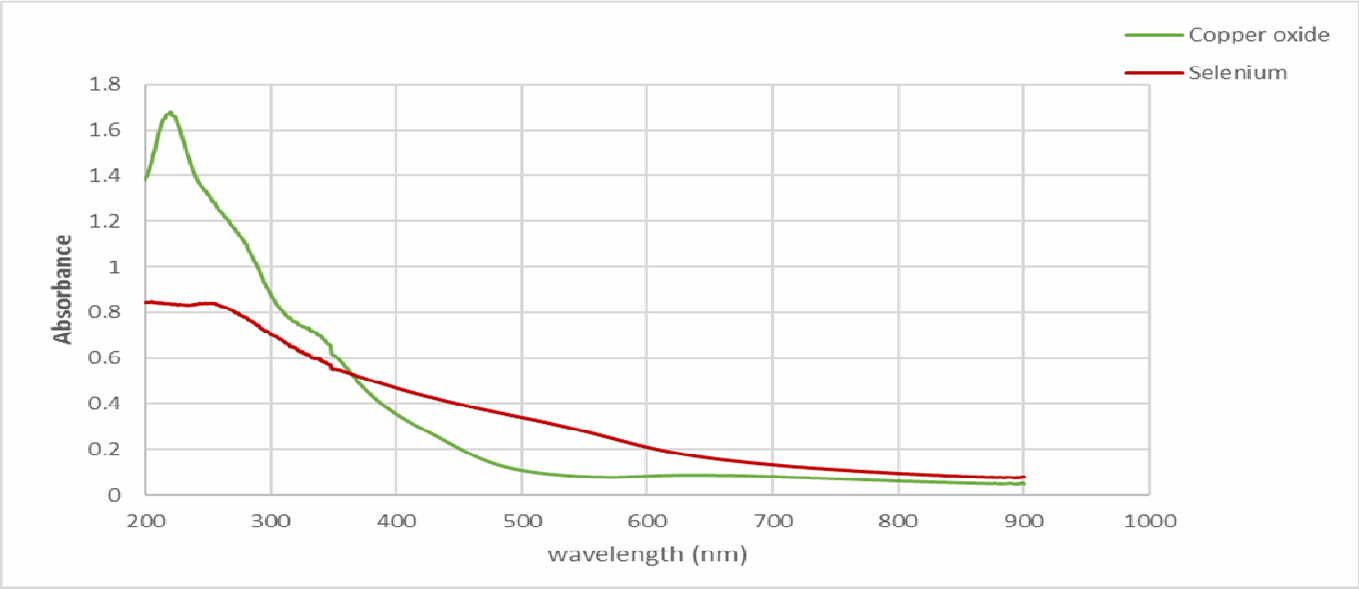

Fig. 2 : UV–visible spectra analysis of CuO and Se NPS

Atomic-force microscopy

Figure (3-a) and (3-b) show the results of the AFM for copper oxide (CuO NPs) and selenium (Se NPs) nanoparticles that have been prepared by laser ablation method. Where the average diameter was 75.56 nm for copper oxide nanoparticles. Where these results are in agreement with Ayad Z. Mohammed (Mohammed, Hamza, and Khaleefah 2016) and Al-Antaki (Al-Antaki *et al*. 2019) who prepared the copper oxide nanoparticles in the same way, As for the selenium nanoparticles, the average diameter was 67.14 nm, which is consistent with the results of (Abdollahnia *et al*. 2020; Geoffrion *et al*. 2020). The image of the atomic force microscope shows the shape of the surface, the shape, and the size of the particles for the samples that have been identified. It also shows a two-dimensional (2D) and three-dimensional (3D) image of the sample.

X-ray diffraction (X-RD)

Analyzed the structure of (CuO NPs) four peaks could be recognized in Figure 4-a where the samples are poorly crystalline apatites according to ASTM standards were (110), (111), (200) and (220). The crystal size is measured and calculated using the Scherrer equation to be equal to 11.10 nm at 2θ =32.51 from the full width of the highest peak = 0.747 for the summit (110). As while as XRD diffraction patterns for selenium nanoparticles were identified in Figure 4-b where the Se NPs structure was analyzed and three peaks were identified as follows (100), (101) and (110). The crystal size was calculated using the Scherrer equation at 2theta = 23.515 and the full width of the highest peak = 0.452 for the summit (100) and found it equal to 15.79 nm.

Field emission scanning electron microscopy

Figures 5-a and Fig. 5-b show FESEM results for both copper oxide and selenium nanoparticles, respectively. Where it is clear that the nanoparticles have an average diameter (20-23 nm) for selenium nanoparticles and (19-23 nm) for the copper oxide nanoparticles.

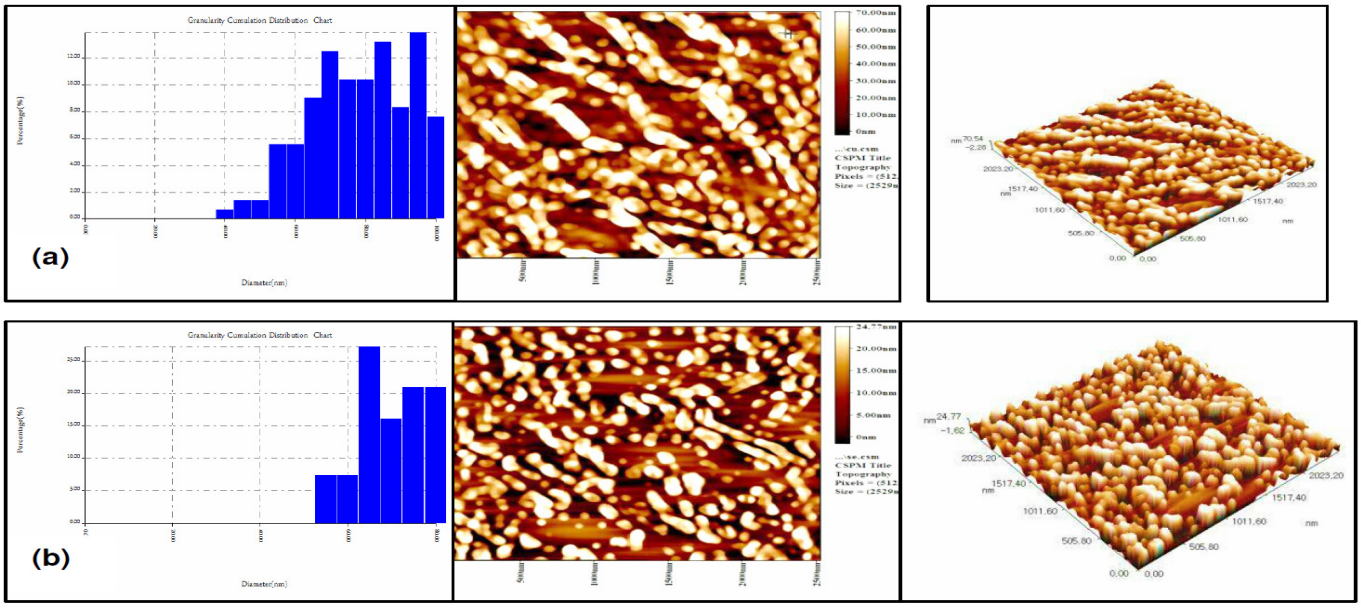

Fig. 3 AFM images and size distribution histogram of **(a)** CuO NPs and **(b)** Se NPs

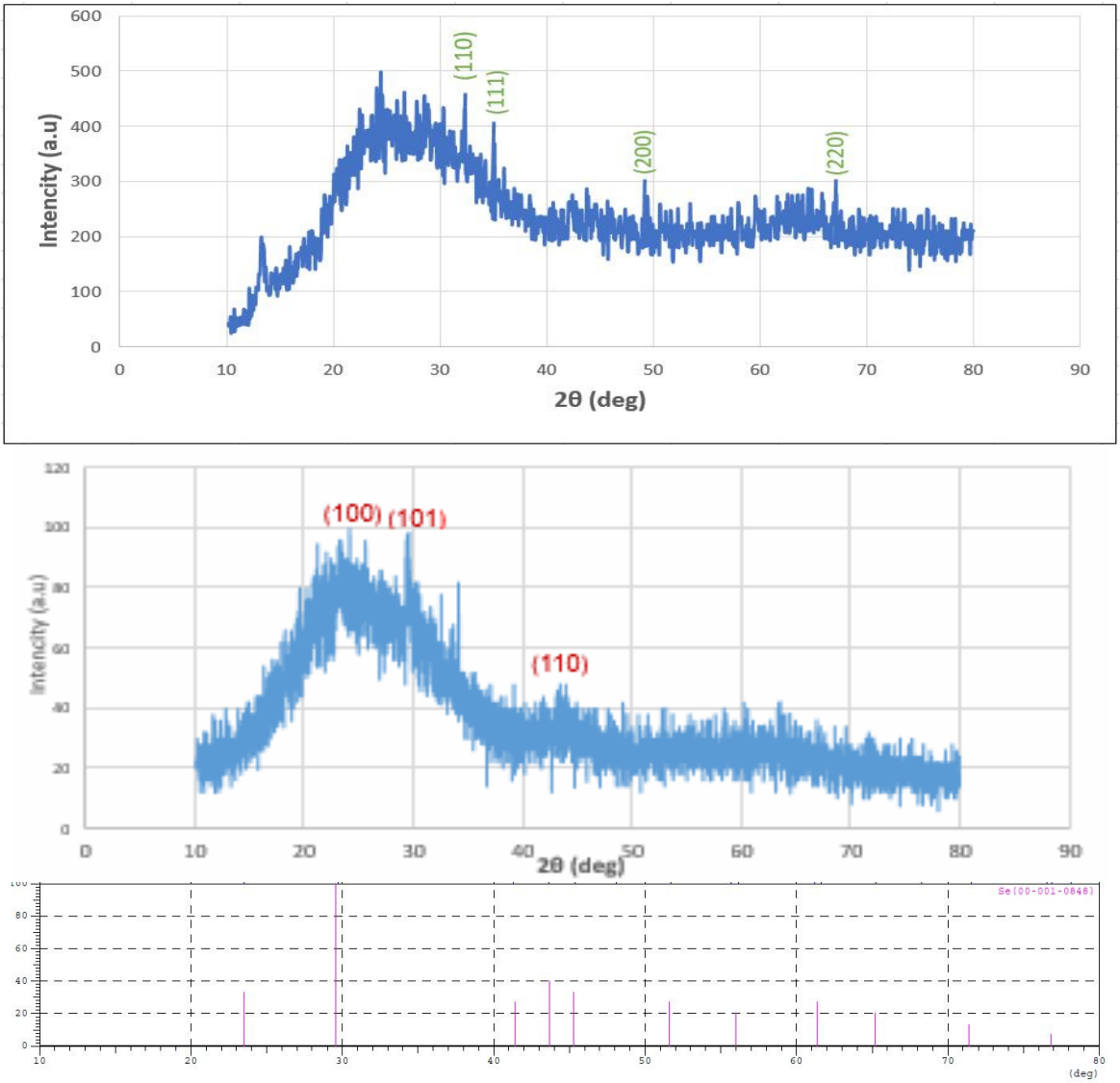

Fig. 4 : X-ray diffraction pattern of**1 (a)** CuO NPs and **(b)** Se NPs

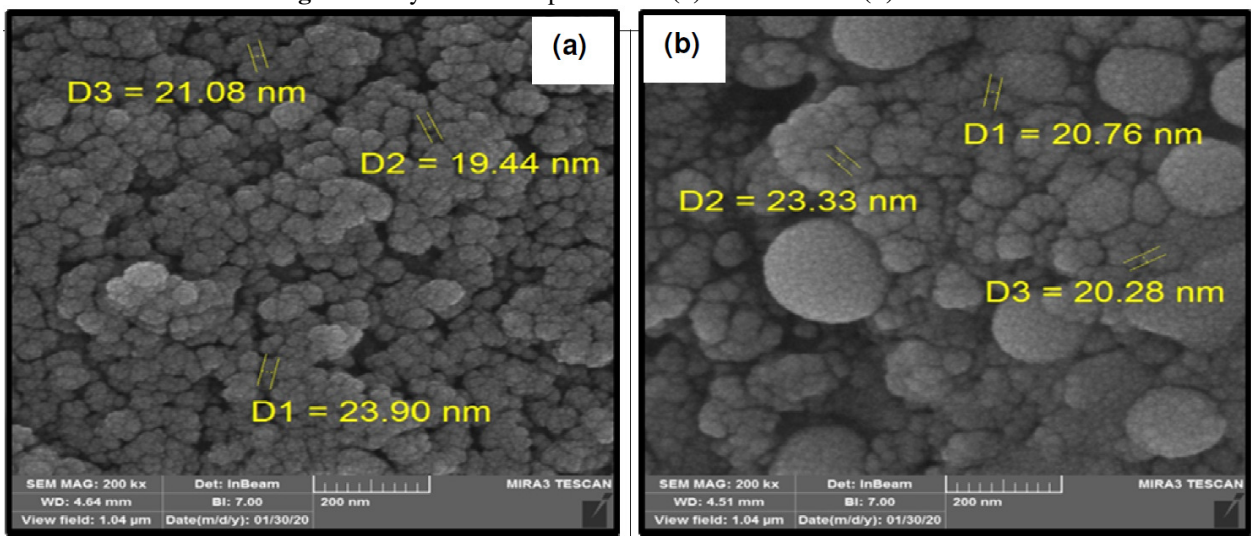

Fig. 5 : FESEM of **(a)** CuO NPs and **(b)** Se NPs

Table 1and figure 6 shows the levels of vitamin D in both patient groups and compared them with the vitamin D levels of the control group. As it appeared that the percentage of those who suffer from a deficiency in the levels of vitamin D for the group PP is 86.66%, 100% for SP and 73.33% for the control group. As for the proportions of those who are satisfied with the levels of vitamin D, they are 13.33% for PP and 26.66 for the control group. also show the vitamin D levels of the two groups of patients and their comparison with the control group, where the PP(16.92 ±2.448), (15.31 ±2.371) for SP and (17.27 ±3 .45) for group C, it was not significant with a value of P>0.05 (P=0.185). The results of this study with respect to vitamin D indicate that there is no relationship between the percentage of vitamin D and

infertility in women, as the cause of infertility may be PCOS or others, and this is consistent with Mogili KD, Sadhir M and Shapiro (Mogili *et al*. 2018; Sadhir, Kansra, and Menon 2015; Shapiro *et al*. 2018). There are studies that indicate that the deficiency in vitamin D negatively affects the process of artificial fertilization and that those who do not suffer from this deficiency have a greater success rate for this process (Ozkan *et al*. 2010; Paffoni *et al*. 2014).

Effect of nanoparticles on vitamin D levels in patient groups

Table 2 with Figures 7 and 8 show the effect of these nanoparticles on vitamin D in both groups of female infertile patients for this study. As the results indicate a significant effect of copper oxide nanoparticle on vitamin D levels in infertility patients for both groups where the mean was before adding the nanoparticle (mean = 16.92 ng/ml for PP) and (mean= 15.31 ng/ml for SP), but after adding the nanoparticle it was (mean = 19.1 ng/ml for PP) with a value of p = 0.003 (p <0.05) and with a percentage of activation equal to 13%, (mean = 18.81 ng/ml for SP) with a value of p = 0.000 (p <0.01) and with a percentage of activation equal to 23%, where these results indicate that these bodies have a positive effect on vitamin D levels at a higher percentage in the SP group. As for selenium nanoparticles, there was no significant effect, where after adding the nanoparticle it was (mean = 17.60 ng/ml for PP) with a value of p = 0.138 (p >0.05) and with a percentage of activation equal to 4%, (mean = 16.04 ng/ml for SP) with a value of p = 0.060 (p >0.05) and with a percentage of activation equal to 5%. That is, the selenium nanoparticles have no effect on vitamin D levels. Among the possible causes of vitamin D deficiency in a large proportion of the population, especially those who are overweight or obese, is the fact that the fat tissue has blocked vitamin D within it (McKay *et al*. 2020; Pourshahidi 2015). As *Leilei Guo et al* (Guo *et al*. 2020), *Reham Z. Hamza et al* (Hamza *et al*. 2020) and *Saleh Al-Quraishy et al* (Al-Quraishy, Adkhil, and Moneim 2015), mentioned, selenium nanoparticles reduce levels of triglycerides, cholesterol and low density lipoprotein, while increasing levels of high density lipoproteins. Which may explain the high levels of vitamin D after adding these particles to the serum, given the possibility of release of vitamin D from the fatty tissue. Given that activation was not significant, this may be because the concentration of nanoparticles was not sufficient. Where it was not possible to take more than one concentration due to lack of financial capabilities. As for the copper oxide nanoparticles, *Rama Narsimha*(Anreddy 2018), mentioned that these particles increase the lipid peroxidation process, which in turn may be the reason these nanoparticles affect the elevation of vitamin D levels, which has an effect on fats.

Table 1: Distribution of vitamin D for patients and control groups

Parameter		C group n=30		PP group n=30		SP group n=30	
		NO.	%	NO.	%	NO.	%
Vitamin D (ng/ml)	<20(deficiency)	22	73.33	26	86.66	30	100
	21-29 (insufficiency)	8	26.66	4	13.33	0	0
	≥30(sufficiency)	0	0	0	0	0	0
	Mean± SD	17.27±3.45		16.92±2.448		15.31±2.371	
	P value	0.185					

Table 2: Distribution of the effect of selenium and copper oxide nanoparticles on vitamin D in patient groups

Statistics	Vitamin D (ng/ml)			
	PP		PS	
	PP + CuO NPs	PP + Se NPs	PS + CuO NPs	PS + Se NPs
Mean± SD (ng/ml)	16.92±2.448		15.31±2.371	
	19.10±3.458	17.60±3.558	18.81±2.952	16.04±2.238
P value	0.003	0.138	0.000**	0.060*

(PP): Primary patient, (SP): Secondary patient, (C) : control
P> 0.05: not significant, (*): significant P< 0.05, (**) highly significant P<0.01

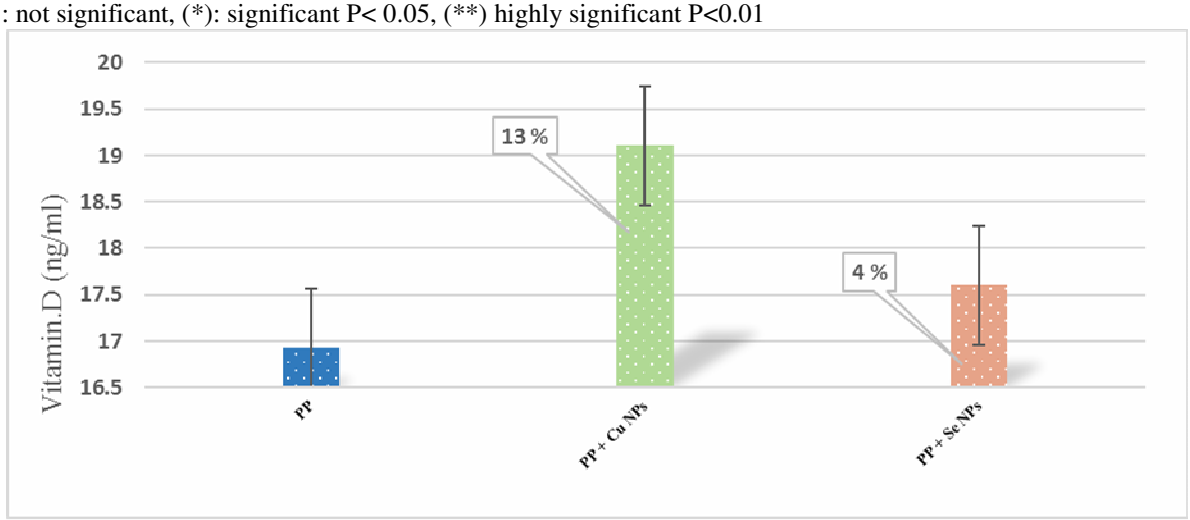

Fig. 6 : Vitamin D levels of patients and control groups

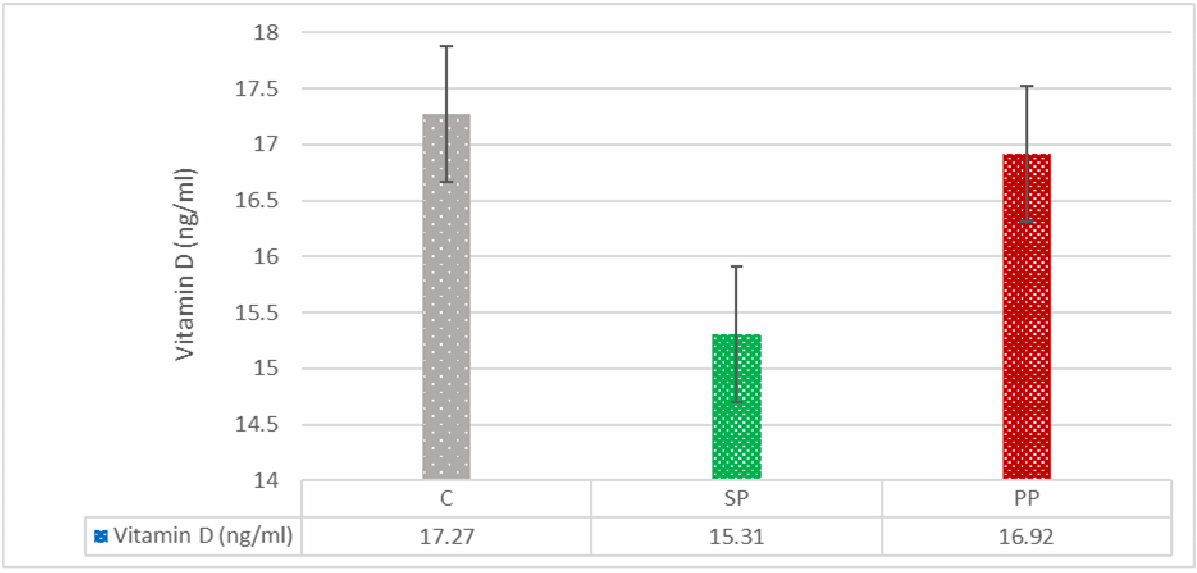

Fig. 7 : The effect of selenium and copper oxide nanoparticles on vitamin D levels of PP groups

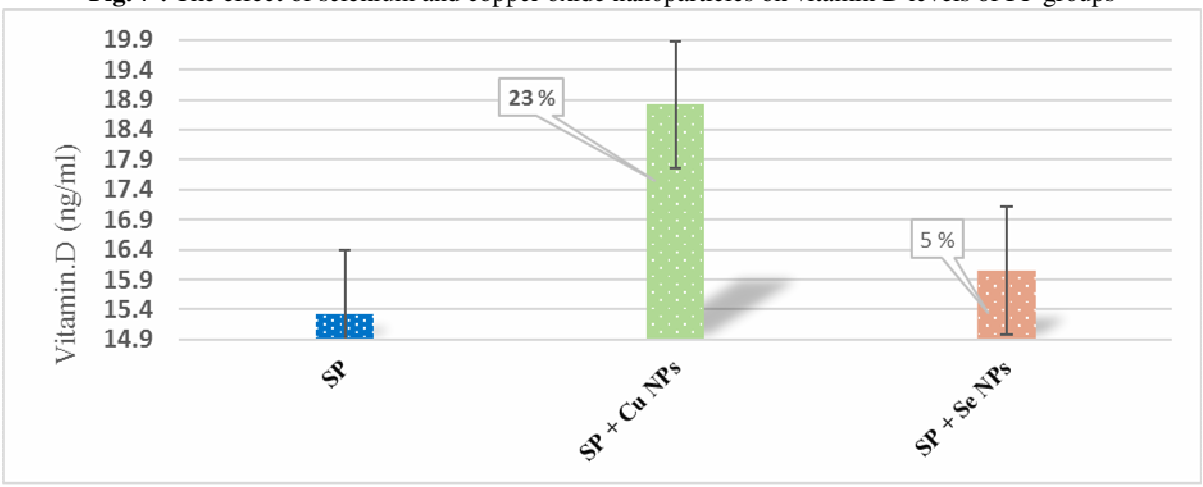

Fig. 8 : The effect of selenium and copper oxide nanoparticles on vitamin D levels of SP groups

Conclusion

The laser ablation method used to synthesize nanoparticles in this study is an easy, straightforward and pure method, as it is easy to use in biological applications. The results of the FESEM examination showed that the size of the nanoparticles of copper oxide was (20-23 nm) according to a test whereas the selenium nanoparticles (19-23 nm) were in agreement with the results of X-ray diffraction which showed that the average crystalline size was 12 nm with respect to copper oxide and 15. 3 nm selenium nanoparticles, In addition, results of AFM, Where the average diameter was 75.56 nm for copper oxide nanoparticles and for the selenium nanoparticles, the average diameter was 67.14 nm. All infertile and healthy patients in this study suffer from a deficiency in vitamin D levels and this may be due to the influence of the geographical area or the effect of the lifestyle. The effect of nanoparticles on vitamin D levels was a stimulating effect, as it had a significant effect of copper oxide NPs for both groups (p=0.00 for PP, p=0.003 for SP), while selenium nanoparticles had no significant effect (p=0.060 for PP, p=0.138 for SP). Among the possible causes of vitamin D deficiency in a large proportion of the population, especially those who are overweight or obese, due to the fact that the fat tissue has blocked vitamin D within it(McKay *et al.* 2020; Pourshahidi 2015). (Guo *et al.* 2020), Reham Z. (Hamza *et al.* 2020) and (Al-Quraishy, Adkhil, and Moneim 2015), mentioned, selenium nanoparticles reduce levels of triglycerides, cholesterol and low density lipoprotein, while increasing levels of high density lipoproteins. Which may explain the high levels of vitamin D after adding these particles to the serum, given the possibility of release of vitamin D from the fatty tissue.

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