

**FULL PAPER**

# The effect of selenium nanoparticles with fenugreek extract on oxidative stress related to polycystic ovary syndrome

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In recent years, there has been growing interest in using Nanosystems in different biomedical applications. Among all metal nanoparticles, selenium nanoparticles have attracted the attention of many researchers due to its low toxicity and nutritional supplementation value. The purpose of the current study was designed to examine the possible effect of selenium nanoparticles in combination with fenugreek leaves extract (an edible herb with good medicinal properties) in the treatment of oxidative stress status-related to polycystic ovary syndrome in letrozole-induced PCOS (an imbalance of reproductive hormones that causes infertility) in adult female rats. Cold plasma was used in the preparation of selenium nanoparticles subsequently the produced nanoparticles were characterized. Thirty rats were divided into six equal groups, including a healthy rat handled with distilled water given orally. To induce the PCOS, rats were given letrozole (1 mg/kg) B.W daily for 21 days, (the letrozole was dissolved in 1% carboxymethylcellulose. The second group was left without any treatment (PCOS group), and the rats in the other 4 groups were treated orally and daily for 30 days using the following treatments: metformin, fenugreek extract only, SeNPs only, and fenugreek extract with SeNPs, where used SeNPs at 10 min of exposure to plasma. Biochemical tests (amylase, superoxide dismutase, and malondialdehyde) levels as well as histopathological examination were performed. The outcomes of the present study show the effective effect selenium nanoparticles in combination with fenugreek leaves extract for the PCOS treatment which can be suggested as a new drug in the PCOS management.

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**KEYWORDS**

Polycystic ovary syndrome; selenium nanoparticles; cold plasma; fenugreek extract; oxidative stress.

**Introduction**

Nanomedicine is the application of nanotechnology-based techniques and methods in medical study and clinical practice for the diagnosis, treatment, and control of biological systems [1,2]. In recent

years, there has been growing interest in using nanosystems in different biomedical applications such as photoablation therapy, bioimaging, biosensors, antibacterial agent, and targeted drug delivery [3-5]. Nanoparticles solve various pharmacokinetic and biopharmaceutical problems associated

with many drugs in a variety of disease classes, they can also cross physiological barriers (blood-brain barrier and placental barrier) due to their absorptive and size-dependent properties [6,7]. Among all metal nanoparticles, selenium nanoparticles (SeNPs) have attracted the attention of many researchers due to its low toxicity and nutritional supplementation value [8,9]. SeNPs find enormous applications since SeNPs show lowering in risk compared with selenium, such as an anticancer [10], antidiabetic [11], and antimicrobial [12] agents. The most common endocrine disease (Polycystic ovary syndrome; PCOS) affects approximately 10% of all women. The majority of women with PCOS suffer from obesity and insulin resistance [13]. A lot of research has shown that oxidative markers are increased in PCOS women compared with normal, also are considered as a possible inducement of this syndrome pathogenesis [14]. Oxidative stress is used to describe the imbalance between the production of reactive oxygen species (ROS) and the protection of the body by antioxidants. It has a central role in the pathophysiology of several different diseases, including PCOS. High oxidative stress levels can affect fetal growth restriction, miscarriages, or fetal death [15]. Hyperglycemia, obesity, and insulin resistance are factors in PCOS that increase oxidative stress; however, non-obese PCOS women without insulin resistance are also reported to have high production of ROS, suggesting that other factors may contribute to increased oxidant status in these women [16]. Currently, because there is no certain and ideal treatment for hormonal disorders and associated clinical manifestations, thereby alternative treatment, especially phytotherapy, can be considered instead of commercially available drugs. Various medicinal plants are used as anti-infertility therapeutic agents. These plants possess different types of anti-infertility activities since it contains active compounds and have

no major adverse effects, they have attracted great attention in recent years. For example, Fenugreek (*Trigonella foenum-graecum L.*) is one of these plants and contains large amounts of certain phytoestrogens such as biochanin A, daidzein, genistein, and formononetin. Fenugreek is a plant of the *Fabaceae* family, different countries around the world including Iraq cultivate and utilize the fenugreek leaves as well as seeds of this plant for different purposes in many traditional systems as a dietary supplement also an antidiabetic agent, gastric stimulant, antibacterial agent [17], antioxidant activity, and anti-inflammatory effects [18]. Fenugreek is rich in numerous valuable biologically active secondary metabolites like alkaloids, phenolics, volatile oils, saponins, vitamins, and carbohydrates [19]. The fenugreek anti-androgenic property causes a reduction level of androgen in PCOS-induced rats [20]. The purpose of the current study is to examine the possible effect of selenium nanoparticles in combination with fenugreek leaves extract in the treatment of oxidative stress status-related to polycystic ovary syndrome in letrozole-induced PCOS in adult female rats.

## Material and methods

### *Preparation of fenugreek extract (FE)*

Fenugreek leaves were obtained from a local market in Baghdad city, Iraq. The plant was air-dried, and then 2 g of sample were mixed with 20 mL of distilled water in conical flasks, shaken well, and covered with aluminum foil. A hot air oven was used to achieve 100 °C for 10 min by using a gas oven. After the extraction processes, the solutions were filtered and kept for future work [21].

### *Preparation and characterization of selenium nanoparticles*

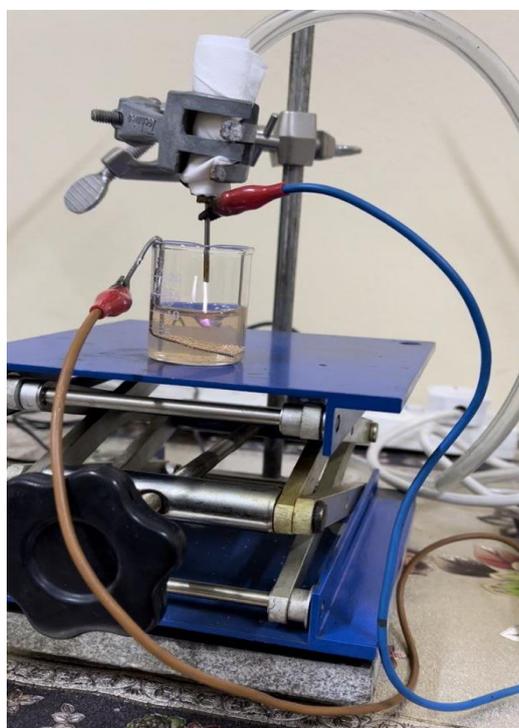
#### *Selenium nanoparticles Preparation*

To prepare SeNPs cold plasma system was used, as displayed in Figure 1. The system consisted of Argon gas and gas flowmeter for gas intake controlling. They connected to the power supply (cathode), which equipped with intermittent and continuous high voltage (25 kV). They had a stainless steel conductive that connect to the anode. In addition, metal tube holder, which carried the

glass beaker that contained the solution. The experimental conditions are mentioned in Table 1. A volume of 10 mL of 0.5 mM concentration from selenium nitrate was prepared [22,23]. The prepared form was placed in a 25 mL beaker and exposed at different times to plasma (6, 8, 10, and 12) minutes.

**TABLE 1** Experimental conditions

Voltage	25 kV
Operating gas	Argon
Concentration	0.5 mM
Frequency	25 kHz
Exposure time	6, 8, 10, 12 minutes



**FIGURE 1** The cold plasma system using to prepare SeNPs

#### Characterization of Selenium nanoparticles

##### UV-Visible spectroscopy (UV- Vis) analysis

Absorption spectra of the synthesized selenium nanoparticles were obtained using a double beam UV-Visible spectrophotometer (Shimadzu UV-Vis 1800 spectrophotometer, Japan). The UV-visible spectra of the synthesized SeNPs were achieved at a wavelength ranging from (200-800) nm.

##### X-ray diffraction (XRD) analysis

The X-ray diffraction (XRD) patterns of the synthesized SeNPs were assessed using (Podwe XRD, 2700AB HAOYUAN Co, China). To get XRD patterns of the SeNPs, operating at 45kV and 40 mA with 2-theta configuration.

### Animals

Thirty female rats (mean body weight 170-200 g) were caged from the animal's house at the University of Al-Nahrain. The animals have been kept in plastic cages and the foodstuff was given as pellets. In this experiment, 30 rats were divided into six equal groups, including a healthy rat (negative control group) of 5 rats handled with distilled water given orally. To induce the PCOS, rats were given letrozole (1 mg/kg) B.W daily for 21 days, (the letrozole was dissolved in 1% carboxymethylcellulose (CMC) [24]. The second group was left without any treatment (PCOS group), and the rats in the other 4 groups were treated orally and daily for 30 days using the following treatments: metformin (100 mg/kg; metformin group), fenugreek extract only (2mL/kg; FE group), SeNPs only (2mL/kg; SeNPs group), and Fenugreek extract with SeNPs (1:1 ratio, 2mL/kg; FE+SeNPs group), where used SeNPs at 10 min of exposure to plasma.

### Determination of serum amylase, SOD, and MDA levels

Serum Amylase level assay by rat Amylase ELISA kit (SunLong Biotech, China) Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels were measured by using ELISA kits (SunLong Biotech, China).

### Histopathological analysis

The histological investigations were carried out due to the standard methods according to [25]. All sections were assessed by an optical microscope (NIKON) at a magnification of  $\times 100$ .

### Statistical Analysis

All statistics were done by GraphPad Prism (v 7.00) "GraphPad Software, USA". In data

analysis, the star symbols state the degree of significance as follows: \*, \*\*, \*\*\*, and \*\*\*\* were representing: ( $P \leq 0.05$ ), ( $P \leq 0.01$ ), ( $P \leq 0.001$ ), and ( $P \leq 0.0001$ ), respectively.

## Results

### Selenium nanoparticles tests

In the current research, SeNPs were prepared using cold plasma, and their formation was observed by color-changing the solutions from colorless to orange (Figure 2). The surface plasmon resonance causes these colors change in the selenium nitrate solution and the selenium particles.



**FIGURE 2** Selenium nanoparticles after exposure to plasma

The UV-Vis spectral analysis was used to prove the SeNP formation in different exposure times to plasma (6,8,10,12) minutes. The result exposed a peak at 290 nm at a range between (200-800) nm. This proves the formation of SeNP (Figure 3). This result is in good agreement with those obtained by the other authors, who reported the presence of a UV-Visible absorption maximum between 200-400 nm during the SeNPs synthesis [26-28].

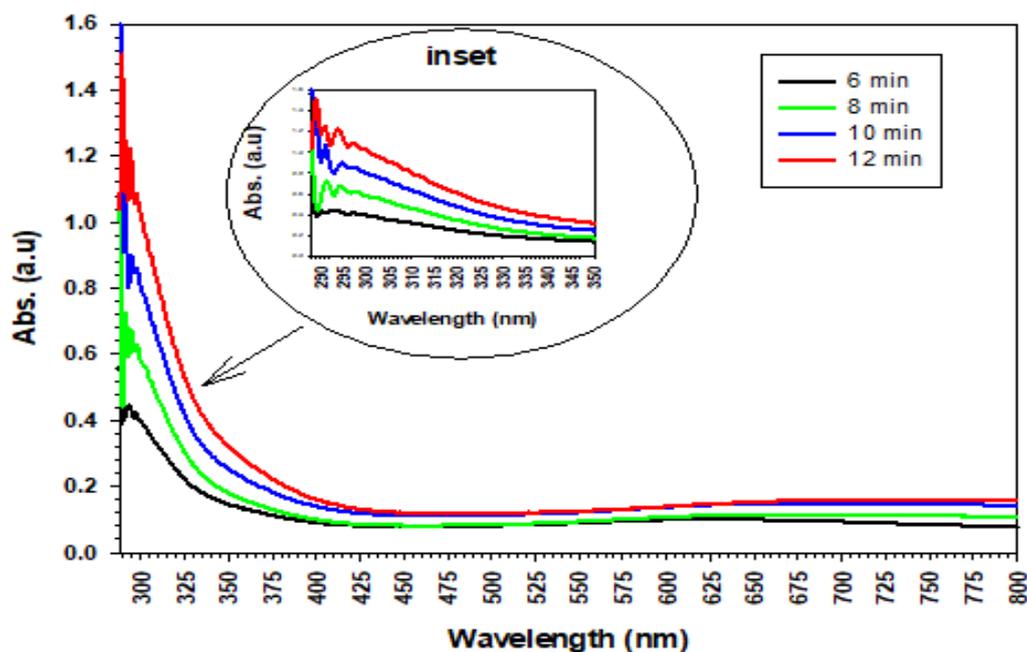


FIGURE 3 UV-Vis spectra of selenium nanoparticles

The XRD analysis of dried selenium particles prepared using cold plasma was done to confirm the presence of nanocrystalline selenium particles. This XRD analysis was mainly performed to confirm the presence of elemental selenium nanoparticles. The peaks of XRD  $2\theta$  are

observed at  $30^\circ$  after 8 and 10 min of exposure to plasma (Figure 4). This indicates the presence of selenium nanoparticles. This result is in agreement with those reported by other authors [27,29,30]. The best results were at 10 min of exposure to plasma.

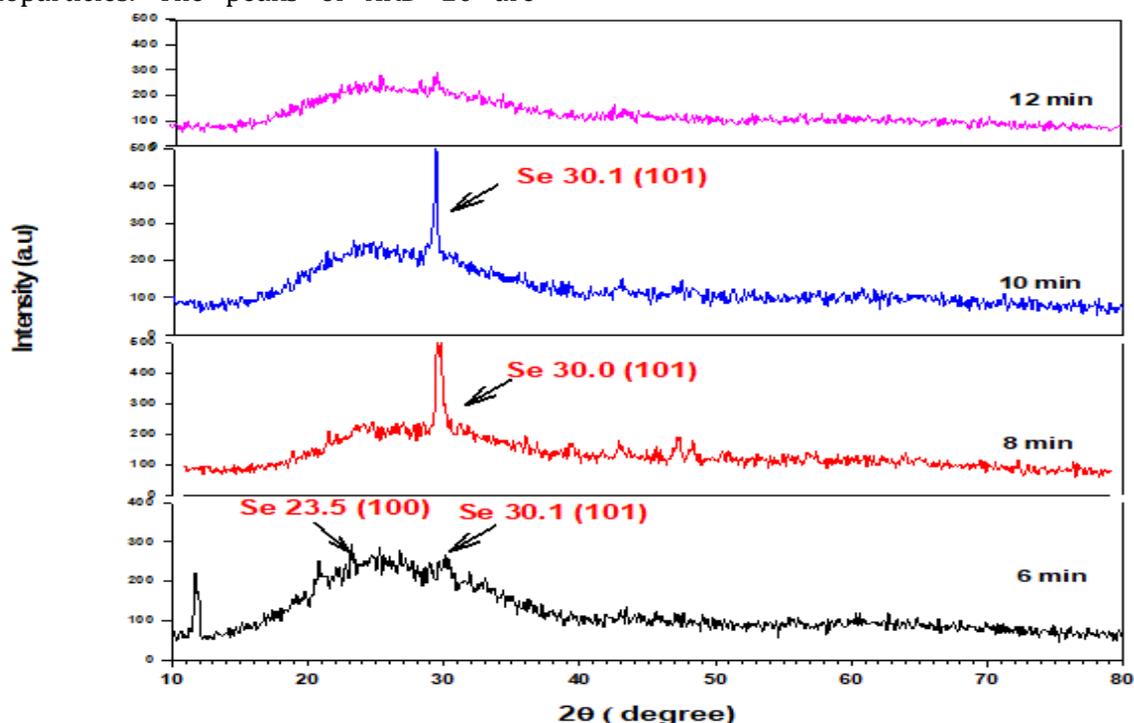
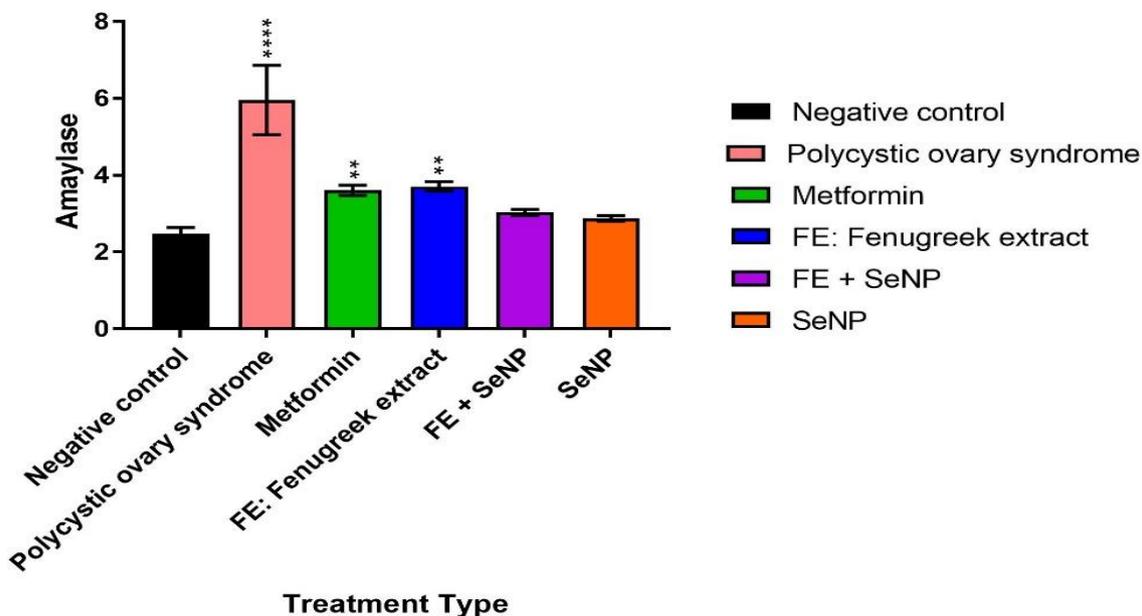


FIGURE 4 X-ray pattern of selenium nanoparticles

*Effect of fenugreek extract, selenium nanoparticles, and metformin on amylase levels*

Figure 5 displays that PCOS group showed a significant increase in amylase levels, as

compared to the control, and then a significant decrease in the levels of amylase in the fenugreek extract, selenium nanoparticles, and metformin treated groups, as compared with PCOS group.

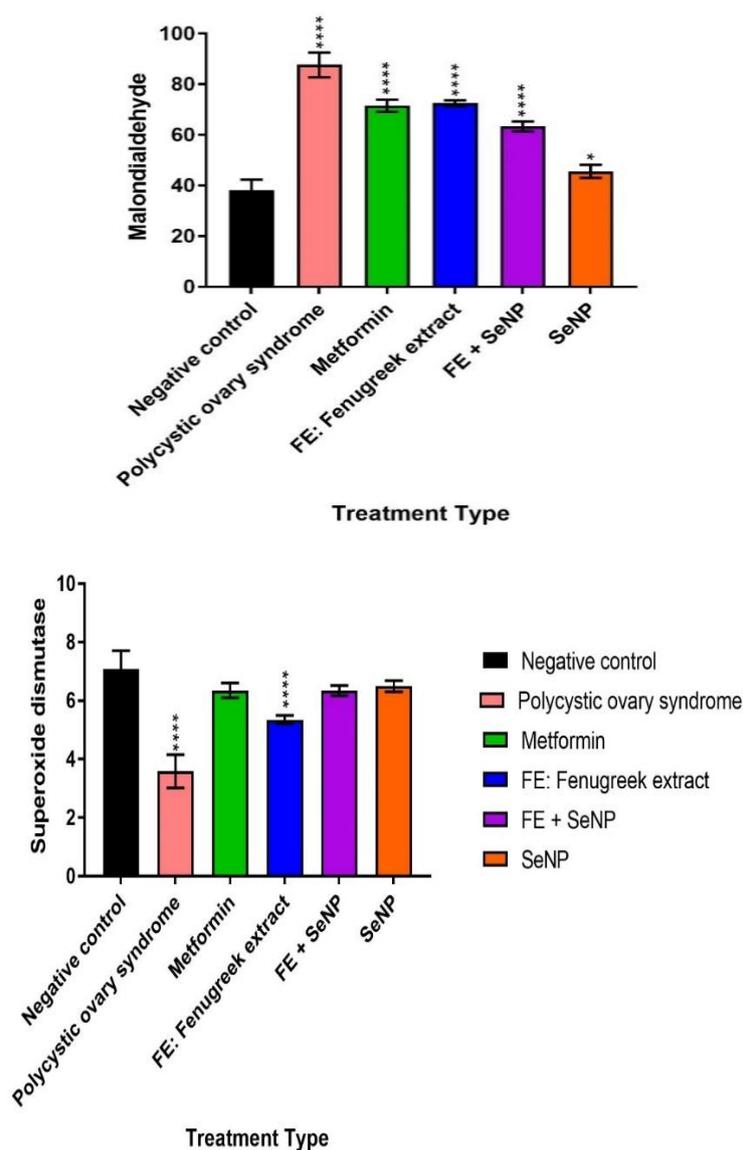


**FIGURE 5** Effect of FE, SeNPs, and metformin on Amylase levels

*Effect of fenugreek extract, selenium nanoparticles, and metformin on antioxidant profile*

In Figure 6A, MDA value of PCOS group was significantly high compared with control, while there was a significant decrease

observed in FE, SeNPs, and metformin treated groups. While in Figure 6B., for PCOS group, the activity of SOD was reduced significantly, as compared with control. Whereas there was also significant increase in SOD observed in FE, SeNPs and metformin treated groups.



**FIGURE 6** Effect of FE, SeNPs, and metformin on (A) MDA (B) SOD

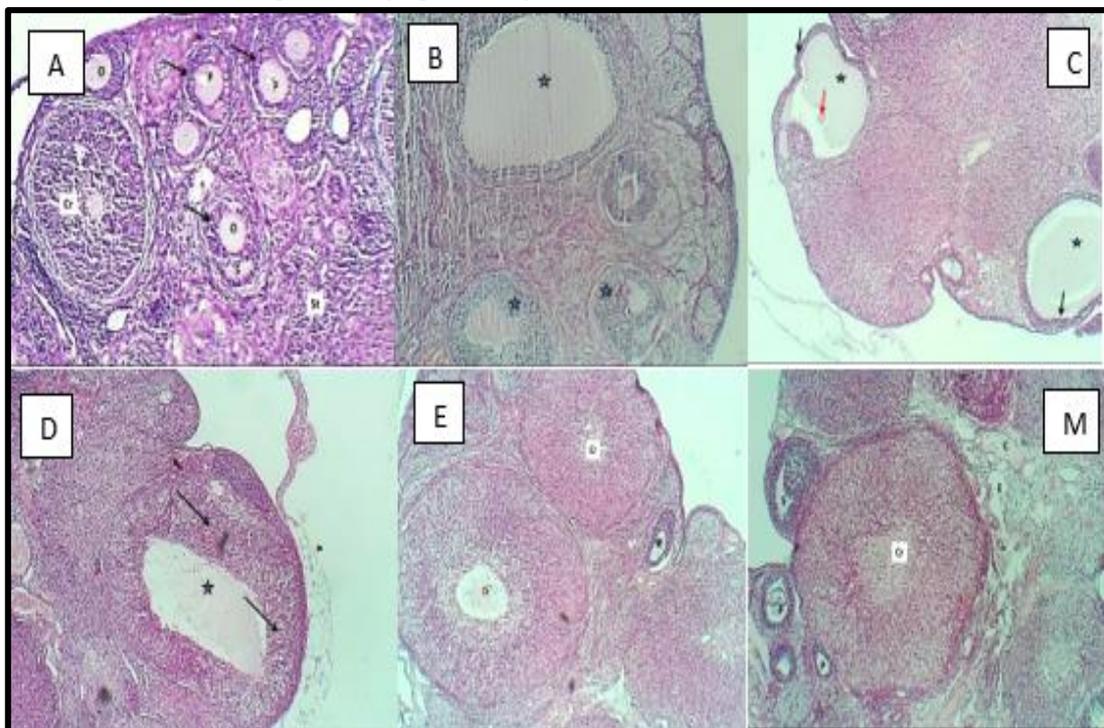
*Effect of fenugreek extract, selenium nanoparticles, and metformin on histopathology of ovaries*

Figure 7 displays the histological investigation of rats' groups, the ovary section of control group showed the outer cortex covered by germinal epithelium, number of primary, secondary, and a single tertiary follicles and corpus leutium (Figure 7A), whereas in the PCOS group (Figure 7B) showed several follicular cysts in variables sizes and a very thin layer of granulosa cells with fibrous layer (Figure 7C). This ovary

section of rat was treated with fenugreek extract displayed one follicular cyst in intermediate-sized with thick granulosa cells layer, primary, and secondary follicles as well as several corpus leutium. (Figure 7D) of rats treated with selenium nanoparticles exposed multiple small-size follicular cysts that created from secondary follicles with thin granulosa layer. In addition, there were many corpus leutium. The ovary section of rats treated with Fenugreek extract and Selenium nanoparticles (Figure 7E) exposed single large-sized follicular cyst and a thick granulosa cells layer, and also shows normal

tertiary follicle, primary follicle, and small sizes secondary follicle. The cortical stromal tissue showed mild per follicular stromal edema and vascular congestion. (Figure 7M)

of metformin-treated rat exposed follicle and the corpus luteum with many layers of granular cells.



**FIGURE 7** Histopathology of ovaries (A) Control (B) PCOS (C) Fenugreek extract (D) Selenium nanoparticles (E) Fenugreek extract and Selenium nanoparticles (M) Metformin

## Discussion

The findings of our present study showed that serum amylase level was higher in the PCOS group compared with control group, serum amylase levels are increased in PCOS which reflect metabolic abnormalities and abnormal glucose metabolism, both of which are associated with insulin action due to the insulin resistance [31]. Serum amylase level decreased after treating with metformin, Fenugreek extract, and SeNPs, as compared with the PCOS-induced group. The presence of polyphenols and flavonoids in fenugreek extract might be responsible for such an activity [32]. Amylase is considered as an essential cofactor in the metabolic rate of carbohydrates. Thus, amylase inhibiting is a more effective way to reduce the level of blood glucose. Meanwhile, our finding showed that selenium nanoparticles decrease

serum amylase levels. Similarly, it has been mentioned that green synthesized selenium nanoparticles that prepared from sodium selenite demonstrated  $\alpha$ -amylase inhibitory [33].

Antioxidant enzymes are an important defensive mechanism that controls the overproduction of reactive oxygen species (ROS) which usually results in the normal functioning of cells and their biological activities [34]. In the present study, the PCOS group showed the increased levels of MDA indicating the excessive production of ROS leading to molecular damage and disturbance of cellular structure [14,35]. The MDA level in the metformin-treated group was decreased which indicated its antioxidant ability and inactivation of an enzyme called NADPH oxidase [36]. In our study, the SOD values decreased significantly in the PCOS group. Metformin, FE, and SeNPs treated groups

showed a significantly elevated value of SOD. FE and SeNPs treatment also reduced the levels of oxidative stress which further depict that it has antioxidant activity. As in the previous studies, it has been observed that SeNPs play a key role in the antioxidant balance and it is considered to be important in the development and function of the reproductive system [37]. All the results indicate that fenugreek extract may improve the SOD activity [38].

A healing effect of ovaries and reduced in the number of follicles cysts was seen in SeNPs and fenugreek extract treated groups [39]. Histopathology of ovarian tissues exposed that there is a resemblance in human and rat PCOS when induced with letrozole. The histopathology results indicate that the anovulation might be due to the active FSH and LH levels and the reduced relationship between cells of ovaries as theca cells and granulosa cells [24]. A thin layer of granulosa cells line up the sub capsular cysts which result in hyperplasia of theca cells; these findings were comparable to the previous studies [40]. The abnormal level of androgen hormone in ovaries leads to the increased follicular atresia and decreased follicular growth [41]. The number of corpus luteum increased significantly in PCOS group as compared with the control group in the present study which is in relation with the previous studies [42]. In the present study, the post-treatment of metformin, FE, and SeNP showed decreased the number of corpus luteum and the number and diameter of cystic follicles which are also in relation to the previous studies [20]. In recent studies, the Se-based herbal medicines were used for PCOS patients, which depicted that Se is best known for a reduction in ovarian cysts [43].

## Conclusion

The present study proved the effect of selenium nanoparticles prepared by a speed and safety method (cold plasma) with

fenugreek extract in treatment of PCOS. Because of the fenugreek leaves high beneficials, it might be used in the medicate of endocrine and ovarian derangements in the PCOS treatment. Meanwhile, SeNPs and the aqueous extract of fenugreek leaves could serve as an anti-infertility therapeutic agent by reducing the number of ovarian cysts, restoring the normal ovarian architecture and improving the equilibrium between antioxidant enzymes and ROS. Further studies are needed to investigate the therapeutic potential of SeNPs so that it can be used for the PCOS treatment to lessen the side effects of the other modern drugs.

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## Conflict of Interest

There are no conflicts of interests.

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