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RESEARCH PAPER

Effect of Selenium Nanoparticles with Cress Extract on Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

Selenium is naturally present in the human body, animals, and plants, and is one of the important elements in their growth and maintenance. Recently, the nanoform of selenium has attracted attention due to its low toxicity and a high degree of adsorption compared to its organic and inorganic forms. The current study aimed to examine the effect of Cress leaves (Lepidium sativum L.) extract in combination with selenium nanoparticles in alleviating polycystic ovary syndrome in letrozole-induced PCOS in adult female rats. Nonthermal or cold plasma was used in the synthesis of selenium nanoparticles. Subsequently, the produced nanoparticles were identified, the 30 rats were divided into 6 equal groups, the first group was healthy (negative control); handled with distilled water given orally. To induce PCOS, rats were given letrozole (1 mg/kg) B.W daily for 21 days, The second group was left without any treatment (PCOS group), while the rats in the other 4 groups were treated orally and daily for 30 days using the following treatments: metformin (metformin group), Cress extract only (CE group), Cress extract with SeNPs (CE+SeNPs group), and SeNPs only (SeNPs group), respectively. Biochemical tests (Follicle stimulating, luteinizing, testosterone, and estrogen hormones as well as glucose and insulin levels) and histopathological analyses were performed. The results of the current study show a significant decrease in glucose and insulin levels, and a highly significant increase in LH, FSH, and testosterone levels while a significant decrease in estrogen levels in the Cress extract, SeNPs and metformin-treated groups with respect to PCOS induced group. The present study demonstrated the effect of SeNPs and Cress extract in the treatment of PCOS. SeNPs and Cress leaves extract improved ovarian dysfunction and reduced the number of ovarian cysts.

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INTRODUCTION

The most common endocrine disease (Polycystic ovary syndrome; PCOS) affects approximately 15-20% of women of reproductive age. It is one of the main causes of infertility [1] in which the clinical and biochemical factors of the syndrome are hyperandrogenism and an increase in testosterone hormone levels. It is related to hyperinsulinemia and insulin resistance and is linked to metabolic syndrome and abnormalities. Metabolic syndrome includes a group of symptoms like obesity, high blood pressure, high glucose and insulin levels in

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the blood, as well as insulin resistance [2,3]. When the two syndromes (polycystic and metabolic) are combined, they confer an increased risk of cardiovascular disease and diabetes type 2 [4].

Selenium (Se) is naturally present in the human body, animals, and plants, and is one of the important elements in their growth and maintenance [5]. Recently, the nanoform of selenium has attracted attention due to its low toxicity and a high degree of adsorption compared to its inorganic and organic forms [6]. Its nanoparticles (SeNPs) are important in numerous physiological processes that are very important for the survival of human beings, such as reproduction, growth and immunity [7]. In medical treatments and pharmaceutical fields and due to their lowering risk compared to Se, SeNPs provide various applications such as the following: a nutritional supplement, an antioxidant, an anticancer agent, an antimicrobial agent [8], and an antidiabetic agent [9]. For PCOS women, Se supplementation had beneficial effects on insulin levels [10]. From a previous study, it has been found that SeNPs with fenugreek leaf extract have a beneficial effect in treating oxidative stress in letrozole-induced PCOS in adult female rats [11]. Therefore, selenium could provide a novel therapeutic strategy for PCOS. Cress (Lepidium sativum L.) is a small herbaceous plant with a height of 50 cm. Cress is an annual, fast-growing, and edible herb belonging to the family (Brassicaceae) and the genus (Lepidium). Furthermore, Cress contains a high percentage of minerals as well as vitamins A and C which are useful for purifying blood and treating anemia. It is considered an edible vegetable in addition to its medicinal uses. In addition, Cress is used in many countries to treat diabetes, hypertension [12], and kidney disease [13]. in the literature, there is a lack of linking the plant to the syndrome under study. This is the first study to demonstrate the effect of Selenium nanoparticles prepared by cold plasma in combination with aqueous Cress fresh leaf extract on polycystic ovary syndrome (PCOS)

Since PCOS is one of the major causes of infertility, finding a treatment for it is needed; the primary purpose of treatment is to return reproductive function and improve metabolic complications. The current study aimed to examine the effect of Cress leaves (Lepidium sativum L.) extract in combination with selenium nanoparticles in alleviating polycystic ovary syndrome in letrozole-induced PCOS in adult female rats.

MATERIAL AND METHODS

Preparation of Cress extract (CE)

Cress fresh leaves (Lepidium sativum L.) were obtained from a local market in Baghdad city. then washed with distilled water (D.W.) and residual moisture was evaporated at room temperature. A weight of 1 g of the plant sample was boiled in distilled water (100 ml) for 10 min. After cooling, the aqueous extract was filtered via a Millipore filter to eliminate particulate matter and kept at $4C^0$ for future work [14].

Preparation and Characterization of Selenium Nanoparticles

Linking the system

Nonthermal or cold plasma was used in the synthesis of selenium nanoparticles [15-17]. The plasma system consisted of five parts as follows: Argon gas and gas flowmeter with 1–5 min calibrator /1 for gas intake controlling. This flowmeter was in connection with A stainless steel hole metal tube 10 cm×1mm. They are connected to the power supply (cathode) equipped with intermittent and continuous high voltage. The tubes could produce up to 25 kV voltage and cutting of 25 kHz voltage. They had a stainless steel conductive that connect to the anode (length of 7 cm and width of 5 mm and strip ends with a 1 × 1 flat end). The fourth part was a metal tube holder, which carried the glass beaker that contained the solution. This tube was placed vertically by a 1mm diameter catcher; for gas regulation, its upper end connects with a rubber tube. The fifth part was the selenium nitrate solution that was placed in a 25 ml beaker on a movable holder under the metal tube.

Preparation of SeNPs

For SeNPs preparation, 10 ml of selenium was nitrated with 0.5 mM concentration. As mentioned above, the prepared form was placed on the holder under the metal tube. For different exposure times to plasma (6, 8, 10, 12) minutes, the distance was 1 mm (between the liquid surface and the nozzle of the tube). Gas in the metal tube was regulated by the flowmeter to control the gas flow. The supplied voltage of the system was gradually increased until the plasma was generated between the surface of the liquid

and the tube.

Characterization of SeNPs

For the synthesized SeNPs, X-ray diffraction (XRD) patterns were obtained on a Podwe XRD, 2700AB HAOYUAN co, China. Radiation with the X-ray generator was operated at 45 kV and 40 mA with a 2-theta configuration.

Animals

Thirty female rats' weights (170-200) grams were cached from the animal's house at the University of Al-Nahrain, they were reserved underneath the appropriate environmental condition of the moisture between (50;50) in dark, while in the light and dark' was (45 -55%), and 22-25°C. The animals were kept in (25×30×50 cm) plastic cages (which were altered every week), and the foodstuff was given as pellets. The 30 rats were divided into 6 equal groups, the first group was healthy (negative control); 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) [18]. The second group was left without any treatment (PCOS group), while the rats in the other 4 groups were treated orally and daily for 30 days using the following treatments: metformin (100mg/kg; metformin group), Cress extract only (2ml/kg; CE group), Cress extract with SeNPs (1:1 ratio, 2ml/kg; CE+SeNPs group), and SeNPs only (2ml/kg; SeNPs group), where used SeNPs at 10 min of exposure to plasma. the letrozole-treated rats were euthanized after 30 days after the treatment.

Determination of relative rat weight (RRW)

Changes in body weight were measured using a sensitive balance every week for all groups throughout the experiment. The relative rat weight (RRW) was also calculated using the following equations: [15].

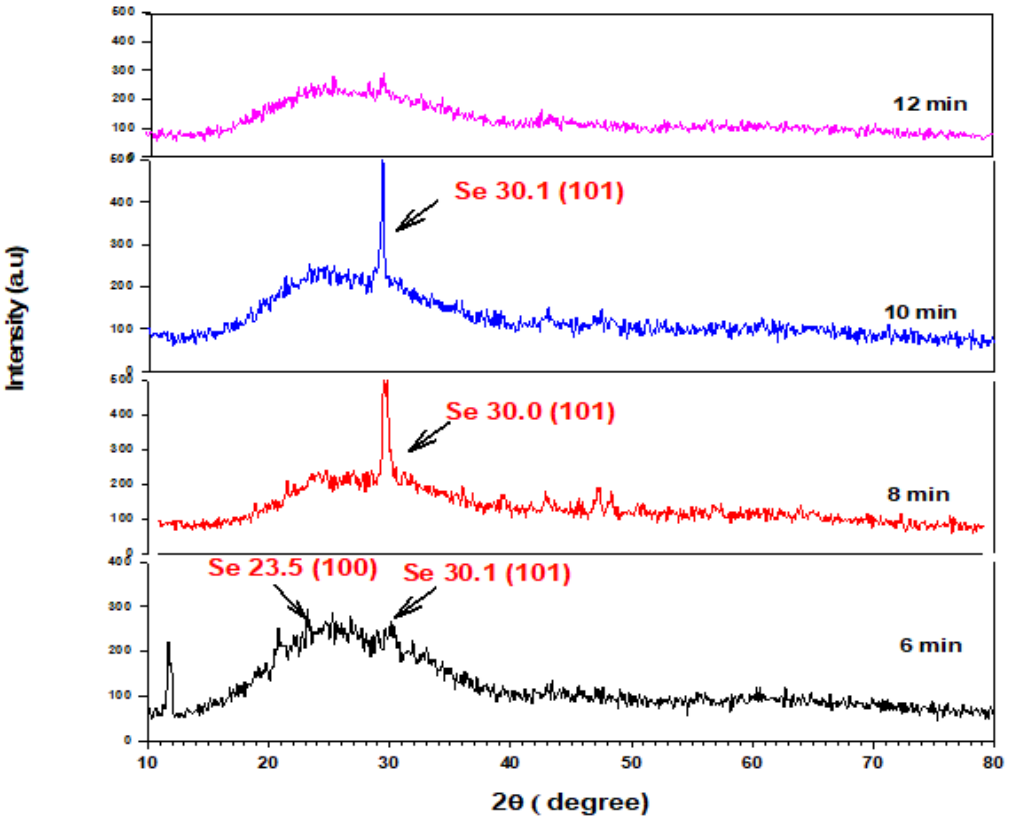

Fig. 1. X-ray pattern of selenium nanoparticles

$$W_R = \frac{W\,(di)}{W\,(do)} \quad (1)$$

Where: W_R represents RRW, W(di) denotes the rat weight on an i^{th} day, and $W(d_0)$ is the rat weight on the day the treatment started.

Determination of some biochemical parameters

By ELISA (enzyme-linked immunosorbent assay), serum insulin concentration (mIU/mL) was evaluated using a commercial kit specified for rats *(*AccuBind, USA*).* Serum glucose level was measured using an Enzymatic colorimetric determination (Agappe, India). The value was expressed as mg/dL

Serum hormone levels of Follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estrogen were determined using an ELISA kit (AccuBind, USA).

Histopathological analysis

The histological examinations were carried out based on standard methods according to [19]. The ovarian samples were fixed in the formalin 10% solution for 48 h. After fixation, the tissues were processed by dehydration, embedding and sectioning, then stained by the H&E staining routine, then evaluating the sections of each ovarian tissue from the cortex of the ovary to the medulla in a spiral and clockwise direction. All sections were assessed by an optical microscope (NIKON).

Statistical Analysis

All statistics were carried out by GraphPad Prism v 7.00 “GraphPad Software, USA”. In data

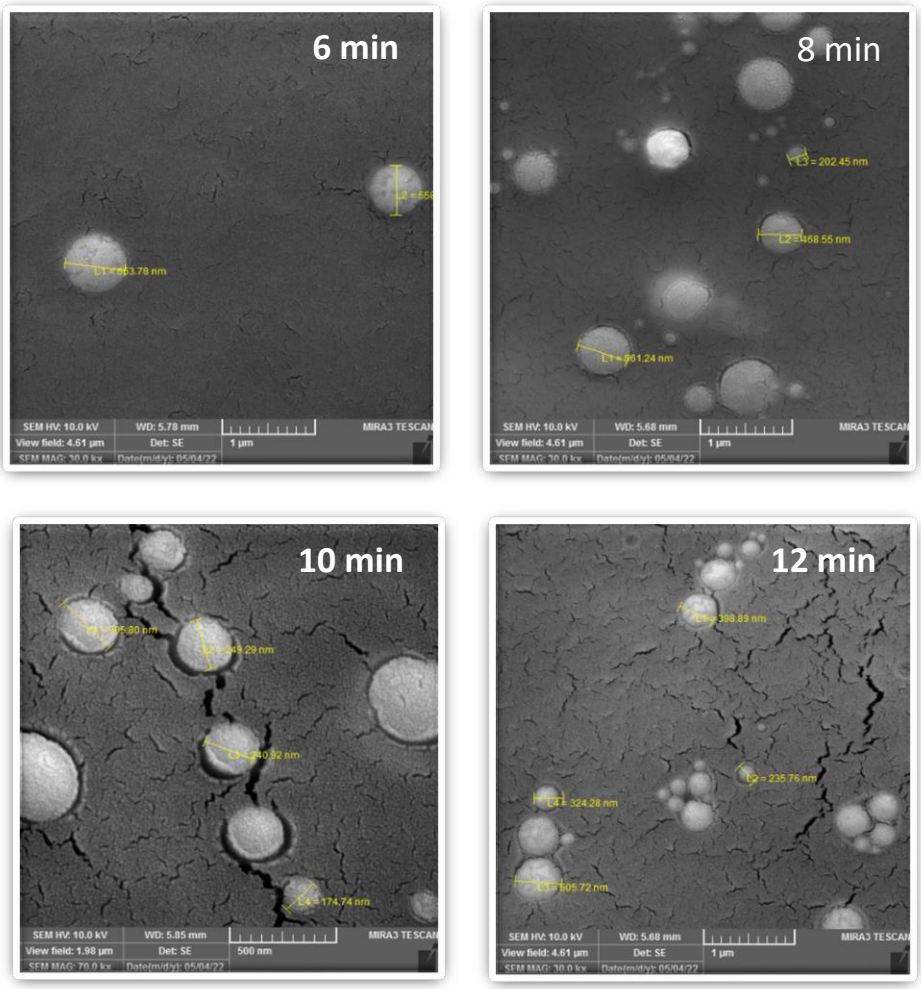

Fig. 2. FE-SEM images of Selenium Nanoparticles with different exposure times.

analysis, the star symbols stated the degree signification as follows: *, **, ***, **** were representing: (P≤0.05), (P≤0.01), (P≤0.001), (P≤0.0001), respectively.

RESULTS AND DISCUSSION

Characterization of the synthesized SeNPs

In the current research, SeNPs were prepared using cold plasma, and their formation was observed by color-changing the solutions from colorless to orange. The surface plasmon resonance causes these colors to change in the selenium nitrate solution and the selenium particles, as it is clear in the figure, the color darkness differs based on the exposure duration time from 6 to 12 min.

To confirm the presence of nano-crystalline selenium particles, the XRD analysis for dried selenium particles was prepared using cold plasma. The peaks of XRD 2θ were observed at 30° after 8 and 10 min of exposure to plasma (Fig. 1). This indicated the presence of selenium nanoparticles. This result is in agreement with those reported by other authors [20].

The particle size of SeNPs were estimated by a field emission scanning electron microscope (FE-SEM); the results are shown in (Fig. 2). FE-SEM micrographs of SeNPs revealed a size distribution of 174-600 nm for (6, 8, 10, 12) min exposure to plasma, this result is in agreement with those reported by other authors [21,22].

The nanoparticle's size can be easily measured from the Atomic Force Microscopy (AFM) image by determining the height of the nanoparticles' image [23]. The AFM image is a representation of three-dimensional data, therefore the height of the nanoparticles can be quantitatively measured. Fig. 3. shows 3D AFM image distribution of the selenium nanoparticles. Generally, the image indicated that SeNPs were spherical with an average height are (90, 50, 18, 70) nm for (6, 8, 10, 12) min exposure to plasma respectively, which is in agreement with a previous study [24].

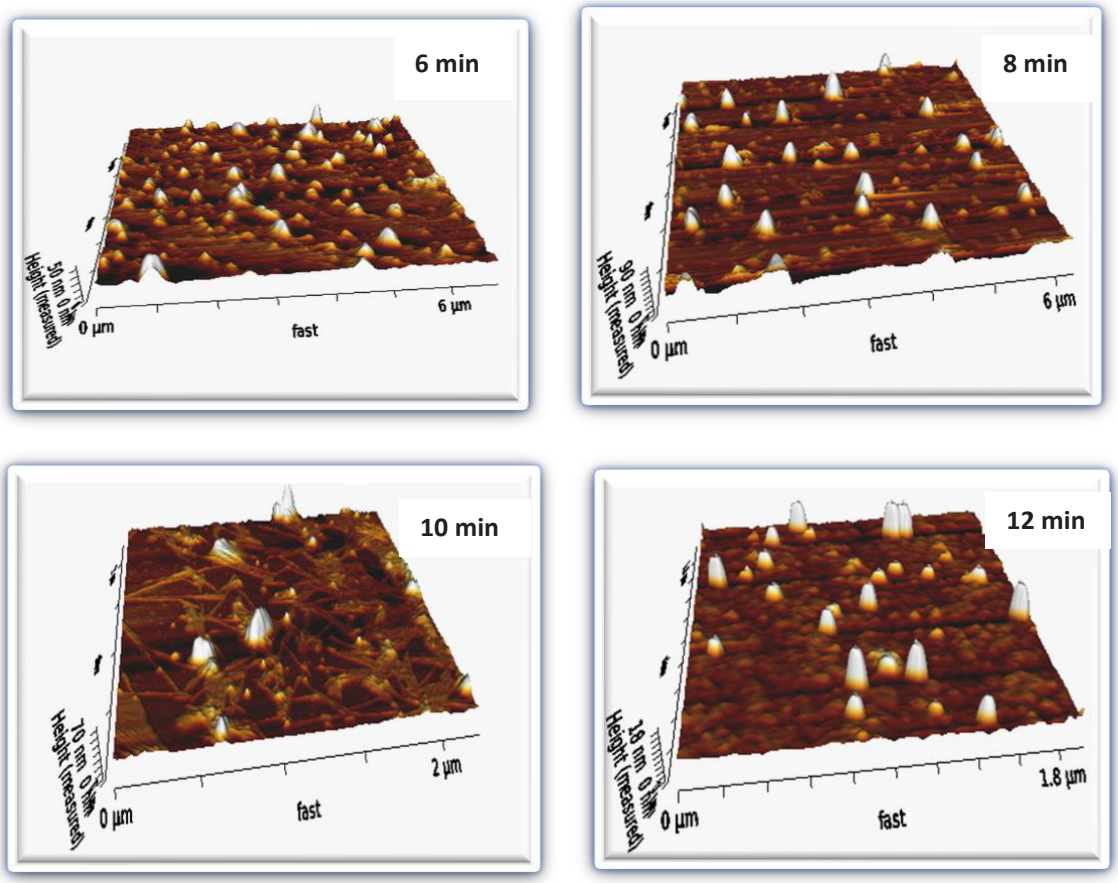

Fig. 3. Atomic Force Microscopy of selenium nanoparticles

Effect of CE, SeNPs, and metformin on Relative rat weight (RRW)

Administration of letrozole to induce PCOS significantly increased the body weight of the PCOS group as compared to the control. In treated groups, (CE, SeNPs, and Metformin) showed a constant increase in body weight as compared to the control.

Effect of Cress extract, Selenium Nanoparticles, and Metformin on parameters

Fig. 4. Shows that the PCOS group exhibited a significant increase in glucose and insulin levels compared to the control group. On the other hand, a significant decrease in glucose and insulin levels was observed in the Cress extract, SeNPs and metformin treated groups with respect to PCOS induced group.

The PCOS-induced group showed a highly significant increase in LH, FSH, and testosterone levels while a significant decrease in estrogen levels compared to the control group was observed. On the other hand, the treated groups (CE, SeNP, and Metformin) showed a significant decrease in LH, FSH and testosterone levels and a significant increase in estrogen levels with respect to PCOS induced group (Fig. 5).

Effect of CE, SeNPs, and metformin on histopathology of ovaries

Fig. 6. Illustrates the histological examination of all groups of rats. The section of ovaries in the control group showed the outer cortex which was covered by germinal epithelium, and stromal tissue that revealed a number of primary and secondary follicles in addition to a single tertiary follicle and corpus leutium (Fig. 6A). Whereas, in the PCOS-induced group Fig. 6B shows multiple variables sizes follicular cysts with a very thin layer of granulosa cells and supported by fibrous layer. Fig. 6C of rat treated with Cress extract shows multiple small, intermediate, and large sizes follicular cysts with very thick granulosa cells layer and numerous corpus lutium. Rat treated with SeNPs (Fig. 6D) revealed multiple small-size follicular cysts that originated from secondary follicles composed of a thin granulosa layer, additionally, there were numerous corpus leutium. The sections of the ovaries of rats treated with CE and SeNPs (Fig. 6E) showed a normal appearance of the tertiary follicle, primary follicle, small secondary follicles, and corpus lutium. Rats treated with metformin (Fig. 6M) showed corpus luteum and follicle with multiple layers of granular cells.

The purpose of the current study was to examine

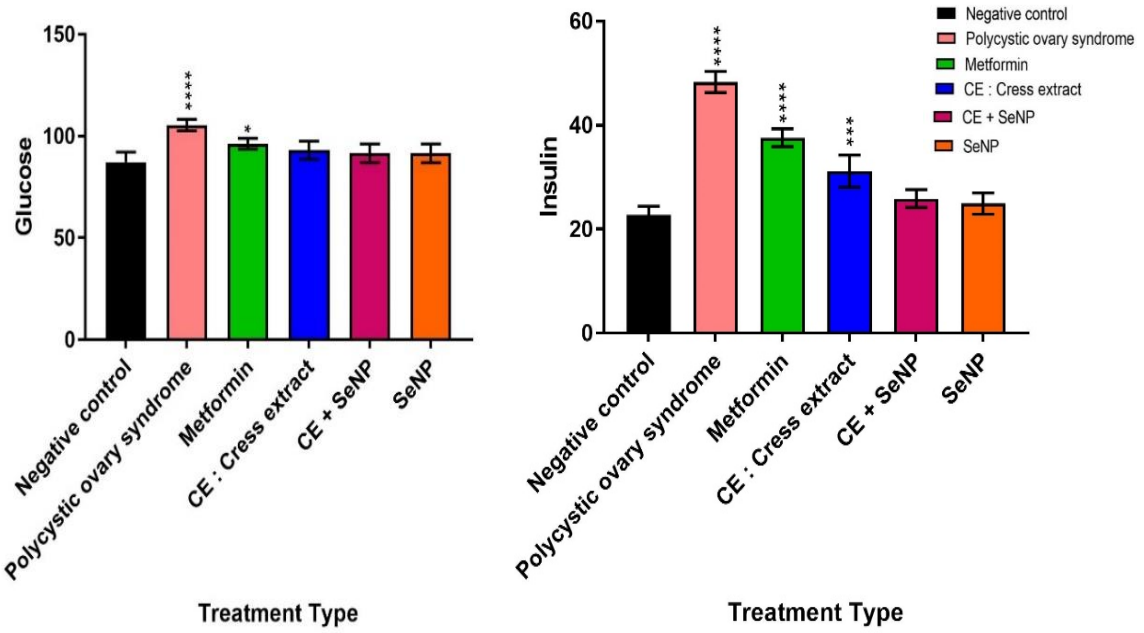

Fig. 4. Effect of CE, SeNPs, and metformin on (A) Glucose (B) Insulin levels.

the effect of Cress leaf extract in combination with selenium nanoparticles in alleviating polycystic ovary syndrome in letrozole-induced PCOS in adult female rats.

Administration by letrozole to induce polycystic ovary in rats caused an increase in body weight, a previous study showed that the model rats with PCOS generally gained more weight than the control rats [24,25]. letrozole could be induced increasing in organs' weight, such as the heart,

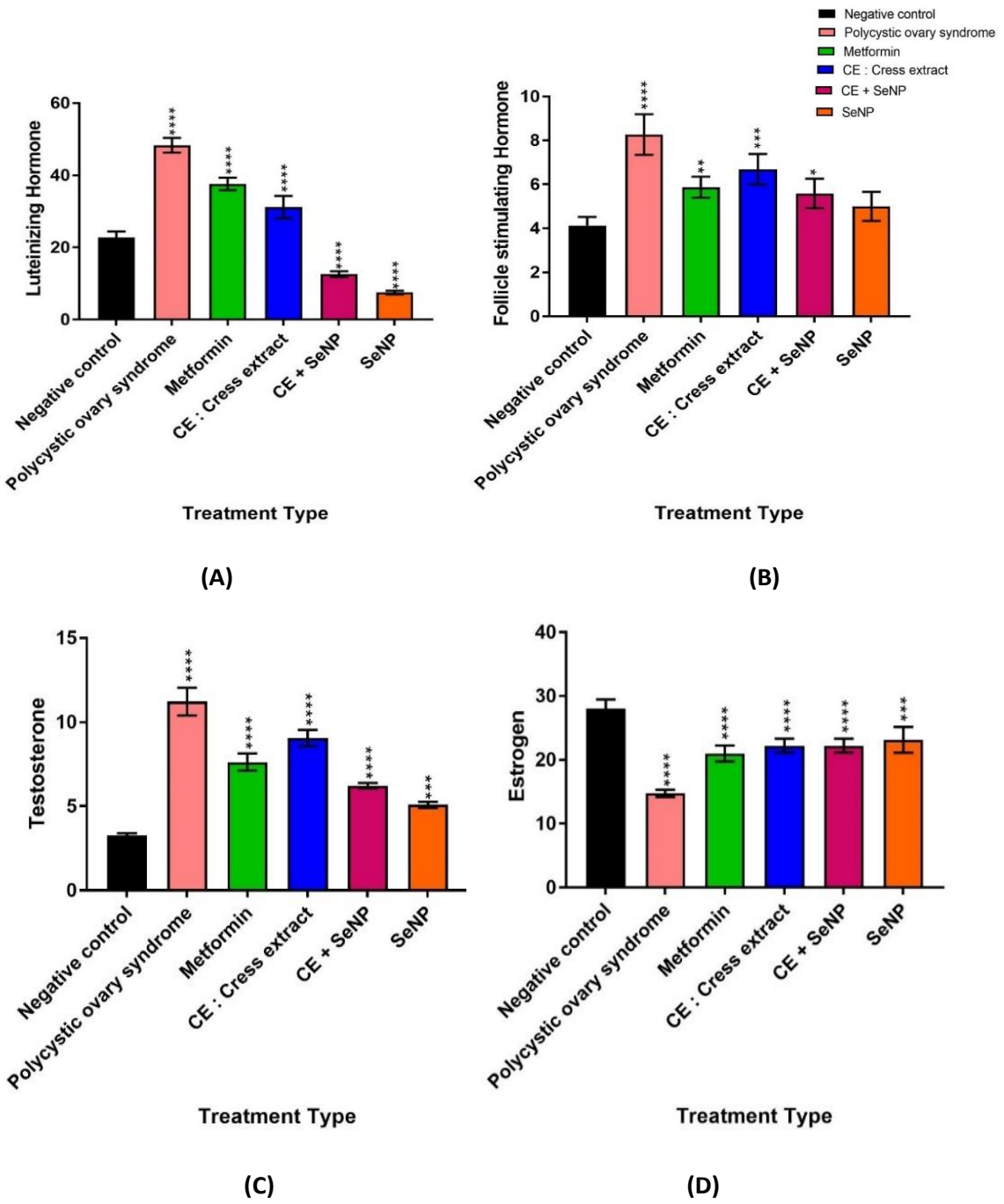

Fig. 5. Effect of CE, SeNPs, and metformin on (A) luteinizing hormone, (B) Follicle stimulating hormone (C) Testosterone (D) Estrogen hormonal levels.

liver, spleen, and kidney [26]. Another study reported that the reduced levels of circulating estrogen can induce lipid metabolic changes, resulting in an increase in the body weight of letrozole-induced rats [27,28]. These could be the reasons why the PCOS rat group elevated their weight after administration with letrozole.

Our results indicated raised glucose and insulin levels in PCOS-induced rats compared to the control group, this agreement with previous studies [2,29-31]. Whereas we found a reduction in glucose and insulin levels after treatment with Cress leaf extract, SeNPs, and metformin. From previous studies, it has been found that Selenium plays important role in the functioning of islets of Langerhans, gastrointestinal tract, and ovaries, Se also has an activity of initiating insulin signaling cascades in the cell, glucogenesis [32], and glucose uptake in adipocytes [33]. This also suggests that SeNPs cause hypoglycemia through enhanced glucose uptake activity [34]. Another study also demonstrated administration of cress seed extract significantly decreased blood sugar in diabetic rats due to the presence of alkaloids, flavonoids, cysteine, and glycine [13]. Thus, CE and SeNPs treated groups showed an improvement

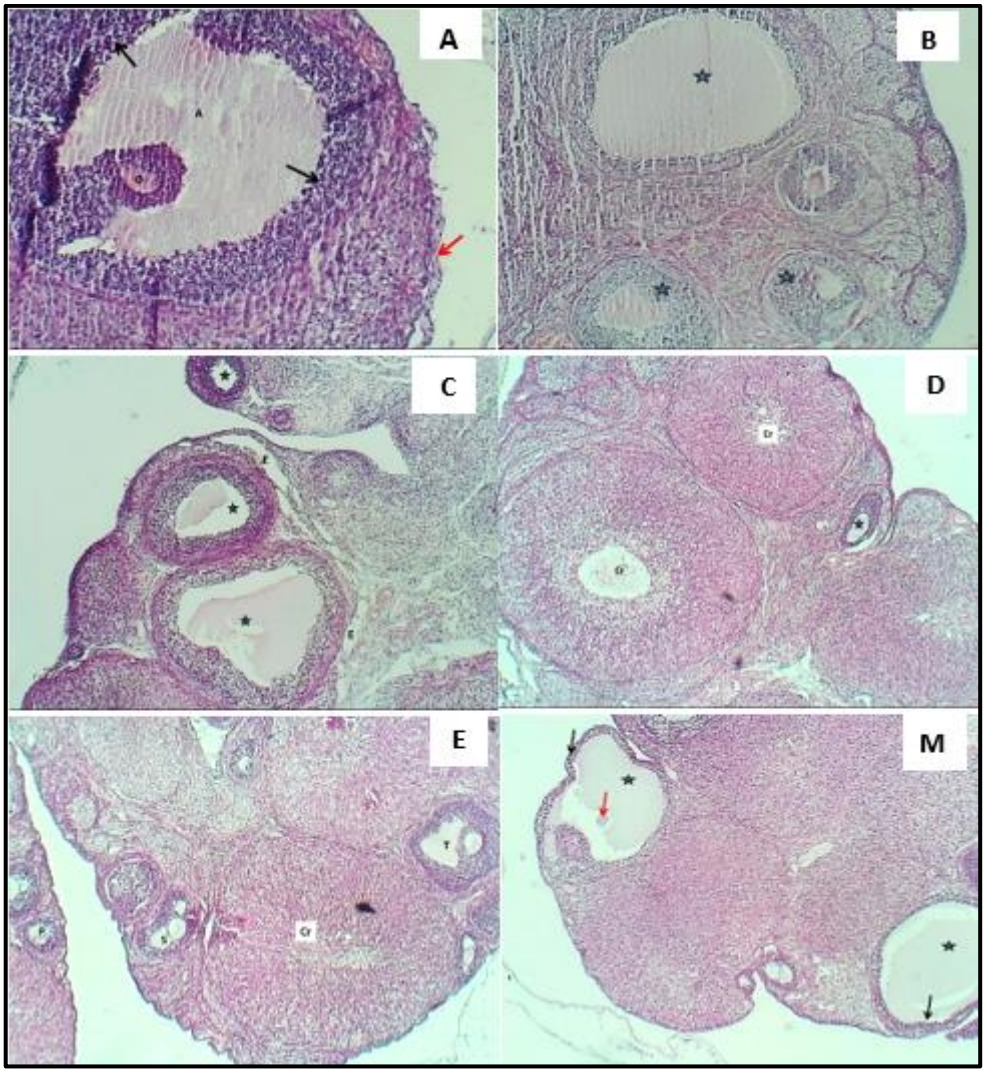

Fig. 6. Histopathology of ovaries (A) Control, H&E stain.400x (B) PCOS, H&E stain.100x (C) Cress extract, H&E stain.40x (D) Selenium nanoparticles, H&E.100x (E) Cress extract and Selenium nanoparticles, H&E stain.40x (M) Metformin, H&E stain.40x

in symptoms associated with the metabolic syndrome, including body weight, insulin, and glucose blood levels.

Our results showed a significantly high level of FSH and LH in PCOS-induced rats in contrast to the control, this agreement with a previous study [35,36]. The elevated LH levels are explained by an increased pituitary sensitivity to hypothalamic gonadotropin-releasing hormone (GnRH), and increased pulse frequency of GnRH which may cause enhanced LH secretion [37]. According to previous studies, it was stated in PCOS women, high levels of FSH caused to form the of ovarian cysts, in fact, elevated androgens in this condition led to an increase in GnRH neurons activities and finally caused to increase in FSH secretion from the pituitary [38]. Increased LH stimulates theca cells of ovaries to undergo rapid proliferation which further results in increased steroidogenic capacity and hence increased androgen production [39]. However, after being treated with CE, SeNPs, and metformin, all FSH and LH show a significant decrease in their levels [40].

A high concentration of testosterone hormone in the PCOS group showed that the androgen level increased because letrozole blocks the conversion of androgen substrate into estrogen [41]. In the meanwhile, the metformin, CE, and SeNPs treated groups assisted in the reduction of testosterone levels in PCOS rats compared to the PCOS-induced group which showed an improvement in androgen levels [42]. Testosterone and Se have a negative correlation as the Se increases as the testosterone level decreases and vice versa [43]. In the metformin, CE and SeNPs treated groups the estrogen concentration significantly increased, as Se is responsible for the excessive release of estrogen by acting on granulosa cells [44]. this result was similar to previous studies[45,46].

A healing effect of ovaries and a decrease in the number of cystic follicles were noticed in CE and SeNP-treated groups. Histopathology of ovarian tissues revealed that there was a resemblance in human and rat PCOS when induced with letrozole. The results of histopathology indicated that the anovulation might be due to active FSH and LH levels and the reduced interplay between cells of ovaries such as theca cells and granulosa cells [16]. A thin layer of granulosa cells lined up the sub capsular cysts which resulted in hyperplasia of theca cells. these findings were similar to the previous studies [47].

Abnormal levels of androgen hormone in ovaries led to increased follicular atresia and decreased follicular growth [48]. In the present study, the post-treatment of metformin, CE and SeNPs showed a decrease in the number of corpus luteum and the number and diameter of cystic follicles which were also in relation to the previous studies [49]. In recent studies, Se-based herbal medicines were used for PCOS patients, which depicted that Se was best known for a reduction in ovarian cysts [50-53].

CONCLUSION

The present study demonstrated the effect of SeNPs and Cress extract in the treatment of PCOS. SeNPs and Cress leaves extract improved ovarian dysfunction and reduced the number of ovarian cysts. This study showed protective potentials that improved the hormonal concentrations of testosterone, estrogen, LH, and FSH in female rats, and also displayed a significant role in reducing the hyperglycemic and hyperandrogenic conditions denoting that the possible ameliorative medication for the treatment of clinical and biochemical characteristics of the polycystic ovarian syndrome. Further studies are needed to investigate the therapeutic potential of SeNPs so that can be used for the treatment of PCOS to lessen the side effects of other modern drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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