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## Ameliorative effects of selenium nanoparticles on letrozole induced polycystic ovarian syndrome in adult rats

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**Abstract:** Selenium (Se) is an essential trace element playing vital role in the physiological processes. It was ignored as a therapeutic agent in the past, but in 1950s it was revealed that it must be obtained from diet as it cannot be produced by organisms. The present study was designed to investigate the anti-androgenic and metabolic effects of selenium nanoparticles (SeNPs) on letrozole induced PCOS using female rats. All rats were administered orally with letrozole (1 mg/kg) in CMC (0.5%) for 36 days. Rats were provided with metformin (2 mg/kg), Se-I (250 mg/kg), Se-II (500 mg/kg), SeNPs-I (50 mg/kg) and SeNPs-II (100 mg/kg) for 15 days. On 37th day animals were sacrificed and biochemical tests, antioxidants and histopathological analysis were performed. The results of the current study depict the anti-androgenic potentials of Se and SeNP for the treatment of PCOS that can be a new drug in the management of PCOS.

**Keywords:** selenium; selenium nanoparticles; biochemical test; histology.

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## 1 Introduction

Selenium (Se) is an essential trace element. It was ignored as a therapeutic agent until the 1950s, when it was revealed that it must be obtained from diet as it cannot be produced by organisms (Rayman et al., 2008; Haug et al., 2007). The main dietary sources of Se are nuts, shellfish, hams, crabs, salmon fish. Intake of Se rich diet depends upon its bioavailability from food sources (Marval-León et al., 2014). Recently, it was revealed that Se is an important constituent, maintaining the functioning of proteins called selenoproteins (Pascual and Aranda, 2013). Selenocysteine is the 21st amino acid of selenoprotein that contains Se as an active site (Shetty et al., 2014). It is well known for its antiviral (Rayman, 2000), antibacterial (Cihalova et al., 2015), antifungal (Guisbiers et al., 2017), antimutagenic (Peng et al., 2016), anticarcinogenic (Ahmad et al., 2015) and antiparasitic activities (Dkhil et al., 2016). On the other hand, it protects cells and tissues from oxidative stress by initiating the activity of seleno-enzyme-glutathione peroxidase (Peters, 1999; Benton, 2012). The relationship of heavy metals and Se depicts that it acts as detoxifying agent and its antagonistic effects to lead, cadmium and mercury has been reported (Flora et al., 2008; Flora, 2009; Pelletier, 1986; El-Demerdash, 2001).

Nanotechnology is a revolutionary tool in many fields of research such as energy, environmental, pharmacy, manufacturing, chemical industries, genomics and medical treatments (Roco et al., 2011; Miyazaki and Islam, 2007; Roco, 2003; Chen and Yada, 2011). Nanomaterials have unique physical and chemical properties, due their nano size in living organisms they have been reported to have a superior and indispensable nature which help them in the treatment of diseases (El-Sayed et al., 2005; Niemeyer, 2001; Nowack and Bucheli, 2007; Veiseh et al., 2010; Duncan, 2011). Nanoparticles can also cross physiological barriers (blood brain barrier, blood testis barrier and placental barrier) due their absorptive and size-dependant properties (Oberdörster et al., 2005; Nel et al., 2006). Recently, nanoscale elemental selenium attracted attention due to its antioxidant

property, low toxicity, excellent bioavailability and scavenging properties of free radicals (Wang et al., 2012; Chen et al., 2015; Dhanjal and Cameotra, 2010; Wu et al., 2013). Previous studies reveal that SeNPs have more efficacies with least toxic effects (Wu et al., 2013; Gao et al., 2002; Nazıroğlu et al., 2017). PCOS is a one of the complex endocrine, reproductive and metabolic disorders present in women worldwide (Teede et al., 2010; Norman et al., 2007; Goodarzi et al., 2011). PCOS is characterised by hyperandrogenism, insulin resistance, dyslipidemia, amenorrhoea, oligoamenorrhea and polycystic ovaries as the key diagnostic features (Goodarzi et al., 2011; Apridonidze et al., 2005; Walters et al., 2012). There is also a great chance for the development of chronic anovulation, estrogen replete amenorrhoea, hormonal imbalance and reduced fertility (Goodarzi et al., 2011; Lobo and Carmina, 2000; Pasquali et al., 2011). In PCOS patients it has been noticed that the oxidative stress cause disturbances in antioxidant enzyme activity leading to the elevation of reactive oxygen species (ROS) including endometriosis, preeclampsia, infertility, mutation in ovarian epithelium's DNA, disturbances in cell signaling and increased apoptosis (Riley and Behrman, 1991; Agarwal et al., 2005, 2012; Gupta et al., 2017; Verit, 2015). Letrozole (Femara) is a PCOS inducer and is also known as an aromatase inhibitor (Smith, 2008; Usadi and Merriam, 2015). It inhibits aromatase enzyme production that reduces the conversion of androgen into estrogen, cause an increase in androgen production leading to PCOS (Smith, 2008; Kafali et al., 2004; Weber, 2015). Metformin is an insulin sensitiser drug and commonly called as glucophage (Masoudi et al., 2003; Bailey and Day, 2004). Metformin inhibits the formation of glucose and decreases the blood glucose level (Foretz et al., 2010; Robertson, 2004; Kim et al., 2008). It is reported previously that metformin induced ovulation in PCOS women reduced insulin level and led to functioning on androgen production of ovaries, glucose uptake, insulin sensitivity and fibrinolysis activity (del Barco et al., 2011; Moghetti et al., 2000; Kolodziejczyk et al., 2000).

Se and SeNPs were chosen as a drug of choice for treatment of hyperlipidemic, antiandrogenic, hyperinsulinemia and oxidative stress consequences of PCOS. Se and SeNPs show a variety of biological activities such as antioxidant, anticarcinogenic, antimicrobial, antihypertensive, antiviral, anti-inflammatory and antilipidemic activities (Baliga et al., 2011; Jaydeokar et al., 2012; Santharam, 2016; Semalty et al., 2017). In the present study, PCOS was induced with letrozole in the rodent model. Se and SeNPs were selected to be used as a therapy against polycystic ovary syndrome and its comparative effects with metformin were assessed. The present study was also designed to scrutinise whether Se and SeNPs are effective in treating the endocrine, oxidative and reproductive dysfunctions in letrozole induced PCOS and whether it can assess the prognostic power of metformin in improving the clinical and biochemical features of PCOS.

## 2 Material and methods

### 2.1 Chemicals

Letrozole, carboxymethyl cellulose (CMC), NaCl, absolute ethanol, glacial acetic acid, hydrogen peroxide ( $H_2O_2$ ), ethylenediaminetetra acetic acid (EDTA), potassium phosphate monobasic ( $KH_2PO_4 \cdot 2H_2O$ ), guaiacol, nicotinamide adenine dinucleotide phosphate (NADPH), NADH, xylenol Orange, sulphosalicylic acid,

2,4-dinitrochlorobenzene (CDNB), ferric chloride, ascorbic acid, sodium azide, trichloroacetic acid (TCA), sodium acetate buffer, ferrous sulphate ( $\text{FeSO}_4$ ), thiobarbituric acid (TBA), phenazine methosulphate, DEPPD sulphate, sodium selenite, fructose and formaldehyde are all chemicals used in the experiment and were purchased from Sigma-Aldrich (Germany). All the chemicals were stored at  $4^\circ\text{C}$  for long term use.

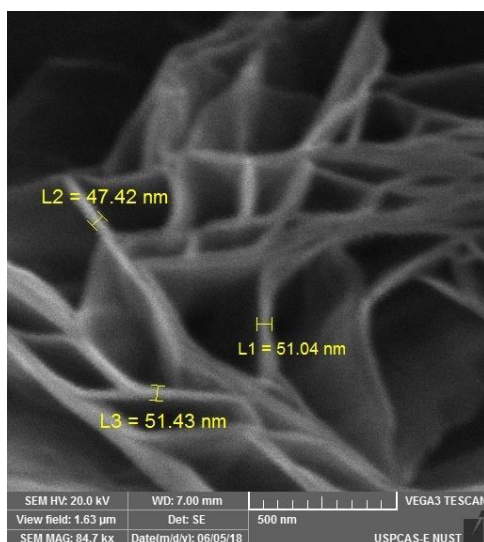
## 2.2 *Synthesis and characterisation of selenium nanoparticles*

SeNP were synthesised by using method developed by Vieira et al. (2017). The 5 ml of 1.0 mmol/L of sodium selenite aqueous solution was dripped into 10 ml of D-fructose solution of 1.0 mmol/L. The mixture was then stirred continuously for 15 min at  $45^\circ\text{C}$  until the red coloured nanoparticles appeared in the mixture. After 20 min, these particles changed into black colour. The whole mixture was centrifuged at 13000 rpm for 10 min and then supernatant was discarded and pellets were obtained and further centrifuged in distilled water for 30 mins. This procedure was repeated three times for removal of organic substances present in the solution. The pellets were then dried for characterisation before experiment.

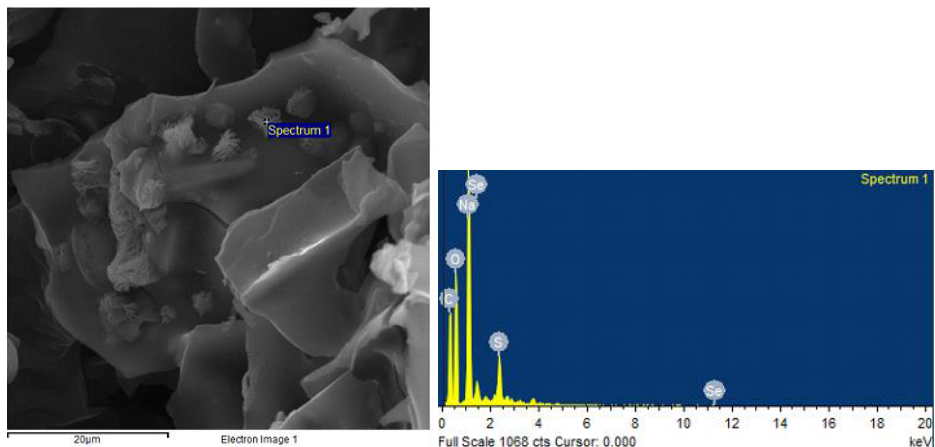
## 2.3 *Scanning electron microscopy and energy dispersive X-ray observation of selenium nanoparticles*

Scanning electron microscopy (SEM) images were taken using Hitachi S 3400N instrument. Samples were first dried, filtered and coated with gold using Ion Sputter Coater Hitachi E1010. Size of SeNPs observed was in range of 52 nm. Energy dispersive X-ray (EDX) analysis was performed by the same instrument and employed to know the elemental compositions of the sample. The results can be observed in Figures 1 and 2.

**Figure 1** Scanning electron microscopy of selenium nanoparticles (see online version for colours)



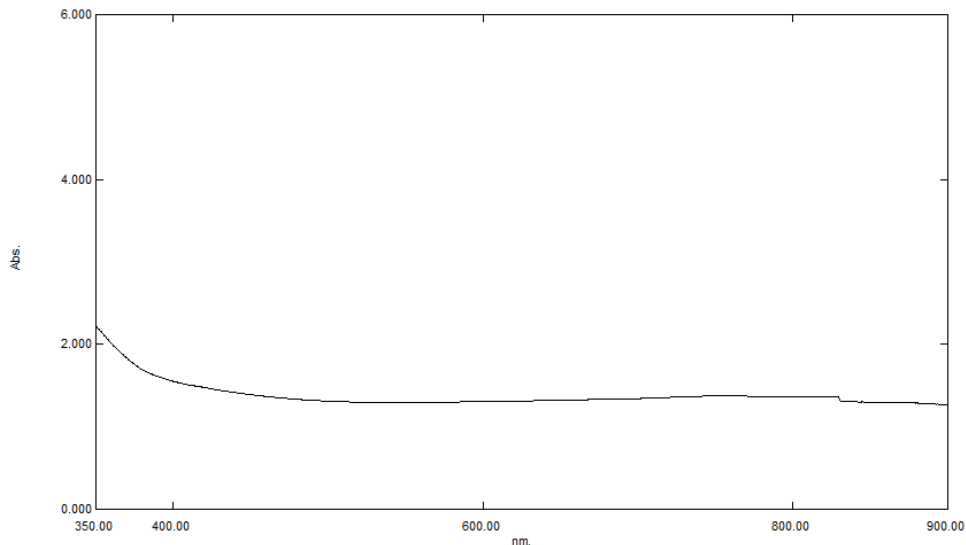
**Figure 2** Energy-dispersive X-ray spectroscopy (EDS) of selenium nanoparticle (see online version for colours)



#### 2.4 Ultraviolet visible spectroscopy

Ultraviolet-visible spectroscopy (UV-VIS) of SeNPs sample was observed by making a suspension of SeNPs in distilled water by using a spectrophotometer of Shimadzu UV-2600. The results can be observed in Figure 3.

**Figure 3** Ultraviolet-visible spectroscopy (UV-VIS) of selenium nanoparticles



## 2.5 Experimental design

Six weeks old adult female *Rattus norvegicus* ( $n = 42$ ), having weight of about  $150 \pm 10$  grams illustrating normal estrous cycle were used. These animals were taken from the animal house of Quaid-i-Azam University, Islamabad. Animals were given feed pellets and clean water for drinking *ad libitum*. The experimental conditions were maintained during the whole experiment of 36 days. Room temperature was kept about  $20 \pm 5^\circ\text{C}$  with the light and dark cycles of 12/12 hours. Group 1 served as vehicle control administered with 0.5% CMC. Group 2 animals were provided with Letrozole (Femara) with daily gavage of 1 mg/kg. Letrozole tablet was dissolved in 0.5% CMC solution and was given for 36 day to induce PCOS. This dose was selected according to previous studies (Kafali et al., 2004). During experiment, for confirmation of induction of PCOS vaginal smear was monitored to analyse the relative proportion of cornified, leukocytes and epithelial cells. Animals in group 3 were allocated as metformin treated group and was administered with 2 mg/kg metformin from day 22 to till the end of experiment. Group 4 animals were treated with Se 250 mg/kg and those of group 5 were treated with Se 500 mg/kg dose. Animals of group 6 were given by 50 mg/kg SeNPs and group 7 were administered 100 mg/kg of SeNPs from day 22 to till end of the experiment.

On day 37 all rats were sacrificed after termination of experiment. Blood was collected from aorta of the rats in heparinised syringes and was centrifuged at 3,000 rpm for 15 min. Blood plasma was separated for biochemical and hormonal analysis and was stored at  $-20^\circ\text{C}$ . Ovaries were taken out for histopathological and antioxidant assessments.

## 2.6 Assessments of experimentally induced PCOS anthropometrical parameters

Changes in body weight were recorded every week in control, PCOS, metformin, selenium and selenium nanoparticles treated groups throughout the experiment. Weight gain was determined during the day of dissection using standard measurement methods.

Ovarian weight was evaluated using usual measurements procedures. To check estrous cyclicity in rats, colypocytological examination was performed every morning from day one till last day of experiment.

## 2.7 Biochemical analysis

The concentration of blood glucose was evaluated on days 1, 21 and 36 of experiment using Accu-Chek glucometer (USA). Total protein content was estimated in ovarian tissues of control and treated animals by using specific protein kit purchased from AMEDA Labordiagnostik GmbH Krenngasse, Graz/Austria (Ullah et al., 2018a). Total cholesterol and high density lipoprotein cholesterol (HDL-C) level in plasma was estimated by using commercially available kits of AMP diagnostic (AMEDA Labordiagnostik GmbH) and were analysed on picco 5 chemistry analyser.

In antioxidant enzymes, reactive oxygen species (ROS) were evaluated using protocol of Hayashi et al. (2007). Thiobarbituric reactive acid substances (TBARS) levels were estimated using Wright and Sutherland (2008). Catalase (CAT), peroxidase (POD) levels were assessed according to Chance and Maehly (1955) method with some modifications. Superoxide dismutase (SOD) was evaluated by protocol of Kakkar et al. (1984). All these

protocols were carried out using Smart Spec TM plus spectrophotometer (USA) using the protocol of Ullah et al. (2018b).

### *2.8 Hormonal analysis*

Serum total testosterone, estradiol and progesterone were quantified by using three different kits purchased from Amgenix Inc (USA). Testosterone concentrations were quantified by using EIA test kits (Amgenix Inc, USA). Estradiol EIA tests kits (Amgenix Inc, USA) were used to determine E2 concentrations (pg/ml) in serum. Progesterone (P4) EIA tests kits (Amgenix Inc, USA) were used to determine P4 concentrations (ng/ml) in the serum.

### *2.9 Histopathological analysis*

The ovaries were processed in paraffin embedding, sectioned at 7  $\mu\text{m}$ , stained with hematoxylin and eosin, and observed under microscope at 40 $\times$  magnification. All the ovarian follicles were examined depending upon their granulosa and morphology using the protocol of Ullah et al. (2017). The presence or absence of corpus luteum and thickness of peripheral theca and granulosa layer were also observed.

### *2.10 Statistical analysis*

All the data were expressed as mean  $\pm$  standard error of mean (SEM). Graph Pad PRISM 5 (San Diego, CA, USA) was used. Comparison of mean was done by using One Way Analysis of Variance (ANOVA), followed by Dunnett's test.

## **3 Results**

### *3.1 Effect of metformin, Se and SeNPs on estrous cyclicity*

Estrous cycle was observed regularly by collecting vaginal smear from all experimental groups for 36 days. Administration of letrozole to rat for 36 days led to estrous cycle irregularity as compared to control while metformin, Se and SeNPs treated groups showed normal estrous cycles.

### *3.2 Effect of metformin, Se and SeNPs on body and ovarian weight*

The initial body weight of included animals was approximately equal while the final body weight showed significant differences in PCOS groups in comparison with control. Administration of letrozole to induce PCOS significantly increased ( $P < 0.001$ ) the body weight of PCOS group. Significant decrease in the body weight gain was observed in metformin treated group as compared to control. The Se-I, Se-II, SeNPs-I and SeNP-II treated groups showed no significant changes in body weight (Table 1).

**Table 1** Mean  $\pm$  SEM of body and ovarian weights of control, PCOS, metformin, Se-I, Se-II, SeNP-I and SeNP-II treated adult female rats during 36 days of experiment

Parameters	Control	PCOS	Metformin	Se-I	Se-II	SeNPs-I	SeNPs-II
Initial body weight (g)	158.5 $\pm$ 0.5	158.3 $\pm$ 0.8	158.4 $\pm$ 0.5	158.5 $\pm$ 0.6	158.1 $\pm$ 0.8	158.3 $\pm$ 0.5	158.2 $\pm$ 0.4
Final body weight (g)	177.3 $\pm$ 0.6	263.1 $\pm$ 0.6***	171.3 $\pm$ 1.4***	175.1 $\pm$ 1.1	177.1 $\pm$ 0.7	175.5 $\pm$ 1.3	178.1 $\pm$ 0.6
Body weight gain (g)	18.8	104.8	12.8	16.6	19.0	17.1	19.9
Ovarian weight (g)	0.06 $\pm$ 0.01	0.12 $\pm$ 0.01**	0.04 $\pm$ 0.02	0.03 $\pm$ 0.04	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.02

Notes: Values are expressed as mean  $\pm$  SEM.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

The ovarian weight of PCOS group showed significant increase (P < 0.01) while there was no significant change observed in ovarian weight of metformin, Se-I, Se-II, SeNPs-I and SeNP-II treated groups as compared to control presented in Table 1.

### 3.3 Effect of metformin, Se and SeNPs on blood glucose concentration and total protein

On the day one the initial blood glucose level was measured which was normal and approximately the same in all animals, but the final blood glucose concentration of PCOS group was significantly different (P < 0.001) as compared to control. In metformin (P < 0.01), Se-I (P < 0.05), Se-II, SeNPs-I (P < 0.01) and SeNP-II treated groups significant decrease in blood glucose concentration was observed when compared with control as presented in Table 2.

In PCOS group the total protein content increased significantly (P < 0.001) while there was significant (P < 0.001) reduction observed in the SeNPs-II treated group when compared with control. No significant change was observed in metformin, Se-I, Se-II and SeNPs-I treated groups as presented in Table 2.

**Table 2** Mean  $\pm$  SEM of glucose concentration, total protein, total cholesterol, HDL of control, PCOS, Metformin, Se-I, Se-II, SeNP-I and SeNP-II treated adult female rats during 36 days of experiment

Parameters	Control	PCOS	Metformin	Se-I	Se-II	SeNPs-I	SeNPs-II
Glucose concentration (mg/dL)	58.4 $\pm$ 1.29	100.6 $\pm$ 2.78***	45.6 $\pm$ 1.57**	50 $\pm$ 2.24*	51.5 $\pm$ 2.18	47.5 $\pm$ 2.36**	52.6 $\pm$ 1.59
Total protein (mg/g of tissue)	25.8 $\pm$ 1.80	44.3 $\pm$ 2.85***	25.4 $\pm$ 1.67	24.6 $\pm$ 1.38	23.3 $\pm$ 1.93	25.0 $\pm$ 1.75	21.5 $\pm$ 2.24***
Total cholesterol (mg/dL)	216 $\pm$ 1.03	234 $\pm$ 1.3***	214 $\pm$ 2.0	210 $\pm$ 2.6	207 $\pm$ 0.9**	211 $\pm$ 2.5	206 $\pm$ 1.02**
HDL (mg/dL)	167 $\pm$ 0.1	158 $\pm$ 0.1	163 $\pm$ 0.3	172 $\pm$ 0.2	175 $\pm$ 0.2	184 $\pm$ 0.1***	196 $\pm$ 0.1***

Notes: Values are expressed as mean  $\pm$  SEM.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.



### 3.4 Effect of metformin, Se and SeNPs on lipid profile

Cholesterol concentration in serum in PCOS group was significantly high ( $P < 0.001$ ) in comparison with control, while it was significantly low in Se-II ( $P < 0.01$ ) and SeNPs-II ( $P < 0.01$ ) treated groups. No significant change was observed in metformin, Se-I and SeNPs-I treated groups (Table 2).

High density lipoprotein cholesterol (HDL-C) concentration did not change in PCOS group, metformin, Se-I and Se-II treated groups but there was significant increase ( $P < 0.001$ ) observed in SeNPs-I and SeNPs-II treated groups (Table 2).

### 3.5 Effect of metformin, Se and SeNPs on antioxidant profile

The results regarding the protective effects of Se and SeNPs against PCOS in rat ovaries and antioxidant enzymes activities such as SOD, POD, CAT, and ROS are shown in Table 3. In PCOS group, the activities of antioxidant enzyme such as SOD, POD and CAT were reduced significantly ( $P < 0.001$ ) as compared to control. There was also significant decrease observed in antioxidant enzyme activities of Se-I ( $P < 0.001$ ), Se-II ( $P < 0.05$ ), SeNPs-I ( $P < 0.001$ ) and SeNP-II ( $P < 0.01$ ) treated groups presented in Table 3.

The ROS values of PCOS group were significantly high ( $P < 0.001$ ) in comparison with control while, there was no significant change observed in metformin, Se-I, Se-II, SeNPs-I and SeNPs-II treated groups. Letrozole increased TBARS activity in PCOS group ( $P < 0.001$ ) as compared to control. Metformin, Se-I and Se-II groups did not show significant change while SeNPs-I and SeNPs-II treated groups exhibited significant reduction ( $P < 0.05$ ) in comparison with control (Table 3).

**Table 3** Mean  $\pm$  SEM of SOD, POD, CAT, ROS and TBARS of control, PCOS, metformin, Se-I, Se-II, SeNP-I and SeNP-II treated adult female rats during 36 days of experiment

Parameters	Control	PCOS	Metformin	Se-I	Se-II	SeNPs-I	SeNPs-II
SOD (U/min)	36.0 $\pm 1.27$	18.2 $\pm 0.8^{***}$	32.0 $\pm 1.06$	28.7 $\pm 1.1^{***}$	30.4 $\pm 1.3^*$	25.4 $\pm 0.6^{***}$	30.1 $\pm 1.6^{**}$
POD (nmole)	3.1 $\pm 0.3$	1.3 $\pm 0.2^*$	2.5 $\pm 0.4$	2.3 $\pm 0.3$	2.5 $\pm 0.3$	2.1 $\pm 0.3$	2.2 $\pm 0.5$
CAT (U/min)	4.8 $\pm 0.4$	2.5 $\pm 0.2$	4.5 $\pm 0.5$	4.0 $\pm 0.4$	4.2 $\pm 0.3$	4.0 $\pm 0.2$	4.2 $\pm 0.3$
ROS (U/min)	1.6 $\pm 0.1$	2.7 $\pm 0.3^{***}$	1.4 $\pm 0.1$	1.3 $\pm 0.1$	1.2 $\pm 0.07$	1.3 $\pm 0.1$	1.2 $\pm 0.06$
TBARS (nmole/min/mg)	4.2 $\pm 0.1$	13.1 $\pm 0.7^{***}$	3.5 $\pm 0.5$	3.4 $\pm 0.5$	3.1 $\pm 0.8$	2.6 $\pm 0.9^*$	2.3 $\pm 1.0^*$

Notes: Values are expressed as mean  $\pm$  SEM.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

### 3.6 Effect of metformin, Se and SeNPs on serum hormonal concentration

The testosterone concentration in serum was significantly high ( $P < 0.001$ ) in PCOS group as compared to control while, no significant change was observed in metformin, Se-I and Se-II, SeNPs-I and SeNPs-II treated groups. Serum estradiol concentration of

PCOS group was significantly reduced ( $P < 0.05$ ) but significantly increased ( $P < 0.05$ ) in Se-II and SeNPs-II treated groups. The metformin and Se-I and SeNPs-I treated groups showed no significant change. Letrozole administered in the PCOS group, Se-II and SeNPs-II treated groups resulted in significant reduction ( $P < 0.001$ ,  $P < 0.01$  and  $P < 0.01$  respectively) in progesterone concentration as compared to control. Se-I and SeNPs-I treated groups did not show any significant change in progesterone concentration as compared to the control (Table 4).

**Table 4** Mean  $\pm$  SEM of testosterone, estradiol and progesterone concentrations of control, PCOS, metformin, Se-I, Se-II, SeNP-I and SeNP-II treated adult female rats during 36 days of experiment

<i>Parameters</i>	<i>Control</i>	<i>PCOS</i>	<i>Metformin</i>	<i>Se-I</i>	<i>Se-II</i>	<i>SeNPs-I</i>	<i>SeNPs-II</i>
Testosterone (mg/ml)	4.1 $\pm 0.2$	7.2 $\pm 0.4^{***}$	3.8 $\pm 0.2$	3.5 $\pm 0.4$	3.7 $\pm 0.3$	3.5 $\pm 0.3$	3.2 $\pm 0.4$
Estradiol (mg/ml)	4.0 $\pm 0.2$	2.6 $\pm 0.2^*$	4.5 $\pm 0.3$	3.2 $\pm 0.3$	5.4 $\pm 0.3^*$	3.2 $\pm 0.5$	5.4 $\pm 0.3^*$
Progesterone (mg/ml)	3.0 $\pm 0.2$	1.7 $\pm 0.2^{***}$	2.8 $\pm 0.1$	2.6 $\pm 0.1$	2.4 $\pm 0.07^{**}$	2.6 $\pm 0.07$	2.4 $\pm 0.06^{**}$

Notes: Values are expressed as mean  $\pm$  SEM.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

### 3.7 Effect of metformin, Se and SeNPs on histopathology of ovaries

#### 3.7.1 Diameter of follicles

The diameter of corpus luteum and cystic follicles depict no significant change among all treated groups when compared to the control. Diameter of atretic follicles of metformin and Se-I treated groups showed significant decrease ( $P < 0.001$ ) as compared to control (Table 5 and Figure 4).

#### 3.7.2 Number of cystic follicles

Number of cystic follicles increased significantly ( $P < 0.001$ ) in PCOS group as compared to the control. No significant change was observed in metformin, Se-I and Se-II, SeNPs-I and SeNPs-II treated groups as compared to control presented in Table 5 and Figure 4.

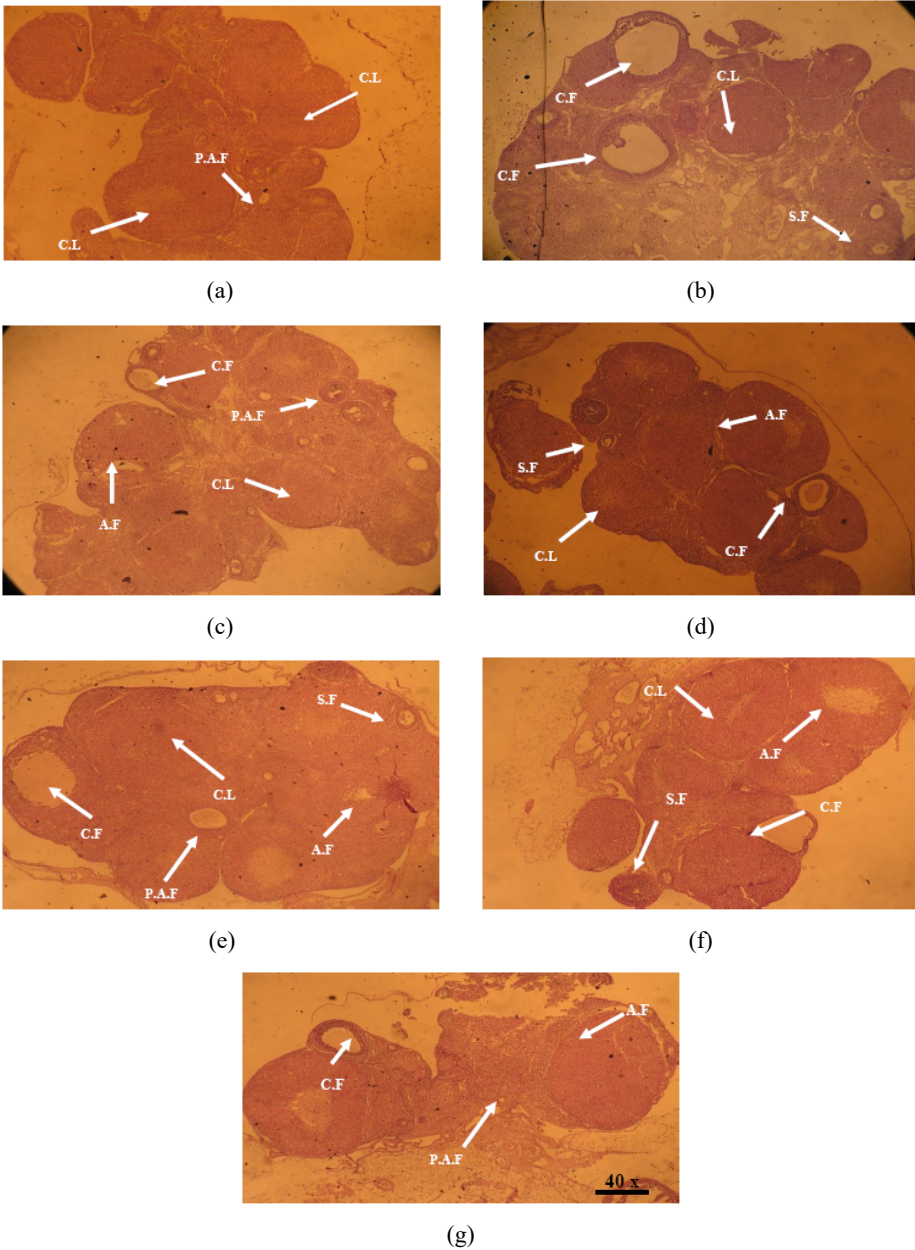
#### 3.7.3 Number of atretic follicles

There was no significant change observed in the number of atretic follicles in all treated groups when compared to the control (Table 5 and Figure 4).

#### 3.7.4 Number of corpus luteum

In the number of corpus luteum there was also no significant change observed in all treated groups as compared to the control (Table 5 and Figure 4).

**Figure 4** Histopathological features of letrozole induced PCOS in adult rats, (a) control (b) PCOS group (c) PCOS + metformin group (d) PCOS + Se-I (e) PCOS + Se-II (f) PCOS + SeNP-I (g) PCOS + SeNP-II treated adult female rats (see online version for colours)



Notes: C.F – cystic follicle, A.F – atretic follicle, P.A.F – preantral follicle, C.L – corpus luteum, S.F – secondary follicle.

**Table 5** Mean  $\pm$  SEM of diameter and number of follicles of control, PCOS, metformin, Se-I, Se-II, SeNP-I and SeNP-II treated adult female rats during 36 days of experiment

Parameters	Control	PCOS	Metformin	Se-I	Se-II	SeNPs-I	SeNPs-II
Diameter of corpus luteum	95.4 $\pm$ 5.4	110.2 $\pm$ 2.9	84.6 $\pm$ 7.2	85.8 $\pm$ 3.5	80.4 $\pm$ 9.3	92.5 $\pm$ 9.6	89.4 $\pm$ 9.3
Diameter of cystic follicle	0.0	75.0 $\pm$ 7.7	67.2 $\pm$ 3.2	66.6 $\pm$ 5.4	61.8 $\pm$ 12.5	60.0 $\pm$ 5.2	57.5 $\pm$ 4.2
Diameter of atretic follicle	105.1 $\pm$ 6.1	107.4 $\pm$ 4.0	60.5 $\pm$ 4.0***	66.1 $\pm$ 5.3***	84.9 $\pm$ 7.9	86.6 $\pm$ 5.8	99.4 $\pm$ 9.21
Number of corpus luteum	7.0 $\pm$ 0.3	1.7 $\pm$ 0.2	3.3 $\pm$ 0.2	4.3 $\pm$ 0.2	4.3 $\pm$ 0.2	4.6 $\pm$ 0.3	3.3 $\pm$ 0.6
Number of cystic follicle	0.0	13.9 $\pm$ 0.4***	5.1 $\pm$ 0.2	4.7 $\pm$ 0.2	1.7 $\pm$ 0.2	0.9 $\pm$ 0.2	2.0 $\pm$ 0.2
Number of atretic follicle	2.4 $\pm$ 2.4	4.7 $\pm$ 0.2	2.4 $\pm$ 0.2	4.7 $\pm$ 0.2	1.9 $\pm$ 0.2	1.9 $\pm$ 0.2	1.3 $\pm$ 0.2

Notes: Values are expressed as mean  $\pm$  SEM.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

#### 4 Discussion

The present study was designed to investigate the possible ameliorative effect of Se and SeNP in alleviating PCOS by reduction of oxidative stress, insulin resistance and dyslipidemia in letrozole induced PCOS in adult female rats. From previous studies it has been found that Se plays important role in functioning of islets of Langerhans, gastrointestinal tract and ovaries (Campbell et al., 2008; Eguchi and Manabe, 2013; Tapiero et al., 2003; Brigelius-Flohé, 1999; Roman et al., 2014; Pieczyńska and Grajeta, 2015; Nazıroglu et al., 2004). On the basis of previous knowledge we have targeted insulin resistance through oral administration of Se and SeNPs for the treatment of letrozole induced PCOS in female rats, as it is evident that Se is also known for its activity of initiating insulin signalling cascades in the cell (McKenzie et al., 2002), glucogenesis (Becker et al., 1996) and glucose uptake in adipocytes (Ezaki, 1990; Fujii et al., 2005; Mistry et al., 2012). In the present study we observed that blood glucose level gets to normal after treatment with metformin, Se and SeNPs as compared to the PCOS. This also suggests that SeNPs might be a potential agent for glycaemic control through increased glucose uptake induced by insulin signalling pathway (Kim et al., 2012; Beckett and Arthur, 2005; Shepherd and Kahn, 1999). The total protein values increased significantly (P < 0.001) in the PCOS group and this might be due to high androgen level in PCOS that leads towards inflammation and infection. In the meanwhile, non-significant reduction in total protein concentration by Se and SeNP treated groups is much comparable to metformin treated groups which revealed that this might have anti-androgenic effects (Oktenli et al., 2007; Bosco et al., 2011). Antioxidant enzymes are an important defensive mechanism which controls the overproduction of reactive oxygen species (ROS) that usually results in normal functioning of cells and their biological activities (Matés et al., 1999; Mittler, 2002). In the present study, the PCOS group showed increased levels of ROS indicating the excessive production of ROS leading to molecular damage and disturbance of cellular structure (Halliwell, 1991;

Houstis et al., 2006; Apel and Hirt, 2004). In the present study, ROS levels in the metformin treated group was decreased which indicated its antioxidant ability and inactivation of an enzyme called NADPH oxidase (Piwkowska et al., 2010). Se and SeNP treatment also reduced the levels of oxidative stress which depict that it has antioxidant activity as well. As in the previous studies it has been observed that Se plays a key role in antioxidant balance and it is considered to be important in development and function of the reproductive system (Mirone et al., 2013; Agarwal and Prabakaran, 2005; Pařízek and Ošťádalová, 1967). Recently, Similar studies depicted that SeNP has more antioxidant activity as compared to Se (Gao et al., 2002; Hosnedlova et al., 2017).

Catalase is well-known antioxidant enzyme (Lobo et al., 2010). It is responsible for the conversion of  $H_2O_2$  into water and oxygen (Devi and Chitra, 2010). On the other hand, SOD converts  $H_2O_2$  into water through GPx, and POD works against oxidative damage of lipid membranes. In our study, the values of SOD, CAT and POD decreased significantly in the PCOS group. Metformin, Se and SeNP treated groups showed no significant change in values of SOD, CAT and POD which suggests that Se as an inducer of antioxidant enzyme and insulin resistance plays role in the hyperglycemia and obesity (Ullah et al., 2017; Hassanin et al., 2013). It was reported that Se has ability to mimic insulin activities and is responsible in metabolic processes like glycolysis and gluconeogenesis (Becker et al., 1996; Nordberg and Arner, 2001).

Lipid profile assay is the study of abnormal changes in lipid contents which comprise cholesterol, HDL, LDL, VDL, non-HDL and triglycerides (Warnick et al., 2001; Liu et al., 2005). The present study showed that cholesterol levels were high in PCOS group in comparison with the other treated groups. Metformin has the anti-lipidemic effect, its administration decreased the cholesterol level, this result was similar to Pai and Majumdar (2014). The Se-II and SeNP-II treated groups also showed significantly decreased cholesterol level as Se is responsible for a reduction of cholesterol. HDL-C is considered as a good cholesterol but it was noticed that HDL-C was decreased non-significantly in PCOS group which was similar to previous studies (Maliqueo et al., 2012; Ebrahimi-Mamaghani et al., 2015). In the meanwhile, SeNP treated groups showed significant increase HDL-C which clearly depicted that Se and its compounds might have therapeutical effects and hence restore the level of good cholesterol (Duntas and Benvenega, 2015; Combs, 2001).

High concentration of testosterone hormone in PCOS group showed that the androgen level increased due to inhibition of aromatase enzyme (Corbin et al., 1999; Garcia-Velasco et al., 2005). In the meanwhile, the metformin, Se and SeNP treated groups show decline in testosterone concentration as compared to PCOS which showed an improvement in androgen level. T and Se have a negative correlation as the Se increased as the T level decreased and vice versa (Coskun et al., 2013; Oluboyo et al., 2012; Behne et al., 1996). Low level of estradiol hormone in PCOS group was initiated by treatment with metformin, Se and SeNPs in comparison with the control group. In Se and SeNP treated groups the estradiol concentration significantly increased, as Se is responsible for the excessive release of estradiol by acting on granulosa cells (Basini and Tamanini, 2000; Gürgen et al., 2013; Ceko et al., 2014). In contrast to this, the progesterone concentration showed significant decrease in PCOS group indicating a cause of anovulation, this result was similar to previous studies (Ullah et al., 2017; Jahan et al., 2016). A significant decrease was noticed in Se and SeNP treated groups which

showed the healing effect of ovaries and a decreased a number of cystic follicles (Campbell et al., 2008).

Histopathology of ovarian tissues revealed that there is a resemblance in human and rat PCOS when induced with letrozole. The results of histopathology indicate that the anovulation might be due to active FSH and LH levels and the reduced interplay between cells of ovaries as theca cells and granulosa cells (Kafali et al., 2004). A thin layer of granulosa cells line up the sub capsular cysts which result in hyperplasia of theca cells; these findings were similar to the previous studies (Baravalle et al., 2006). Abnormal level of androgen hormone in ovaries leads to increased follicular atresia and decreased follicular growth (Homburg, 2009). The number of corpus luteum increased significantly in PCOS group as compared to the control group in the present study which is in relation with the previous studies (Anderson et al., 1998; Humaidan et al., 2006; Heijnen et al., 2005). The post-treatment of metformin decreased the number of corpus luteum in the present study and the number and diameter of cystic follicles in PCOS group increased, whereas metformin, Se and SeNP treated groups showed decreased numbers of these follicles which are also in relation to the previous studies (Kafali et al., 2004; Farin et al., 1988; Rajakoski, 1960; Brawer et al., 1986). In recent studies, Se-based herbal medicines were used for PCOS patients, which depicted that Se is best known for a reduction in ovarian cysts (Rezvanfar et al., 2012; Fauser et al., 2012; McCook et al., 2005; Broekmans et al., 2008).

## 5 Conclusions

The present study demonstrated the anti-androgenic effect of Se and SeNPs in treatment of PCOS. Selenium and selenium NPs improved ovarian dysfunction, reduced the number of ovarian cysts, improved equilibrium between antioxidant enzymes and ROS and also improved lipid profile to normal level. In this study Se and SeNP showed protective potentials that improved the hormonal concentrations of testosterone, estradiol and progesterone in female rats. Se and SeNP also displayed a significant role in reducing the hyperglycemic condition which means the possible ameliorative medication for treatment of clinical and biochemical characteristics of polycystic ovarian syndrome. Further studies are needed to investigate the therapeutic potential of Se and SeNP so that it can be used for treatment of PCOS in order to lessen the side effects of other modern drugs.

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