WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 11, 113-125. Research Article ISSN 2277– 7105

EFFECT OF SILVER NANOPARTICLES ON LEVELS OF SERUM FSH, LH AND ESTRADIOL IN PCO-INDUCED FEMALE MICE

Prof. Dr. Muhammed-Baqir M-R. Fakhrildin[1], Lec. Assis. Suzan I. Bajilan*[2] and Prof. Assis. Dr. Gazwa Al-Naqeeb[3]

[1]Jabir Ibn Hayyan Medical University, Dep. Physiology, Al-Kufa-Iraq.

[2]College of Nursing, Basic Science Dep. University of Baghdad, Baghdad-Iraq.

[3]College of Science for Women, Biology Dep. University of Baghdad, Baghdad-Iraq.

Article Received on
29 July 2017,
Revised on 19 August 2017,
Accepted on 10 Sep. 2017
DOI: 10.20959/wjpr201711-9483

***Corresponding Author**
Prof. Suzan I. Bajilan
College of Nursing, Basic Science Dep. University of Baghdad, Baghdad-Iraq.

ABSTRACT

This study was conducted to investigate the effect of two concentrations (0.5 and 1ppm) of silver nanoparticles on levels of serum FSH, LH and E_2 in PCO-induced female mice. A number(n=80) of mature female mice were intraperitoneally injected with human Chorionic Gonadotropin (hCG) at a dose of (10 IU/ mL) three days a week for two weeks in order to induce PCO. Then, these mice were divided into two groups, according to the duration of treatment (30, 45 days). Each group was subdivided to subgroups according to the substance administrated. Two subgroups were treated with (0.5 and 1ppm) of silver NPs solution by (i.p) injection twice a week and the third subgroup was treated with metformin at a dose of (1mg/day) orally three days a week. Results: there was a significant reduction ($P<0.05$) in serum levels of FSH in the PCO group treated with (1ppm) of silver NPs for 30 days as compared to the control. While, a significant elevation ($P< 0.05$) was observed in levels of E_2, for the same group as compared to the control. The PCO group treated with (0.5ppm) recorded a significant reduction ($P <0.05$) in serum levels of E_2 for the same duration. But longer duration (45 days) exhibited a significant elevation ($P< 0.05$) in levels serum of FSH in the PCO-induced mice treated with (0.5ppm) of silver NPs which, was similar to the effect of metformin. A significant elevation ($P> 0.05$) in serum levels of FSH was observed in the PCO group treated with (0.5ppm) of silver NPs along 30 days as compared to their similar group that was treated along 45 days but, non significant alteration ($P> 0.05$) were observed in serum levels of LH or E_2 between groups of the (30 days) duration as compared to their similar groups of the second duration (45 days). Conclusion:

There were significant alterations in levels of serum FSH, LH and E_2 in the PCO-induced mice treated with both concentrations of silver NPs as compared to the control.

KEYWORDS: Polycystic Ovary (PCO), Silver NPs, Metformin.

INTRODUCTION

According to the ASRM/ESHRE consensus meeting on the polycystic ovarian syndrome (PCOS), in Rotterdam, ovarian polycyst (PCO) included the presence of 12 or more follicles measuring 2-9 mm in diameter.[1,2] PCO is one of the main characteristics of polycystic ovarian syndrome (PCOS) which also includes chronic anovulation, hyperandrogenemia, altered LH: FSH ratio (> 2/3:1)[3] and /or ovarian volume that, exceed 10 cm^3.[4] The major endocrine systems involved in polycystic ovarian disease (PCOD) are the hypothalamic-pituitary axis, the ovary, the adrenal gland and peripheral tissue.[5]

The hormonal imbalance in PCO patients, makes the follicles reserves to increases in size and becomes cystic, this will lead to an increase in ovarian volume (OV) which becomes ≥ 10mL.[6] An elevated serum LH pulsatility and decreased FSH secretion with elevated LH/ FSH ratio resulting from abnormal GnRH pulses, represents an important etiologic factors in the pathogenesis of PCOS.[7] Besides, low intrafollicular estrogen concentration in association with the high intra-ovarian androgen, might compromise the maturation of the developing follicles thereby creating a cohort of small antral follicles.[8] The dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis and related neurochemical systems can be considered as another cause of PCOS.[9] Moreover, PCOS patients have a disruption of the neuroendocrine mechanisms including; a deficiency of hypothalamic dopamine regulating both gonadotropin- realizing hormone (GnRH) and prolactin release.[10]

Although, there are no specific treatments available for the PCOS patients, in general, treatments aimed at decreasing insulin and androgen levels. This encompasses drug therapy including insulin lowering and anti androgen medications or oral contraceptives, and life style interventions.[11] Women with PCOS have higher basal and glucose-stimulated insulin levels than weight-matched controls.[12] Insulin increases the testosterone bioavailability in PCOS women by reducing hepatic production of sex hormone binding globulin (SHBG). Further, it has been suggested that metformin reduces hyperandrogenism through its effect on both the ovary and adrenal gland suppressing their androgen production, reducing pituitary luteinizing hormone and increases the production of sex hormone binding globulin by the

liver.[13] Metformin can modulate the reproductive axis affecting the release of GnRH and LH as a result, serum levels of LH, LH/FSH, androstenedione, DHEA-s and progesterone will decrease.[14]

Nanomaterials in general, have unique physiochemical properties, such as ultrasmall size, large surface area to mass ratio, and high reactivity, such properties can be used to overcome some of the limitations found in traditional therapeutic and diagnostic agents.[15] According to the definition used by scientific communities relating to nano-technology, a nanoparticle is defined as a particle with dimension between 1 and 100 nano-meters.[16] One of these nanomaterials is Nanosilver or silver nanoparticles that is under the most scrutiny today, its release and effects are studied increasingly.[17]

The small size of the particles, increases the total surface area of the silver exposed in solution, resulting in the highest possible effect per unit of silver.[18,19] In general, nanostructures can enter the body through six principle routs intravenous, dermal, subcutaneous, inhalation, intraperitoneal and orally.[15] The picking up of s NPs silver by the cell, depends on the cell type and the specific analysis method applied, the surface proprieties and size of silver NPs also are important factors.[20] The effect of oral administration of silver NPs solution and in liposomal forms with a dose of 10µg /kg /day revealed an increase in serum levels of E_2 on day 14 of gestation in rabbits.[21] Therefore, this study was conducted to investigate the effect of silver NPs on hormone levels of FSH, LH and estradiol E_2 in PCO-induced female mice. A comparison also was achieved between effect of metformin and silver NPs on levels of these hormones for two durations (30 and 45 days).

MATERIALS AND METHODS

a-Animals

Mature female mice (no.80), weighting about (20-25) gm were purchased from Animal House of the High Institute for Infertility Diagnosis and Assisted Reproductive Technology /Al-Nahrain University. Animals were retained under standard conditions of temperature (25-28) °C and 12 hours light dark cycle throughout the period of experiments. In order to induce polycystic ovaries (PCO), these mice were intraperitoneally injected (i.p) with human Chorionic Gonadotropin hCG (Choriomon) at a dose of (10 I.U); three times a week for two weeks.[22] The stage of estrous cycle was not determined at the beginning of the experiment because, many of the mechanisms involved in the induction of ovarian follicular cysts seemed to be independent of the stage of development of the ovary.[23]

b-Preparation of human Chorionic Gonadotropin Hormone (hCG) solution

This solution was prepared by diluting one ampoule of Choriomon (5000 I.U.) in 10 mL of normal saline as a stock solution, then (0.1) mL of this solution was taken and diluted again with (0.4)mL of normal saline. Each mouse was intraperitonialy injected with (0.1) mL of the last preparation.

c- Preparation of silver nanoparticles (Ag NPs) solution

Silver nanoparticles solution was prepared by Iranian Nanoparspanda Company. The concentration of this solution was (4000) ppm and the size of these particles were about (50-100) nanometer. The characteristics of this solution was confirmed by using Scanning Probe Microscope (SPM) at department of Chemistry /College of Science /University of Baghdad. Then the solution was activated by using ultrasound sonicator at department of Physics/ College of Science /University of Baghdad every two weeks. In this study, two dilutions (0.5 and 1ppm) were prepared from this solution according to a study conducted to assess the effect of silver nanoparticles on ovarian features via intraperitoneal injection (i.p).[24] These doses were prepared according to the formula : $C_1V_1=C_2V_2$.[25]

d- Preparation of Metformin drug solution

One pill of metformin (500) mg was crashed using a manual mortar, the powder then was dissolved in (25)mL of normal saline. This suspension was considered as **Stock 1**.One mL of Stock 1 was taken and diluted with (19) mL normal saline and this solution was considered as **Stock 2**. Preparation of stock 2 was repeated every time when animals treated with the drug. However, (0.1)mL at a dose of (1mg/mL) was taken from stock 2 and administrated orally to each female mouse three times a week throughout the duration of each experiment.[26] PCO-induced mice then were divided into two main groups according to days of treatment (30 and 45 days) respectively. Each group has the following subgroups: the first and second subgroups were treated with (0.5 and 1ppm) respectively by i.p. injection twice a week while, the third subgroup was treated orally with metformin three days a week. At the end of each experiment, female mice were sacrificed, blood samples were collected by direct cardiac puncture in eppendorf tubes, serum was isolated by using cooling centrifuge at (14)°C at a speed of 2500 rpm, and preserved at freezing temperature for hormonal analysis. Serum levels of FSH, LH and E_2 was measured by the use of enzyme linked immuno sorbent assay (ELISA) technique at a private laboratory.

Statistical Analysis

The Statistical Analysis System- SAS[27] program was used to study the effect of different factors for all study parameters. Least significant difference (LSD) test was used to compare between means of groups in this study.

RESULTS

Table (1) represents levels of serum FSH, LH and E_2 for the control and the treated groups of the first duration (30 days). Concerning levels of FSH, there was a significant reduction ($P \leq 0.01$) in the PCO group as compared to the control. Similarly, the PCO group treated with metformin revealed a significant reduction ($P \leq 0.01$) as compared to control group. Both PCO groups treated with (0.5ppm and 1ppm) demonstrated a significant reduction ($P \leq 0.01$) in serum levels of FSH as compared to the control group. Regarding serum levels of LH, the PCO group revealed a significant reduction ($P \leq 0.01$) as compared to the control group. While, the PCO group treated with (1ppm) of silver NPs recorded a significant decrease ($P \leq 0.05$) as compared to the control and a significant elevation ($P \leq 0.05$) as compared to the PCO group treated with (0.5ppm) of silver NPs. Whereas, serum levels of E_2 appeared as the following, the PCO group revealed a significant decrease ($P \leq 0.05$) as compared to the control. But, the PCO group treated with (1ppm) of silver NPs recorded a significant elevation ($P \leq 0.01$) when compared to the control group.

Table (2) represents levels of serum FSH, LH and E_2 for the control and the treated group for a duration of (45 days). Non significant difference ($P > 0.05$) was observed in serum levels of FSH between the PCO group treated with metformin and the control group. But, a significant elevation ($P < 0.05$) in levels of FSH was seen in the group treated with metformin. Similarly, (0.5ppm) of silver NPs caused a significant elevation ($P < 0.05$) in serum levels of FSH but, (1ppm) of silver NPs caused a significant reduction ($P < 0.05$) when compared to the control group. Serum levels of LH was significantly reduced ($P<0.05$) in the PCO group as compared to the control group. But, the following treatments did not reveal a significant alteration ($P > 0.05$) in serum levels of this hormone in comparison to the control group.

A significant elevation ($P< 0.05$) in serum levels of E_2 was seen in the PCO group treated with metformin as compared to the control. While, the PCO group treated with (0.5ppm) of silver NPs revealed a significant reduction ($P< 0.05$) as compared to the PCO group treated with metformin and the PCO group treated with (1ppm) of silver NPs.

A comparison in the levels of serum FSH, LH and E_2 between the 30 days duration and the 45 days duration was achieved. Note that, the control group and the PCO group was shared between these two experiments. Serum levels of FSH was significantly low ($P<0.05$) in the PCO group treated with metformin for 30 days as compared to the same group that was treated for 45 days. Similarly, a significant reduction($P< 0.05$) was observed in levels of serum FSH in the PCO group treated with (0.5ppm) of silver NPs when compared to their analogous group that was treated for 45 days. But, non significant differences ($P> 0.05$) was seen in serum levels of FSH between the PCO group treated with (1ppm) of silver NPs for 30 days and the same group which was treated for 45 days.(Figure 1).

There was a significant reduction ($P<0.05$) in levels of serum LH in the group treated with metformin for 30 days as compared to the same group that was treated for 45 days. While, both doses of silver NPs (0.5 and 1ppm) seemed to cause non significant alteration ($P> 0.05$) in levels of serum LH neither along 30 days nor 45 days of treatment. Figure (2).

There was a significant reduction ($P<0.05$) in levels of serum E_2 in the PCO group treated with metformin for 30 days as compared to their parallel group which was treated for 45 days. While, non significant alteration($P > 0.05$) was seen in levels of serum E_2 in the PCO group treated with (0.5ppm) of silver NPs for 30 days as compared to their similar group that was treated for 45 days. In addition, silver NPs at a dose of (1ppm) seemed to cause non significant alteration($P> 0.05$) in levels of serum E_2 neither for 30days duration nor for 45 days, as represented in the figure (3).

Table. (1): Effect of metformin, silver NPs (0.5 and 1ppm) and duration of treatment (30 days) on serum levels of FSH, LH and E_2 in the PCO induced female mice.

	Serum levels of hormones (mean ± S.E)		
Groups	**FSH**	**LH**	**Estradiol (E_2)**
Control	6.6 ± 1.79 a	5.20 ± 2.67 a	48.03 ± 3.48 b
Polycystic ovary (PCO)	1.31±0.9 b	1.32 ± 0.72 b	53.64±2.31b
PCO+ metformin	0.55 ± 0.02 b	1.04 ± 0.43 b	52.55 ± 4.31b
PCO+ silver NPs (0.5ppm)	0.975 ±0.44 b	1.91 ± 0.52 b	54.18 ±4.32 b
PCO+ silver NP (1ppm)	1.23 ±0.09 b	3.38 ± 0.41 b	73.33 ± 6.29 a
LSD values	4.512**	2.677**	18.653**

Means with different subscripts within each column are significantly different ($P<0.05$).

Means with similar subscripts within each column are non significantly different ($P> 0.05$).

Table. (2): Effect of metformin, silver NPs (0.5 and 1ppm) and duration of treatment (45 days) on serum levels of FSH, LH and E_2 in the PCO induced female mice.

	Serum levels of hormones (mean ± S.E)		
Groups	**FSH**	**LH**	**Estradiol (E_2)**
Control	6.6 ± 1.79 a	5.20 ± 2.67 a	48.03 ± 3.48c
Polycystic ovary (PCO)	1.31± 0.9 b	1.32 ± 0.72 b	53.64±2.31c
PCO+ metformin	8.90 ± 2.64 a	6.30 ± 2.49 a	62.08 ± 5.61 b
PCO+ silver NPs (0.5ppm)	8.20 ± 2.05 a	3.10 ±1.05 a	56.76 ± 5.02 c
PCO+ silver NP (1ppm)	0.90 ± 0.36 b	3.90 ±0.88 a	72.16 ± 7.51a
LSD values	6.784**	2.903**	13.962**

Means with different subscripts within each column are significantly different (P<0.05).

Means with similar subscripts within each column are non significantly different (P> 0.05).

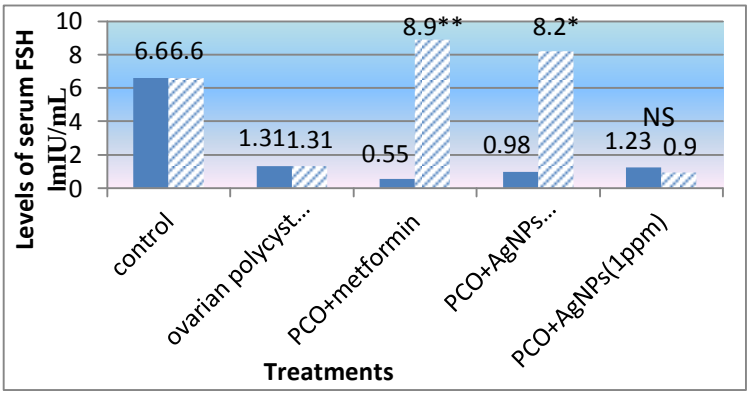

Figure (1): Effect of two doses of silver NPs (0.5 and 1ppm), metformin and days of treatment (30 and 45 days) on serum levels of FSH in PCO induced female mice. full columns: 30 days, striated columns: 45 days. ** significant difference (P<0.01), * significant difference (P< 0.05), NS non significant difference (P > 0.05).

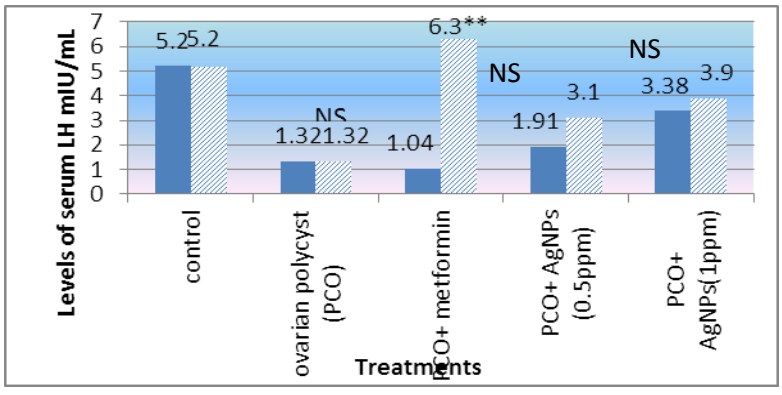

Figure (2): Effect of two doses of silver NPs (0.5 and 1ppm), metformin and days of treatment (30 and 45 days) on serum levels of LH in PCO induced female mice. full columns: 30 days, striated columns: 45 days. ** significant difference (P<0.01), NS non significant difference (P > 0.05).

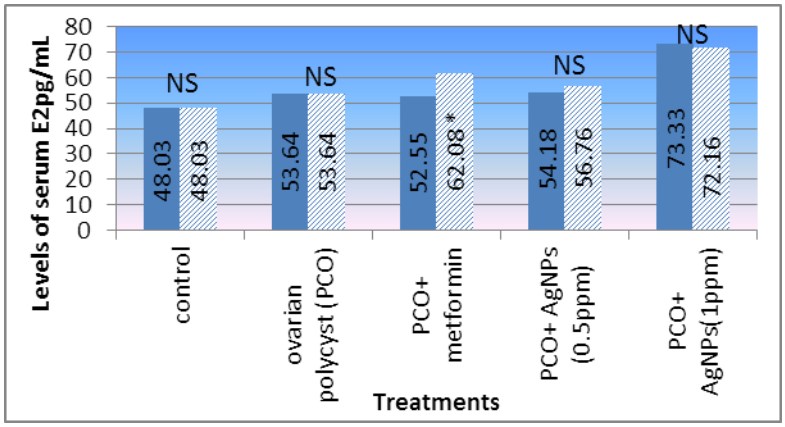

Figure (3): Effect of two doses of silver NPs (0.5 and 1ppm), metformin and days of treatment (30 and 45 days) on serum levels of E_2 in PCO induced female mice. Blue columns: 30 days, striated columns: 45 days. * significant difference (P< 0.05), NS non significant difference (P > 0.05).

DISCUSSION

Serum levels of FSH in the PCO group, was significantly low (P≤ 0.05) as compared to the control. These results were in consistence with Shriock[5] who reported that small follicles and relatively low levels of FSH in addition to elevated androgens were the main characteristics of PCO women. Whereas, an increase in serum levels of FSH and LH were considered among causes of PCO.[28,29] This relatively low levels of FSH in the PCO group might contribute to the inhibition of the aromatase reaction in the ovary.[5] Many reports have suggested that unknown factors could be involved in the lack of *in vivo* aromatase function which is one of the main features of the PCOS granulosa cells.[8] Aromatase is considered as another key enzyme in ovarian steroidogenesis found in granulosa cells and participated in conversion of androgens to estrogens.[30]

Serum levels of LH was significantly reduced in the PCO-induced mice compared to the control. This result came in line with Mukherjee and Maitra[3] who reported that, altered LH: FSH ratio (> 2/3:1) and polycystic ovaries were among the main characteristics of PCOS, in addition to chronic anovulation, hyperandrogenemia. But previous study by Ota[31] indicated that, continues long term treatment (80 days) of hCG by subcutaneous injection could induce polycystic ovaries and cause a tonic secretion of LH. Moreover, raised levels of serum LH, testosterone and androstenedione, in association with low or normal levels of FSH were described as a diagnostic endocrine profile for polycystic ovary syndrome (PCOS).[32] In this study, hCG was administrated in a discontinuous manner for two weeks which might cause

imbalance in hormone levels and reduced serum levels of LH. Treatment, with metformin for 30 days did not show a significant alterations in serum levels of the three hormones LH, FSH and E_2. But, longer duration of treatment revealed a significant elevation in levels of serum FSH as a result of treatment with metformin (table 2). This result came in line with Mittal[33] who observed a significant increase in FSH levels and a reduction in LH levels and LH/ FSH ratio after treating PCOs patients with metformin for six months. A significant increase in levels of serum FSH for women with PCOs after(4-6) months of treatment with metformin also, was observed.[34] In contrast, metformin as compared to genseng extract caused a reduction in FSH and progesterone levels in non obese PCO women.[35] On the same way, a significant reduction in serum levels of FSH in PCOs women was observed after treatment with metformin for (6-12) months.[14]

Similarly, silver NPs at (0.5ppm) for longer duration caused a significant elevation in levels of serum FSH, table (2). This result was in consistence with Kong[36] who reported that treatment with Nickel nanoparticles(Ni-NPs) exerted an elevation in serum levels of FSH and LH, and a reduction E_2 associated with significant and dose-dependent in females. These results might be attributed to inhibin protein. This protein with both types, inhibin A and inhibin B are produced by granulosa cells of normal and PCOS follicles and act in an endocrine manner to suppress FSH and locally to enhance follicle development.[37] Whereas, the high dose of silver NPs caused a significant reduction in serum levels of FSH for 30 days and for 45 days. This result was in line with Baki[16] who noticed a non significant reduction in serum levels of FSH in male rats which, were treated with oral administration of silver nanoparticles (60nm) with four doses (25, 50**,** 100 and 200) for 45 days**.** Moreover, gonadotropins (LH and FSH) have protein structure and decreased by injection of nanoparticles which, inhibit function of the endocrine system by blocking of pituitary-hypothalmus axis.[38] Nanoparticles of (1–100 nm) in size can easily cross the blood–brain barrier (BBB) and/or produce damage to the barrier integrity by altering endothelial cell membrane permeability.[39,40]

A highly significant elevation in serum levels of E_2 was observed in the PCO-induced mice treated with (1ppm) of silver NPs as compared to the control for both durations (30 and 45 days). These results were in line with Syrvatka[41] who reported that, silver nanoparticles can suppress the ovarian steroidogenesis through the output of silver ions. While, 10nm of gold-nanoparticles(GNPs) can enter rat ovarian granulosa cells (GC) and localize in lipid droplets

and mitochondria, these particles modulate GC estrogen production and affect certain enzyme genes in the steroid biosynthesis pathway and cause subtle alteration in the intact ovarian culture model.[42] Similar results were observed on concern Ni-NPs and levels of E_2 in females rats.[36] These results were compatible to levels of serum LH for both periods. According to the Hypothalmic-pituitary axis, sex steroids secreted by the gonads have a negative feedback effect on the secretion of GnRH (gonadotropin-releasing hormone) and on the secretion of gonadotropins.[41]

CONCLUSION

This *in vivo* study demonstrated a significant alterations in levels of serum FSH, LH and E_2 in the PCO-induced mice treated with both concentrations of silver NPs as compared to the control. These results might be beneficial in case of PCO because it is a condition characterized by elevated serum LH pulsatility and decreased FSH secretion with elevated FSH/LH ratio and subsequently low levels of E_2.

ACKNOWLEDGEMENT

Grateful and appreciations are submitted to all persons who offer their help and support to achieve this approach.

REFERENCES

1. Chang PL, Lindheim SR, Lowre C, Ferin M, Gonzalez F, Berglund L, Carmina E, Sauer MV. and Lobo RA. Normal Ovulatory Women with Polycystic Ovaries Have Hyperandrogenic Pituitary-Ovarian Responses to Gonadotropin-Releasing Hormone-Agonist Testing. J. of Clinical Endocrinology & Metabolism, 2000; 85(3): 995-1000.
2. Balen A. The current understanding of polycystic ovary syndrome. The Obst. & Gyn., 2004; 6: 66-74.
3. Mukherjee S. and Maitra A. Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome. Indian. J. Med. Res., 2010; 131: 743-760.
4. Bachanek M, Abdalla N, Cendrowski K, Sawicki W. Value of ultrasonography in the diagnosis of polycystic ovary syndrome – literature review. J. Ultrason, 2015; 15: 410–422.
5. Schriock E, Martin MC, Jafee RB. Polycystic Ovarian Disease. Medical Progress, 1985; 142. 4: 519-522.
6. Usmani A, Rehman R, Qamar A. Effect of age on uterine and ovarian morphology with polycystic ovaries. J. Pak. Med. Assoc, 2014; 64(10): 1119-1122.

7. Krishna BM, Laloraya M. Insulin Pardox and Polycystic Ovarian Syndrome: Implications on Mechanism and Pathogenesis. Health Sci., 2013; 2(1): 1-12.
8. Andereani CL, Pierro E, Lazzarin N, Lanzone A, Caruso A, Mancuso S. Effect of follicular fluid on granulosa luteal cells from polycystic ovary. Human Reproduction, 1996; 11(10): 2107-2113.
9. Chang RJ. The Reproductive Phenotype in Polycystic Ovary Syndrome. Nature. Reviews. Endocrinology, 2007; 3: 688-695.
10. Hassan JS, Ali AH, Fakhridin M-B M-R. Role of Prolactin in Iraqi Obese Women with Polycystic Ovary Syndrome. Americ. J. Med& Med. Sci., 2015; 5(3): 135-139.
11. Mansour A, Hosseini S, Larijani B, Reza M, Tehrani M. Nutrients as Novel Therapeutic Approaches for Metabolic Disturbances in Polycystic Ovary Syndrome. EXCLI Journal, 2016; 15: 551-564.
12. Pasquali R, Pelusi C, Raggazini C, Hasanaj R, Gambineri A. Glucose Tolerance, Insulin Secretion and Insulin Sensitivity in Polycystic Ovary Syndrome. JOP. J. Pancreas (online), 2002; 3(1): 1-7.
13. Bailey CJ and Turner RCT. Metformin. The New England J. Medicine, 1996; 334(9): 574-579.
14. Valija-Asimi Z. Evaluation of endocrine changes in women with the polycystic ovary syndrome during metformin treatment. Bosn. J. Basic. Med Sci., 2013; 13(3): 180-185.
15. El-Ansary A, Al-Daihan S. On the Toxicity of Therapeutically Used Nanoparticles: An Overview. J. Toxicol, 2008; 2009: 1-9.
16. Baki ME, Miresmaili SM, Pourentezari M, Amraii CE, Yousefi V, Spenani HR, Talebi AR, Anvari M, Fazilati M, Fallah AA, Mangoliu E. Effects of silver nano-particles on sperm parameters, number of leydig cells and sex hormones in rats. Iran. J. Reprod. Med., 2014; 12(2): 139-144.
17. Nowack B, Krug HF, Height M. 120 Years of Nanosilver History: Implications for Policy Makers. Environ. Scien. &Techn. ACS Publications. American Chemical Society, 2010; 1-7.
18. Panyala NR, Peña-Měndez EM,Havel J. Silver or silver nanoparticles : a hazardous threat to the environment and human health? J.Appli. Biomed, 2008; 6: 117-129.
19. Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MM. Nanosilver particles in medical applications: synthesis, performance, and toxicity. Int. J. Nanomed, 2014; 9: 2399-2407.
20. Bartlomiejczyk T, Lankoff A, Kruszewski M, Szumiel I. Silver nanoparticles-allies or adversaries? Annals of Agricultural and Environmental Medicine, 2013; 20(1): 48-54.

21. Syrvatka V, Rozgoni I, Slyvchu Y, Milovanova G, Hevkan I, Matyukha I. Effects of Silver Nanoparticles in Solution and Liposomal Form on Some Blood Parameters in Female Rabbits During Fertilization and Early Embryonic Development. J. Microb. Biotech & Food Sci., 2013; 3(4): 274-278.
22. Bogovich K. Follicle –Stimulating Hormone Plays a Role in the Induction of Ovarian Follicular Cysts in Hypophysectomized Rats. Bio. Rep., 1992; 47: 149-161.
23. Bogovich K. Induction of Ovarian Follicular Cysts in the Pregnant Rat by Human Chorionic Gonadotropin. Bio. Rep., 1991; 45: 34-42.
24. Ghorbanzadeh V, Moshtaghian SJ, Habibian S, Ebadi A-G, Vandechali OB. Influence of nanosilver on secondary follicles of ovary via intraperitoneal injection in rats. Europ. J. Experiment. Bio., 2012; 2(4): 1367-1369.
25. Skoog DA, West DA, Holler FJ, Crouch SR. Fundamental of analytical chemistry.4th ed. Thomson learning Inc. USA, 2004; 10.
26. Elia E, Sanders V, Luchetti CG, Solano ME, Giralmo GD, Gonzalez C, Motta AB. The mechanism involved in the action of metformin in regulating ovarian function in hyperandrogenized mice. Mol. Hum. Rep., 2006; 12(8): 475-481.
27. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA, 2012; 20-25.
28. Hussein B, Alalaf S. Prevalence and characteristics of polycystic ovarian syndrome in a sample of infertile Kurdish women attending IVF infertility center in maternity teaching hospital of Erbil City. Open Journal of Obst. & Gyn, 2013; 3: 577-585.
29. Hashemi AH, Mozdarani H, Naghavi A. Comparison of the Levels of LH and FSH, TSH, Prolactin, Progesterone and Estradiol Hormones between Iranian Infertile Women with Polycystic Ovary Syndrome and Healthy Women. Int. J. Med. Res. & Health. Sci., 2016; 5(12): 370-375.
30. Marca A la, Morgante G, Palumbo M, Cianci A, Petraglia F, Leo VD. Insulin-lowering treatment reduces aromatase activity in response to follicle stimulating hormone in women with polycystic ovary syndrome. Fertil & Steril, 2002; 78(6): 1234-1239.
31. Ota H, Fukushima M, Maki M. Formation of Polycystic Ovary in Mature Rats by The Long-Term Administration of Human Chorionic Gonadotropin. Tohoku. J. exp. Med., 1987; 151: 33-40.
32. Balen AH, Loven JSE, Tan S-L. and Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definition. Hum. Rep. Update, 2003; 9(6): 505-514.

33. Mittal JAS, Kumar S, Misra R, Dadhwal V, Vimala N. Metformin therapy in women with polycystic ovary syndrome. International Journal of Gynecology and Obstetrics, 2004; 87: 237—241.
34. Morin-Papunen LC, koivunen RM, Ruokonen A, Martikainen HK. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil. & Steril, 1998; 69(4): 691-696.
35. Masha SG, Naeimeh D, Hamed DP, Hossien KJ, Nazanin SJ. Copmarison of gensing extract and metformin on improvement of Polycystic Ovary Syndrome (PCO). J Entom. & Zool. Studies, 2015; 3(3): 501-505.
36. Kong L, Tang M, Zhang T, Wang D, Hu K, Lu W, Chao WC, Liang G, Pu Y. Nickel Nanoparticles Exposure and Reproductive Toxicity in Healthy Adult Rats. Int. J. Mol. Sci., 2014; 15: 21253-21269.
37. Welt CK, Taylor A, Martin KA, Hall JE. Serum Inhibin B in Polycystic Ovary Syndrome: Regulation by Insulin and Luteinizing Hormone. J. Clinic. Endo. & Meta, 2002; 87(12): 5559–5565.
38. Reza EH, Mohammad F, Leila S, Vahid YB, Somayeh B, Esmail A. Investigation the Zinc Oxide Nanoparticle's Effect on Sex Hormones and Cholesterol in Rat. Intern. Res. J of Bio. Sci., 2013; 2(8): 54-58.
39. Sharma HS, Hussain S, Schlager J, Ali SF, Sharma A. Influence of Nanoparticles on Blood–Brain Barrier Permeability and Brain Edema Formation in Rats. Brain. Edema, 2009; XIV: 359-364.
40. Attia AA. Evaluation of the Testicular Alterations induced by Silver Nanoparticles in Male Mice: Biochemical, Histological and Ultrastructural studies. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2014; 5(4): 1558-1589.
41. Syrvatka VJ, Slyvchuk YI, Rozgoni II, Gevkan II, Shatapenko OV. Effect of Silver Nanoparticles on Maturation of Rabbit's Oocytes Co-Cultured with Granulosa cells *in vitro*. Studia Biologica, 2015; 9(1): 57-66.
42. Hutz RJ, Carvan MJ, Larson JK, Liu Q, Stelzer RV, King-Heiden TC, Baldridge MG, Shahnoor N, Julien K. Familiar and novel reproductive endocrine disruptors: xenoestrogens, dioxins and nanoparticles. Cur. Trends Endocin, 2014; 7: 111–122.
43. Fox SI. Human Phyiology. 14th. ed. McGraw-Hill Education. New York. USA, 2016; 727-730.