The effect of silver nanoparticles prepared from the aqueous extract of the fenugreek plant on some of the hormones associated with polycystic ovary syndrome in female laboratory rats

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ABSTRACT

Background: This study dealt with an assessment of the effect of the nanoparticles of the fenugreek plant and the metformin of 50 mg/kg on female laboratory rats infected with the updated ovarian cyst syndrome using the letrazole with a concentration of 1 mg/kg of body weight (except for the healthy group), as ovarian affiliation is a common endocrine disorder that affects 3-13% of women at the age of pregnancy and childbearing. **Objective**: to assess the effectiveness of medicinal plants represented by the Fenugreek extract with nanoparticles and its ability to treat female rats infected with ovarian cyst syndrome**. Materials and Methods**: Twenty-four animals of white laboratory rats were tested, and the groups were randomly divided into the following ways: 1- The control group. 2- A group of animals infected with ovaries. 3- A group of animals with cysts was given orally the nano-fenugreek extract 2 mg/ kg. 4- A group of animals infected with cysts and treated by adding metformin 50 mg/ kg of body weight. After the end of the experiment period (30 days), the blood was withdrawn by heart puncture to conduct laboratory tests for hormones that are affected by the new disease, such as Lutein hormone, Follicle-stimulating hormone, Prolactin, Progesterone, and Estrogen. **Results** showed the following: 1- The injection of litrazole 1 mg/ kg led to an ovarian cyst and negative effects on the animal represented in a moral increase in levels, of lutein hormone and prolactin, while the injury led to a moral decrease in the hormone concentration that stimulates follicles, progesterone, and estrogen. 2- The treatment of an oral dosage of 2 mg/ kg of body weight with the nanoparticle fenugreek extract led to positive effects in the body's hormones represented in a moral increase in FSH, progesterone, estrogen while the same treatment led to a moral decrease in the lutein hormone and prolactin in the body at a level ($P > 0.05$). For follicles and decrease in prolactin and lutein hormone in the body at the probability level ($P > 0.05$). **Conclusion**: The current study has found that the Nano - extract fenugreek can be prepared with the Green synthesis by the use of Nanoscopic silver particles, which is an effective treatment to reduce the strikes in the hormones of the body with ovarian stinging syndrome, as a result of the small surface area to the size of the nanoparticles, so the absorption of cells has more efficient and faster than The normal size of the plant.

Keywords: PCOS, aqueous extract, fenugreek, silver nanoparticles.
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1-INTRODUCTION

Polycystic ovary syndrome (PCOS) is a disorder that occurs in the endocrine glands. Studies have indicated that polycystic ovary syndrome is the primary cause of poor ovulation, which affects 3-13% of women of childbearing age (1). Women with polycystic ovary syndrome suffer from metabolic disorders. Hormonal and psychological factors increase the potential risks of pregnancy, such as gestational diabetes, premature birth, and miscarriage (2). In recent years, the effective role of some types of herbal medicinal plants that have medicinal effects has been found to treat polycystic ovary syndrome. The most famous of these plants that have been known in nature for centuries is: Fenugreek (*Trigonella foenum-graecum*), whose origins go back to the Mediterranean, was used by the ancient Egyptians to treat many diseases without knowing the basic components of the plant (3). Nanotechnology is one of the modern technologies of great importance and is intertwined with most types of science. The other is that nanotechnology deals with ultra-small particles of size 1-100 nanometers to create materials with unique properties different from their raw material counterparts as a result of the high surface-to-volume ratio and other physical and chemical properties (4). Therefore, in this study, silver nanoparticles manufactured using the green method from the aqueous extract of fenugreek seeds were used, and the effectiveness of this method was studied in treating induced polycystic ovary syndrome in female albino laboratory rats.

2- MATERIAL AND METHODS

The study was conducted at the Center for Biotechnology Research, Al-Nahrain University / Baghdad. Twenty-four females (Sprague Dawely) ages (4-6) months and weights (200-250) g. The animals were randomly divided into four groups. Each group includes 6 rats. The first is a healthy control group and the other four groups were injected with Letrazol at a concentration of 1 mg/kg for induce polycystic ovary syndrome the silver nanoparticles were prepared by the green method by dissolving 0.017gm of silver nitrate in 100 ml of distilled water with continuous, vigorous stirring, resulting in a colorless solution. Then 6 ml of aqueous extract of *T foenum-graecum* was gradually added to the solution formed by Stirring vigorously for 20 minutes at a temperature of 75 degrees Celsius, the color of the solution gradually changed to brown, which confirmed the decrease of Ag ions, and the formation of the green color of silver nanoparticles. The groups were divided according to the transactions as follows: -

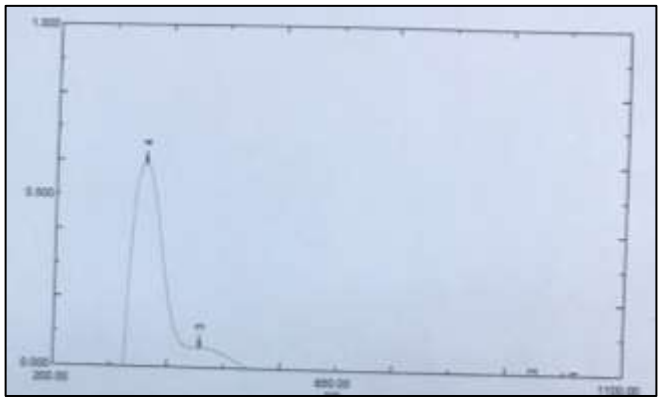
N	Group	Treatment	Feed components
1	Non-Infected control	Standard	The usual diet
2	Infected control	Standard	The usual diet
3	Infected	dose of Nano fenugreek extract	2 mg /kg /30 days
4	Infected	Dosage with Metformin	50 mg /kg/30 days

After completing the preparation of the Nano-extract of the fenugreek, each group was dosed for 30 days the animals were fasted for 12 hours, after which the blood was drawn from the animal (3 –5) ml by cardiac puncture with the insulin syringes (5 ml) coated from the inside with heparin to prevent blood clotting. The blood was placed in a dedicated Eppendorf tube and inserted into the centrifuge to isolate the serum from the blood and was stored in the freezer until use. Then, the necessary tests were carried out for the experiment based on the working method attached to each test kit.

Statistical analysis: To analyze the data statistically, the Social Sciences Statistical Program (SPSS) version 16 was relied upon to analyze the primary data, and the analysis of variance (ANOVA) test was used, using the least significant difference at ($P \geq 0.05$).

3-RESULTS

1. **Ultraviolet-Visible Spec (UV):**The results of the spectroscopic analysis of the fenugreek plant extract shown in Figure (1) showed that the spectrum of the sample was obtained for the wavelength range between 200-1100 nm and our results indicated the presence of an absorption band at 320- 450, and the absorption peak obtained was at 343 nm.



Figure(1): UV absorption spectrum of silver nanoparticles with aqueous extract of fenugreek seeds

2. **Field Emission Scanning electron microscope (FE-SEM):** It was revealed from microscopic imaging that is shown in the figure. (2) The silver nanoparticles that were manufactured using the aqueous extract of fenugreek seeds were of high density. The average size of the manufactured nanoparticle ranged between 8.932 and 33.50 nanometers. The microscopic images also showed that the nanoparticles took an irregular spherical shape, the average size of the manufactured nanoparticle.

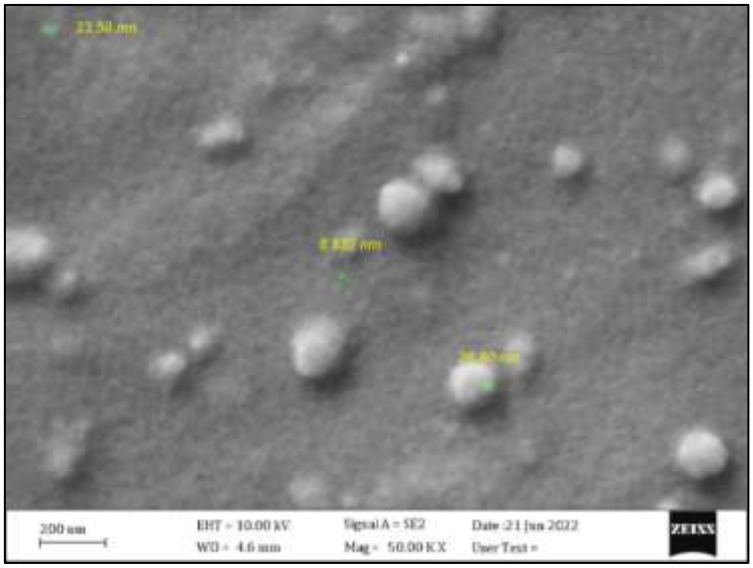


Figure (2): FESEM of silver nanoparticles manufactured from aqueous extract of fenugreek seeds at 75°C after stirring for 20 minutes and at 100,000X magnification

3. **Luteinizing hormone (LH):** It is clear from the current results shown in Figure (3) that the introduction of polycystic ovaries has led to a significant increase in the concentration of luteinizing hormone (LH) compared to the healthy control group. They also showed that dosing with metformin led to a moral increase in the level of luteinizing hormone, and this result agreed with (5). It was also clear from the results that there was a significant decrease in the level of luteinizing hormone concentration when the nano-extract of fenugreek was added orally to infected animals.

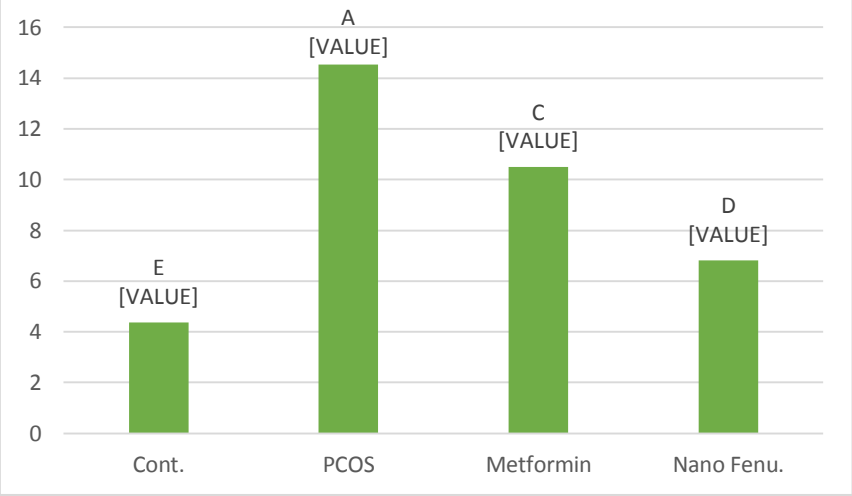


Figure (3:) The effect of polycystic ovary syndrome, treatment with metformin, and fenugreek nano-extract on the level of luteinizing hormone in female rats.

4. **Follicle-stimulating hormone (FSH):** The results shown in Figure (4) showed that infection of rats with polycystic ovaries led to a significant decrease in the level of follicle-stimulating hormone compared to the group of healthy control animals. So, the results also showed that giving metformin to animals with polycystic ovary syndrome It led to a significant increase in FSH concentration and ovarian follicles This result agreed

with (6). The results also showed that the use of nanoparticles synthesized from the aqueous extract of the fenugreek plant led to a noticeable increase in the level of FSH compared to the group of untreated infected animals throughout the duration of the experiment.

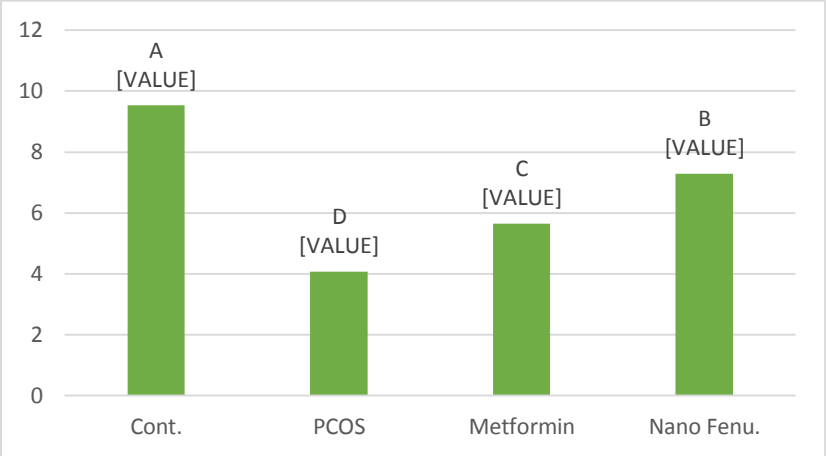
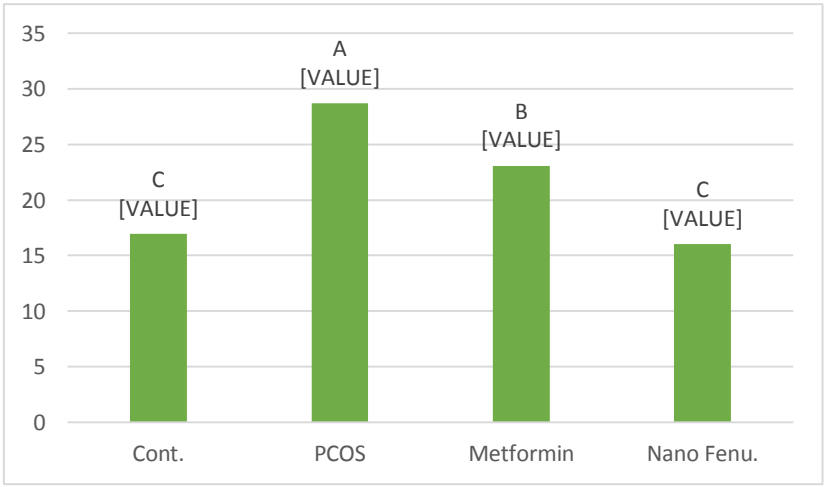


Figure (4): The effect of polycystic ovary syndrome, metformin treatment, and fenugreek nano extract on the level of follicle-stimulating hormone in female rats.

5. **Prolactin Hormone:** The results in Figure (5) showed that a significant increase in the level of prolactin in group infection of female rats with polycystic ovaries compared with a group of healthy control animals. While group treated with nano-extract fenugreek showed a significant decrease in the concentration of prolactin at the probability level ($P \leq 0.05$) compared to the PCOS-infected and untreated animals.



Figure(5): The effect of polycystic ovary syndrome, metformin treatment, and fenugreek nano extract on the level of prolactin hormone in female rats.

6- Progesterone Hormone: The results of the current study shown in Figure (6) showed that the concentration of progesterone decreased significantly in cases of polycystic ovary syndrome compared to the control group. It was also clear from results that giving the drug metformin 50 mg/kg orally to rats with polycystic ovary syndrome led to a significant increase in the concentration of the hormone progesterone compared to the untreated infected group

throughout the experiment. The results also showed that the use of a nano-extract of the fenugreek plant made from silver nanoparticles led to a significant increase in the level of progesterone when compared with the group of animals infected with cysts and untreated for the duration of the experiment.

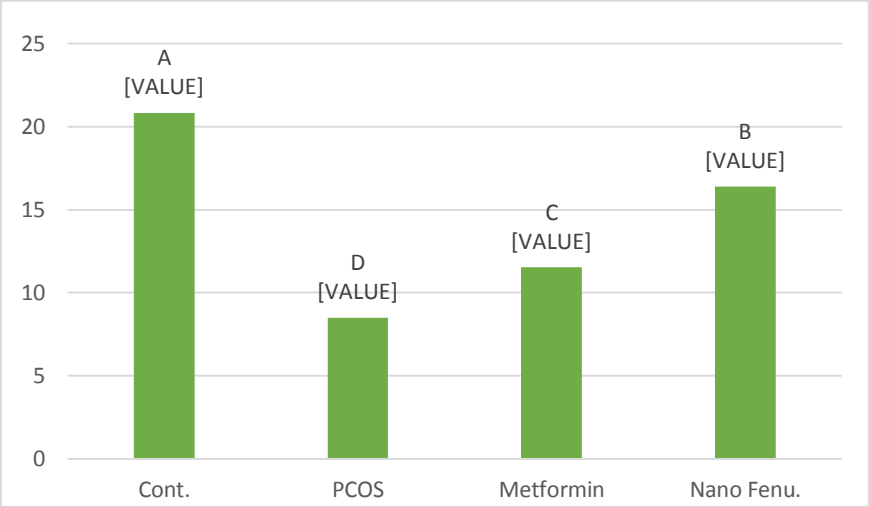


Figure (6): The effect of polycystic ovary syndrome, metformin treatment, and fenugreek nano extract on the level of progestrone hormone in female rats.

7- Estrogen Hormone: Results shown in Figure (7) showed that stimulating polycystic ovaries in female laboratory albino rats led to a significant decrease in estrogen levels compared to the control group. It was also clear from current results that the addition of silver nanoparticles manufactured from the aqueous extract of the fenugreek plant led to a significant increase in the concentration of estrogen compared to the group of cyst-infected and untreated animals.

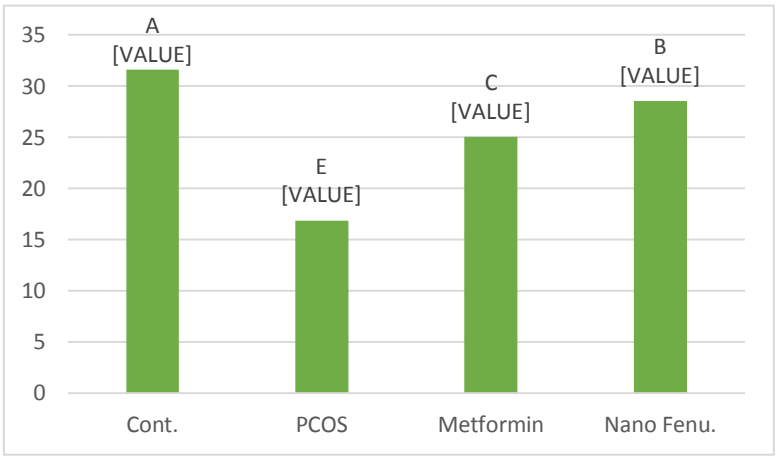


Figure (7): The effect of polycystic ovary syndrome, metformin treatment, and fenugreek nano extract on the level of estrogen hormone in female rats.

4-DISSCUSION

1- Ultraviolet-Visible Spec (UV): The degree of absorption and its peak obtained from the UV examination is due to the so-called surface plasmon resonance (SPR) phenomenon, which silver nanoparticles exhibit when they oscillate when exposed to electromagnetic radiation. This oscillation gives a typical value for the absorption peak that occurs (7). Results agreed with (8) in his study on celery and myrtle extracts using UV-Vis spectroscopy, that the highest absorption peak was at wavelengths 408-410, and this is within the absorption limits of silver. The metal ranges between 400-450. Nm.

2- Field Emission Scanning electron microscope (FE-SEM): The result obtained from the examination agreed with the researcher (9), who proved that the biomolecules present in the fenugreek extract, such as protein, act as a covering agent to support the formation of nanoparticles to adhere to the surfaces of other spherical nanoparticles. It is worth noting that the colors of some plant extracts appear similar. It was found (10) that the color of the ivy plant extract appears similar to the color of the licorice plant extract, but when the examination was conducted with the (FESEM) device, a color difference appeared between the two plants.

3- Luteinizing hormone (LH): The reason for the increase in luteinizing hormone when suffering from polycystic ovary syndrome is due to enhancing the sensitivity of the pituitary gland to increased secretion of GnRH, or imbalances may occur in its production from the hypothalamus is a result of the imbalance occurring in the hormone balance when contracting the disease. This result is consistent with (11), who indicated that an increase in the level of luteinizing hormone leads to an imbalance or lack of ovulation in the stages of the follicular cycle because LH works to stimulate the process of ovulation and the corpus luteum. On the synthesis of steroid hormones, a high level of the hormone inhibits the effectiveness of aromatase and egg growth. Or perhaps the reason for the increase in luteinizing hormone is due to the decrease in the level of progesterone when suffering from polycystic ovary syndrome, as happened in our current results (.(12 As for the reason for the decrease in luteinizing hormone when animals are given the nano-extract of fenugreek, it is consistent with (13) who explained that fenugreek has high effects on the body's hormones and reduces the rate of Luteinizing hormone, relative to follicle-stimulating hormone (LH/FSH), reduces ovarian cysts and improves irregular menstrual cycles in women with polycystic ovary syndrome.

4- Follicle-stimulating hormone (FSH) : The decrease in the level of follicle-stimulating hormone when suffering from polycystic ovary syndrome was consistent with This result (14) and the reason is due to the disturbances occurring in the body upon infection. Polycystic ovary syndrome is caused by an increase in the pulsating frequency secreted by gonad-stimulating hormones (GnRH), which leads to an increase in the secretion of luteinizing hormone (LH) relative to follicle-stimulating hormone (LH/FSH) (15). So, the method of administering the nano-extract of fenugreek to animals suffering from cysts was also consistent with the result of (16) who noted an increase in Follicle-stimulating hormone after 8 weeks of treatment with fenugreek, in addition to increasing the hemoglobin level in mice with polycystic ovary syndrome. This may be due to fenugreek containing active compounds such as flavonoids and phytoestrogens, which have an effect similar to estrogen, which is one of the main hormones regulating ovulation. The reason may also be due to the aqueous extract of fenugreek containing diosgenin, which is known for its effective role in its action similar to that of sex hormones (17).

5- Prolactin Hormone: - The reason for the increase in the hormone prolactin when suffering from polycystic ovary syndrome is due to the close connection between the disease and an increase in milk hormone (hyperprolactinemia) due to an imbalance of hormones in the body. As a result of the increase in the level of androgens and the occurrence of a defect in the pituitary gland, which is responsible for secreting prolactin, which is the most well-known symptom of polycystic ovary syndrome (18). The reason for the decrease in the level of the hormone prolactin when using the nano-extract of fenugreek was consistent with the result (19) which indicated a decrease in prolactin in Experimental mice when treated with nanoparticles of silver due to the small size of the particles, which allows the cells to absorb the active substance more efficiently and faster than the normal size.

6- Progesterone Hormone: - It has been shown that polycystic ovary syndrome has led to a decrease in progesterone, and it may return to excessive production of the luteinizing hormone, which stimulates the production of abundant amounts of androgens, which leads to a decrease and then the cessation of the follicular maturation

production of estrogen and the body's inability to reach the follicular maturation stage. Formation of the corpus luteum (20). Progesterone increases when treated with metformin because metformin works to prevent the uterine lining from depigrating, and increasing Its thickness reduces its resistance to the hormone, and its concentration gradually increases within the body (21). Also, treatment with nano-extract of fenugreek led to an increase in the proportion of progesterone in the blood serum of animals with cysts, which is consistent with This is consistent with what was stated in one of the studies that were conducted in 2011 on laboratory mice. This indicated an increase in the hormone progesterone and estrogen, which are known for their main importance in the ovulation process, when given fenugreek daily for 8 consecutive weeks, as fenugreek works to enhance the production of eggs naturally. It is known that fenugreek, due to its effective compounds such as flavones and the many vitamins it contains, has been used for many centuries for the purpose of Increasing fertility and stimulating the ovulation process (22)

7- Estrogen Hormone: - The decrease in estrogen when suffering from cysts agrees with the result (23). This showed that high levels of insulin in the blood contribute to stimulating the synthesis of steroids, in addition to high androgens, which increase the production of estrogen, and therefore the body's metabolism is involved in polycystic ovary syndrome due to its association with high androgens and increased insulin in the blood. Estrogen affects most of the vital processes in the female reproductive system. It maintains the efficiency of reproductive functions, in addition to many other functions in the rest of the body's organs, through hormone receptors in the cytoplasm, nuclei, and mitochondria of the cells. Research has indicated that an imbalance of estrogen affects the growth and development of ovarian follicles, Therefore, more studies are needed to understand the role of estrogen in polycystic ovary syndrome (22). It also turns out that treatment with nano-extract of fenugreek led to an increase in the level of the hormone estrogen, because This may be due to the plant containing important active compounds such as saponins, flavonoids, coumarins, vitamins, and amino acids. It acts as a plant hormone in the body, and studies have indicated that fenugreek acts as a natural estrogen in the body, so it is recommended for women after menopause to alleviate the symptoms that accompany this age period (20).

5-CONCLUSION

The current study has found that the Nano - extract fenugreek can be prepared with the Green synthesis by the use of Nanoscopic silver particles, which is an effective treatment to reduce the strikes in the hormones of the body with ovarian stinging syndrome, as a result of the small surface area to the size of the nanoparticles, so the absorption of cells has more efficient and faster than The normal size of the plant.

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تأثير جسيمات الفضة النانوية المحضرة من المستخلص المائي لنبات الحلبة على بعض الهورمونات المرافقة لمتلازمة تكييس المبايض عند اناث الجرذان المختبرية

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[3]مركز بحوث التقنيات الاحيائية/ جامعة النهرين/ بغداد – العراق

الخلاصة: -:

خلفية حول الموضوع: تناولت هذه الدراسة تقييم تأثير المستخلص النانوي لنبات الحلبة وعقار الميتفورمين 50 ملغم/كغم على إناث الجرذان المختبرية المصابة بمتلازمة المبيض المتعدد الكيسات المستحدث باستخدام عقار الليترازول بتركيز 1 ملغم. /كجم من وزن الجسم (باستثناء المجموعة السليمة) اذ ان تكييس المبايض هو اضطراب غدد صماء شائع يصيب 3-13 % من النساء في عمر الحمل والانجاب. **الهدف من هذه الدراسة**: هو تقييم مدى فعالية النباتات الطبية متمثلة بمستخلص نبات الحلبة المحضر نانوياً بواسطة جسيمات الفضة النانوية وقدرته على علاج إناث الجرذان المصابة بمتلازمة تكييس المبايض **المواد وطرق العمل** تم تحضير 24 حيوان من اناث الجرذان المختبرية البيضاء وتم تقسيم المجاميع عشوائياًعلى النحو التالي: -:1- مجموعة السيطرة السليمة. 2- مجموعة الحيوانات المصابة بتكييس المبايض وغير المعالجة طول المدة التغذوية. 3- مجموعة الحيوانات المصابة بالتكييس والتي تم اعطاءها فموياً المستخلص النانوي للحلبة 2 ملغم/ كغم يوماً. 4- مجموعة الحيوانات المصابة بالتكييس والمعاملة بإضافة الميتفورمين 50 ملغم/ كغم من وزن الجسم. وبعد انتهاء فترة التجربة (30 يوما) تم سحب الدم باستخدام حقن مخصصة بطريقة الوخز القلبي لغرض اجراء الفحوصات المختبرية للهورمونات التي تتأثر بالمرض المستحدث كالهورمون اللوتيني، الهورمون المحفز للجريبات، البرولاكتين، البروجيسترون والاستروجين. وقد أظهرت **النتائج:** ما يلي: 1- أدى الحقن بعقار الليترازول 1ملغم/ كغم إلى حدوث تكيس المبايض وتأثيرات سلبية على الحيوان تمثلت في زيادة معنوية في مستويات والهورمون اللوتيني والبرولاكتين بينما أدت الإصابة الى انخفاض معنوي في تركيز الهورمون المحفز للجريبات والبروجيسترون والاستروجين. 2- أدت المعاملة بالتجريع الفموي 2 ملغم/ كغم من وزن الجسم بمستخلص الحلبة النانوي إلى تأثيرات إيجابية في هورمونات الجسم تتمثل في زيادة معنوية في تركيزFSH ، البروجيسترون، الاستروجين بينما أدت المعاملة نفسها الى حدوث انخفاض معنوي في تركيز الهورمون اللوتيني والبرولاكتين في الجسم عند مستوى احتمالية.($P > 0.05$)) 3- أدى التجريع عن طريق الفم بعقار الميتفورمين 50 ملغم/ كغم من وزن الجسم يوميا الى حدوث تأثيرات إيجابية على مستوى تركيز الهورمونات في الجسم مقاربة الى تأثيرات المعاملة بالحلبة النانوية وتتمثل بالارتفاع المعنوي في تركيز البروجيسترون والاستروجين والهورمون المحفز للجريبات وانخفاض في البرولاكتين والهورمون اللوتيني في الجسم عند مستوى احتمالية ($P > 0.05$).

الاستنتاج : تم التوصل من الدراسة الحالية ان نبات الحلبة الطبي المحضر نانوياً بالطريقة الخضراء من جسيمات الفضة النانوية هو علاج فعال للتقليل من الإضرابات الحاصلة في هورمونات الجسم المصاب بمتلازمة تكييس المبايض وذلك نتيجة صغر المساحة السطحية نسبة الى الحجم لجزيئات الحلبة النانوية فيكون امتصاص الخلايا لها اكثر كفاءة واسرع من الحجم الاعتيادي للنبات.

كلمات مفتاحية: نبات الحلبة، تكييس المبايض، جسيمات الفضة النانوية