



Investigation the effect of isotretinoin on some level hormones in female rats

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ABSTRACT

The aim of the study Investigation the effect of isotretinoin on serum LH,FSH, Prolactin and AMH level hormones in female rats

The current study Contain 20 rats, each group containing five rats. The first group was the control group given normal saline daily by oral, while the second group was given the drug 15 mg/kg isotretinoin (Pinar *et al.*,2020) and the third group was given 30 mg/kg isotretinoin. The fourth group was given 30 mg/kg isotretinoin and omega-3 400mg/kg. after 30 days of treatment serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, and total and anti- Muller hormone were measured and compared.

After treatment, mean serum LH,FSH,and AMH levels significantly decreased in G2 and G4, but prolactin serum levels increase. While in G4 There was a slight improvement in hormone LH,FSH,and AMH levels.

Introduction

Oral isotretinoin has transformed acne treatment since its FDA approval in 1982. Its off-label benefits are still being discovered (Bagatin & Costa, 2020). Isotretinoin, a powerful synthetic retinoid derived from vitamin A, stands as the sole solution capable of completely eradicating acne vulgaris (Olisova & Shepeleva, 2023). The drug is effective in cases where other treatments, such as topical therapies and antibiotics, have failed, making it a critical option for severe acne cases (Layton, 2023). Its remarkable sebo-suppressive, comedolytic, anti-inflammatory, and potential immunological effects stem from the transactivation activity of nuclear receptors or non-nuclear targets, making it a compelling choice for skin health. Isotretinoin's mechanism of action remains unclear (Agamia *et al.*, 2023). Isotretinoin poses

serious risks during pregnancy. Isotretinoin is highly teratogenic, with exposure during pregnancy leading to a high incidence of congenital anomalies. Studies have shown that isotretinoin exposure during pregnancy results in congenital anomalies in 21-52% of cases, although more recent studies report lower rates (Alay *et al.*,2023). The risk of spontaneous abortion and embryopathy is significant, with estimates suggesting that 40% of pregnancies exposed to isotretinoin result in spontaneous abortion (You *et al.*, 2023).

Experimental Animals:

This study was conducted in the animal house of Veterinary Medicine College \ Tikrit University, during the period 25 October to 25 June, Eighty(80) adult apparently healthy Albino

rats were obtained from animal house of Veterinary Medicine College \ Tikrit University. animals range between (8-10) weeks of age and their weight ranged between (200-250) g with an average of 225g. animals were housed in plastic standard cages of dimensions (46*28*13) cm. they were kept under suitable environmental conditions of 20-25 C°. The current study divided animals into 9 groups used 50 rats use 6 rat in each group (Nabi, 2014).

Materials and methods

The Animals

Contain 20 rats, each group containing five rats. The first group was the control group given normal saline daily by oral, while the second group was given the drug 15 mg/kg isotretinoin (Pinar et al.,2020) and the third group was given 30mg/kg isotretinoin. The fourth group was given 30mg/kg isotretinoin and omega-3 400mg/kg (Sara et al.,2023). daily for 30 days.

General Experimental design

- The first group: (5 rats) this group was given 1 ml of normal saline daily by oral gavage for 30 days.
- The second group: (5 rats) administered orally at 15 mg/kg Isotretinoin every day for 30 days.
- The third group:(5 rats) administered orally at 30 mg/kg Isotretinoin every day for 30 days.

- The fourth group: (5 rats) isotretinoin was taken orally at 30 mg/kg then given Omega3 orally at (400 mg/kg/day) for 30 days.

Blood Collection

The blood samples of all rats were obtained by cardiac puncture under the anesthesia using capillary test tubes to determine biochemical parameters . Blood was centrifuged at 3000 rpm for 15 min. Serum was separated and stored in Eppendorf tubes (0.5 mL) at -20°C until used.

Biochemical Assessment

Determination of (Luteinizing Hormone (LH), follicle-stimulating hormone (FSH), prolactin, and anti-Mullerian hormone (AMH).

Result

Biochemical markers determination

The Figure (1) showed presents data on Luteinizing Hormone (LH) levels across four groups: G2, G3 and G4 and the Con., with $p = 0.01$ showing statistical the significance. The values of LH hormone give the comparative picture about the levels of this hormone in the control group and the experimental groups, G2, G3 and G4 and all the observations depict that: The control group have higher level of this hormone (32.344, 33.459) than the experimental groups (G2 23.660 to 24.805), (G3 18.115 to 19.980) and This may imply that the treatments which have been administered on G2, G3 and G4 can be effective in lowering LH level.

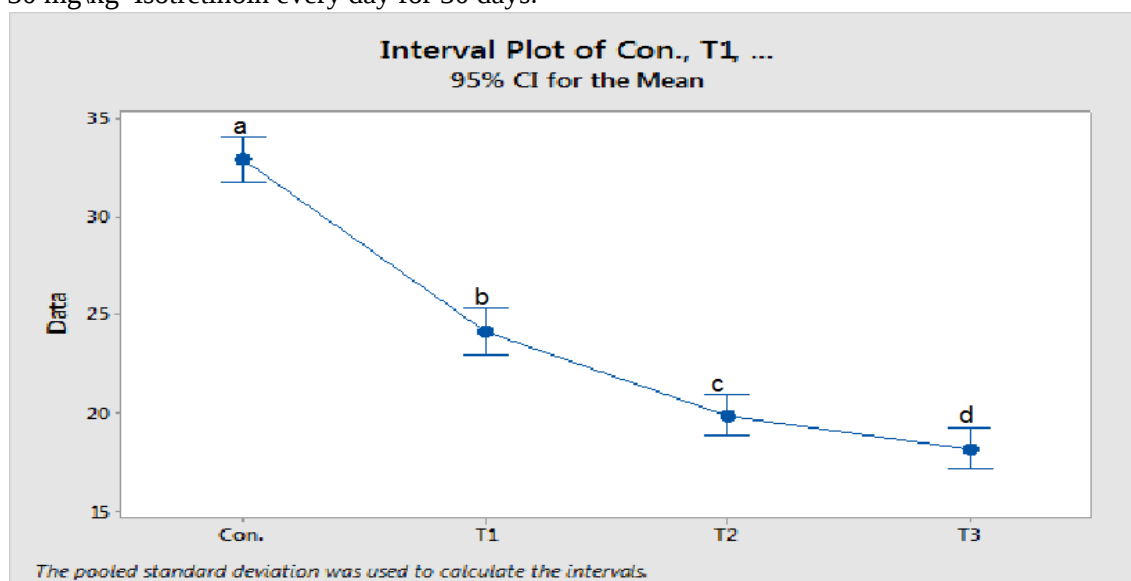


Figure 1: (LH) levels among studies groups

Table 1: (LH) levels among studies groups

Groups	N	Mean	StDev
G1	5	32.923 a	0.595
G2	5	24.119 b	0.466
G3	5	19.833 c	1.489
G4	5	18.157 d	0.528
Pooled StDev = 2.14944			

The Figure (2) presents data on Follicle-Stimulating Hormone (FSH) levels across four groups: G2, G3, G4 and a control group Con. with $p < .001$ in support of a statistical significance. The FSH hormone values measured in the control group are higher and ranged between 32.729 and 39.431 while those of the

experimental groups computerized ranged between G2 17.812 and 17.979, G3 11.513 and 18.317 and G4 8.578 and 14.253. This presented data indicates that treatment as provided to G2, G3, and G4 have proven efficacy to lower FSH than the control group.

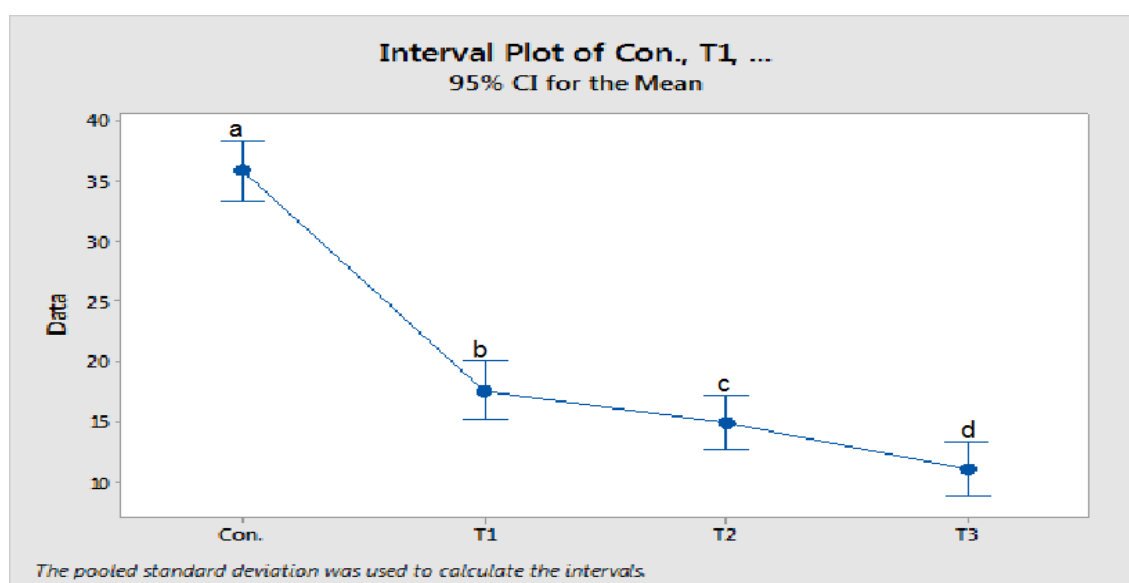


Figure2: (FSH) levels among studies groups

The table presents data on Prolactin levels across four groups: The results show a significant difference based on group comparisons of G2, G3, G4, and a control group (Con.), with $p = 0.01$. The Prolactin levels in the control group is 10.09 – 10.879 while those of

the experimental groups were G2- 8.316 – 8.841, G3-14.327 – 16.614 and G4- 11.532 – 21.463. This pattern indicates that some of treatments used on G2, G3 and G4 might have affected the secretion of Prolactin, and might therefore be higher than that of the control group.

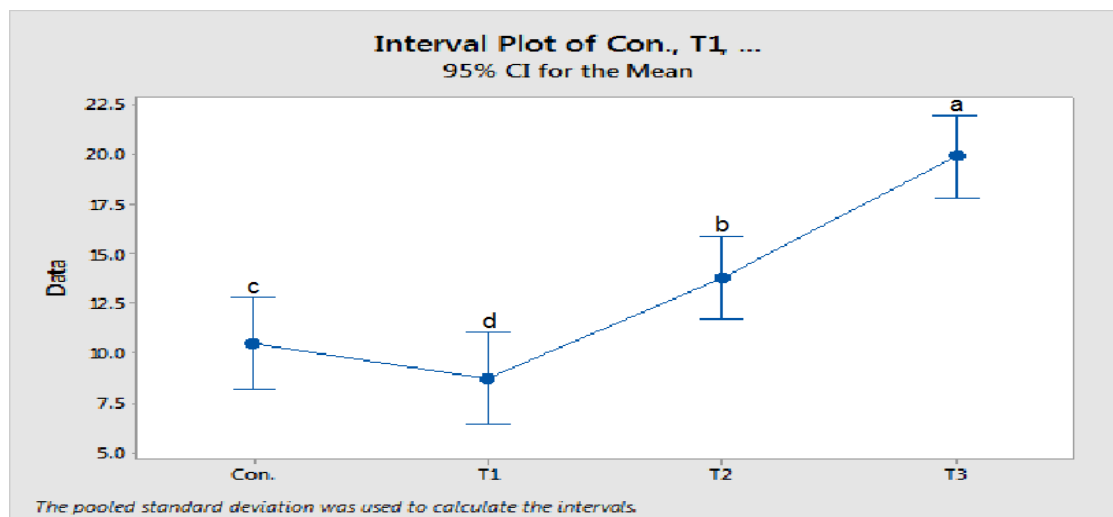


Figure 3: Prolactin levels among studies groups

Table.3: Prolactin levels among studies groups

Groups	N	Means	stDev
G1	5	10.489c	0.334
G2	5	8.724d	0.139
G3	5	13.790b	3.17
G4	5	19.900a	2.46
Pooled StDev = 2.14944			

The table presents data on Anti-Müllerian Hormone (AMH) levels across four groups: Four groups were over expressed; G2, G3, G4 and a

control group (Con.). By using ANOVA test the p value was significant at 0.01 levels. The different AMH levels in the control group average to be 620.441-654.89 while the different groups' average is 526.711 - 566.418 for G2, 349.231 - 389.352 for G3 and 267.352 - 388.511 for G4. In connection with this trend, it can be AMH levels compared with the control group.

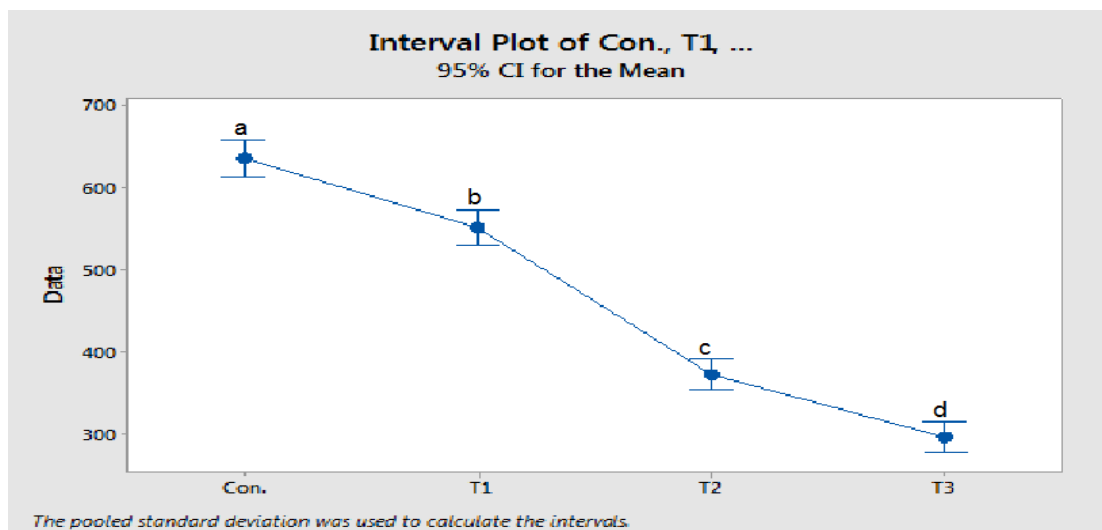


Figure 4: Anti-mullerian hormone AMH levels among studies groups

Table .4: Anti-mullerian hormone AMH levels among studies groups

Groups	N	Mean	StDev
G1	5	635.21 a	16.45
G2	5	551.83 b	17.74
G3	5	372.20 c	14.84
G4	5	296.30 d	26.50
Pooled StDev = 19.7068			

DISCUSSION

Assessment of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels

The results of the current study found that isotretinoin reduce the level of LH and reduction elevated according to increase dose . This results agree with a study carried out by Öztürk et al., 2015 on the post adolescent females having acne reported that the level of the luteinizing hormone (LH) significantly decreased after a course of isotretinoin treatment than the initial level . Another study by Karadag et al., 2015 suggested mild suppression of pituitary hormones secretion and isotretinoin similarly affects LH levels in a dose dependent manner. Highly effective forms of isotretinoin are connected with more considerable changes in hormone levels of the pituitary gland.

Similarity , Akpolat, 2021 , as well as Nasrallah et al., 2019 reported that Isotretinoin

influences the hormonal system and particularly LH in a broader context of hormonal shifts that involve also testosterone and other sexual hormones.es. These modifications may in some way help to explain why the drug usually works to minimise acne signs.

Mendi et al., 2024 revealed that most of the women who were receiving isotretinoin changed their menstrual cycles, and some of the changes were infrequent menses.

In addition Elnagar et al., 2024 found LH decrease and other changes that accompany it may affect reproductive function and fertility and even ovarian function in women. This is especially applicable for ladies who have diseases such as polycystic ovary syndrome (PCOS) for which isotretinoin had been prescribed due to their impact on decreasing the ovarian volume and testosterone level.

A cross-sectional study by Acmaz et al., 2019 on forty five female patients with severe acne who were treated with isotretinoin found that the hormone FSH significantly reduced.t. This study comprised 32 female patients with isotretinoin therapy for 5 to 8 months, Total

hormonal levels of FSH, LH, and estradiol were significantly reduced. While disagree with Haroun et al., 2022 found isotretinoin does not adversely affect ovarian reserve or FSH levels. And disagree with Angioni et al., 2005 isotretinoin also seems to act directly on the gonads (ovaries or testes), thus inhibiting the feedback influences controlling the release of LH and FSH.

The possible way through which isotretinoin impacts pituitary-ovarian axis could be through the activation of p53/FOXO1 proteins, which induce apoptotic effect thus suppressing gonadotropin releasing (Abdelhamed et al., 2021). Also Changes platelet count, alters lens transparency, and influences the nuclear receptors of PPAR γ /RXR of Isotretinoin might also affect the hormonal systems, release and synthesis of pituitary hormonal products (B. Melnik, 2011). As well as Nilsson, 2020 found isotretinoin also act directly on the gonads (ovaries or testes), thus inhibiting the feedback influences controlling the release of LH and FSH. While disagree with Karadag et al., 2011 and indicating non-significant reductions in mean FSH.

Assessment of Prolactin

The findings of the present study supported the elevated level of prolactin in after using isotretinoin. the result of study conducted by Karadag et al., 2015 and Gadelha, Wildemberg and Kasuki, 2022 found isotretinoin affects the pituitary gland, which may lead to increased production of prolactin due to isotretinoin affects dopamine receptors in the brain, which may lead to increased production of prolactin additionally, isotretinoin affects liver function, which may lead to increased levels of prolactin in the blood. Also Aktar et al., 2021 found that isotretinoin affects the levels of other hormones in the body, such as estrogen and teratogenic hormones, which may lead to increased production of prolactin. while the present study disagree with Feily et al., 2020 conducted a study involving thirty-six women aged 18 to 30 with moderate-to-severe acne, administered 20 mg isotretinoin daily for three months, revealing a decrease in blood prolactin levels from 27.29 ± 11.55 pre-treatment to 17.86 ± 7.92 post-treatment. As well as It has been established that isotretinoin interferes with several pituitary hormones, and particularly a decrease in prolactin and Luteinizing hormones after treatment, Also disagree with Karadag et al., 2011

demonstrated that isotretinoin treatment led to reductions in prolactin.

Assessment of Anti-Müllerian Hormone level

AMH has emerged in recent research as a potential biomarker for assessing ovarian reserve post-chemotherapy and exposure to gonadotoxic substances, demonstrating earlier indication of ovarian reserve in the aging process compared to established ovarian hormones such as FSH, inhibin B, or oestradiol, while maintaining stable serum levels throughout the menstrual cycle. Isotretinoin is associated with numerous adverse effects, including demonstrated ovarian toxicity in rats as evidenced (Abali et al., 2013).

The findings of the cureent study found that isotretinoin reduce the Anti-Müllerian Hormone level. this results agree with Can et al., 2020 found that the mean AMH level of the treatment group was significantly lower at month 6 than the level prior to treatment and that of the control group ($p=0.001$, $p=0.013$), despite no substantial difference between the treatment group and the control group prior to treatment ($p=0.967$). At month 6, mean AMH levels in both the low-dose and high-dose groups were significantly lower than before treatment ($p=0.005$, $p=0.001$). When the 6th month AMH levels of the low and high-dose treatment groups were compared to controls, the high dose group's mean AMH level was lower, whereas the low-dose treatment group's mean AMH level was not significantly different from the control group ($p=0.009$, $p=0.097$). Similarity consistent with Aksoy et al., 2015 reported that significant differences were observed in AMH levels [2.20 ng/ml (25th–75th percentile 1.14–4.07) vs. 1.31 ng/ml (0.32–2.28); $p=0.001$] show the range where most people's AMH levels fall after treatment. Additionally Cinar et al., 2017 discovered that while all ovarian reserve (OR) metrics were assessed, the mean AMH levels at baseline and 12 months post-treatment cessation were statistically comparable, despite a significant decline immediately following the treatment.

Conclusions

1-The findings of this study demonstrate that isotretinoin treatment significantly affects hormonal levels in experimental groups. The biochemical analysis revealed that isotretinoin administration led to a substantial decrease in

Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Prolactin, and Anti-Müllerian Hormone (AMH) levels in comparison to the control group. The dose-dependent reduction in these hormone levels suggests that isotretinoin has a suppressive effect on the pituitary-ovarian axis

2-The observed hormonal changes align with previous research, indicating that isotretinoin may impact pituitary hormone secretion and ovarian function, potentially leading to reproductive challenges.

References

- [1] Abadie, R. B., Keller, C. L., Jones, N. T., Mayeux, E. L., Klapper, R. J., Anderson, L., Kaye, A. M., Ahmadzadeh, S., Varrassi, G., & Shekoohi, S. (2023). Review of Teratogenic Effects of Leflunomide, Accutane, Thalidomide, Warfarin, Tetracycline, and Angiotensin-Converting Enzyme Inhibitors. *Cureus*, 15(12).
- [2] Abali, R., Yuksel, M. A., Aktas, C., Celik, C., Guzel, S., Erfan, G., & Sahin, O. (2013). Decreased ovarian reserve in female Sprague–Dawley rats induced by isotretinoin (retinoic acid) exposure. *Reproductive Biomedicine Online*, 27(2), 184–191.
- [3] Abdelhamed, A., El-Dawla, R. E., Karadag, A. S., Agamia, N. F., & Melnik, B. C. (2021). The impact of isotretinoin on the pituitary-ovarian axis: An interpretative review of the literature. *Reproductive Toxicology*, 104, 85–95.
- [4] Abdelmaksoud, A., Lotti, T., Anadolu, R., Goldust, M., Ayhan, E., Dave, D. D., Vestita, M., Ebik, B., & Gupta, M. (2020). Low dose of isotretinoin: a comprehensive review. *Dermatologic Therapy*, 33(2), e13251.
- [5] Abou Zommar, G. O. H., Nofal, A., & Ibrahim, A. S. M. (2022). Possible Side Effects of Isotretinoin Use in Dermatology. *The Egyptian Journal of Hospital Medicine*, 89(1), 4562–4565.
- [6] Acmaz, G., Cinar, L., Acmaz, B., Aksoy, H., Kafadar, Y. T., Madendag, Y., Ozdemir, F., Sahin, E., & Muderris, I. (2019). The effects of oral isotretinoin in women with acne and polycystic ovary syndrome. *BioMed Research International*, 2019(1), 2513067.
- [7] Agamia, N. F., El Mulla, K. F., Alsayed, N. M., Ghazala, R. M., El Maksoud, R. E. A., Abdelmeniem, I. M., Talaat, I. M., Zaki, I. I., Sabah, R. M., & Melnik, B. C. (2023). Isotretinoin treatment upregulates the expression of p53 in the skin and sebaceous glands of patients with acne vulgaris. *Archives of Dermatological Research*, 315(5), 1355–1365.
- [8] Ahmetašević-Demirović, A., Lagumdžija, D., & Pehlivanović Kelle, B. (2023). Application of Isotretinoin in the Treatment of Acne Vulgaris: A Questionnaire-Based Survey. *Mediterranean Conference on Medical and Biological Engineering and Computing*, 368–376.
- [9] Akpolat, D. (2021). Unexpected effects of oral isotretinoin in adolescents with acne vulgaris. *Cureus*, 13(8).
- [10] Aksoy, H., Cinar, L., Acmaz, G., Aksoy, U., Aydin, T., Vurdem, U. E., Oz, L., Idem Karadag, O., & Kartal, D. (2015). The effect of isotretinoin on ovarian reserve based on hormonal parameters, ovarian volume, and antral follicle count in women with acne. *Gynecologic and Obstetric Investigation*, 79(2), 78–82.
- [11] Aktar, R., Gunes Bilgili, S., Yavuz, I. H., Ozaydin Yavuz, G., Aktar, S., Ozturk, M., & Karadağ, A. S. (2021). Evaluation of hirsutism and hormonal parameters in acne vulgaris patients treated with isotretinoin. *International Journal of Clinical Practice*, 75(3), e13791.
- [12] Al Muqarrab, F., & Almohssen, A. (2022). Low-dose oral isotretinoin for the treatment of adult patients with mild-to-moderate acne vulgaris: Systematic review and meta-analysis. *Dermatologic Therapy*, 35(4), e15311.
- [13] Alajaji, A., Alrawaf, F. A., Alosayli, S. I., Alqifari, H. N., Alhabdan, B. M., & Alnasser, M. A. (2021). Laboratory abnormalities in acne patients treated with oral isotretinoin: a retrospective epidemiological study. *Cureus*, 13(10).

دراسة تأثير الأيزوتريتينوين على بعض الهرمونات في إناث الفئران

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الخلاصة:-

هدفت هذه الدراسة إلى دراسة تأثير الإيزوتريتينوين على مستويات هرمونات في مصل إناث الجرذان. LH و FSH و Prolactin و AMH أجريت الدراسة الحالية على 20 جرّاء، كل مجموعة تضم خمسة جرذان. كانت المجموعة الأولى هي المجموعة الضابطة التي أعطيت محلولاً ملحيّاً طبيعياً يومياً عن طريق الفم، بينما أعطيت المجموعة الثانية دواء الإيزوتريتينوين بجرعة 15 ملغ/كغ (بينار وآخرون، 2020)، وأعطيت المجموعة الثالثة دواء الإيزوتريتينوين بجرعة 30 ملغ/كغ. أما المجموعة الرابعة، فقد أعطيت 30 ملغ/كغ من الإيزوتريتينوين وأوميغا 3 بجرعة 400 ملغ/كغ. بعد 30 يوماً من العلاج، تم قياس مستويات هرمون الملوتن (LH) وهرمون تحفيز الجريبات (FSH) والبرولاكتين والهرمون الكلي وهرمون مضاد مولر في مصل الجرذان، ومقارنتها.

بعد العلاج، انخفض متوسط مستويات LH و FSH و AMH في مصل الجرذان بشكل ملحوظ في المجموعتين الثانية والرابعة، بينما ارتفعت مستويات البرولاكتين في مصل الجرذان. بينما في G4 كان هناك تحسن طفيف في مستويات الهرمونات LH و FSH و AMH.

الكلمات المفتاحية: أيزوتريتينوين، الجهاز التناسلي للفئران الإناث، مستوى الهرمونات