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Investigation the effect of isotretinoin on ovary tissues and reproduction in female rats

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ABSTRACT

Background: Oral isotretinoin, a potent synthetic retinoid, derived from vitamin A, uniquely eradicates acne vulgaris. Its multifaceted action on skin health is attributed to nuclear and non-nuclear receptor transactivation. However, isotretinoin's teratogenicity and associated serious adverse effects necessitate careful consideration, particularly regarding its use during pregnancy, where a notable incidence of congenital anomalies and spontaneous abortion has been documented. Isotretinoin is regarded as a highly teratogenic agent. may adversely affect ovarian reserve and female fertility, evidenced by decreased anti-mullerian hormone (AMH) levels. .

Objective: This study aims to elucidate the detrimental effects of toxic isotretinoin doses on reproductive efficacy by investigating its influence on reproductive organs tissue and reproduction.

Materials and methods: forty rats were used in this study, The current study consists of two parts each part used 20 rats which lasted for month. each group contain 5 rats. The first part: Consists of four groups, The first group was the control group, while the second group was given the drug 15 mg/kg isotretinoin and the third group was given 30 mg/kg isotretinoin. The fourth group was given 30 mg/kg isotretinoin and omega-3 for 30 days. the blood samples of all rats were obtained by cardiac puncture under the anesthesia for Assessment of histopathological ovaries for Rat. Then, the rats were sacrificed, and bilateral ovariectomy was performed ovarian tissues were fixed in a 10% formalin solution for histological evaluation. It was noticed after a histological exam in groups 3, and 4 that the ovarian atrophied mucosal with degeneration of epithelial cells cortex contained atrophied ovarian follicles with degenerated Oocytes, the medulla of the ovary contained congested blood vessels hemorrhage in the interstitial connective tissue of the medulla also hemolysis blood of blood capillaries While the second part, the second part was to evaluate the effect of the drug on reproductive efficiency. Four groups were divided, each group containing five rats, and were given the same dose for a month. After treatment, a male was introduced to each group and left to mate with the females. The result was pregnancy and births only in the control group, while no pregnancy occurred in the other group, The administration of isotretinoin, particularly in elevated doses, poses substantial risks to female reproductive efficacy, notably affecting ovarian reserve, a key fertility determinant.

Keywords: *Isotretinoin, acne vulgaris, ovaries, teratogenic agent*

1. 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)

2. MATERIAL AND METHODS

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 jdimensions (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).

General Experimental design

The current study consists of two parts

The first part: 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 (Sara *et al*.,2023). daily for 30 days.

- *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.

The second Part :- The second part was to evaluate the effect of the drug on reproductive efficiency. Consisting of 20 rats, four groups were divided, each group containing five rats, and were given the same dose for a month. After treatment, a male was introduced to each group and left to mate with the females

Assessment: - for Number of pregnant, number of fetuses and their weight

Part1:-Histopathological evaluation

At the end of the experiment, the ovaries were individually immersed in Bouin's fixative, dehydrated in alcohol, and embedded in paraffin. Serial sections of 5µm were obtained, deparaffinized, and stained with hematoxylin-eosin (H and E) following standard techniques (Bancroft and Gamble, 2008).

Part II:-Reproduction evaluation

2 males and 5 females were kept together overnight in plastic cages. The first day of pregnancy was indicated by the presence of spermatozoids in the vaginal-washing smear observed at optical microscopy (Vickery and Bennett 1970).

3. RESULT ;-

Descriptive Histology result of ovary

Figure 1:Histopathological section in normal ovary of control group the ovarian tissue showed we formed by Cortex with the containing the different stage of ovarian follicle a primary follicle witch-had containing or Oocyte surrounded by simple cuboidal epithelium cells also present The secondary follicles around it by two rows of epithelial cell, Tertiary follicle or grrrfian follicle which we containing multiple rows of epithelial follicular cells surrounded Oocyte with the Corona radiate with the presence of antrum filled with follicular fluid the medulla of overly had many congested the blood vessel surrounded by collagen bundle.

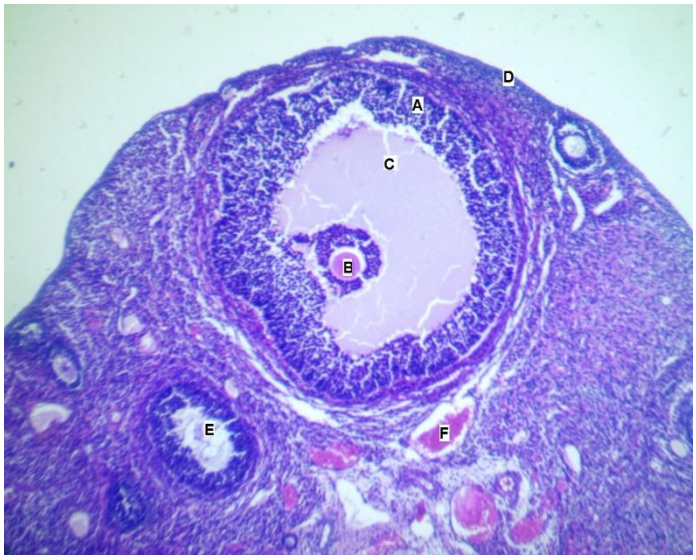


Figure (1): Cortex of ovary graafian follicle with follicle. Cells (A) Oocyte (B) follicular fluid (C). Germinal epithelium (D) secondary follicle (E) blood vessels (F) (CH & EX40)

Figure 2: Histopathological section in normal ovary of control group The cortex of ovary showed had different stage of ovarian development, Such as primary, secondary and tertiary follicle the service of ovary covered by a germinal epithelium.

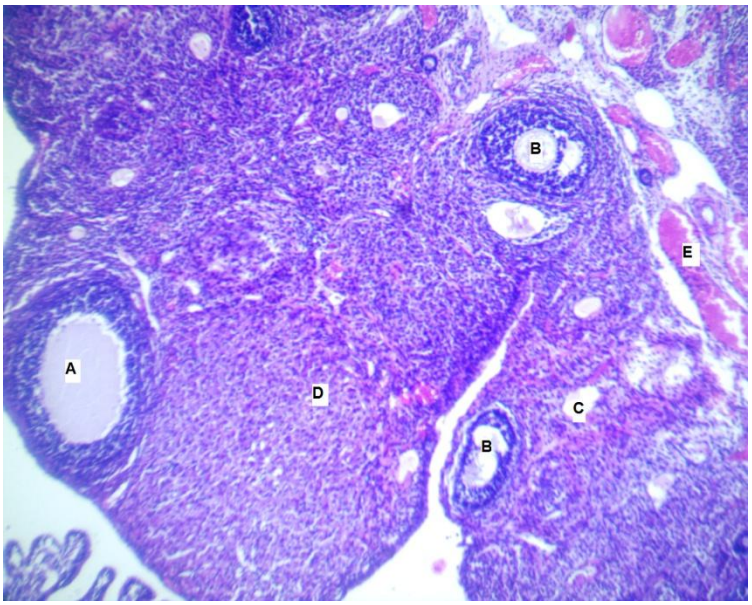


Figure (2): Ovary, growing tertiary follicle (A) secondary follicle (B) primary follicles(C) corpus luteum(D) blood vessels in medulla(E). CH & EX (10)

Figure 3: Histopathological section in normal ovary of control group the cortex of ovary had spherical corpuscle (ovarian

luteal corpora) which had cords of luteal cells surrounded by collagen strands , the center of corpora had homogenized follicular fluid , leutel cells with fat droplets were present in certain leutel cells

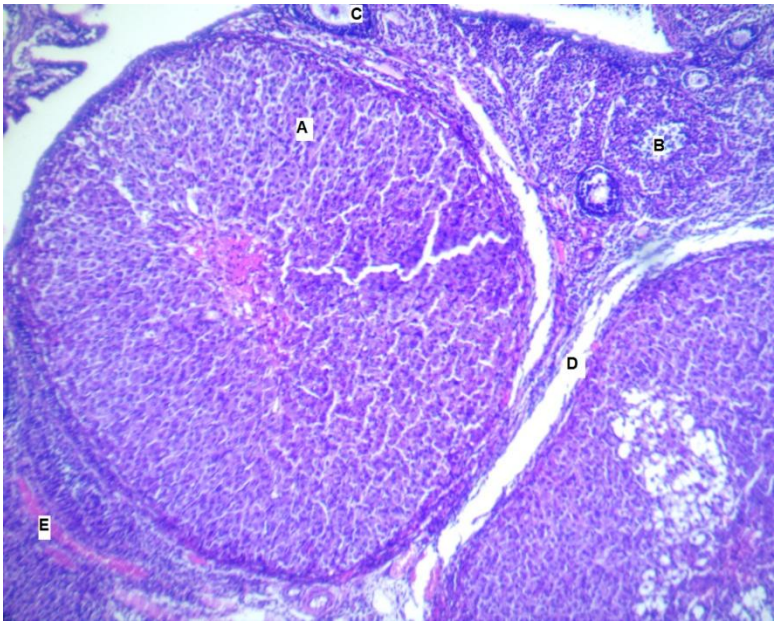
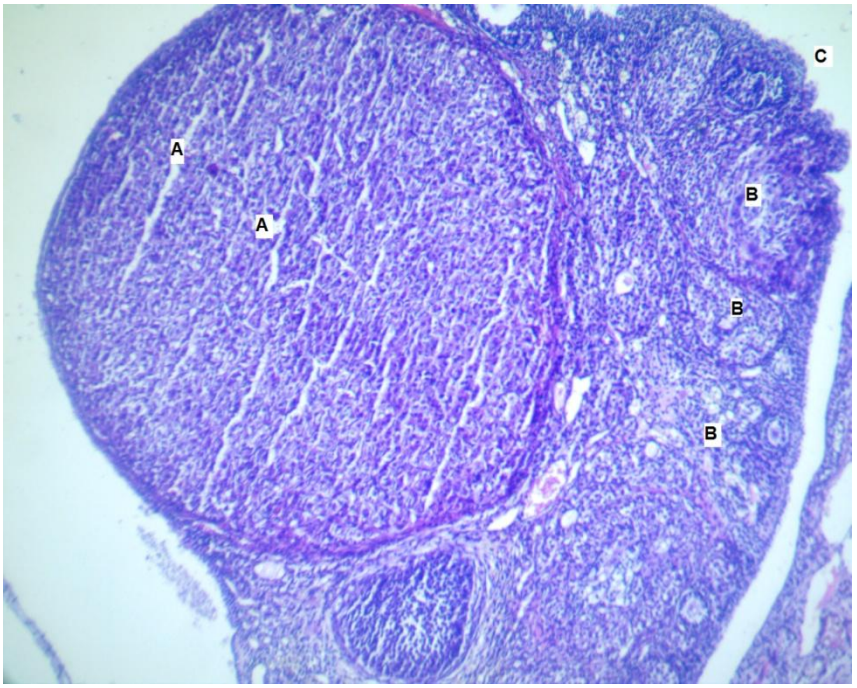


Figure 3: showed the cortex of ovary, corpus luteum with luteal cells (A) secondery follicle (B) primary follicle (C) collagen bundle (D) blood vessels (E) CH2 EX10.

Figure 4:A Histopathological section of the ovary treted with isotretinon (20ml\kg) showing the great copus luteum containing degenerated , atrophied luteal cells the most of primordial follicle had vaculated epithelial cells , the germinal epithelial cells on the surface of ovary was hyperplastic.



Figure(4) showed spherical corpus luteum with degenerated atrophied luteal cell (A) Vaculation of folliculer cells of primordial follicle (B) hyperplasia of the germinal epitheliam (C) CH2 EX10

Figure 5: A histopathological section of the ovary treted with isotretinoin (20 ml\kg) showing most of the ovarian follicle was containing degeneration follicular cells, with presence of congested blood vessels in between the corpus luteum we containing vacuole of the surrounding C.T. of luteum infiltered with WBCs.

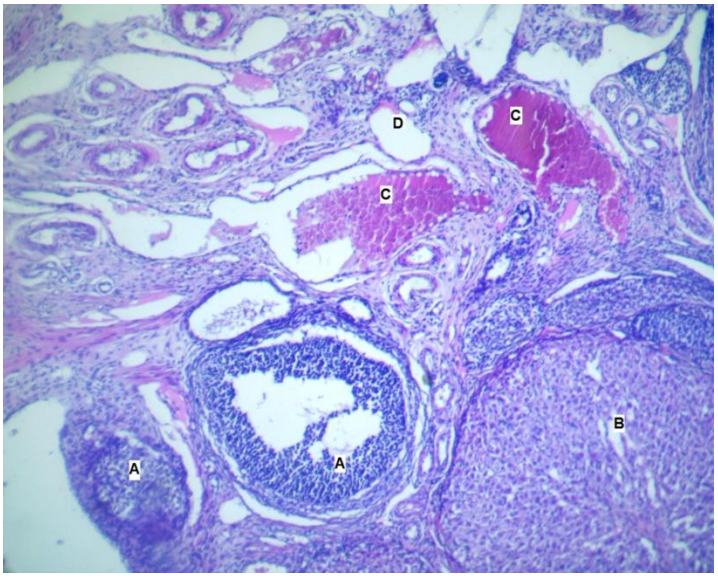


Figure (5): Ovary, degeneration of follicular cells (A) vacillation of luteal cell of corpus luteum (B) blood congestion (C) blood vessels empty from blood (D) (CH&EX 1o)

Figure 6:A histopathological secton of ovary treted with isotretinoin (20ml\kg) showing The mucosal fold of oviduct atrophied of lined with degenerated epithelial cells , WBCs were present in the core of mucosal fold , and around the basal area of fold

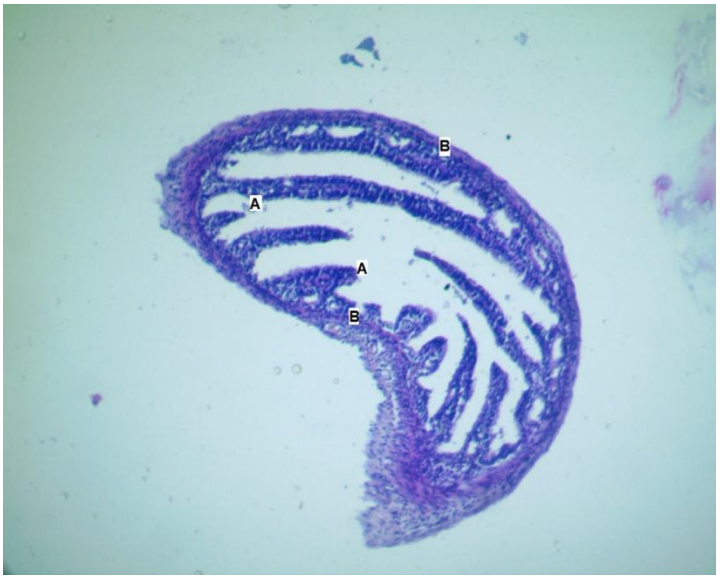


Figure (4.) showed ovary , atrophied mucosal fold with degeneration of epithelial cells (A) white blood cells infiltration (B) (CH2E X10)

Figure 4.:A histopathological section of the ovary treated with isotretinoin (20ml\kg) showing the graafian follicle of the ovarian cortex we containing degenerated follicular cells and the germinal epithelium we dissociated from the surface of ovary.

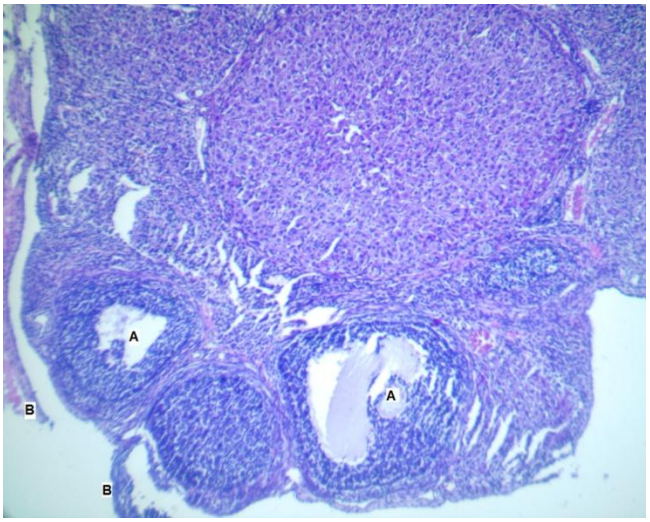


Figure (7.): Grraafian follicle devoid its Oocyte (A) detachment of germinal epithelium (CH&EX10)

Figure 8.: A histopathological section of the ovary treated with isotretinoin (40ml\kg) showing the ovarian cortex was containing atrophied ovarian follicle with degenerated Oocyte, the medulla of ovary we containing congested blood vessels blood hemorrhage in the interstitial connective tissue of medulla also hemolysis blood of blood capillaries were present around the corpus luteum.

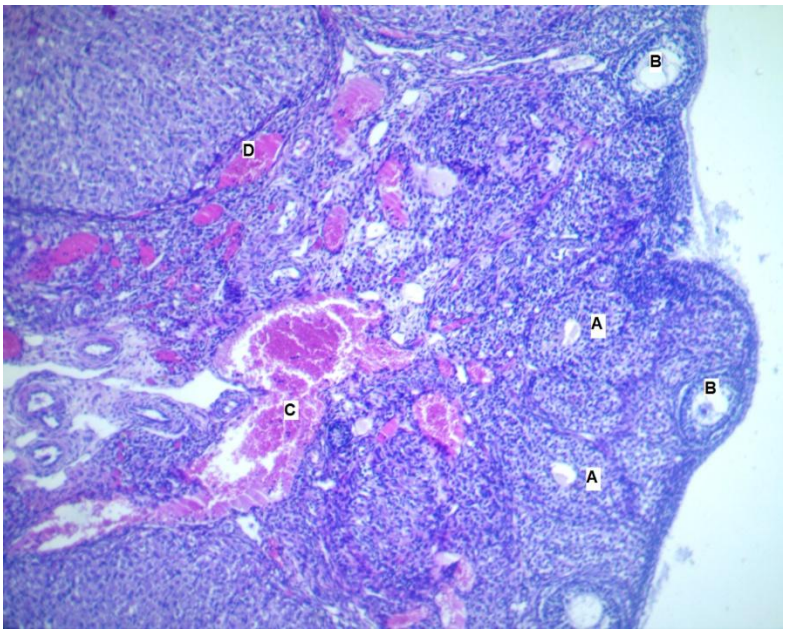
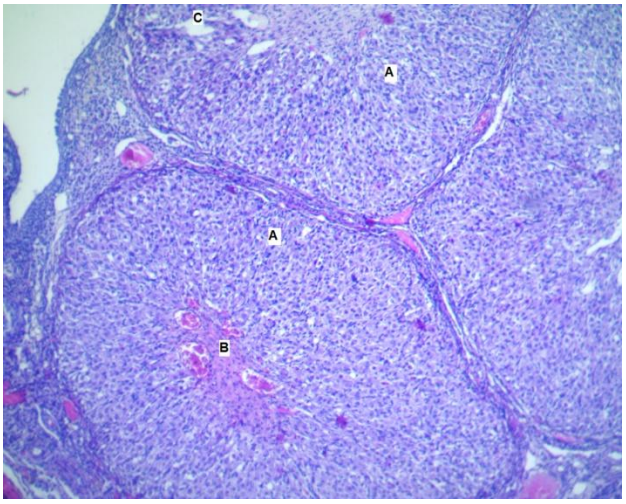


Figure (8): Ovary, atrophy of ovarian follicle (A) degenerated Oocyte (B) blood hemorrhage (C) blood congestion (D) (CH& EX10).

Figure 9:A histopathological section of the ovary treated with isotrtinoin (40 ml\kg) showing the ovarian crtex had great spherical corpora lutea , certain luteal cells had cytoplasmic vacuolation with certain sinuse of blood capillary congestion , bundle of collagen strand were invested each corpus luteum , blood capillary around each corpus were engorged by blood .



Figure(9) figure showed ovarian cortex corpora lutea with cytoplasmic vaculation of luteal cells (A) blood hemolysis (B) sinuse in the core of corpus luteum (C) atrophied of germinal epithelium (D) CH EX10

Figure 10: A histopathological section of the ovary treated with isotretinoin (40ml\kg) showing the Graafian follicle we present in the cortex of ovary which had cavity filled with follicular fluid the follicle devoid for Oocyte degeneration of certain follicular cells.

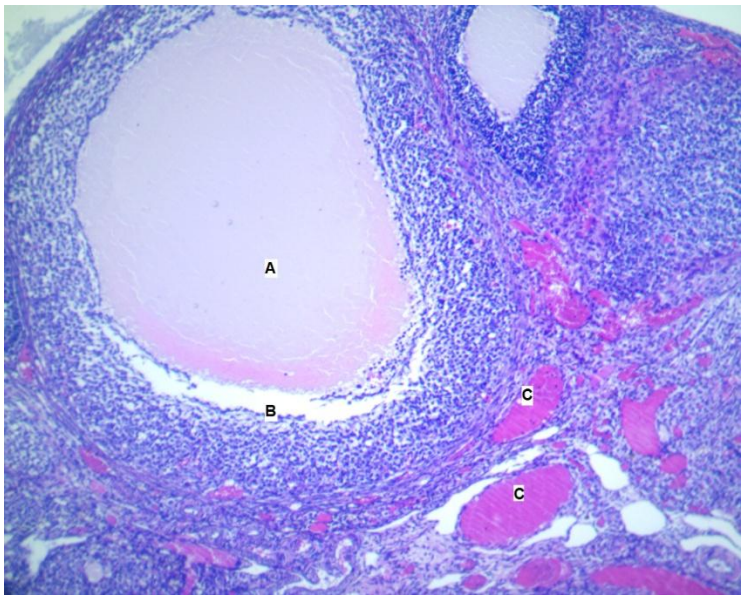


Figure (10): Graafian follicle (A) disappearance of Oocyte in the antram. Degeneration of follicular cells (B) severe blood hemolysis congestion (C). (CH& EX10)

Figure 10:A histopathological section of the ovary treated with isotrtinoin (40ml\kg) showing Degeneration of follicular cyst and corpus luteum, great cavities were present around the corpus luteum and the other follicular Oocyte, masses of blood were present in the interstitial connective tissue, Also presence of inflammatory WBCs around the follicular.

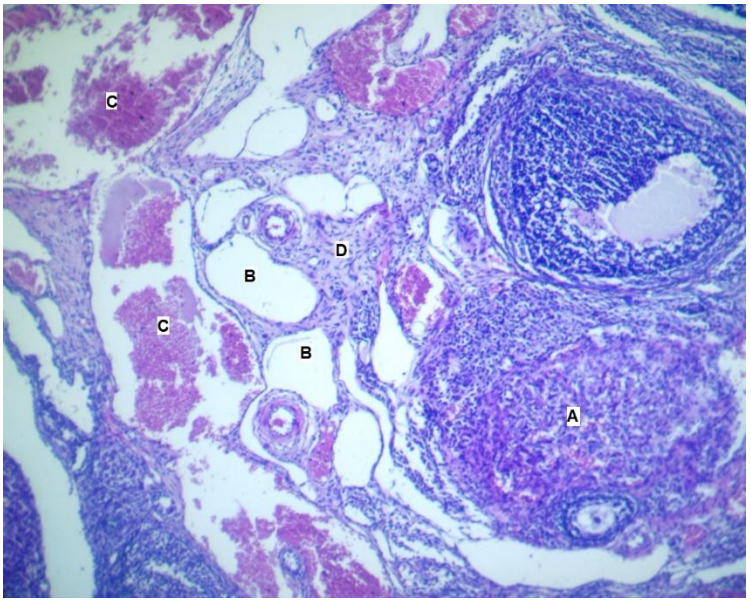


Figure (10): Ovary, degeneration of follicular cysts (A) great cavity around corpus luteum (B) masses of blood (C) in the interstitial connective tissue and inflammatory WBCs CH&EX10).

Figure 11:A histological section of the ovary treated with isotretinoin (40ml\kg) and Omega3 (20 ml\kg) showing The cortex of the ovary had extensive degeneration of development stage of ovarian follicle. Which were the devoid of Oocyte, the group of follicles were surrounded by collagen bundle hemorrhage we evident around the group of ovarian follicle.

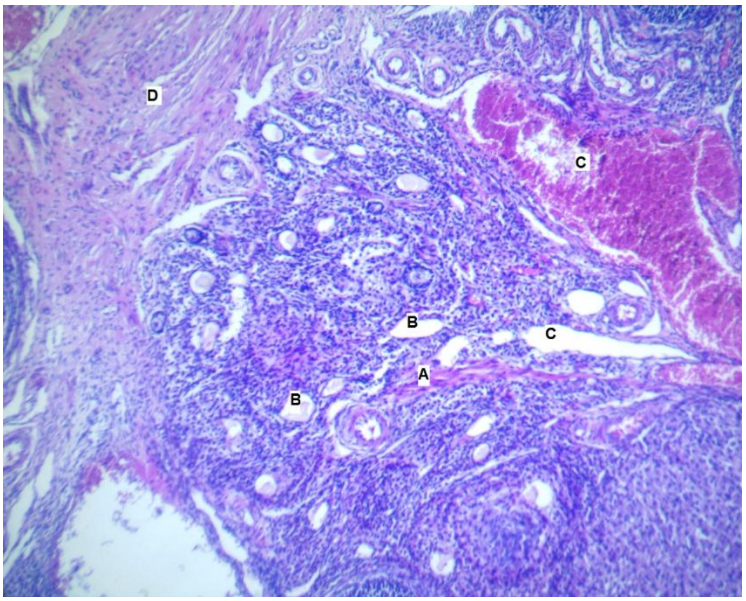


Figure (11): ovary extensive degeneration of the ovarian follicle (A) disappearance of Oocyte (B) extensive blood hemorrhage (C). Collagen bundle (D) (CH & EX10)

Figure 12: A histopathological section of the ovary treated with isotrtinoin(40 ml\kg) and Omega3 (20 ml\kg)showing the secondary follicular of primary follicular were atrophied which were restricted in between corpora lutei also most of the primordial follicle were also atrophied most of the germinal epithelium on the service of ovary we removed.

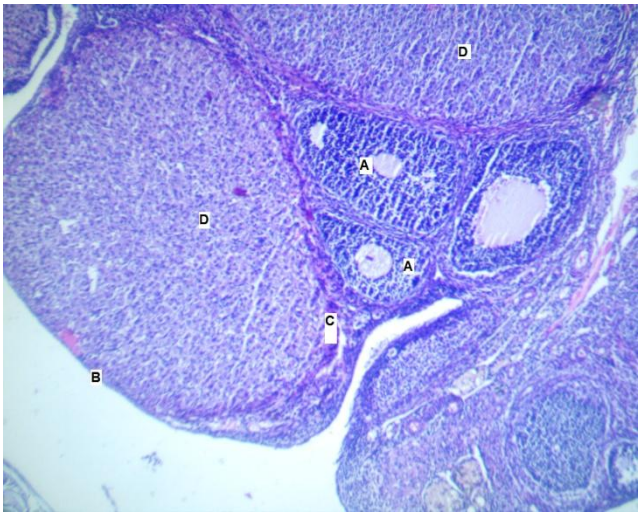


Figure (12): Atrophied follicular cysts (A) atrophy of germinal epithelium (B) congestion of blood capillary (C) around the corpus (D) (CH&EX10)

Figure 13: A histopathological section of the ovary with treated isotretinoin (40ml\kg) and Omega3 (20ml\kg) showing The Lumen of oviduct had detached mucosal folds of presence of atrophied and mucosal fold the connective tissue around the oviduct we thin and delicate

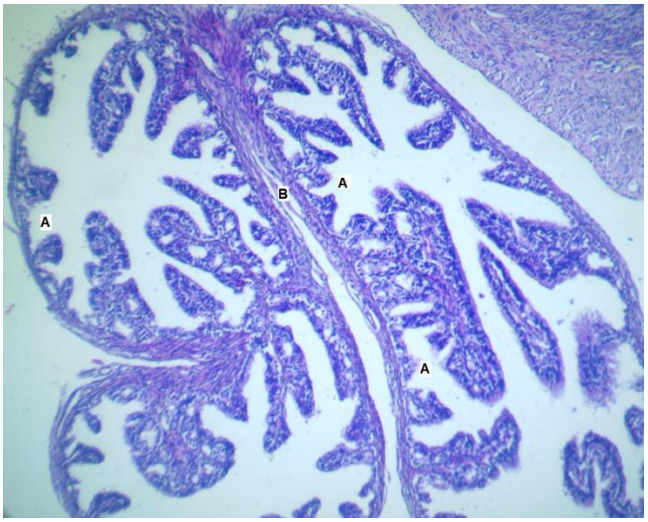


Figure (13): Oviduct with atrophied mucosal fold (A) Detachment of connective tissue in between lumens of oviduct (CH&EX10)

Figure 14:A histopathological section of the ovary with treated isotrtinoin (40 ml\kg)and Omega3 (20 ml) showing The oviduct we containing atrophied mucosal fold with disappearance of other fold, the base of fold had infiltrated with WBCs.

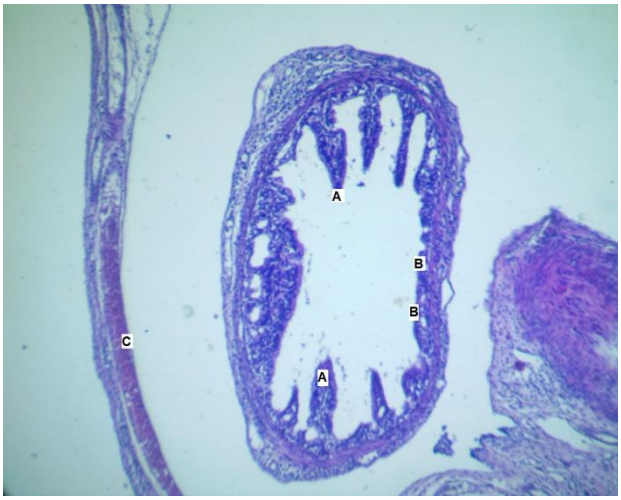


Figure (14): Oviduct with atrophied mucosal fold (A) Disappearance of mucosal fold (B) Detachment of connective tissue (C) (CH&EX10)

Result of the second stage of the experiment

After treating the groups with isotretinoin for thirty days, the male was introduced to mate with the female to evaluate the effectiveness of the female reproductive system in rats. It was noted that the first control group had become pregnant.

The results were the number of births ranged between 6-9. The average number of births for the first group was 7-8, and their weights ranged between 9-11 grams. The average number of fetuses for the first group was 10 pup

While in the treated groups, no pregnancy occurred at all.

4. DISCUSSION

The first phase :- Histological assessment

This study investigated the effects of varying doses of isotretinoin on ovarian morphology and associated histopathological changes in different experimental groups. The second group received isotretinoin at a dose of 20 ml\kg, while the third group was administered a higher dose of 40 ml\kg. The fourth group also received 40 ml\kg of isotretinoin but in combination with Omega-3 fatty acids.

Histological changes in the architecture of the ovaries were observed throughout all the groups in the current study. In the third group, histopathological changes included atrophied Graafian follicles in the ovarian cortex which we observed as having degenerated oocytes.According to the present study these finding scenarios are in accordance with Abali *et al.*, 2013 who reported that increased concentration of isotretinoin results depicted atrophied Graafian follicles and degenerative oocytes which depict negative impact on ovarian follicle.

Furthermore in the present study the medulla of the ovary showed dilated blood vessels and haemorrhage in interstitial connective tissue around the corpora lutea. This implies that isotretinoin at high concentrations may stimulate vascular changes and destruction of follicle; therefore, has the propensity to affect functionality of the ovary.This confirmed with , Yalçın *et al.*, 2024 who stated that congestion of blood vessels and hemorrhage of the ovarian medulla were observed indicating that isotretinoin could cause vascular change

Analysis of the third group revealed the presence of large Graafian follicles with follicular fluid, but no oocytes and the authors pointed out that degeneration of certain follicular cells. This degeneration was accompanied with severe blood hemolysis and congestion implying that isotretinoin was likely to be injurious to the ovary.As the Graafian follicles filled with fluid and without oocytes means the degeneration of follicular cells, Fonseca *et al.*, 2023 found found similar results This study is in accordance with Cinar *et al.*, 2017 demonstrated that isotretinoin reduced ovarian reserve through decrease AMH levels and increased atretic follicles.

The fourth group that received isotretinoin at 0.2 mg combined with Omega-3 showed severe destruction of ovarian follicles. The follicles were always enclosed in collagen fibres and exerted huge hemorrhage. Furthermore, there was some atrophy dissection in both secondary and primary follicles, while atrophy was evident in most of the primordial follicles. This extensive degeneration seems to cast doubt on the effects of isotretinoin on the long-term effects on ovarian reserve and fertility.The oviduct sampled in the fourth group had atrophic mucosal ridges while connective tissue was thin and delicate. This is also an indication of an inflammatory process evident by the infiltration an inflammatory WBCs round the mucosal

folds which could be due to the treatment regimen.This is in accordance with Słomczyński et al., 2024 This agree with Słomczyński *et al.*, 2024 who observed that accumulation of inflammatory white blood cells around the mucosal folds in the oviducts indicate inflammation elicited by isotretinoin.Besides, similar to Mendi *et al.*, 2024 had blunting of mucosal folds, which could suggest certain effects on fertility.

The second phase assessment

In the second phase of this experiment, after mating, revealing that only the first control group achieved pregnancy, with birth numbers ranging from 6 and a pregnancy rate of 7-8, while weights varied between 9-11, and the average number of fetuses in the first group was 10, contrasting with the other groups which exhibited no pregnancies. The current results agree with (Kacamak *et al*., 2020) that isotretinoin has been shown to have significant reprotoxic effects, particularly in female rats. This has implications for oocyte maturation whereby GDF-9 and BMP-15 which are imperative for follicular development and oocyte maturation are downregulated in this study. Moreover, Bas *et al*., 2023 concluded that isotretinoin also contributes to a decrease in healthy primordial follicles, thus suggesting a poor ovarian reserve. Additionally, Tawfiq *et al*., 2020 reported isotretinoin's cytotoxic and genotoxic properties contribute to its reproductive toxicity. It induces apoptosis in various cells, including those in reproductive tissues, which can lead to infertility.

5. CONCLUSIONS

The histopathological examination findings, as isotretinoin-treated groups exhibited significant ovarian alterations, including follicular degeneration, corpus luteum atrophy, and hemorrhagic changes. The presence of atrophied mucosal folds in the oviduct and infiltration of white blood cells in ovarian tissues indicates inflammatory responses and potential impairment of reproductive function. The second stage of the experiment confirmed this, as only the control group achieved successful pregnancies, whereas none of the treated groups were able to conceive.

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I sincerely want to thank my family, especially my mother. Their belief in me has kept my spirit and motivation high during this study.

Lastly, I am grateful to all persons who helped and supported me to complete writing this thesis.

NOVELTY STATEMENT

What distinguishes our study is its focus on the physiologically active ingredient in isotretinoin, which is used as an anti-acne drug, and specifically on the side effects it causes in women, specifically through the reduction of egg reserves, which leads to infertility. Given the existence of safe and effective medications for treating acne, especially for women before and after pregnancy, it is necessary to focus on them and use them as an alternative to the aforementioned treatment.

AUTHORS CONTRIBUTION

The author contributed equally to the work with me.

CONFLICT OF INTEREST

The authors have stated that there is no conflict of interest.

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