

HISTOPATHOLOGICAL AND HORMONAL EFFECTS OF DIFFERENT DOSES OF ASPIRIN ON THE FEMALE REPRODUCTIVE SYSTEM OF ALBINO RATS

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ABSTRACT

The present study was carried out to investigate the effect of aspirin on the female reproductive organs in albino rats. (21) mature female albino rats (150-170gm) were divided into three groups: 1st group which treated as negative control group, it was drenched only 0.2 ml of PBS. 2nd group was drenched with Aspirin (10 mg/Kg of B.W once daily for 14 days). 3rd group was drenched with Aspirin (20 mg/Kg B.W once daily for 14 days). Our results showed that 2nd group and 3rd group demonstrated significantly decrease ($P \leq 0.05$) in the values of plasma LH compared with control group. In values of plasma FSH hormone, the 2nd and 3rd groups showed significantly decreased ($P \leq 0.05$) in the relation with the control group. On the other hand, the microscopic examination of the histopathological sections of uteri of all the treated and control groups showed that the 2nd group demonstrated mild hyperplasia and degeneration in the epithelial cells which lining the uterus and there is few numbers of uteri glands. Also marked hyperplasia and vacuolation of the epithelial cells which lines the uterus. In 3rd group, the histopathological examination of uteri elucidates profuse proliferation and irregular hyperplasia of smooth muscles in the wall of uterus, there is high infiltration of inflammatory cells and there is congestion of blood vessels. Also there is marked vacuolation of uterine epithelial cells and fewer and smaller uterine glands than the 2nd group and 1st (control) group. In ovaries of 2nd group showed markedly few follicular growth wave characterized by primary, secondary follicles and there is congestion and thrombi in the ovarian stroma. Also presence of large corpus luteum. But the histopathological changes in the ovaries of 3rd group animals showed more severity than 2nd group animals in which there are

primary and secondary follicles with severe congestion and hemorrhage, also there is high numbers of corpus luteum. 1st (control) group was showed normal histological feature of uteri and ovaries. We conclude from the present study that different doses of aspirin can cause histopathological effects and hormonal disturbances in FSH and LH hormones.

INTRODUCTION

Aspirin (acetyl salicylic acid ,ASA)has been used as one of the most famous ,cheap, easily available and widely used non steroid anti-inflammatory drug useful as an analgesic to relief minor aches and pains(1,2). It has also an anti-platelets effect which in long time and low dose can prevent heart attacks and thrombus formation in hyper-coaguable states e.g. cancer. On the other hand, it has a side effect, due to its anti-coagulant properties, that increased bleeding in menstruating women (3).

Aspirin (non-steroidal, consistently inhibit ovulation in all mammalian species investigated), likely due to the inhibition of cyclooxygenase 2(COX-2) because COX-2inhibition, has major effects on ovulation, fertilization and implantation (4). And on the reproductive system, however, there are several reports in Women indicating that the use of aspirin before pregnancy associated with decrease risk of ectopic pregnancy (5).

Low-dose of aspirin may improve ovarian responsiveness, uterine and ovarian blood flow velocity, and implantation and pregnancy rates in patients undergoing IVF (6). Also, low-dose aspirin (100 mg/day)may improve uterine perfusion in women with unexplained infertility and impaired uterine blood flow (7).In women, it has been reported that, treatment of young women with aspirin to treat inflammatory joint disease, induces luteinized ruptured follicle syndrome (LUF) (8).Moreover, aspirin or NSAIDs are associated with the recurrent development of this syndrome in young women receiving treatment with those drugs to treat ankylosing spondylitis and rheumatoid arthritis, and normal ovulation can be resumed in these patients following drug withdrawal (9).

MATERIAL AND METHODS

Animals and their care:

(21) Of female albino rats (150-170) gm. were obtained from animal house of Vet. Med. College of AL-Qadisiya University and prior to use were acclimatized for 7 days 12 hr. light/ dark cycle. The animals were housed in plastic cages in an air-conditioned room with temperature maintained at $25 \pm 2^\circ\text{C}$. Rats were given sterile food pellets and water ad libitum. All rats were randomized in to three groups of 7 rats each one and were treated as below for (30 days):

1- First group (1st): which treated as negative control group, it was drenched only 0.2 ml of PBS.

2- Second group (2nd): It was drenched with Aspirin (10mg/Kg B.W once daily for 30 days).

3- Third group (3rd): It was drenched with Aspirin (20 mg/Kg B.W once daily for 30 days).

Chemicals:

Aspirin: this drug was obtained from the market. (400mg) of Aspirin powder (molecular weight 180.157g/mol, Sigma-Aldrich, USA) prepare by dissolving with (100) ml of 4% carboxymethyl cellulose to prepare the stock solution of Aspirin (10).

Blood collection:

Blood collection was done at 30 days of experiment via the abdominal vein. The collected bloods were collected in test tube with anticoagulants that allowed coagulating for 15 min in refrigerator. Serum was separated from coagulated blood samples by centrifugation at 3000 rpm for 15 min, and then kept in the frozen at -20°C until using to estimate LH and FSH hormones.

Estimation of FSH and LH:

FSH and LH hormones were estimated according to radioimmuno- assay (RIA) method by using kit instructions (11).

Histopathology:

After (30) days, the animals were sacrificed by the inspiration of chloroform in the Colton swab put in closed container. Specimens were taken from the uteri and ovaries and the tissues were kept in 10% formaldehyde solution for fixation, and then processed routinely by using the histokinette (SLEE medical/Germany). Tissue

sections were embedded in paraffin wax, and sectioned by rotary microtome and all sections were stained with hematoxylin and eosin stain(12).

Statistical analysis

All the grouped data were statistically read by SPSS program, Version 17 software (2010). Testing methods including one way ANOVA for comparisons among groups followed by least significant differences (LSD) test for comparison between two groups. P values of $p \leq 0.05$ were considered to record statistical significance, all data were expressed as mean $\pm$ standard error (SE) (13).

RESULTS

A-Hormonal estimation:

Our results showed that 2nd group and 3rd group demonstrated significantly decrease ($P \leq 0.05$) in the values of plasma FSH (1.33 ± 0.07) and (0.91 ± 0.08) respectively compared with control group as in table (1). In the values of plasma LH hormone, the 2nd and 3rd groups showed significantly decreased ($P \leq 0.05$) (1.05 ± 0.03) and (0.98 ± 0.04) respectively in the relation with the control, as in table (1).

Table (1): Effect of Aspirin on FSH and LH hormones in studied groups.

Groups	FSH hormone mIv/ml	LH hormone mIv/ml
1 st group	1.68 ± 0.09 a	1.36 ± 0.07 a
2 nd group	1.13 ± 0.07 b	1.05 ± 0.03 b
3 rd group	0.91 ± 0.08 b	0.98 ± 0.04 c

-Results expressed as mean $\pm$ S.E

-Different letters refer to significant differentiations between groups at ($P \leq 0.05$).

-Similar letters refer to no significant differentiations between groups at

($P \leq 0.05$).

-1st group: control group.

-2nd group: first treatment group gavages with Aspirin at a dose 10 mg/Kg of B.W.

-3rd group: second treatment group gavages with Aspirin at a dose 20 mg/Kg of B.W.

B-Histopathological changes:

1- Uteri:

In the 2nd group, the uteri sections showed mild hyperplasia, degeneration and vacuolation of the epithelial cells which lining the uterus and there is few numbers of uteri glands as in fig. (2) & (3), the uterine glands showed fewer numbers and small in size as in fig. (4).

In the other hand: the 3rd group, uteri showed profuse proliferation and irregular smooth muscle hyperplasia in the wall of uterus, there is high infiltration of inflammatory cells and there is congestion of blood vessels as in fig. (5). Also there is Marked vacuolation of epithelial cells which lining the uterine tissue as in fig. (6).

The uteri of control group animals showed no histological changes, there is normal epithelial cells which lining uterus with profuse and mature uterine glands as in fig. (1).

2- Ovaries:

In the 2nd group, there is few follicular growth wave characterized by primary, secondary and there is congestion and thrombi in the ovarian stroma as in fig. (9). Also presence of large corpus luteum as in fig. (10).

On the other hand, the ovarian histological sections of 3rd group animals showed similar changes for 2nd group, there is little follicular growth characterized by primary and secondary with severe congestion and hemorrhage larger than 2nd group, also there is high numbers of corpus luteum as in fig. (11) & (12).

The ovarian histological sections of control group animals showed no or undetectable histopathological changes, there is normal follicular growth wave characterized by presence of graffian follicle, secondary and primary follicles as in fig. (7) & (8).

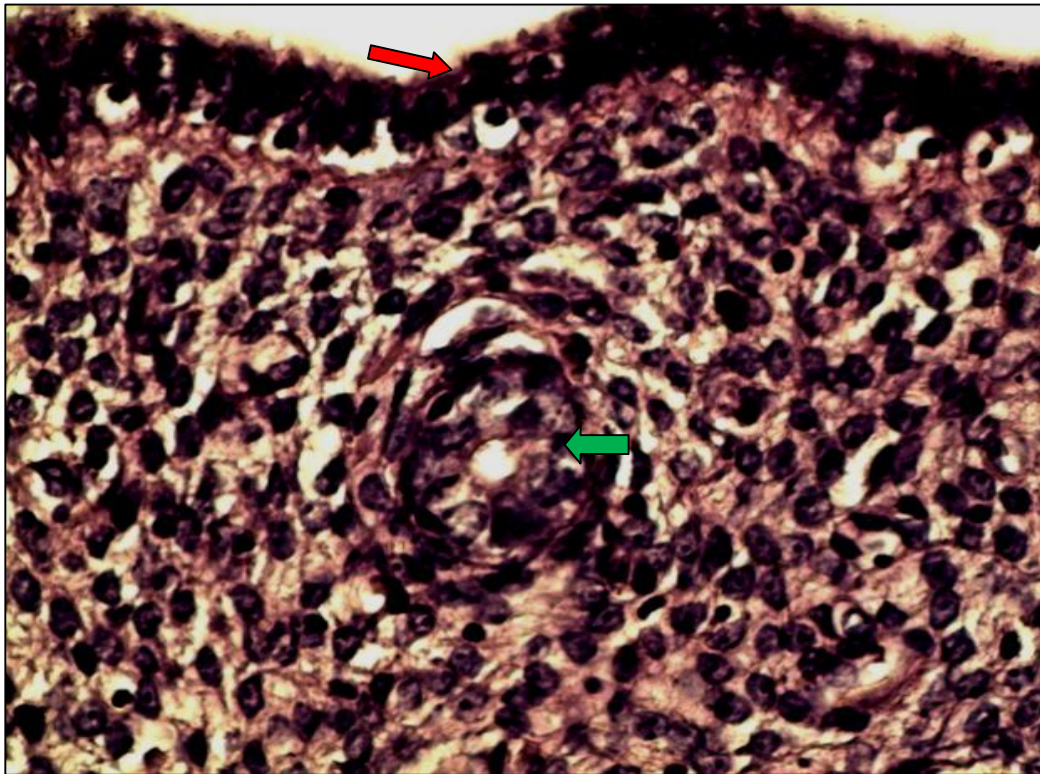


Fig. (1): 1st group is a control uterus: There is normal epithelial cells which lining uterus (red arrows), profuse and mature uteri glands in the uterine wall (green arrow). 40XH&E.

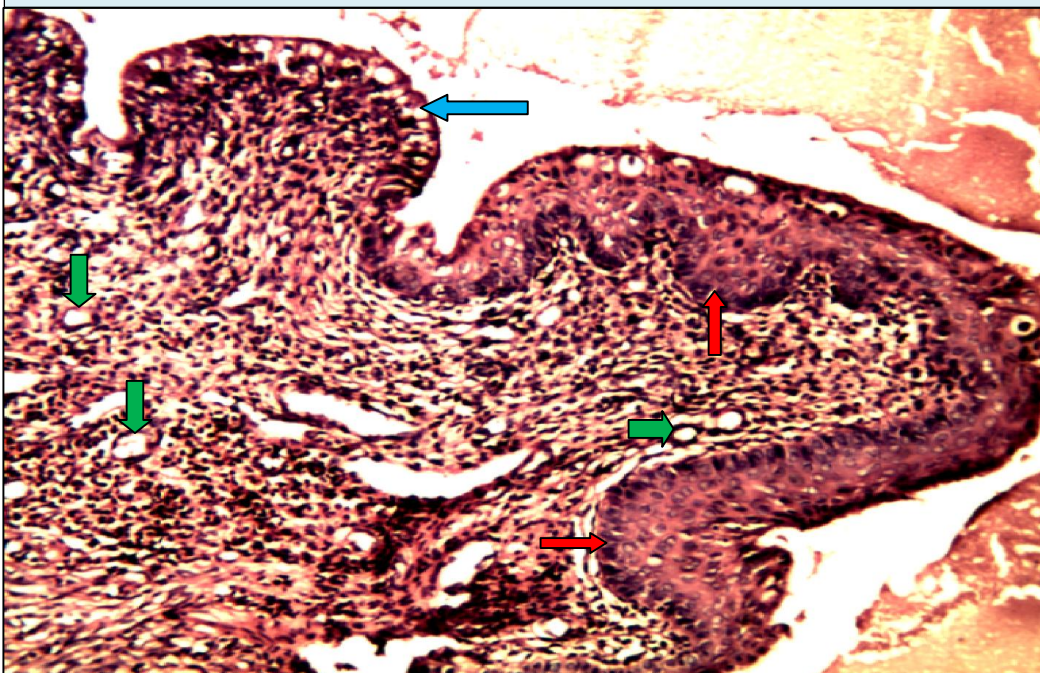


Fig. (2): 2nd group uterus: There is Mild hyperplasia (red arrows) and degeneration in the epithelial cells which lining the uterus (blue arrows) and there is few numbers of uteri glands (green arrows).10XH&E.

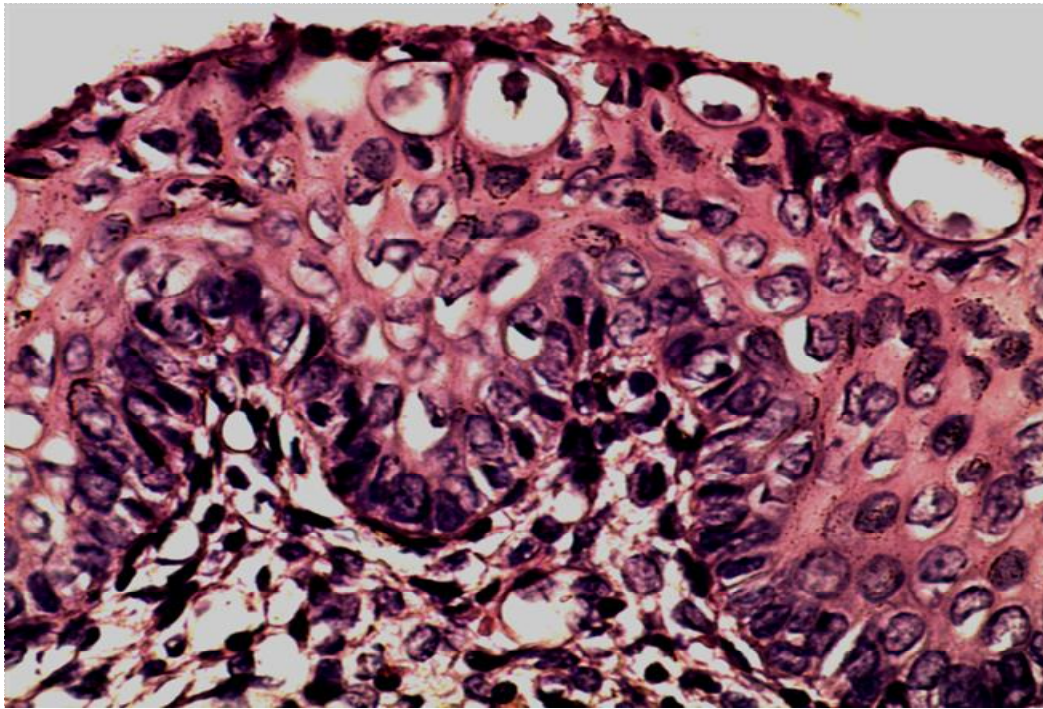


Fig. (3): 2nd group uterus: Higher magnification marked hyperplasia (red arrows) and vacuolation of epithelial cells which lining uterus (thin arrows).40XH&E.

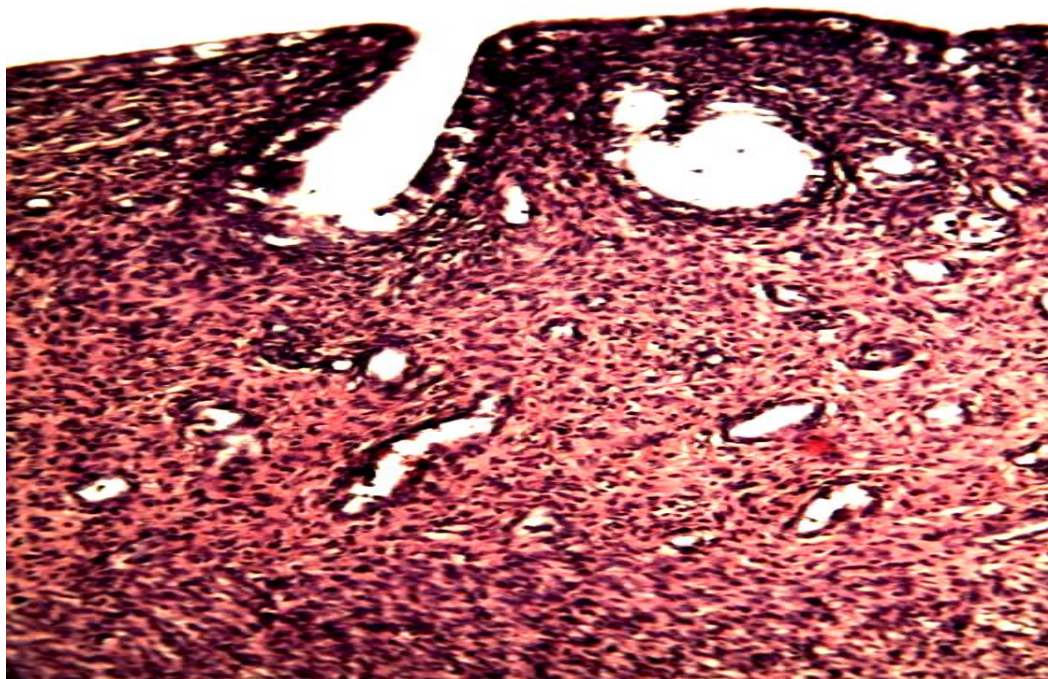


Fig. (4): 2nd group uterus: There is degeneration (blue arrow) of epithelial cells which lining uterus. The uterine glands showed fewer numbers and small in size (thin arrows). 10XH&E.

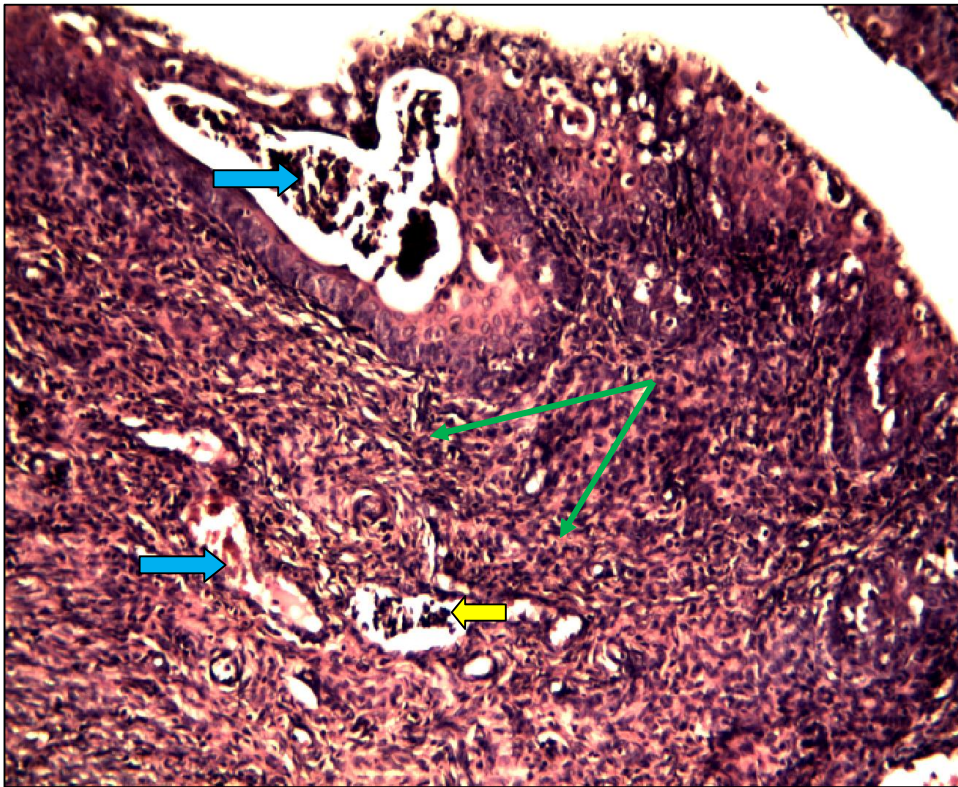


Fig. (5): 3rd group uterus: There is profuse proliferation and irregular smooth muscle hyperplasia in the wall of uterus (thin green arrow), there is high infiltration of inflammatory cells (yellow arrow) and there is congestion of blood vessels (blue arrow). 10XH&E.

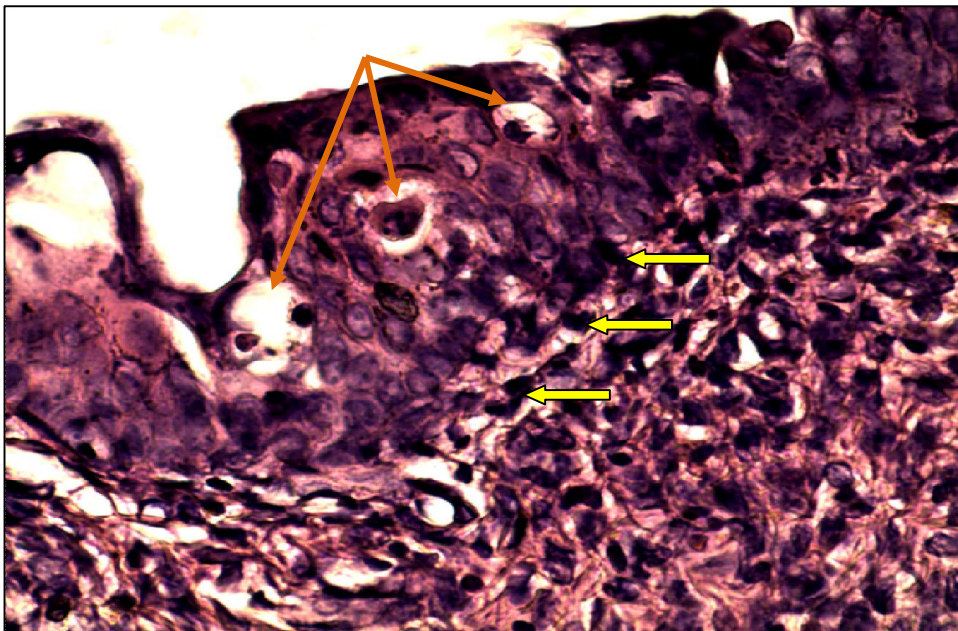


Fig. (6): 3rd group uterus: Higher magnification, Marked vacuolation of epithelial cells which lining uterus, and infiltration of inflammatory cells (yellow arrows). 40XH&E.

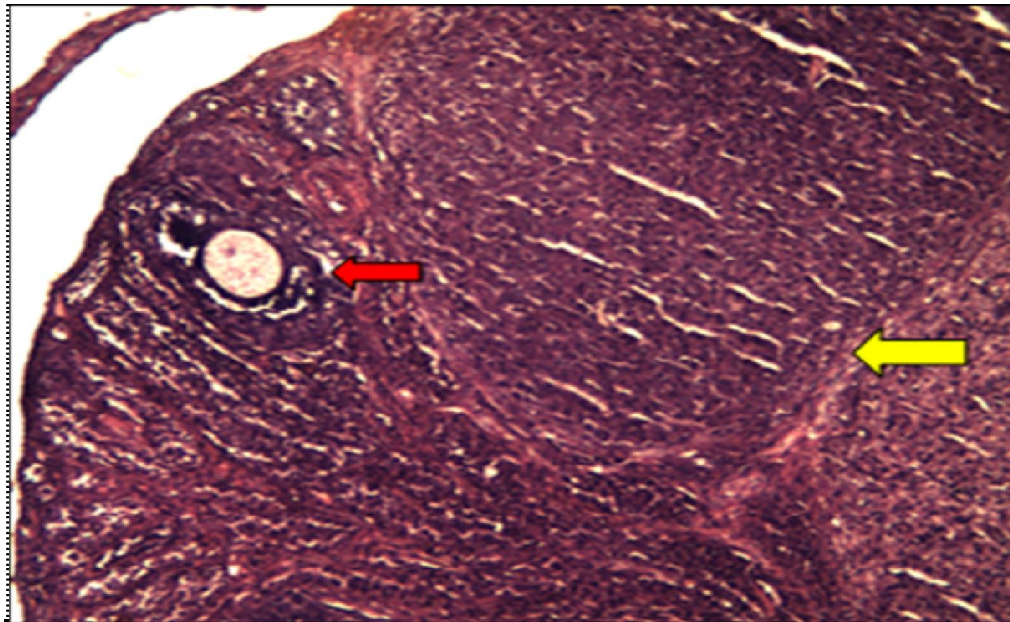


Fig. (7): 1st group is a control ovary: There is normal ovarian stroma characterized by low follicular growth wave only secondary follicle (red arrow) with large corpus luteum (yellow arrow). 4XH&E.

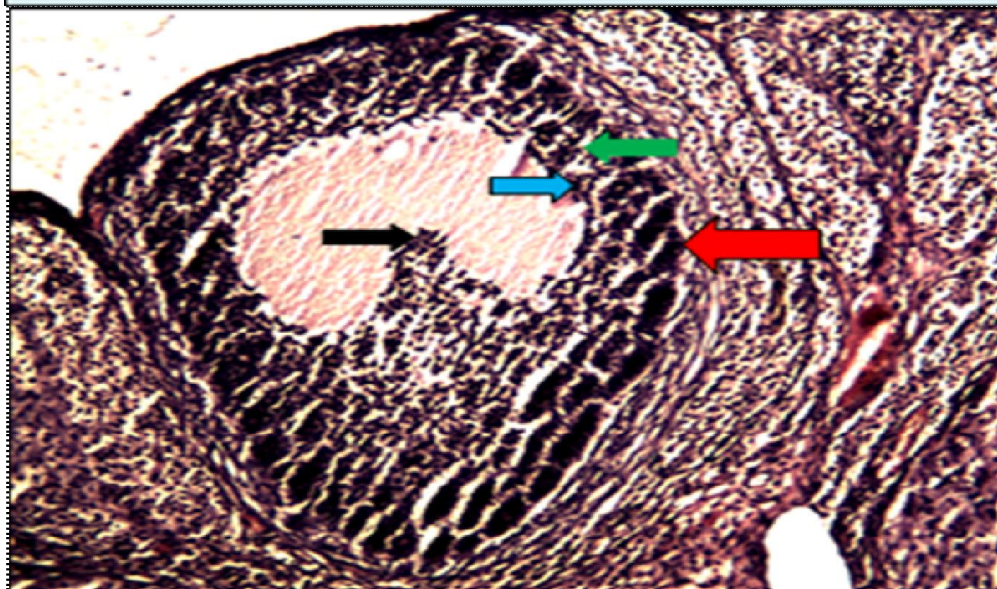


Fig. (8): 1st group is a control ovary: There is graafian follicle (red arrow) which characterized by theca interna (blue arrow) and theca externa (green arrow) surrounding the ova (black arrow). 40XH&E.

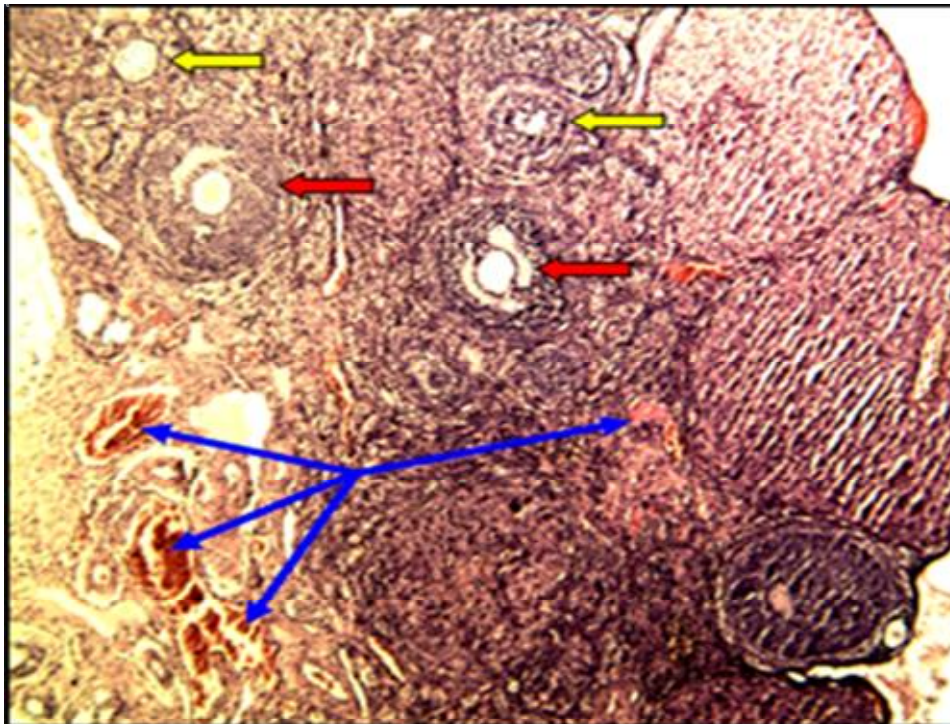


Fig. (9): 2nd group ovary: There is few follicular growth wave characterized by primary (yellow arrows), secondary (red arrow) and there is congestion and thrombi in the ovarian stroma (blue arrow). 4XH&E.

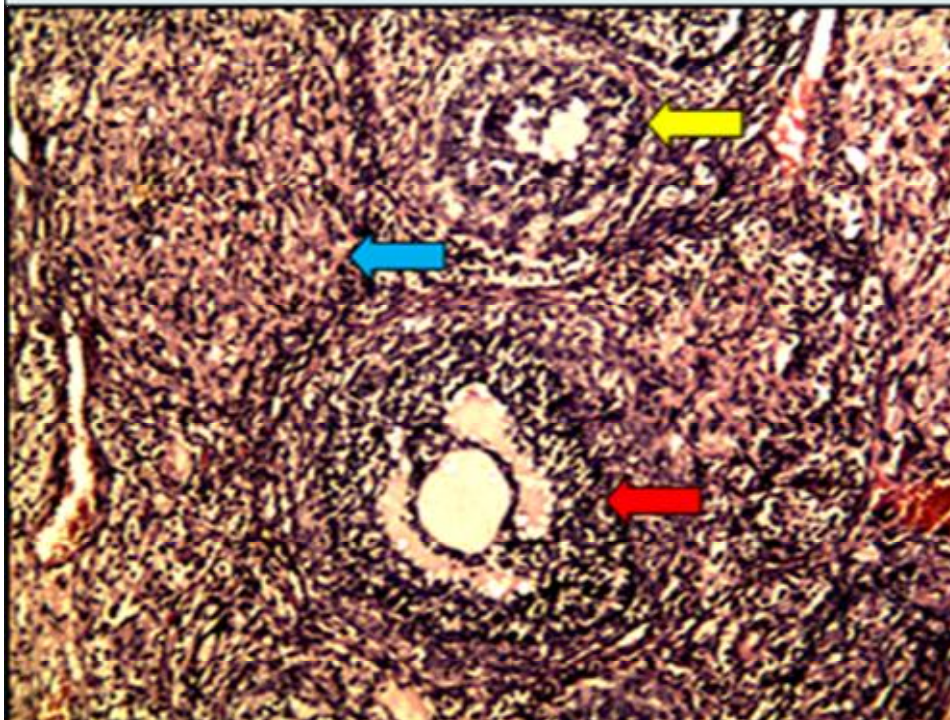


Fig. (10): 2nd group ovary: Higher magnification, there are primary (yellow arrow), and secondary follicles (red arrow) and presence of large corpus luteum (blue arrow). 10XH&E.

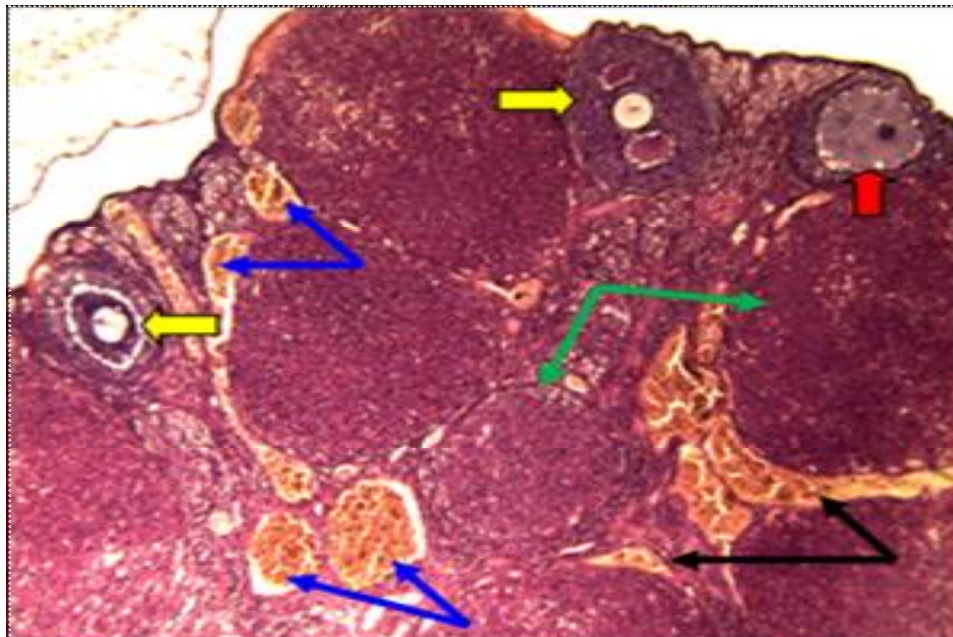


Fig. (11): 3rd group ovary: There is few follicular growth characterized by primary (red arrow), secondary (yellow arrows) with severe congestion (blue arrows) and hemorrhage with ovarian stroma (black arrows) also there is high numbers of corpus luteum (green arrows). 4XH&E.

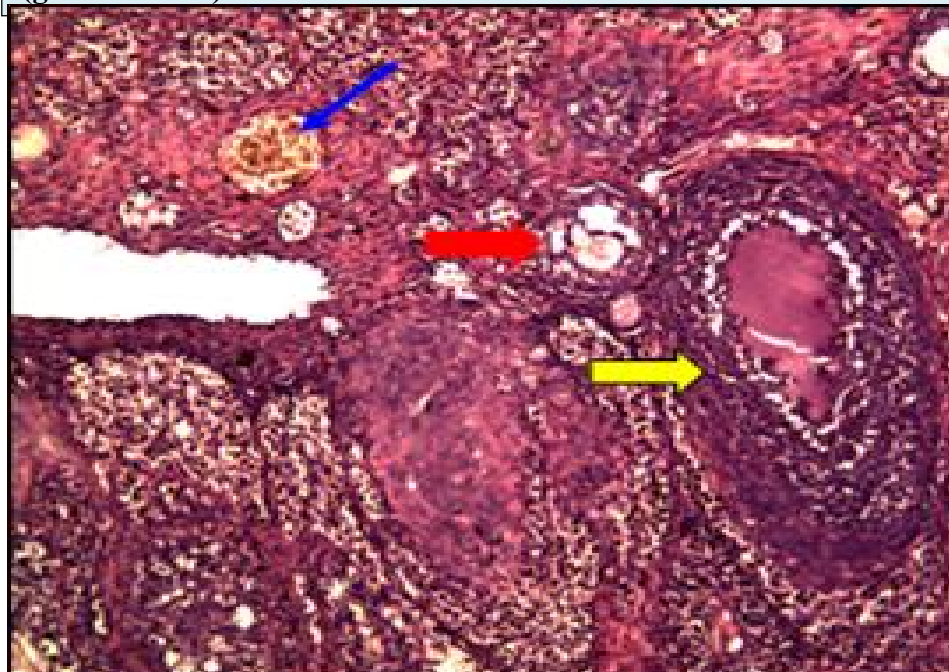


Fig. (12): 3rd group ovary: Low follicular wave characterized by secondary (yellow arrow) and primary follicles (red arrow) and congestion of blood vessels (blue arrow). 10X H&E.

DISCUSSION

Aspirin is widely used drugs in the world in patients with a wide range of therapeutic uses for the treatment of inflammatory joint diseases, prevention of thrombosis and many other causes for its anti-inflammatory analgesic antipyretic and antiplatelet effects (14), and on the reproductive system (15).

Hormonal changes:

Our results showed that 3rd group animal, appeared a significant decrease ($p \leq 0.05$) in the values of FSH and LH compared with control group. The cause of these results due to the effect of high doses of aspirin (20mg/kg B.W), in which Aspirin will increase inhibin A levels by increase PGF2 α which prevents luteolysis and maintenance of corporal leutea and then increase negative feedback mechanism on hypothalamus and pituitary gland and then decrease FSH and LH levels, this evidence was provided by (16) who provided that aspirin administration persistently keep down luteolysis via blocking of prostaglandin f2 α by high level of progesterone hormone.

Also (17) provided that inhibin A will decrease the bioactive FSH blood level and precluded follicular evolve in monkeys.

Histopathological changes:

A-Ovaries:

1-3rd group:

Our present result of the histopathological lesions in the ovaries of 3rd group rats showed few follicular growth wave, the cause of these results due to the long-term administration of aspirin in which decrease blood levels of FSH and LH which inhibit folliculogenesis, the effect of Aspirin either by inhibit inhibin hormone will reduce FSH and LH blood levels or by maintenance of corpora lutea and prevent luteolysis and increase progesterone will cause finally decrease FSH and LH blood levels.

Also our result in ovaries showed circulatory disturbances characterized by severe congestion and hemorrhage in the ovarian stroma, these changes occurred due to the vascular properties of Aspirin. Aspirin can inhibit cyclogenase enzyme, thus it prevents conversion of arachidonic acid into thromboxane A2, Finally vasoconstriction and platelets aggregation may be eschewed. This result agreed with (18) who showed that Aspirin may be inhibiting cyclooxygenase and then inhibit thromboxane A2.

2- 2nd group:

Our present findings of ovaries 2nd group rats showed somewhat similar results of histopathological lesions in the ovaries of 3rd group rats due to the long-term administration of Aspirin (for 30 days), these results agreed with (19), who showed the oral administration of drug acetyl salicylic acid (ASA, Aspirin) caused significant histopathological variation in female reproductive system in rat (ovaries and uteri).

B-Uteri:

1-3rd group:

Our present result of the histopathological lesions in the ovaries of 3rd group rats showed vacuolation of epithelial cells which lining the uterus, the reason of these results was the toxic effect of long-term Aspirin on these cells, Also our results showed few numbers of the uterine glands, so Aspirin can cause decrease in FSH and LH levels and also it cause prevent luteolysis and then increase progesterone and decrease estrogenic level constrains the development of uterine gland.

These findings were consistent with the results of previous studies (20), which showed that during the luteal phase, progesterone reduces the number of oestrogen receptors and overgrows the activity of 17-B hydroxyl steroid dehydrogenase enzyme which facilitate the conversion of estradiol to estrone and finally inhibit the biological effect of estradiol on the endometrium.

Our results in the uteri of 3rd group showed presence of inflammatory response characterized by profuse proliferation and irregular smooth muscles hyperplasia and infiltration of inflammatory cells. The reason of these results due to the degenerative changes of Aspirin in the endometrium, the degeneration and damage within the uterine wall will stimulates the phagocytic cells like neutrophils and macrophage for clearing the cellular debris and degenerated cells. This evidence can agreed with (19), who indicate that Aspirin can cause degenerative changes in endometrium.

Eventually, our results of this group (3rd group) elucidate that there is congestion of the blood vessels in the uterine wall, the reason of these results indicate the effect of long-term aspirin in which decrease the level of PGF2 α which responsible for vasodilation, also Aspirin can cause inhibition of cyclooxygenase enzyme and finally impairment of thromboxane A2 conversion then vasoconstriction and platelets aggregation will be avoided. These results agreed with (21, 22).

2-2nd group:

The histological examination of 2nd group uteri showed slighter changes due to the animals of this group received fewer dose of Aspirin (10 mg/kg B.W) for 30 days. These results indicate that there is hyperplasia of epithelial cells of uterus due to the degenerative effect of Aspirin in which the intact cells divided and proliferate to compensate the reduction in the cell number. Also there are other histopathological changes such few numbers of uterine glands but these changes with less severity than 3rd group due to the little dose of aspirin.

التأثيرات المرضية النسجية والهرمونية لجرع مختلفة من الأسبرين على الجهاز التناسلي الأنثوي في الجرذان البيضاء

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الخلاصة

إجريت الدراسة الحالية للتحري عن التأثيرات المرضية والنسجية والهرمونية الناتجة من استخدام جرعة مختلفة للأسبرين على الجهاز التناسلي الأنثوي في الجرذان البيضاء.

استخدم (21) من إناث الجرذان البيضاء الناضجة ، تراوحت أوزانها ما بين (150-170) غم، حيث قسمت

الحيوانات عشوائياً الى 3 مجاميع: المجموعة الاولى التي عُولت كمجموعة سيطرة سالبة (جرعة 0.2 مل من المحلول الدائري المتعادل طيلة فترة التجربة والبالغة 30 يوم)، مجموعة المعاملة الثانية حيث جرعت بالاسبرين (بتركيز 10 ملغم/كغم من وزن الجسم مرة واحدة يومياً لمدة 30 يوماً) ومجموعة المعاملة الثالثة إذ جرعت بالاسبرين (بتركيز 20 ملغم/كغم من وزن الجسم مرة واحدة يومياً لمدة 30 يوماً).

نتائجنا أظهرت ان المجموعة الثانية والثالثة أظهرت إنخفاضاً معنوياً ($P \leq 0.05$) في قيم الهرمون اللوتيني

البلازمي مقارنة مع مجموعة السيطرة. كذلك في قيم الهرمون المحرض للجريبات البلازمي للمجموعة الثانية والثالثة أظهرت انخفاضا معنوياً ($P \leq 0.05$) بالمقارنة مع مجموعة السيطرة. من جهة أخرى الفحص

المجهري للمقاطع المرضية النسجية للأرحام لجميع المجاميع المعالجة ومجموعة السيطرة ، أظهرت ان المجموعة الثانية أظهرت فرط تنسج بسيط مع تنكس في الخلايا الظهارية التي تبطن الرحم ، وهناك أعداد قليلة من الغدد الرحمية ، وتفجج في الخلايا الظهارية التي تبطن الرحم. في المجموعة الثالثة ، الفحص النسيجي المرضي للأرحام أظهر تكاثر غزير وفرط تنسج غير منتظم للعضلات الملساء. هناك ارتشاح للخلايا الالتهابية وهناك أيضاً احتقان للأوعية الدموية. كذلك تفجج ملحوظ للخلايا الظهارية الرحمية. الغدد الرحمية أصغر وأقل من المجموعة الثانية والمجموعة الاولى (السيطرة). أما في مبايض المجموعة الثانية أظهرت موجة نمو جريبية

أقل بصورة ملحوظة تتميز بوجود جريبات ثانوية وأبتدائية وهناك احتقان وخثر في سدى المبيض. كذلك وجود جسم أصفر كبير. بينما التغيرات المرضية النسجية في مبايض حيوانات المجموعة الثالثة ظهرت بأكثر شدة من حيوانات المجموعة الثانية التي فيها جريبات ثانوية وأبتدائية مع احتقان شديد ونزف ، وكذلك وجود أعداد كبيرة من الأجسام الصفراء. حيوانات المجموعة الأولى (السيطرة) أظهرت صورة نسجية طبيعية في الأرحام والمبايض. نستخلص من الدراسة الحالية ان الجرعة المختلفة للأسبرين يمكن أن تسبب تغيرات مرضية نسجية واضطرابات هرمونية في الهرمون اللوتيني والهرمون المحرض للجريبات.

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