Original Article

Effect of Estrogen and Progesterone on Irritable Bowel Syndrome in Ovariectomized Albino Rats: A Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Irritable bowel syndrome (IBS) is a functional disorder characterized by dysregulated immune and inflammatory pathways. Its symptoms are most prominent in postmenopausal women suggesting that female sex hormones withdrawal may contribute to it.

Objective: To evaluate the effect of estrogen and progesterone on the ileum of experimentally induced IBS in ovariectomized rats using histological and immunohistochemical methods.

Materials and Methods: Sixty adult female albino rats were divided equally into 6 groups: Group I: control group, Group II: IBS group, Group III: ovariectomized (OVX) IBS group, Group IV: OVX IBS Estrogen group, Group V: OVX IBS Progesterone group, Group VI: OVX IBS Estrogen & Progesterone group. Bilateral ovariectomy was performed in groups III, IV, V & VI to induce experimental menopause. In control and IBS groups, rats underwent the same surgical procedure but without removal of the ovaries. Two weeks after surgery, all rats except control were submitted to water avoidance stress (WAS) exercise for 10 days to induce IBS. The same procedure was done for control rats in an empty water tank. Simultaneously, groups IV, V & VI were given daily subcutaneous injections of estrogen or progesterone or both respectively for continuous 14 days. Then, rats were sacrificed and paraffin ileum sections were prepared and stained with H&E, Alcian blue and Toluidine blue stains and immunohistochemical staining against claudin-1. This was followed by morphometric and statistical analysis.

Results: Groups II & III showed extensive damage in ileum structure associated with depletion of goblet and Paneth cells and a significant increase in mast cells and claudin-1 level. On the other hand, groups IV, V and VI revealed apparently normal ileum structure and increased goblet and Paneth cells with a significant decrease in mast cells and claudin-1 level.

Conclusion: Estrogen and Progesterone had regenerative effects on histological structure of the ileum against experimentally induced IBS in ovariectomized rats.

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Key Words: Claudin 1, estrogen, IBS, ovariectomy, progesterone.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder with a world-wide prevalence of 10 – 25% and a 1% greater risk of colorectal cancer than the general population. It is associated with structural abnormalities and characterized by abdominal pain or discomfort with defecation and/or a change in bowel habits[1,2].

The underlying pathogenesis of IBS is multifactorial. However, dysregulation in immune and inflammatory pathways is considered an important pathophysiological mechanism contributing to IBS. Immune cell populations e.g. mast cells and T-lymphocytes act as major presented cells in IBS[3,4].

The prevalence of IBS in women is 67% higher than in men with the most prominent symptoms in postmenopausal women suggesting that female sex hormones withdrawal may contribute to IBS[5,6].

Female sex hormones affect pro- and anti-inflammatory pathways and influence peripheral and central regulatory mechanisms of the brain-gut axis[7,8]. However, the exact effect of hormonal replacement therapy (HRT) on IBS symptoms during menopause is still unclear and showed conflict results[9,10]. It was found that HRT reduces abdominal symptoms of IBS in postmenopausal women[11] while, in another study[12] HRT was found to increase the prevalence and worse the condition of IBS in postmenopausal women.

So, this study was designed to evaluate the effect of estrogen and progesterone on the ileum of experimentally induced IBS in ovariectomized rats using histological and immunohistochemical methods.

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MATERIALS AND METHODS

Animals

Sixty adult female albino rats (200-240g) were purchased from the Animal House of Faculty of Medicine, Zagazig University. They were housed in steel wire cages (50cm X 60cm X 60cm) (5 rats per cage) at room temperature, light cycle (12h dark and 12h light) and hygienic conditions. Free access to water and standard rat chow were provided to all rats. Animals were left for one week prior to the beginning of the experiments to adapt laboratory conditions. The experimental protocol was approved by physiology department and by local medical ethics committee in Faculty of Medicine of Zagazig University (The Institutional Animal Care and Use Committee, Zagazig University, ZU-IACUC) with approval number: ZU-IACUC/3/F/166/2019. Experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

Experimental Design

Rats were randomly divided into six equal groups (n = 10 rats for each):

Group I (control): rats underwent sham operation (the same surgical procedure as group III without removal of ovaries). After 2 weeks, they underwent water avoidance stress technique in an empty water tank and received daily subcutaneous injections of 0.1 ml sesame oil for 14 days.

Group II (IBS): rats underwent sham operation as group I. After 2 weeks, they underwent water avoidance stress technique and received daily subcutaneous injections of 0.1 ml sesame oil for 14 days.

Group III, ovariectomized (OVX) IBS group (OVX/IBS): rats underwent bilateral ovariectomy. After 2 weeks, they were subjected to water avoidance stress technique and were given daily subcutaneous injections of 0.1 ml sesame oil for 14 days.

Group IV (OVX/IBS/Estrogen group) (OVX/IBS+E): rats were subjected to bilateral ovariectomy. After 2 weeks, they underwent water avoidance stress technique and were given daily subcutaneous injections of 17β-estradiol dissolved in 0.1 ml sesame oil for 14 days.

Group V (OVX/IBS/Progesterone group) (OVX/IBS+P): rats underwent bilateral ovariectomy. After 2 weeks, they were subjected to water avoidance stress technique and received daily subcutaneous injections of progesterone dissolved in 0.1 ml sesame oil for 14 days.

Group VI (OVX/IBS/Combined Estrogen and Progesterone group) (OVX/IBS+E&P): rats underwent bilateral ovariectomy. After 2 weeks, they were subjected to water avoidance stress technique and were given daily subcutaneous injections of 17β-estradiol and progesterone dissolved in 0.1 ml sesame oil for 14 days.

Experimental Procedures

Bilateral ovariectomy was performed in rats to induce experimental menopause: ketamine was used for anesthesia (100 mg/kg, intraperitoneal injection) (Sigma Chemicals)[13]. Then, a transversal dorsolateral incision of skin was made between the last rib and pelvis, muscles were dissected to expose the abdominal cavity. The periovarian fat was grasped to lift and exteriorize the ovary and the ovaries were removed by cutting above the clamped area. The muscle and the skin incision were closed with nylon suture, covered by garamycin cream and sterilized gauze[14]. In control and IBS groups (groups I and II), rats underwent the same procedure but, the ovaries were exposed and then put back in the abdominal cavity.

Induction of IBS by water avoidance stress (WAS) technique: after 2 weeks of ovariectomy, each rat was placed on the top of a cube (6 x 6 x 9.5 cm3) which was placed in the middle of the water tank (25 x50 x 25 cm3) for 1 hour daily for 10 consecutive days. The water was filled up to 2 cm away from the top (at room temperature)[15]. The same procedure was done for control rats without adding water.

Hormonal replacement method: after 2 weeks of ovariectomy, rats of group IV and V received daily subcutaneous injections of 17β-estradiol (10µg /kg/ day) and progesterone (10mg /kg/day) (Misr CO. pharm. Ind. S.A.A. Materia. Cairo-A.R.E.), respectively, dissolved in 0.1 ml sesame oil as a vehicle (ADWIC Laboratory Chemicals, Egypt) for 14 days[16,17]. Rats of group VI received both 17 β-estradiol and progesterone in the same way as groups IV and V. Control, IBS and OVX /IBS groups received daily subcutaneous injections of 0.1 ml sesame oil for 14 days.

Light microscopic techniques

Animals from all groups were sacrificed at the end of the experiment using chloroform inhalation[18]. Specimens from the distal ileum were fixed in 10% buffered formalin. Paraffin-embedded serial sections were cut at 5-7 µm thickness and were subjected to:

Histological staining

- Hematoxylin and Eosin stain[19].
- Alcian blue stain for demonstration of mucus in goblet cells[20].
- Toluidine blue stain for demonstration of mast cells[20].

Immunohistochemical staining

Immunohistochemical stain[19] for Claudin 1 protein (a tight junction protein). It is a concentrated rabbit polyclonal antibody (Abcam Laboratories, catalogue number ab15098). Claudin 1 immunopositive reaction appears as brown membranous and cytoplasmic deposits. A photomicrograph of positive control from skin immunostained with Claudin 1 was obtained from Abcam Laboratories.

Steps of immunohistochemical staining

Immunostaining required pre-treatment by boiling for 10 minutes in 10Mm citrate buffer pH 6 (cat no AP 9003) for antigen retrieval and leaving the sections to cool at room temperature. Then, the sections were incubated for one hour with the diluted primary antibodies 1:500 (this step was omitted in negative control sections). Immunostaining was completed by the use of Ultravision detection system (cat no TP - 015- HD). Slides were then counterstained using Mayer's haematoxylin (cat no TA-060- MH). Citrate buffer, Ultravision detection system and Mayer's haematoxylin were purchased from Lab Vision Corporation Laboratories USA.

Morphometric Study

Image analysis was done using Leica Qwin 500 LTD software image analysis computer system (Cambridge, England) in Histology Department, Faculty of Medicine, Cairo University. All measurements were done with an objective lens x10 magnification in 10 non overlapping randomly chosen fields for each animal. The following parameters were measured:

a. The area % of alcian blue positive staining.

b. The number of mast cells stained with toluidine blue.

c. The area % of claudin 1 immune expression.

Statistical Analysis

Morphometric results were recorded and statistically analyzed. Data were expressed as mean and standard deviation (SD) for the quantitative variable. Data were statistically analyzed using statistical package SPSS version 16 (SPSS Inc., Chicago, USA). Comparisons between groups were done using ANOVA (analysis of variance) followed by a post hoc test for multiple comparisons between each 2 groups. The results were considered significant when $p<0.05$[21].

RESULTS

Histological Results

Hematoxylin and eosin stained sections

Group I showed normal architecture of the ileum. Villi were intact covered by continuous surface epithelium (Figure 1a) and crypts containing Paneth cells identified by their dense acidophilic granules (Figure 4a). Group II showed disturbed architecture with moderate inflammatory infiltrate and dilated congested blood vessels. The villi were interrupted and lost their surface epithelium (Figure 2a). Crypts contained few Paneth cells with sparse acidophilic granules (Figure 4b). Group III showed the same findings as group II with marked inflammatory infiltrate, completely destroyed some crypts (Figure 2b) and few Paneth cells (Figure 4c). Groups IV & V showed apparently nearly normal architecture with moderate inflammatory infiltrate, congested capillaries (Figures 3 a,b) and numerous Paneth cells (Figures 4 d,e). Group VI showed apparently normal architecture with minimal inflammatory infiltration (Figure 1b) and numerous Paneth cells (Figure 4f).

Alcian blue stained sections

Group I & VI showed marked spread of alcian blue reaction in goblet cells (Figures 5 a,f) while group II & III displayed mild spread (Figures 5 b,c) and Group IV & V exhibited moderate spread (Figures 5 d,e).

Toluidine blue stained sections

Group I & VI showed no mast cells in all layers (Figures 6 a,b) while group II & III displayed multiple mast cells (Figures 7,8). Group IV & V exhibited few mast cells (Figures 9,10). Mast cells had coarse granules and most of them were degranulated.

Immunohistochemical Results

Anti-claudin 1 immunohistochemical-stained sections

Group I & VI showed mild membranous & cytoplasmic immunoreactivity in epithelial cells of the mucosal villi and crypts (Figures 11 a,b). Group II & III displayed marked immunoreactivity (Figures 12 a,b) while, Group IV & V exhibited moderate immunoreactivity (Figures 13 a,b) in epithelial cells of the mucosal villi and crypts.

Morphometric Results (Figure 14)

The area % of alcian blue positive staining

Mean values of alcian blue area % showed a significant decrease in both groups II & III as compared to control. Moreover, group III showed a significant decrease when compared to group II. Mean values of groups IV, V & VI showed a significant increase as compared to groups II & III. However, groups IV & V showed a significant decrease when compared to the control group. Meanwhile group VI showed a nonsignificant difference when compared to the control group.

The number of mast cells stained with toluidine blue

Mast cells were not detected in groups I & VI. They were detected in groups II & III and the mean values of their numbers showed a significant decrease in groups IV & V when compared to groups II & III.

The area % of Claudin 1 immune expression

Mean values of Claudin 1 area % showed a significant increase in both groups II & III as compared to control. Moreover, group III showed a significant increase when compared to group II. Mean values of groups IV, V & VI showed a significant decrease as compared to groups II and III, however these groups showed a significant increase when compared to the control group.

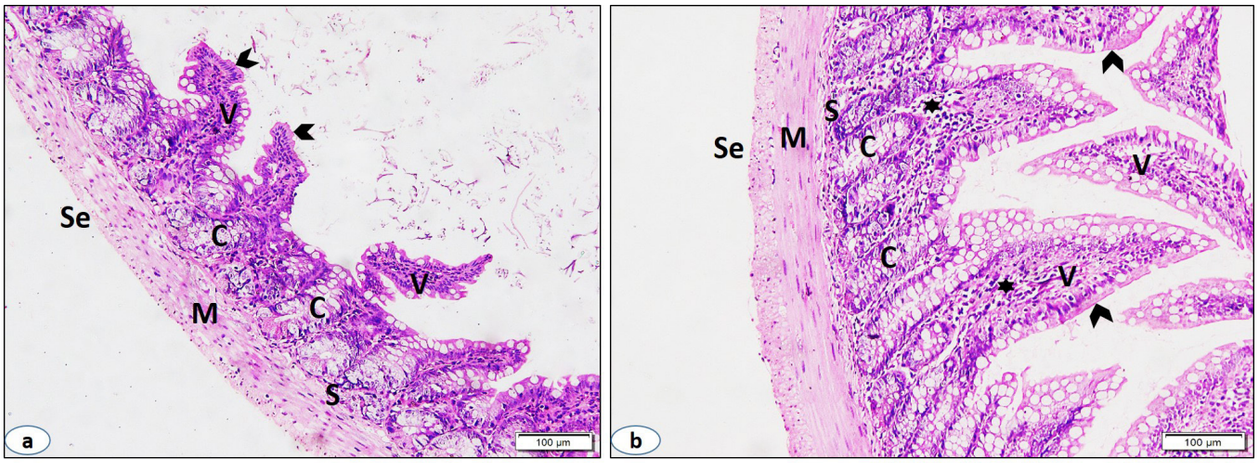

Fig. 1: Photomicrograph of a section in the ileum from: **(a)** Group I showing normal architecture with intact mucosa formed of intact villi (V) covered by continuous surface epithelium (arrowheads) and crypts (C). The Submucosa (S), musculosa (M) and serosa (Se) show normal structure. **(b)** Group VI showing apparently normal architecture with mild inflammatory infiltrate (asterisks). (H&E, x100)

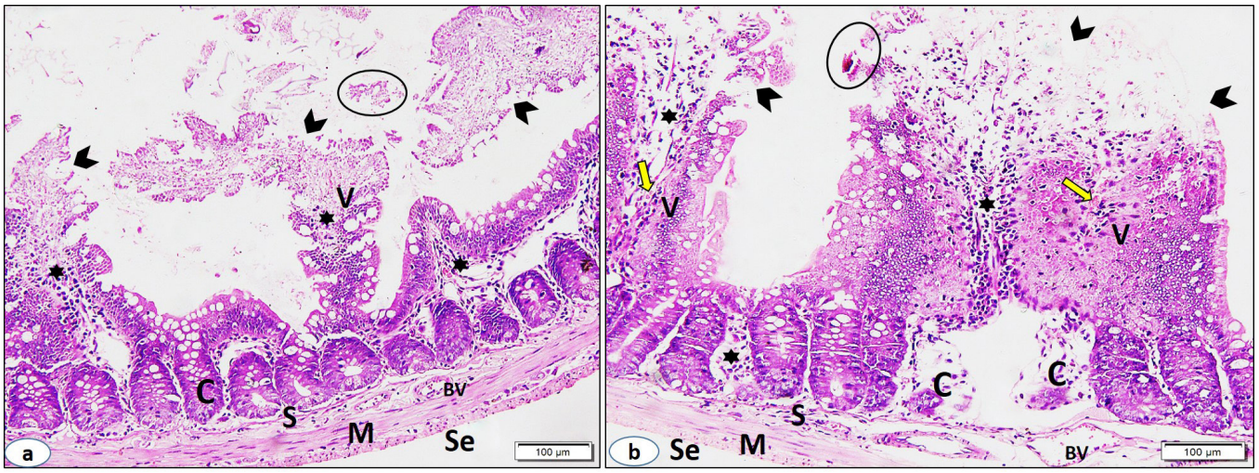

Fig. 2: Photomicrograph of a section in the ileum from: (a) Group II and (b) Group III showing disturbed architecture with dilated congested blood vessels (BV) and capillaries (yellow arrows). Inflammatory infiltrate (asterisks) is moderate in group II and marked in group III. The villi (V) are interrupted and their surface epithelium (arrowheads) is detached into the lumen (oval). Some crypts (C) are completely destroyed in group III. The Submucosa (S), musculosa (M) and serosa (Se) show apparently normal structure. (H&E, x100)

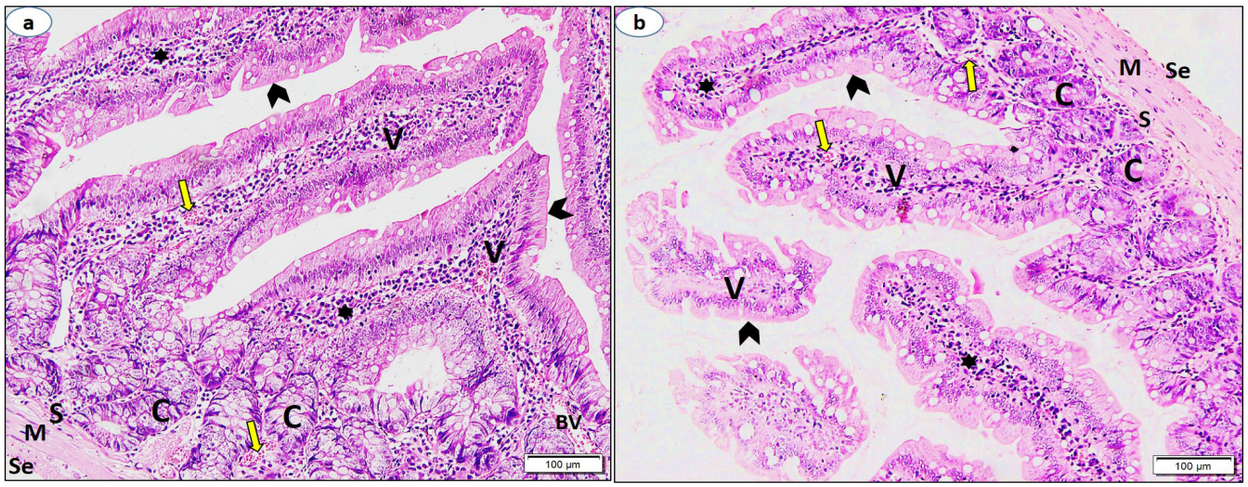

Fig. 3: Photomicrograph of a section in the ileum from: (a) Group IV and (b) Group V showing apparently nearly normal architecture with moderate inflammatory infiltrate (asterisks) and congested capillaries (yellow arrows). (S): Submucosa, (M): musculosa, (Se): serosa, (V): villi, (arrowheads): surface epithelium, (C): Crypts, (BV): blood vessels. (H&E, x100)

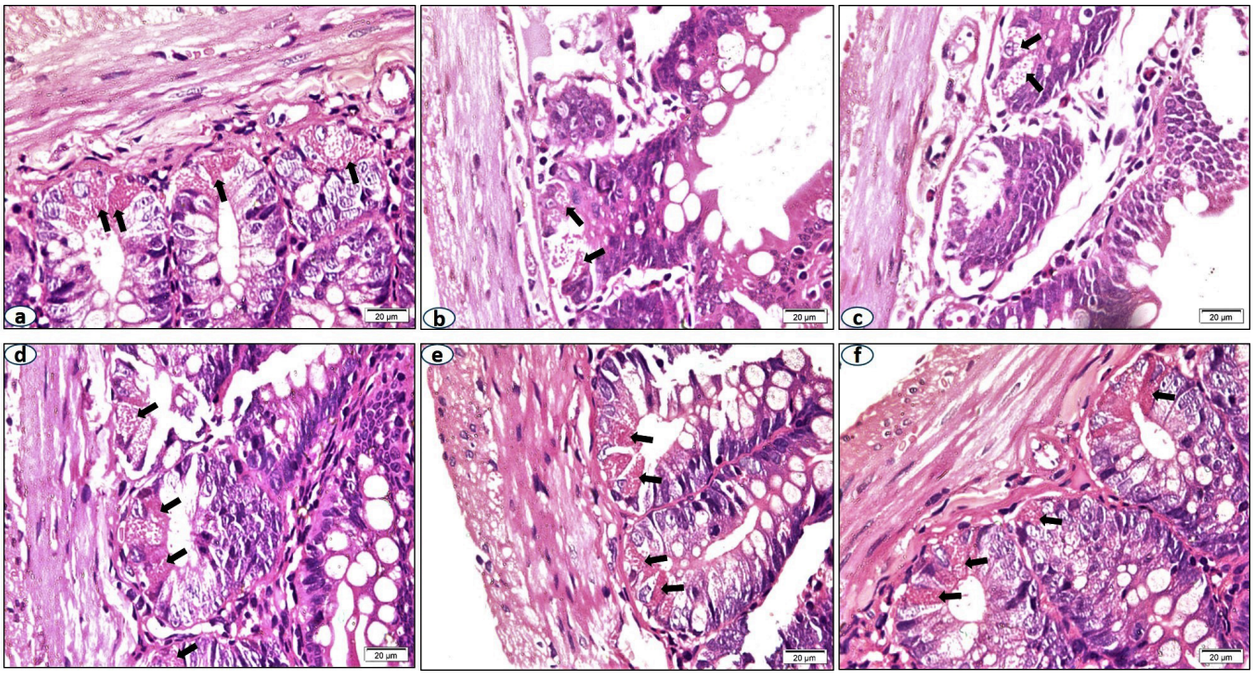

Fig. 4: Photomicrograph of a section in the ileum from: (a) Group I, (d) Group IV, (e) Group V and (f) Group VI showing normal Paneth cells (arrows) with dense acidophilic granules. (b) Group II and (c) Group III showing few Paneth cells (arrows) with sparse acidophilic granules. (H&E, x200)

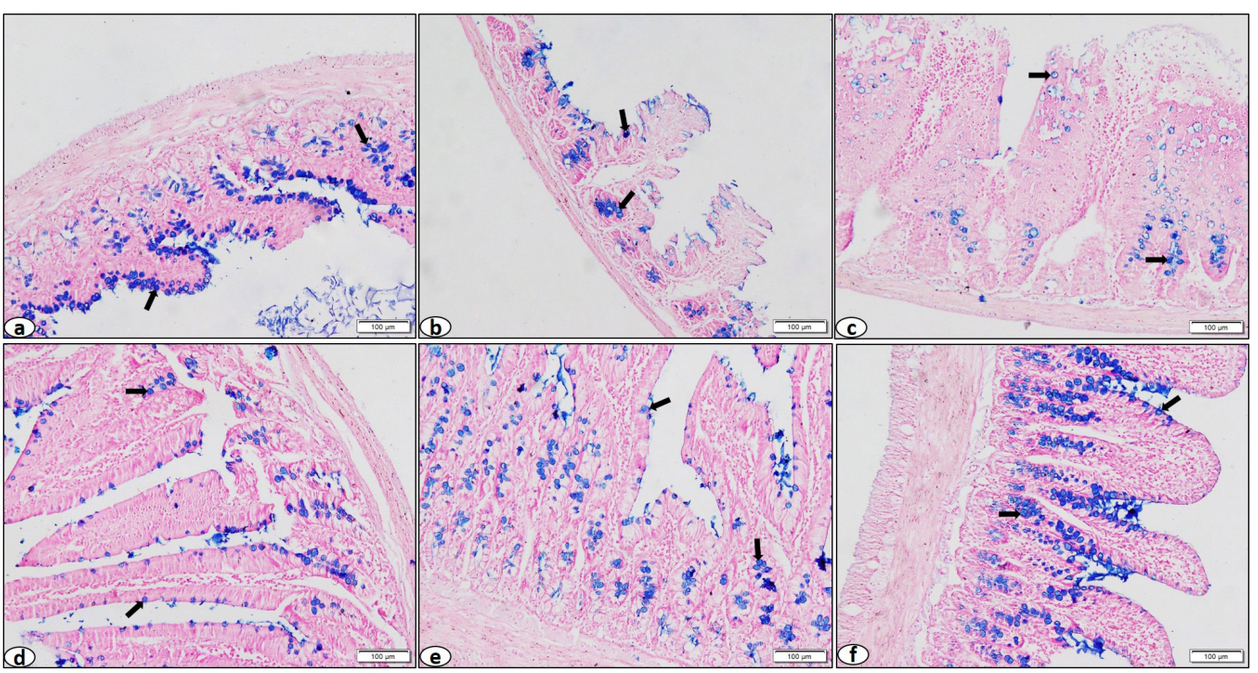

Fig. 5: Photomicrograph of a section in the ileum from: (a) Group I and (f) Group VI showing a marked spread of alcian blue reaction in goblet cells (arrows). (b) Group II and (c) Group III displaying a mild spread of alcian blue reaction in goblet cells. (d) Group IV and (e) Group V exhibiting a moderate spread of alcian blue reaction in goblet cells. (Alcian blue, x100)

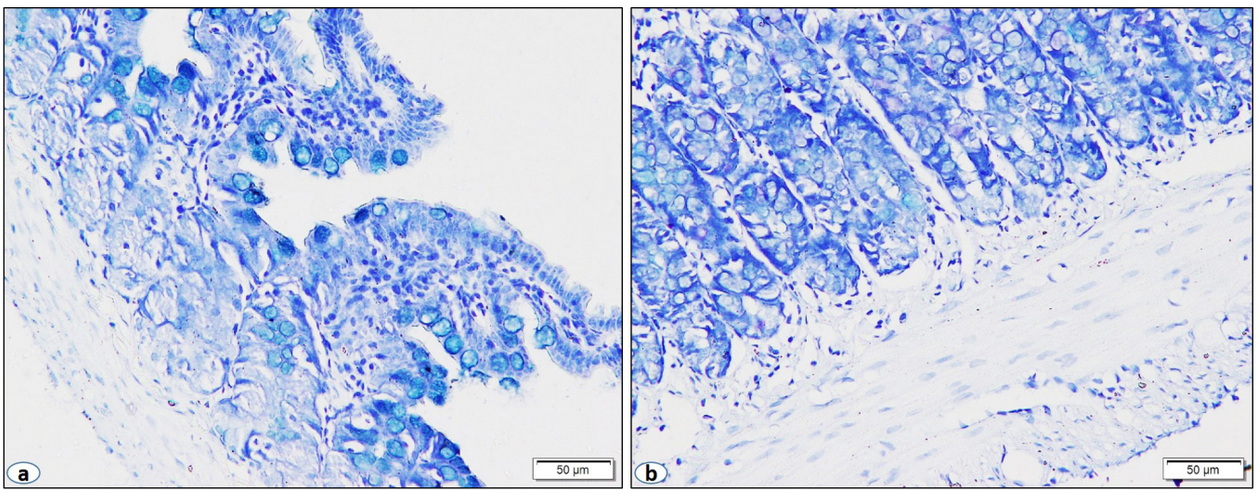

Fig. 6: Photomicrograph of a section in the ileum from: (a) Group I and (b) Group VI showing no mast cells in all layers. (Toluidine Blue a&b x200)

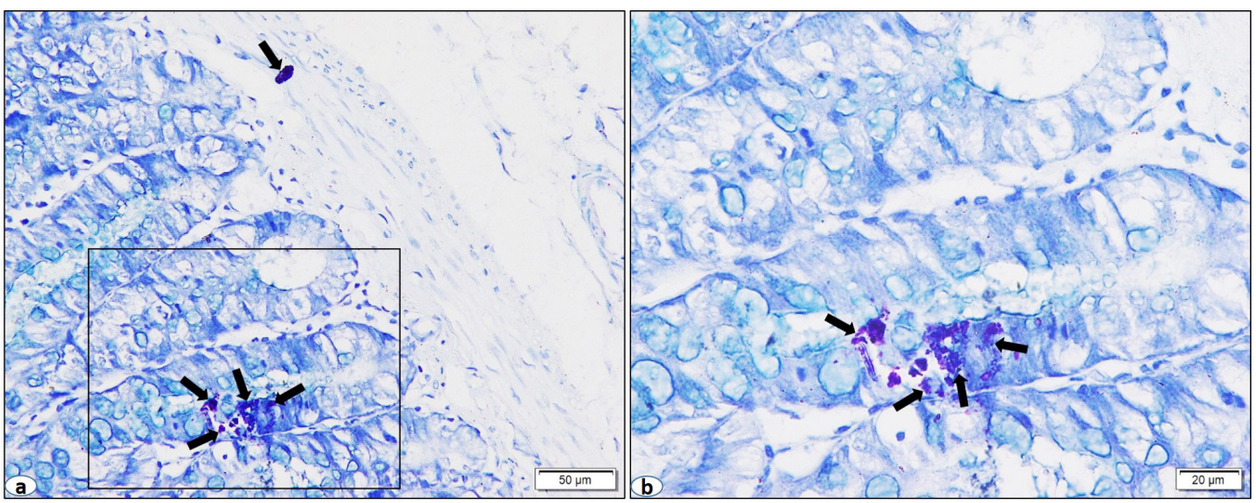

Fig. 7: Photomicrograph of a section in the ileum from Group II: (a) showing multiple mast cells with their coarse granule (arrows) in the mucosa, submucosa. (b) Higher magnification of the rectangle in (a) showing degranulation of mast cells (arrows). (Toluidine Blue, a x200; b x400)

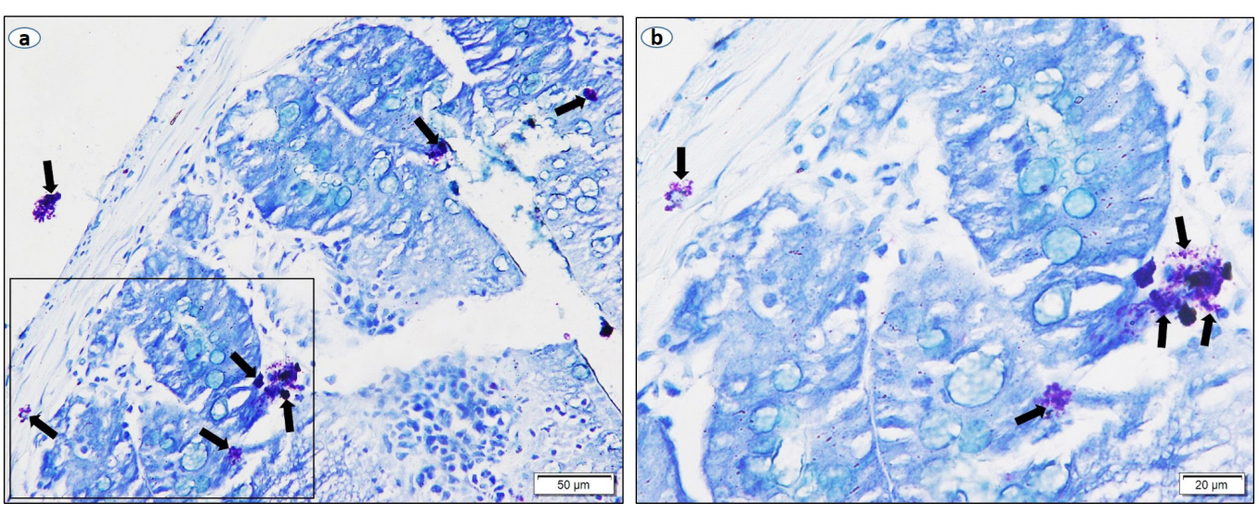

Fig. 8: Photomicrograph of a section in the ileum from Group III: (a) showing multiple mast cells with their coarse granule (arrows) in the mucosa, submucosa and serosa. (b) Higher magnification of the rectangle in (a) showing degranulation of mast cells (arrows). (Toluidine Blue, a x200; b x400)

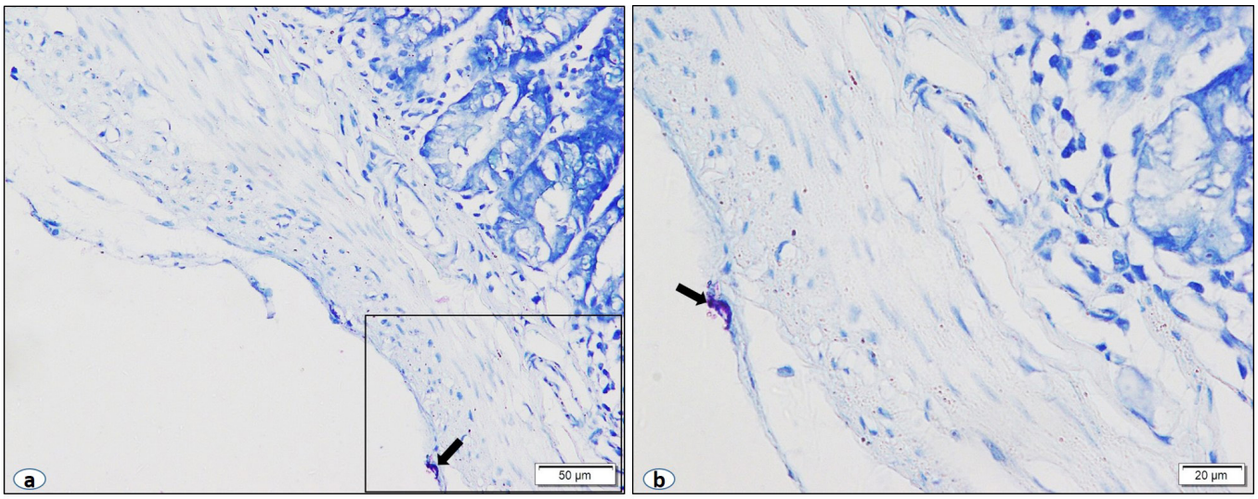

Fig. 9: Photomicrograph of a section in the ileum from Group IV: (a) showing one mast cell in serosa (arrow). (b) Higher magnification of the rectangle in (a) showing coarse granules of mast cells (arrow). (Toluidine Blue, a x200; b x400)

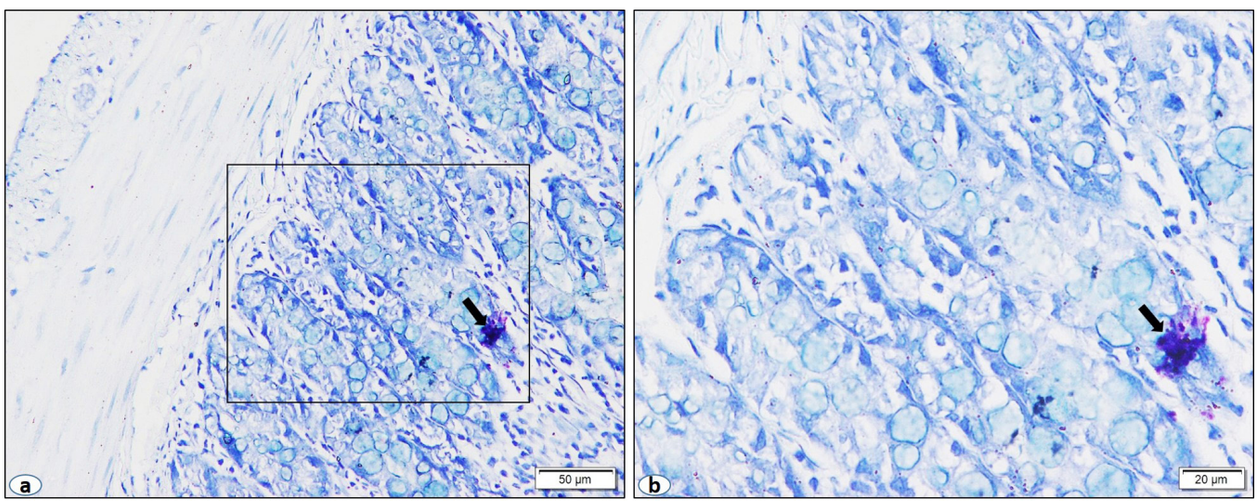

Fig. 10: Photomicrograph of a section in the ileum from Group V: (a) showing one mast cell in the mucosa (arrow). (b) Higher magnification of the rectangle in (a) to show coarse granules of mast cells (arrow). (Toluidine Blue, a x200; b x400)

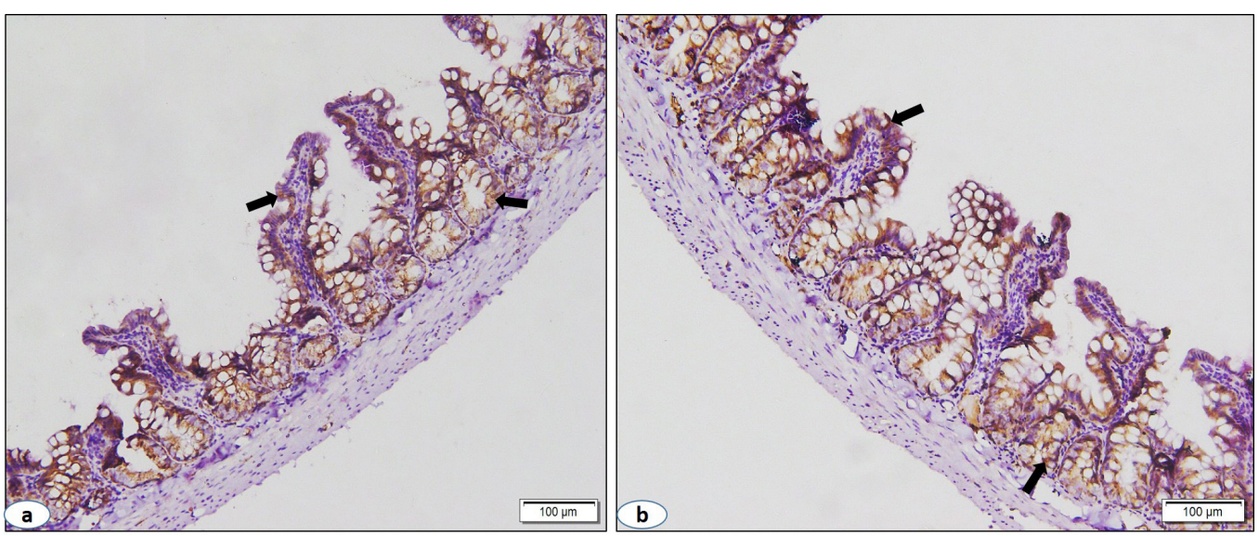

Fig. 11: Photomicrograph of a section in the ileum from: (a) Group I and (b) Group VI showing mild membranous and cytoplasmic immunoreactivity (arrows) in epithelial cells covering the mucosal villi & lining the crypts. (Claudin 1, x100)

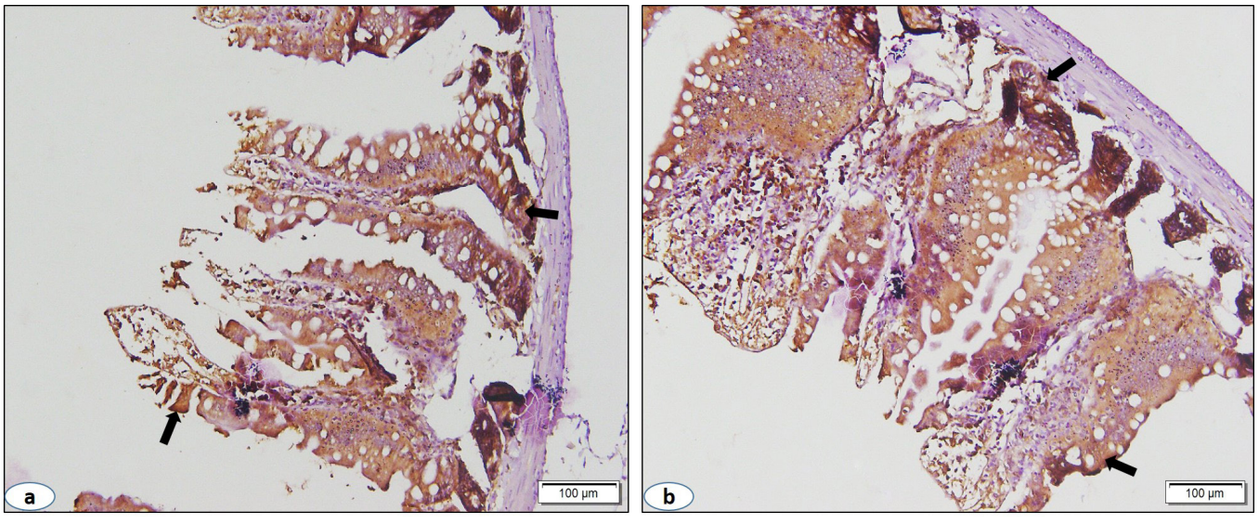

Fig. 12: Photomicrograph of a section in the ileum from: (a) Group II and (b) Group III displaying marked membranous and cytoplasmic immunoreactivity (arrows) detected in epithelial cells covering the mucosal villi & lining the crypts. (Claudin 1, x100)

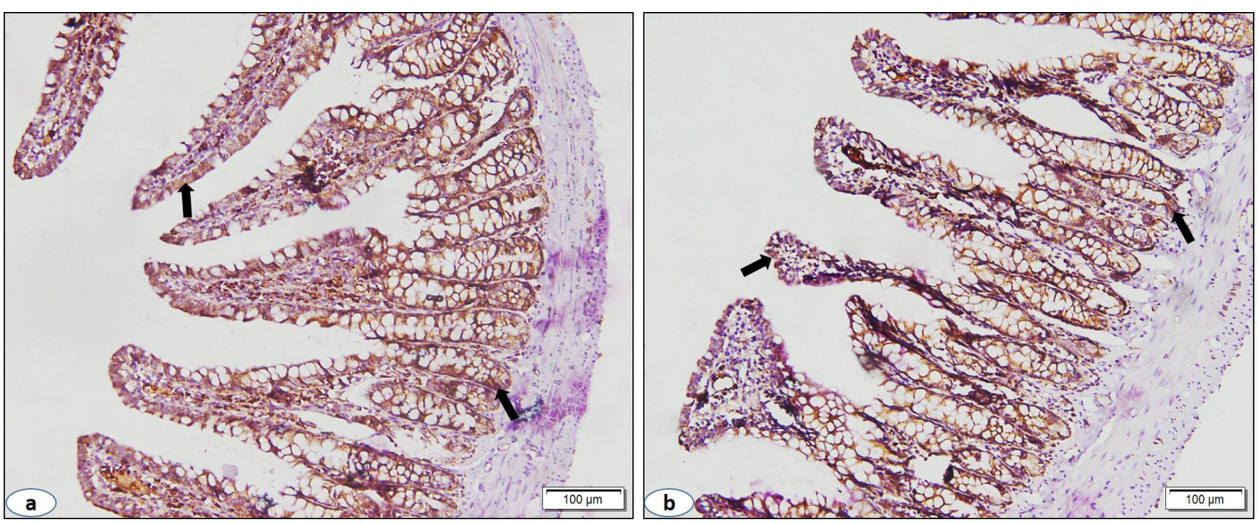

Fig. 13: Photomicrograph of a section in the ileum from: (a) Group IV and (b) Group V exhibiting moderate membranous and cytoplasmic immunoreactivity (arrows) detected in epithelial cells covering the mucosal villi & lining the crypts. (Claudin 1, x100)

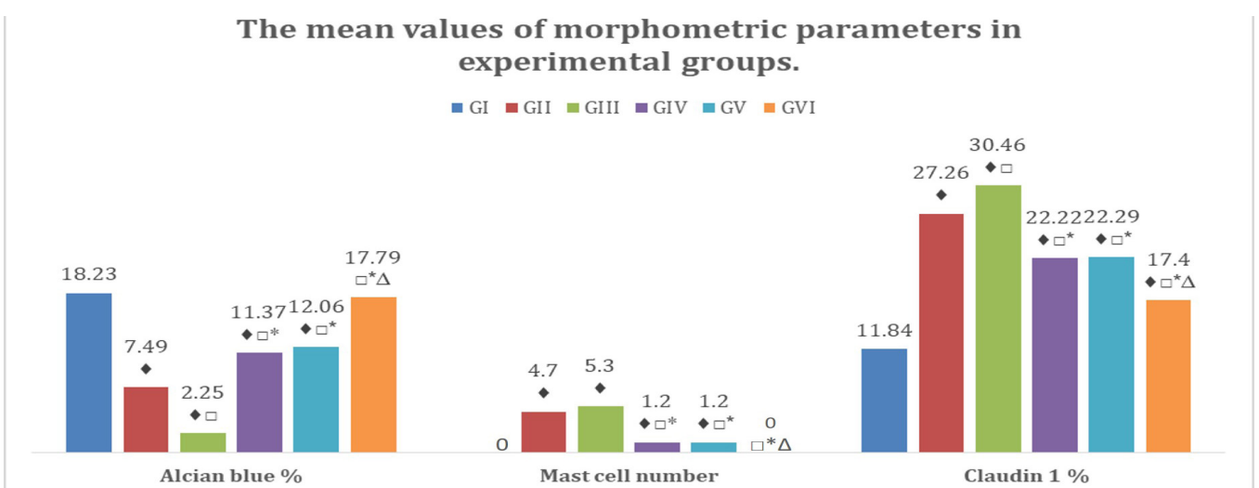

Fig. 14: The mean values of morphometric parameters in experimental groups. (♦ Significant when compared with GI, □ Significant when compared with GII, * Significant when compared with GIII, Δ Significant when compared with GIV and GV)

DISCUSSION

Irritable bowel syndrome is a chronic bowel disorder characterized by gastrointestinal tract (GIT) and psychological symptoms (stress, anxiety, and depression) that lead to impaired quality of life[22,23]. Low ovarian hormone levels correlate to different GIT symptoms in patients with IBS indicating that ovarian hormone status could modify the IBS symptoms[24].

The present work aimed to examine the effect of estrogen and progesterone on the ileum of experimentally induced IBS in ovariectomized rats using histological methods. Female Rats were used in this study ,as rats showed the same cyclic hormonal changes as human[25] and the prevalence of IBS in females is 67% higher than in males with the most prominent symptoms in post menopause[5,6].

In this study, the ileum was chosen as it is one of the most common areas of GIT affected in IBS[26]. In addition, ovariectomy was chosen as a method to attenuate the ovarian hormonal level as it induces similar changes to the postmenopausal state in humans[27]. Furthermore, water avoidance stress was used as a method of chronic stress as it is well known to induce IBS through inflammatory, permeability and structural changes[1].

In the present work, the effect of HRT was evaluated after 2 weeks according to a previous study[16] in which authors reported that HRT can reverse the ovariectomy response after 14 days.

Hematoxylin and eosin staining of ileum in IBS group II showed disturbed structure with dilated congested blood vessels (BV), inflammatory infiltration and few Paneth cells in intestinal crypts. These results could be attributed to the damaging effect of chronic stress. Such a result was in agreement to a previous study[28] in which the role of stress on mucosal barrier and inflammatory products was studied. The authors concluded that chronic stress enhanced ileum mucosal permeability leading to increased passage of antigens and bacteria that produced mucosal injury and intraepithelial lymphocytes infiltration. Bacterial infection in IBS is associated with increased proinflammatory cytokines and CD4+ T helper cell in the gastrointestinal mucosa which drives gut's cellular inflammation and adaptive immune response[2]. In addition, inflammatory mediators such as corticotrophin-releasing factors secreted by enterochromaffin cells together with mediators of degranulated mast cells enhance ion and water secretion and epithelial permeability[28] leading to more passage of bacteria.

Paneth cells play important roles in intestinal cells immunity and stem cell proliferation. They enhance mucosal repair and improve intestinal permeability by restoring intestinal epithelial cells proliferation. Failure of intestinal barrier function and bacterial translocation may be due to lack of antimicrobial peptides from Paneth cells. Loss of Paneth cells leads to visceral hypersensitivity, which is an important sign of IBS[29].

The previous histological changes were more evident in OVX /IBS group III that showed marked mucosal damage and inflammatory reaction suggesting the role of deficient female sex hormones in ileum injury in IBS. These results agreed with a previous study[30] in which researchers found that reduced levels of E2 in old age, particularly during female menopause can potentially increase epithelial permeability and microbial translocation.

Receiving estrogen and progesterone in groups IV and V respectively regained nearly normal ileum architecture with moderate inflammation and many Paneth cells, while the best findings were found in group VI that received both estrogen and progesterone. It showed normal mucosal histological configuration with minimal inflammation and many Paneth cells. These findings were in agreement of a previous study[31] in which the authors reached a conclusion that both estrogen and progesterone markedly improved intestinal mucosal inflammation by attenuating cytokines IL8, IL6 and TNFα released by endoplasmic reticulum in stressful states. In addition, authors in another study[30] provided an evidence that increased E2 in rats induced the expression of transjunctional protein occludin via binding of estrogen receptor β leading to decreased intestinal epithelial permeability and microbial translocation. Furthermore, these findings could be explained by increased Paneth cells in this study which could improve intestinal barrier function and decrease bacterial translocation as mentioned before.

On the other hand, there was a conflict on the effect of female hormones on gut structure and function. It was found that different types of contraceptive pills (OC) e.g. drospirenone and levonorgestrel had a high risk to develop IBS symptoms. In addition, receiving 17 β estradiol and progesterone in ovariectomized rats, had led to a significant decrease of spontaneous colonic contractile activity (CCA). However, dehydroepiandrosterone sulfate, which is a precursor of estrogens and androgens, was found to decrease visceral hypersensitivity and colonic permeability in rat models of IBS induced by lipopolysaccharide and repeated water avoidance stress[32].

In this study, alcian blue staining of ileum in both IBS and OVX/IBS groups II, III showed mild staining of goblet cells. This was confirmed by measuring the area % of alcian blue positive staining that showed a significant decrease of groups II, III as compared to the control group. The same result was shown by a previous study[33] in which researchers mentioned that goblet cells and mucin secretion were depleted in the intestine of IBS rats. Mucin 2, secreted by goblet cells, along with small amounts of mucin-related proteins and water, polymerizes into a gel that provides the mucosal surfaces with an insoluble thick mucus barrier. This barrier serves to protect the intestinal epithelium and limit interactions with luminal microbes. Decreased goblet cells and disturbance of mucin secretion, lead to infiltration of the epithelium by luminal flora[34].

Groups IV, V and VI receiving E, P and both respectively exhibited a significant increase of mucus and goblet cells compared to group II, III. These findings agreed with results from a previous study[35] in which researchers showed that female hormones regulate the expression of mucin genes MUC2 and MUC4 within the goblet cells in both the small and large intestine. Researchers in another study[36] found that the small intestinal mucosal barrier was damaged greatly in ovariectomized rats, and intake of progesterone improved the small intestinal mucosal barrier function and restored goblet cell number in the rats with bilateral ovariectomy.

Examination of mast cell using toluidine blue stain and measuring its number, revealed a significant increase in number and degranulation in the IBS and OVX/IBS groups as compared to the control. However, groups IV, V and VI receiving E, P and both respectively revealed a significant decrease compared to groups II and III. Moreover, mast cells were not detected in group VI similarly as control group.

Mast cells have been reported to participate in the development of visceral hypersensitivity by releasing inflammatory mediators such as histamine and protease. Moreover, they were located close to the nerve terminal innervating intestine where they release these mediators causing irritation of pain nerve terminals. This mechanism was supported by the fact that anti-histamine and protease inhibitors were found to attenuate hypersensitivity. Furthermore, histamine, chymase, and prostaglandin D2 secretion by disturbed mast cells was associated with marked disturbance in intestinal motility, permeability and secretion through stimulating water and chloride secretion in the intestinal epithelium[37].

A marked conflict was noticed on the effect of female hormones on the mast cells production and their activity as authors in a previous study[38] stated that decreased estrogen level in ovariectomized rats produced marked attenuation of mast cells and their histamine expression induced by stress. While, in another study[30] researchers showed that 17β estradiol acted on E2 receptors attenuated upper GIT tissue damage in ovariectomized rats via a reduction of mast cell-mediated cytotoxicity and cytokine.

Progesterone was found to regulate mast cells production and reduce their degranulation through inhibiting mast cells activator substance p and Ig E. This was further supported by electron microscopic study that exhibited almost all the secretory granules were intact in mast cells obtained from female rat peritoneum treated with progesterone[39].

In this study, mild membranous and cytoplasmic claudin-1 immunoreactivity was detected in epithelial cells of the mucosal villi and crypts. The immunoreactivity of both IBS group II and OVX /IBS group III revealed a significant increase as compared to the control group, while there was a significant decrease in hormonal treated groups IV, V and VI when compared to groups II and III. Claudin-1 protein is one of the tight junction proteins that represent the most important component of the constitutive intestinal barrier present in epithelial cells. The intestinal barrier separates the gut lumen from the inner host and is composed of mucus, epithelial layer, humoral elements, immunological and neurological elements. Epithelial proteins which include occludin and claudins regulate the permeability of the barrier by linking adjacent cells to the actin cytoskeleton[40].

Claudin-1 protein is thought to play a role in maintaining the gut barrier and decreasing intestinal permeability. There is a large conflict regarding the relation between the level of claudin-1 and IBS inflammatory and gut permeability. Claudin-1 was found to be decreased in IBS associated with diarrhea while it increased in IBS associated with constipation and mixing type IBS[41,42]. However, claudin-1 level was found to be increased in some inflammatory condition like inflammatory bowel disease (IBD) as researchers in a previous study[43] demonstrated that claudin-1-overexpression in mice increased susceptibility to experimental colitis, impaired goblet cell differentiation, delayed epithelial recovery, epithelial hyperplasia and sustained inflammation. Researchers in another study[44] displayed that Claudin-1 was localized to not only in the region of the tight junction but also along the lateral membrane and within cytoplasmic granules of colonocytes in control tissue. Moreover, they mentioned that claudin-1 expression showed progressive increase, with more expression correlated with inflammatory activity grade in IBD.

Upregulated claudin-1 activated Notch-signaling, which inhibited the goblet cell differentiation leading to decreased mucin-2 which enhances mucosal inflammation[45]. Moreover, increased proinflammatory cytokines and T-helper cells led to Claudin-1 dysregulation, impaired barrier function, bacterial leakage and more increased inflammation[43].

Estrogen deficiency in postmenopausal women had led to chronic unregulated inflammation[46]. This may explain the increase in claudin-1 following ovariectomy in group III. In the same study, it was found that estrogen treatment had a significant anti-inflammatory activity that decreased degenerative diseases associated with menopause. This anti-inflammatory activity could explain the decreased claudin-1 level in group IV that received estrogen. Similarly, progesterone anti-inflammatory activity may explain the decreased claudin-1 level in group V[47].

Authors in another study[48] concluded that claudin-1 was decreased by estrogen treatment in human invasive breast cancer. Furthermore, the attenuating effect of progesterone on claudin-1 in ovine uterus was highlighted[49].

CONCLUSION AND RECOMMENDATION

Conclusion: Our experimental results indicated that administration of estrogen and progesterone counteracted most of the harmful histological ileum alterations induced

by IBS in ovariectomized rats through preserving Paneth cells and goblet cells with its mucus production that acts as a mucosal protective coat. Moreover, they regulated mast cell number and the epithelial barrier protein claudin 1. Furthermore, estrogen and progesterone were equal in their efficacy nevertheless, their combination provided the best results.

RECOMMENDATION

Further experimental studies with increasing duration of estrogen and progesterone intake more than 2 weeks may give better results. Further studies to guide human use of estrogen and progesterone for menopausal females with IBS are also recommended.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الاستروجين والبروجستيرون على متلازمة القولون العصبي في الجرذان البيضاء بعد إستئصال المبيض: دراسة هستولوجية وهستوكيميائية مناعية

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مقدمه: تعتبر متلازمة القولون العصبي اضطراب وظيفي تتميز بمسارات التهابية ومناعية غير منتظمة. تظهر أعراضه بشكل أكثر وضوحًا عند النساء بعد انقطاع الطمث مما يشير إلى أن انسحاب الهرمونات الجنسية الأنثوية قد يساهم في ذلك

- الهدف من البحث: هو تقييم تأثير هرموني الاستروجين والبروجستيرون علي اللفائفي في متلازمة القولون العصبي المستحث تجريبياً في الجرذان بعد إستئصال المبيض باستخدام طرق هستولوجية وهستوكيميائية مناعية.

- مواد وطرق البحث: قسمت ستين أنثى بالغة من الجرذان البيضاء بالتساوي إلى ٦ مجموعات: المجموعة الأولي: المجموعة الضابطة، المجموعة الثانية: جرذان متلازمة القولون العصبي، المجموعة الثالثة: جرذان متلازمة القولون العصبي بعد إستئصال المبيض، المجموعة الرابعة: جرذان متلازمة القولون العصبي بعد إستئصال المبيض وتلقي الإستروجين، المجموعة الخامسة: جرذان متلازمة القولون العصبي بعد إستئصال المبيض وتلقي البروجستيرون، المجموعة السادسة: جرذان متلازمة القولون العصبي بعد إستئصال المبيض وتلقي الإستروجين والبروجستيرون معا. تم إستئصال المبيضين في المجموعات الثالثة والرابعة والخامسة والسادسة للحث على إنقطاع الطمث التجريبي. في مجموعتي الضابطة و متلازمة القولون العصبي ، خضعت الجرذان لنفس الإجراء الجراحي ولكن دون إستئصال المبيضين. بعد إسبوعين من الجراحة ، خضعت جميع الجرذان باستثناء المجموعة الضابطة إلى تمرين إجهاد تجنب الماء لمدة ١٠ أيام للحث على القولون العصبي. تم عمل نفس الإجراء لجرذان المجموعة الضابطة في وعاء خالي من الماء. في نفس الوقت، حقننت المجموعات الرابعة والخامسة والسادسة يوميا تحت الجلد الإستروجين أو البروجستيرون أو كليهما على التوالي لمدة ١٤ يومًا متواصلة. بعد ذلك ضحي بالجرذان وتم اعداد شرائح بارافين للفائفي وصباغتها بصبغات الهيماتوكسيلين والإيوسين و ألشيان الأزرق و التوليودين الأزرق والصبغة الهستوكيميائية المناعية ضد كلودين -١. تبع ذلك تحليل إحصائي ومورفومتري.

نتائج البحث: أظهرت المجموعة الثانية والثالثة تلفًا واسعًا في بنية اللفائفي مع استنفاد خلايا الكأس والبانيث وزيادة ذات دلالة إحصائية في الخلايا البدينة ومستوى كلودين-١. بينما، كشفت المجموعات الرابعة والخامسة والسادسة عن بنية طبيعية ظاهريا للفائفي وزيادة خلايا الكأس والبانيث مع انخفاض ذو دلالة إحصائية في الخلايا البدينة ومستوى كلودين-١.

الإستنتاج: كان للإستروجين والبروجستيرون تأثيرات تجديدية على البنية النسيجية للفائفي ضد القولون العصبي المستحث تجريبياً في الجرذان بعد استئصال المبيض.