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New Possible Approach in Treatment of Experimental Induced Vaginal Atrophy by Bone Marrow-derived Mesenchymal Stem Cells in Treatment of Induced Vaginal Atrophy in Adult Female Albino Rats (Histological, Immunohistochemical and Biochemical Study)

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Abstract

Women after menopause have a lot of complains that negatively distress their life. In the modern years, a lot of conduction methods have been presented to relief undesirable symptoms. Bone marrow mesenchymal stem cells (BM-MSCs) can be recently used as a new therapeutic method in treatment of many diseases and avoidance the hormonal sides effects that happened after menopause.

Aim: The aim of this research is to show a new approach in modification of the structure of vaginal mucosal atrophy by uses of (BM-MSCs) in induced ovariectomized rats.

Methods and results: Fifty-five female albino rats were used and divided randomly into five groups: control group, ovariectomized group, ovariectomized group plus estrogen (20 ug/kg/day for 4 weeks), ovariectomized with BM-MSCs (107 MScs/rat intravenously) and ovariectomized rats with stem cells/BM-MSCs Intra-vaginal group, the expression of genes for *GAPDH, iNOS* and *TGF-β* were done and vaginal biopsies were taken for histological and immunohistochemical studies. In ovariectomized group there was inflammation, ulceration and re-epithelialization with irregularity in collagen fibers, decreased estrogen receptors expression and expression of *TGF-β, GAPDH* and *iNOS* were very high. While the rate of healing of epithelium was increased with increased in the vasculatures of vaginal mucosa and the estrogen receptors expression was high with decreased expression of *GAPDH, iNOS* and *TGF-β* in ovariectomized rats that treated with intra-vaginal BM-MSCs.

Conclusions: Using the BM-MSCs could be used intravaginal safely in case of vaginal atrophy as they modify the structure of vaginal mucosa than estrogen hormone therapy alone.

Keywords: Vaginal atrophy; BM-MSCs; Estrogen

Introduction

Females suffer from general and local changes which appear after menopause. These changes are due to decrease in the level of estrogen [1]. This may be physiologically at menopause or occur accidentally after surgical removal of ovaries [2]. This decrease in estrogen level has many physiological changes as vasomotor instability, mood changes, an increased risk of osteoporosis and vaginal atrophy [3]. Vaginal atrophy is a common and affects more than 40% of postmenopausal females. This atrophy causes thinning and shrinking in the vaginal epithelial wall and decrease in smooth muscle fibers with less elasticity of vaginal wall [4]. Vaginal atrophy causes burning, dryness, irritation, and dyspareunia [5]. These symptoms do not improve with time and are not resolve without treatment [6]. The good level of estrogen from puberty is essentially for good blood supply for vaginal mucosa and its lubrication [7]. The vaginal wall rugae, wall thickening and lubrication are depending on presence of estrogen [8]. Vagina is estrogen dependent organ as estrogen is responsible for induction of proliferation of vaginal epithelium layers, smooth muscles and collagen fibers so maintains vaginal rugae [9]. Experimental bilateral ovariectomy was done to know the effects of decrease in hormonal levels and their activity in female rats [10]. This experimental ovariectomy has an important role to understand the pathophysiological changes and to help in developments of therapy [11]. So, to improve pathophysiological changes after ovariectomy, estrogen therapy was used as a replacement therapy, but there are many limitations for hormonal uses because its side effects and hazards as, cancer specially, breast, ovaries or uterus. Also, causes cardiovascular hazards or thrombosis [12]. These side effects can be controlled through good monitoring, mammography and endometrial thickness measurement [13]. To avoid risk of hormone replacement searches done to establish other line of treatment. Embryonic and somatic stem cells proved that they can be differentiated into female germ cells [14]. Somatic cells also appear to differentiate into granulosa and theca cells of the ovarian follicles which are responsible for production of estradiol [15]. The aim of this study is to explore the role of estrogen hormone in restoring histological changes of vagina after ovariectomy and to assess other lines of therapy as MSCs to improve histopathological changes in ovariectomized rat.

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Material and Method

Animals

Fifty adult female rats weighing 160 ± 200 g were obtained from the Animal House, Moshtohor Faculty of Veterinary Medicine, Benha University. Rats were kept under observation for 1 week before the beginning of the experiment for accommodation. They were maintained in a temperature- and humidity-controlled room and given free access of water and food. All animal experiments were conducted in accordance to approved protocols and the recommendations for the proper care and use of laboratory animals.

Experimental design

Fifty-five female rats (eight to ten weeks old) were taken and divided into five groups each group 10 rats and five rats were prepared for isolation and culture of MSCs.

Group I: (-ve control): no surgical operation.

Group II: (ovariectomized group): In this group the rats under grow ovariectomy operation [15].

Group III: (ovariectomized group plus estrogen): rats treated by natural estrogen (estradiol) injected subcutaneously in a dose of 20 ug/kg/day for 4 weeks [16].

Group IV: (ovariectomized rats with BM-MSCs): (rats were injected once by 10^6 MScs/rat intravenously (through tail vein) and scarified after 6 weeks.

Group V: (ovariectomized rats with stem cells/BM-MSCs Intra-vaginal group): rats were injected once with 10^6 MScs/rat into the subepithelial space intravaginal and scarified after 6 weeks [17].

Ovariec tomy technique: The rats were anesthetized by ether inhalation. Under sterile conditions, a 2–3 cm ventral lower midline incision was made into the skin and muscle (to expose the ovaries). After good homeostasis, the ovaries were removed after tying off and cut from the oviduct. Antibiotics were applied locally before suturing the muscles and the skin to close the incision. The anoestrus phase was considered to be occurring 15 days after surgery. To confirm this phase, the oestradiol level was tested 15 days after surgery, to compare oestradiol hormonal levels pre and post-surgery. At the time anoestrus was confirmed in all groups we began the treatment by estradiol or stem cells [15].

Isolation, culture, identification of BM-MSCs and labeling stem cells with GFP

All procedures were performed according to "Guide for the Care and Use of Laboratory Animals" in which bone marrow samples were taken from tibiae and femurs of five female white albino rats and flushing with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL), then were added to 10% foetal bovine serum (GIBCO/BRL) and the nucleated cells were isolated with a density gradient (Ficoll/Paque (Pharmacia)) and were suspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO_2 for 14 days as primary culture to formation of large colonies. When large colonies developed (80~90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were suspended with serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures [18]. MSCs in culture were characterized by their adhesiveness and fusiform shape [17]. At passage 6, cellular expression of CD29, CD44 (HPA005785 Sigma-Aldrich, St. Louis, MO, USA) were evaluated by flow cytometry.

For MSCs labelling the cell were taken at passage 4, and labeled with GFP (AAA27722 Sigma-Aldrich, St. Louis, MO, USA). MSCs were infected by using the MSC Nucleo fector Kit and a plasmid encoding the fluorescent protein GFP. Cells were centrifuged, washed twice in serum free medium, and then put in nucleo fector solutions. A final concentration of 4-5 × 10^5 cells/100 µl nucleo fector solutions was applied. The sample was placed in cuvette of electroporation transfection instrument at program U-23 (for high transfection efficiency) or C-17 (for high cell survival). 24 hours post-nucleo fection cells were analyzed by fluorescence microscopy. Transfection efficiencies of around 80% can be reached with GFP. Labeled cells were injected intravenously in ovariectomized rats. All rats at end of experiment were sacrificed and vaginal tissue was prepared d for histological, immunohistochemical examination and real time PCR analysis vaginal tissue was examined with a fluorescence microscope (Leica, Germany) to detect the cells stained with GFP.

Hormonal assay of serum FSH and E2

Blood samples were taken and collected from tail vein then centrifuge 5.000 rpm for 15 min to separate serum to estimate FSH and E2 hormones using ELISA kits (CUSABIO, USA). This estimation of hormones done every fifteen days to estimate ovarian function till the end of experiment [19].

Histological examination

Vaginal tissue samples were divided into two sections. The first section was examined by fluorescent microscope for tracing of injected labeled cells with GFP. The second sections were processes for paraffin block and stained with hematoxylin and eosin (H&E) and sirius red (for demonstration of collagen fibers) [20].

Immunohistochemistry

Immunohistochemical staining for estrogen receptors were performed on 5-µm, formalin-fixed, paraffin-embedded sections by using the streptavidin-biotin detection system (DAKO). They were obtained from Sigma Company and kit (SRP2163-4UG). Human prostate (taken from the pathology department in our institution) served as a positive control according to Manufacturer Company. Negative control slides were prepared by the same steps, except they were incubated with the antibody diluent instead of primary antibody. Positive reaction for estrogen receptors appeared in the form of brown nuclear staining [21].

RNA Extraction and Quantitative real-time polymerase chain reaction (qRT-PCR)

Vaginal tissues of all studied groups were homogenized and total RNA was isolated with RNA easy Mini Kit (Qiagen) then analyzed for quantity and quality with Beckman dual spectrophotometer (USA). Quantitative real-time polymerase chain reaction was done as following, 200 ng of total isolated RNA from each sample were used for DNA synthesis by reverse and transcription method by using High capacity cDNA Reverse Transcriptase kit (Applied Biosystem, USA) to measure the quantitative amount of *Inos*, *GAPDH* and *TGF-β genes*. Then the DNAs were amplified with the Syber Green I PCR Master Kit (Fermentas) in a 48-well plate using the Step One instrument (Applied

Bio-system, USA). Primers sequence for each gene demonstrated as *Ions:*

CCACCCATGGCAAATTCCATGGCA (Forward),

TCTACACGGCAGGTCAGGTCCACC (Reverse);

GAPDH:

CAGGAGGATGGTGGTTTGAT (Forward),

TGCCACTTTATCCCATTCAG (Reverse);

TGF-β:

AAGTCATCCATCCCTTCAGC (Forward),

AGCCCACCTGAGCCCTATAA (Reverse).

Morphometric study

By using image analyzer (Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA), from each group sections were measured in ten non-overlapping fields by high power field:

- Mean vaginal epithelial thickness in each group [22].
- Mean area percent of collagen fiber content (±SD) in each group.
- Mean area percent of estrogen immunostaining was quantified.

Statistical analysis

One-way study of variance (ANOVA) was used to measure up differences between the groups and by using IBM SPSS Statistics software for Windows, Version 20 (IBM Corp., Armonk, NY, USA) we recorded and analyzed collected data from each group, all data was put across as the mean value, standard deviation (SD) and differences were considered to be significant at $p<0.01$.

Results

BM-MSCs identification and homing

Undifferentiated (BMSCs) were appear as spindle and fibrocyte-like by inverted microscope as shown in Figure 1A while flow cytometry analysis of surface molecule for CD29, CD44 for confirmation of mesenchymal cell phenotypes as shown in Figure 1B. Florescence microscopy examination of vaginal cells treated by mesenchymal cells indicated that the GFP-transduced injected cells were localized within the vaginal tissue as shown in Figure 1C and 1D.

Hormonal assay results

E2 levels were assessed 15 days and 30 days after injection of estrogen and MSCs. Results showed that there was a significant decrease in E2 levels in ovariectomized group compared to control (12.5 ± 1.72 after 15 days versus 60.32 ± 12.8 in control rats, $p<0.001$ and 10.1 ± 3.5 after 30 days versus 62.1 ± 12.4 in control rats $p<0.001$). Use of either estrogen or MSCs led to a significant increase in E2 levels with more superior therapeutic effects with intravaginal MSCs as compared to MSCs (40.5 ± 10.3, $p<0.05$, 50.1 ± 10.2, $p<0.01$ and 55.7 ± 19.2 respectively after 15 days versus 60.32 ± 12.8 in control rats and were more significant with estrogen or MSCs treated groups respectively after 30 days (50.9 ± 10.2, 53.2 ± 1.4 and 60.1 ± 11.8, $p<0.01$) versus control group 62.1 ± 12.4 as shown in Figure 2.

FSH levels were assessed 15 days and 30 days after injection of estrogen and MSCs.

There was a significant elevation of FSH levels in ovariectomized group compared to control group (85.6 ± 19.1 versus 32.6 ± 9.0 in control rats, $p<0.001$ after 15 days, 92 ± 14.2 versus 35.6 ± 9.4 in control rats after 30 days respectively, $p<0.001$). Use of estrogen and MSCs led to a significant decrease in FSH levels as compared to ovariectomized group (40.1 ± 9.2 and 45.3 ± 11.7) after 15 days, $p<0.05$ and 38.2 ± 5.1 and 43.3 ± 6.2 after 30 days. Use of intravaginal MSCs did not lead to

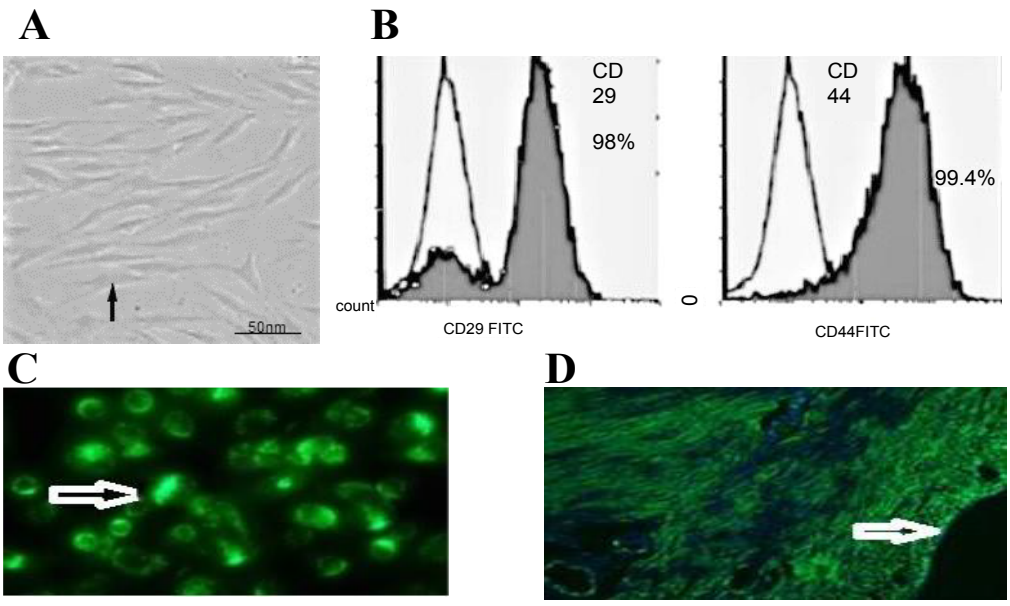
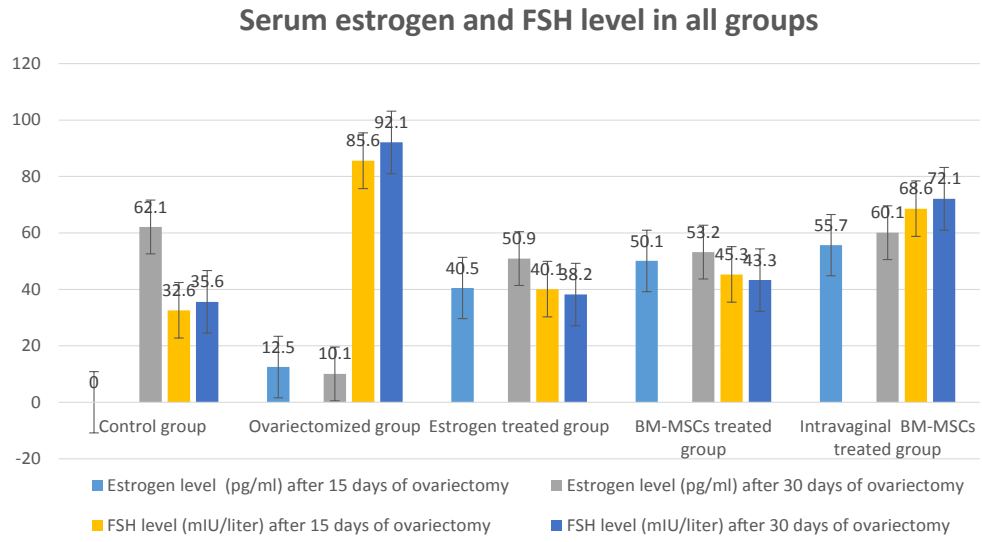


Figure 1: Photomicrographs of identification of bone marrow-derived mesenchymal stem cells. (A) Spindle-shaped like cells (arrow) on two weeks from primary culture (inverted microscope) (×200). (B) Image analyzer positive foe CD29 and CD44. (C) Homing of green fluorescent labeled MCSs (arrow) from stem cells group *in vitro*. (D) Labeled MSCs in vaginal tissue1A (arrow) *in vivo* (fluorescent microscope) (×1000).



Group	Estrogen level (E2) (pg/ml) after 15 days of ovariectomy	Estrogen level (E2) (pg/ml) after 30 days of ovariectomy	FSH level (mIU/liter) after 15 days of ovariectomy	FSH level (mIU/liter) after 30 days of ovariectomy
Control group	60. 2 ± 1.8	62.1 ± 0.4	32.6 ± 1.0	35.6 ± 0.4
Ovariectomized group	12.5 ± 1.12*	10.1 ± 0.5*	85.6 ± 1.1*	92.1 ± 1.2*
Estrogen treated group	40.5 ± 0.3#	50.9 ± 0.2#	40.1 ± 0.2#	38.2 ± 1.1#
BM-MSCs treated group	50.1 ± 1.2#	53.2 ± 1.4#	45.3 ± 1.7#	43.3 ± 0.2#
Intravaginal BM-MSCs treated group	55.7 ± 1.2#	60.1 ± 1.8	68.6 ± 1.9#	72.1 ± 0.5

Figure 2: Serum E2 and FSH levels in all groups. #Significant as p value <0.05 versus ovariectomized group. *Significant as p value <0.05 versus control group.

decrease in FSH levels as compared to control groups. (68.6 ± 13.9 after 15 days, p<0.01 and 72.1 ± 6.5 after 30 days, p<0.01 versus 32.6 ± 9.0 and 35.6 ± 9.4 in control group, p<0.05).

Histological results

Hematoxylin and Eosin and sirius red results: H&E examination of vaginal tissue from control group revealed normal non keratinized stratified squamous epithelium with multiple papilla, and cells appeared, densely packed, vacuolated with dark nuclei as shown in Figure 3A. The underlying connective tissue appeared thick with regular packed bundles of collagen fibers as shown in Figure 3B. In ovariectomized group the epithelium appeared thin desquamated with apparent many dilated blood vessels and inflammatory cells infiltration in underlying connective tissue as shown in Figure 3C. The connective tissue was irregular arrangement and decrease in amount with dilated blood vessels as shown in Figure 3D. In estrogen treated group the covering epithelium appeared normal with well recognized basal layer with multiple dilated blood vessels as shown in Figure 3E, while the connective tissue appeared normal with regular bundles of collagen fibers with moderate in amount (Figure 3). In BM-MSCs treated group showed many protrusions in the basal surface of the epithelium as shown in Figure 3G, also connective tissue increase in amount with regularly appearance as shown in Figure 3H. The intravaginal BM-MSCs treated group showed many protrusions in the basal part of epithelium that appear as feet fixing the epithelium to the basement membrane indicated functionally vaginal epithelium as shown in Figure 3I, also their connective tissue differentiated into thick regular arrangement bundles of collagen fibers as shown in Figure 3J.

The morphometric result of mean vaginal epithelial thickness revealed of 189.10 ± 10.26 mm, 27.80 ± 2.14 mm, 128.19 ± 5.98 mm, 165.74 ± 1.30 mm and 180.79 ± 3.90 in Groups I, II, III, IV, and V respectively. The mean thickness of the vaginal epithelium of rats in Group I (permanent estrus) was significantly greater than that of animals in Groups II, III (p=0.0001), with no statistically significant differences between groups, IV and V. (p=0.0709).

The morphometric result of Mean area percent of collagen fiber content (±SD) in the studied groups revealed a significant decrease in groups II, as compared to the control group and values recorded for groups III, IV and V represented a statistically significant decrease, as compared to the ovariectomy group. However, the values were not statistically significant as compared to the control as shown in Figure 4.

Immunohistochemical results

To assess the estrogen receptors of vaginal cells after MSC transplantation, strong estrogen receptors expression was detected in the epithelium and in connective tissue of control group as shown in Figure 5A, while the expression of estrogen was minimal in ovariectomized group as shown in Figure 5B, also moderate expression in the epithelium of estrogen treated group as shown in Figure 5C, mild to moderate in group BM-MSCs treated group as shown in Figure 5D and severe expression in group treated with BM-MSCs intravaginal as shown in Figure 5E. The mean area percentage of estrogen for all groups was represented in Figure 4. There was a significant increase in mean area percent of estrogen immuno-expression of groups IV, V compared with group II.

Quantitative gene expression

The expression of *genes* for *GAPDH*, *iNOS* and *TGF-β* in all groups were quantified by real-time PCR (Table 1).

The expression of *TGF-β* was very low in healthy control vaginal tissue. After ovariectomy, the expression of *TGF-β* was very high. The treatment with BM-MSCs reduced *TGF-β* expression. The highest reduction of *TGF-β* expression was seen in group treated with BM-MSCs intravaginal.

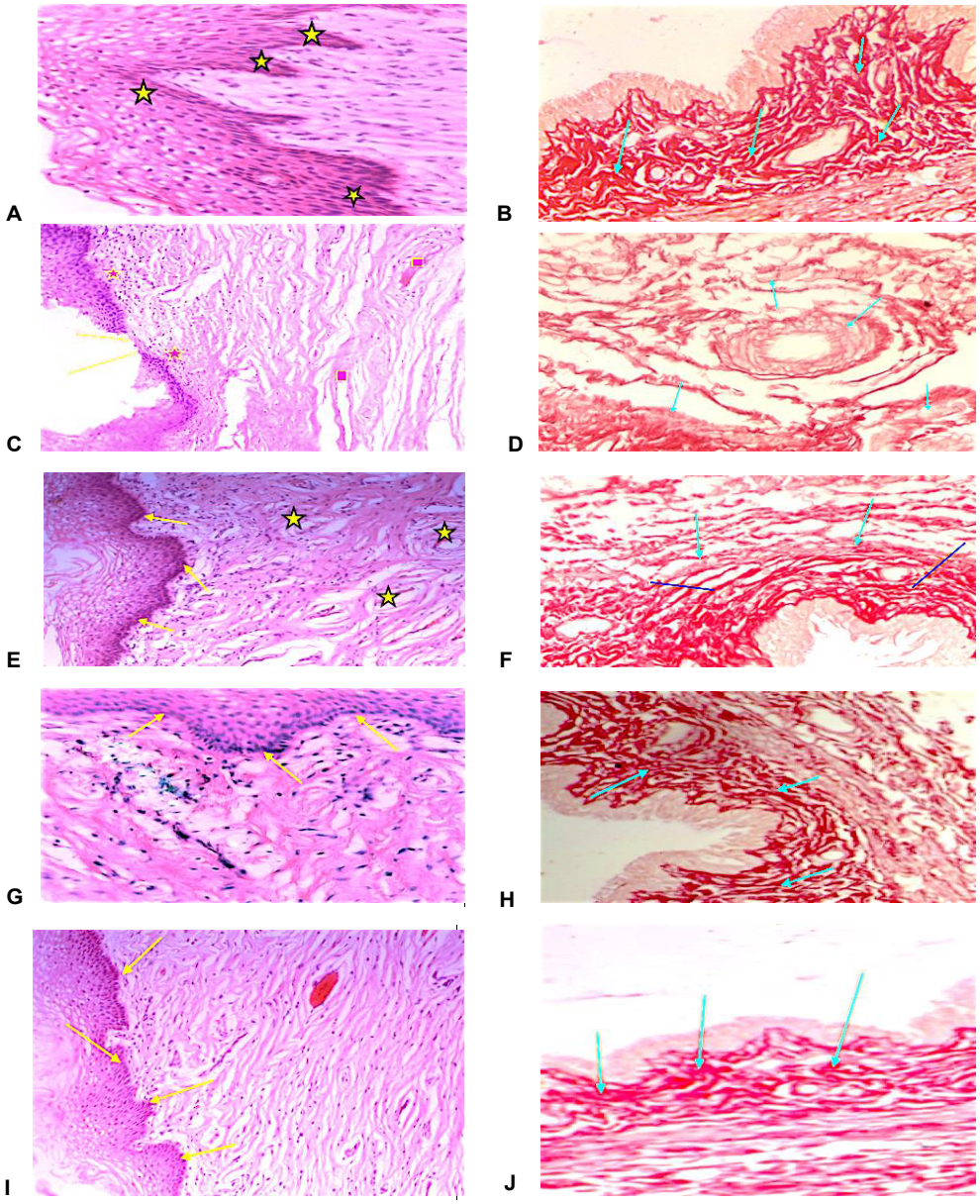


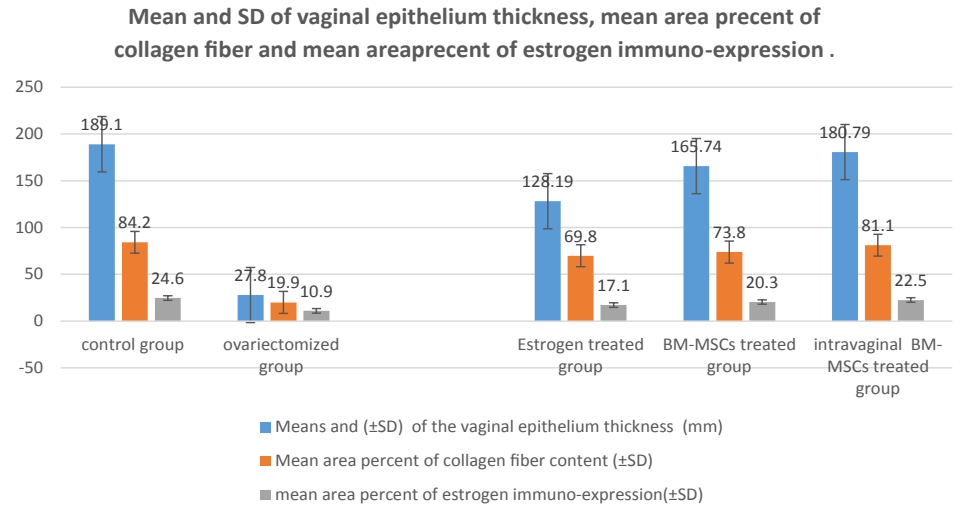
Figure 3: (A) A photomicrograph of a section of the vagina of adult control group (group I) showing normal stratified squamous epithelium with multiple papilla (stars). Notice that the cells are densely packed, vacuolated with dark nuclei (arrows) H&E ×400. (B) The connective tissue is intensely stained with Sirius (arrows) Sirius, ×400. (C) The epithelium of group II showing desquamation (arrows) with many dilated blood vessels in underlying dispersed connective tissue (squares). Notice inflammatory cells under epithelium (stars). (D) The connective tissue is faintly stained by Sirius (arrows) with regular arrangement of collagen fibers, Sirius, ×400. (E) The epithelium of group III showing normal epithelial cells with well recognized basal layer (arrows) with multiple dilated blood vessels (stars) H&E, ×400. (F) The connective tissue is moderate staining with Sirius red (arrows). Notice that the regular arrangement of collagen fibers (blue lines) Sirius, ×400. (G) The epithelium of group IV showing many protrusions in the basal surface (arrows) H&E, ×400. (H) The connective tissue is moderate to strong staining by Sirius red (arrows) Sirius, ×400. (I) The epithelium of group V showing many protrusions in the basal surface (arrows) H&E, ×400. J) The connective tissue is strongly staining by Sirius red (arrows). Sirius, ×400.

The expression of *Ions* and *GAPDH* were absent in healthy control group while its expression increased after ovariectomy to reach very high expression after 6 weeks of ovariectomy. The treatment of ovariectomized rats with BM-MSCs reduced *iNOS* expression and significantly decreased in group treated with BM-MSCs intravaginal.

Discussion

Menopause is a very important state in the life of females as it is associated with many changes in their health. Estrogen hormone deficiency after menopause or surgical removal of ovaries is responsible for these health hazards [23].

Ovary is the main organ for production of estrogen so ovariectomy causes decrease of this hormone [24]. Decrease of estrogen is associated with elevation of follicular stimulating hormones (FSH). This increase of (FSH) is due to failure of negative feedback on pituitary effect as



Group	Means and (±SD) of the vaginal epithelium thickness (mm)	Mean area percent of collagen fiber content (±SD)	mean area percent of estrogen immuno-expression(±SD)
Control group	189.10 ± 1.26	84.2 ± 1.3	24.6 ± 1.9
Ovariectomized group	27.80 ± 0.14*	19.9 ± 05*	10.9 ± 1.8*
Estrogen treated group	128.19 ± 2.98#	69.8 ± 0.2#	17.1 ± 0.2#
BM-MSCs treated group	165.74 ± 1.30#	73.8 ± 1.9#	20.3 ± 1.7#
Intravaginal BM-MSCs treated group	180.79 ± 1.90#	81.1 ± 0.2	22.5 ± 1.9#

Figure 4: Means and standard deviations (±SD) of the vaginal epithelium thickness, mean area percent of collagen fiber content (±SD) and mean area percent of estrogen immuno-expression of female rats in all groups. #Significant as p value <0.05 versus ovariectomized group. *Significant as p value<0.05 versus control group. (Group I exhibited a significantly greater epithelial thickness than Groups II, III and IV (p<0.0001) and no difference was observed between Groups II, III and IV (p=0.0809).

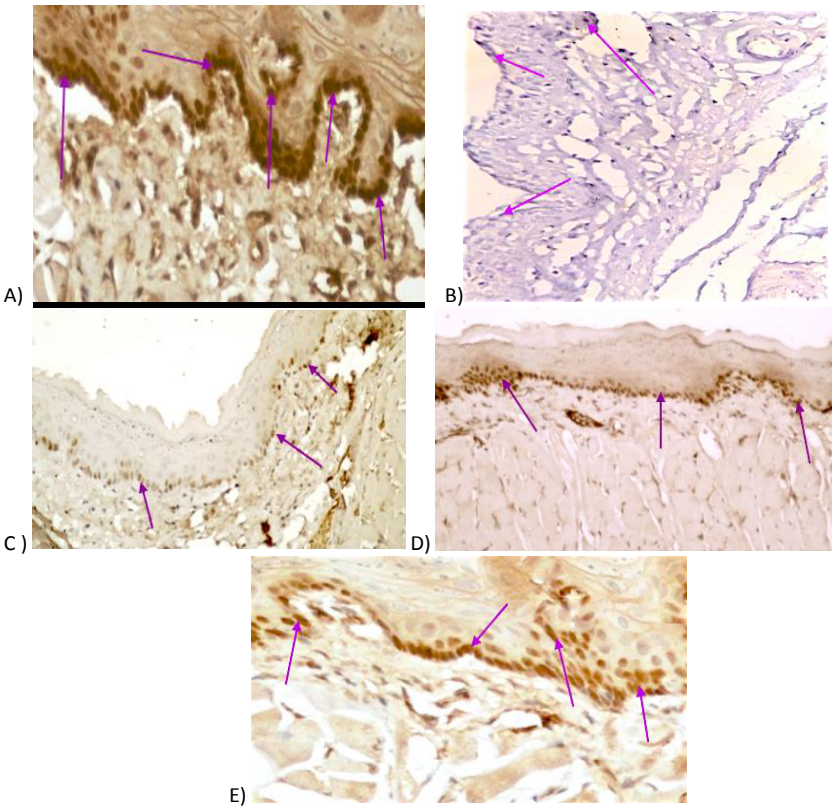


Figure 5: (A) A photomicrograph of a section of the vagina of adult control group (group I) showing strong positive estrogen receptor in epithelium (arrows). (B) The epithelium of group II showing negative estrogen receptor (arrows). (C) The epithelium of group III showing mild to moderate estrogen expression (arrows). (D) The epithelium of group IV showing moderate estrogen expression (arrows). (E) The epithelium of group V showing moderate to severe estrogen expression (arrows) Estrogen immunostaining ×400).

Group	Means ± SD of the TGF-β gene expression	Means ± SD of Ions gene expression	Means ± SD of GAPDH gene expression
Control group	11.7 ± 1.26	13.2 ± 1.3	14.3 ± 1.9
Ovariectomized group	22.14 ± 0.14*	29.3 ± 0.9*	28.6 ± 2.1*
Estrogen treated group	12.19 ± 1.28#	17.2 ± 1.2#	18.1 ± 0.2#
BM-MSCs treated group	14.24 ± 0.52#	15.8 ± 0.8#	15.3 ± 1.2#
Intravaginal BM-MSCs treated group	12.17 ± 0.70#	14.5 ± 0.5	14.9 ± 0.1#

Table 1: Mean and SD of TGF-β, Ions and GAPDH genes expression for all groups. #Significant as p value<0.05 versus ovariectomized group. *Significant as p value<0.05 versus control group.

result of the decrease in ovarian hormones [25,26]. Estrogen defects begin 15 days after ovariectomy [27].

In this study hormonal assay was done at 15 and 30 days after ovariectomy and showed that E2 levels were assessed and the results showed that there was a significant decrease in E2 levels in OVX group as compared to the control.

Use of either estrogen or MSCs injection led to a significant increase in E2 levels with more superior therapeutic effects with intravaginal MSCs as compared to MSCs intravenous injection. As regard FSH level there was a significant elevation of FSH levels in OVX group compared to control group.

Use of estrogen and MSCs led to a significant decrease in FSH levels as compared to ovariectomized group. Use of intravaginal MSCs did not lead to decrease in FSH levels as compared to control groups.

The level of follicle stimulating hormone (FSH) more than 40 mIU/ml indicates that there is an ovarian failure [28].

The previous results showed that levels of estrogen and FSH after treatment of OVX rats with MSCs injection were near to control level. This indicates that MSCs has an endocrine functions and effects on FSH through communication with pituitary gland [29]. The study suggests that BM-MSCs can recover the function and structure of injured tissues [30].

Many studies reported that the ovarian granulosa and theca cells which were differentiated from stem cells lead to secretion of estrogen in response to elevation of the level of FSH level in OVX rats and subsequent increase estradiol and suppress the level of FSH nearly to normal [31]. So BM-MSCs used to restore levels of ovarian hormone and could reactivate folliculogenesis in animal model of premature ovarian failure (POF) due to the use of chemotherapy [32].

Studies done on cases of premature ovarian failure (POF) detected that injection of BM-MSCs could differentiate to many types of cells as theca cells, granulosa cells, and corona radiate cells. This lead to ovarian function recovery specially its endocrine and steroidogenesis [33].

The deficiency of ovarian hormones as a result of various factors (POF, menopause or surgical ovariectomy) leads to structural changes of the vagina which become narrow, short, thinner with no folds [33,34].

Estrogen hormone is vital in maintaining of vaginal structures and functions. As vaginal wall thickness and rugae are depending on estrogen [35,36].

The present study showed that in ovariectomized group the epithelium appeared thin desquamated with apparent many dilated blood vessels and inflammatory cells infiltration in underlying connective tissue. The connective tissue was irregularly arranged and decrease in its amount.

Ovariectomy is the cause of a significant decrease in vaginal epithelial thickness and in its glycogen content. This decreasing in vaginal epithelium, its layers and defects in stratification makes it liable to abrasion [37].

All this above reasons lead to decrease the power of protection against bacterial infection [38].

The effects of estrogen on vaginal mucosa is due to that the estrogen increases the blood supply of vagina and its vascularization [39,40].

This study showed that there were increases in mean thickness in the treated groups. From our result we found that the injection of estrogen produced a fast and good response on the vaginal epithelium as regeneration occur more with intravaginal MSCs injection. This response is due to presence of large number of estrogenic receptors in the genital tract [41]. Estrogen produces their effects (cellular proliferations) through receptors which are present in various tissues as uterus, breast, and vagina [2].

In this study assessment of estrogen receptors showed that there are strong estrogen receptors expression in the epithelium and in connective tissue of control group while the expression of estrogen was minimal in OVX group (group II). Moderate expression in the epithelium of group III while mild to moderate in group IV. In group V there were severe expressions of the receptors.

Normally the estrogenic receptors are of large numbers in vagina which react rapidly with estrogen either natural or synthetic [7].

As estrogen maintains the thickness of vaginal epithelium, it also maintains the production of glycogen. Estrogen hormone has an important role in connective tissue maintenance. As the receptors for estrogen are identified in the connective tissue nuclei in vaginal wall [42].

The estrogen defect causes defect in collagen fibers. This leads to change of vaginal pH which is normally low [43].

Commencellas microorganisms (Lactobacilli) found in vagina need glycogen to produce lactic acid and keep low vaginal PH about 3.5 to 4.5. This low PH protects the vagina against infections [44].

The decrease of collagen also is the cause of weakening of vaginal wall and may predispose prolapse in postmenopausal women [45].

The morphometric result of Mean area percent of collagen fiber in this study revealed a significant decrease in groups II, as compared to the control group with good improvement of vaginal epithelium thickness and connective tissues in OVX rats after treatment by estrogen and BM-MSCs injection, but the best effects were after intravaginal BM-MSCs.

BM-MSCs have proved that they have direct great effects in the soft tissue regeneration [46]. Recent study showed that transplantation of these cells leading to new tissue growth and deposition of collagen [47].

These cells can help in repair of tissues as they able to differentiate to many types of cells as connective tissues cells [48].

In case of decrease ovarian hormones especially estrogen due to various causes (POF, normal menopause or surgical removal of ovaries) the symptoms as vaginal atrophy and its complications can be avoided or minimized by use of the hormone [49]. But WHO study published in 2002, reported that increase risk of many diseases after hormone replacement therapy as stroke or heart disease. Also increasing estrogen level leads to proliferation of epithelium and predisposing to cancers as cancer breast and endometrial carcinoma [50].

Conclusion

BM-MSCs can be used to treat vaginal atrophy and avoid the uses of hormonal therapy and their effects on vagina are through elevation of estrogen hormone or through direct effect on vaginal epithelium and connective tissues.

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