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

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Abstract

Women after menopause have a lot of complaints that negatively distress their life. In the modern years, a lot of conductions methods have been presented to relief of undesirable symptoms. Bone marrow mesenchymal stem cells (BM-MSCs) can be recently used as a new therapeutic method to treat and avoid the hormonal therapy symptoms and complications that used after menopause.

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

Methods and Results: Fifty female albino rats were used and divided randomly into five groups: control group, ovariectomized group, ovariectomized group plus estrogen (20 μg/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 delayed re epithelialization with irregularity in collagen fibers, decrease estrogen receptor expression and expression of TGF-β, GAPDH and iNOS were very high, while the rate of healing of epithelium with increase, the vasculatures of vaginal mucosa, the estrogen receptors expression were high and decrease expression of GAPDH, iNOS and TGF-β in ovariectomized rats with stem cells/BM-MSCs Intra-vaginal group.

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

Keywords: Vaginal atrophy; BM-MSCs; Estrogen; Embryonic and somatic stem cells

Introduction

Females suffer from general and local changes which appear after menopause. These changes are due to decrease the level of estrogen [1]. This may be physiologically at menopause or occur accidentally after surgical removal of ovaries (surgical menopause) [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 shrink inner of vaginal epithelial wall, smooth muscle and the wall become less elastic [4]. Vaginal atrophy causes burning, dryness, irritation, and dyspareunia [5]. These symptoms do not improve with time and are not resolve without treatment [6]. Estradiol which is a very important estrogen that are produced by ovary and a good level of estrogen from puberty is essentially for good blood supply for vaginal mucosa and keep lubrication [7]. The vaginal wall rugae, wall thickening and lubrication are depending on presence of estrogen [8]. Estrogen is responsible for induction of proliferation of layers of vaginal epithelium, smooth muscles and collagen fibers so maintains vaginal rugae, so the vagina is estrogen responsive organ [9]. To know the effects of decrease in hormonal levels and their activity experimentally this through bilateral ovariectomy in female rats [10]. This experimental ovariectomy has an important role to understand the pathophysiological changes and help in developments of therapy [11]. To improve pathophysiological changes after ovariectomy replacement by estrogen hormone is done. But there is limitation 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 to improve histopathological changes in ovariectomized rat vagina as MSCs.

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 female rats were divided into five groups each group 10 rats.

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

Group II: (Ovariectomized group): In this group the rats under grow sham operation where rats were anesthetized and followed by antiseptic cleaning of their skins. Small surgical incisions were made in the lower midline before suturing it back [15].

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

Group IV: (ovariectomized rats with BM-MSCs): (rats were injected by 107 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 with 107 MScs/rat intra vaginal and scarified after 6 weeks.

Ovarietomy 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 anoestrous 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 anoestrous 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 8-week-old female white albino rats and flushing with Dulbecco’s modified Eagle’s medium (DMEM, Gibco/ BRL), then were added to 10% fetal bovine serum (Gibco/ BRL) and the nucleated cells were isolated with a density gradient (Ficoll/Paque [Pharmacia]) and suspended in complete culture medium supplemented with 1% penicillin-streptomycin (Gibco/ BRL). Cells were incubated at 37°C in 5% humidified CO₂ for 14 days as primary culture to formation of large colonies. When large colonies developed (8090% 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 cm² culture flask (Falcon). The resulting cultures were referred to as first-passage cultures [17]. MSCs in culture were characterized by their adhesiveness and fusiform shape [17]. At passage 6, cellular expression of CD29, CD44, were evaluated by flow cytometry.

For MSCs labelling the cell were taken at passage 4, and labeled with GFP (amaxa GmbH, amaxa Inc. Europe/World USA Scientific Support). MSCs were infected by using the MSC Nucleo factor Kit and a plasmid encoding the fluorescent protein GFP. Cells were centrifuged, washed twice in serum free medium, and then put in nuclo factor solutions. A final concentration of (4-5) × 105 cells/100 μl nuclo factor solutions was applied. The sample was placed in cuvette of elactroporation transfection instrument at program U-23 (for high transfection efficiency) or C-17 (for high cell survival). 24 hours post-nucleofection 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 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 [18].

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 [19].

Immunohistochemistry

Immunohistochemical staining for estrogen receptors were performed on 5 μm, formalin-fixed, paraffin-embedded sections by using the streptavidin-biotin detection system (DAKO). Positive and negative control sections were used for each assay [20].

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), TCTACACGGAGGTCAGGTCCACC(Reverse); GAPDH: CAGGAGGATGGTGGTTTGAT (forward), TGCCACTTTATCCCATTCAG(Reverse); TGF-B: AAGTCATCCATCCCTTACGC(forward), AGCCCACCTGAGCCCTATAA(Reverse).

Morphometric study
By using image analyzer (Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA), we measure in five non-overlapping fields from each rat of each group:

- Mean vaginal epithelial thickness in each group [21].
- 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.

Result

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

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 62.1±12.4; non-significant difference and 50.9±10.2, 53.2±1.4 and 60.1±11.8, p<0.01 after 30 days versus 62.1±12.4 in control rats respectively) (Table 1).

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 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) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Estrogen level (E2) (pg/ml) after 15 days of ovariectomy</th>
<th>Estrogen level (E2) (pg/ml) after 30 days of ovariectomy</th>
<th>FSH level (mIU/liter) after 15 days of ovariectomy</th>
<th>FSH level (mIU/liter) after 30 days of ovariectomy</th>
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</thead>
<tbody>
<tr>
<td>control group</td>
<td>60.3±12.8</td>
<td>62.1±12.4</td>
<td>32.6±9.0</td>
<td>35.6±9.4</td>
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<tr>
<td>ovariectomized group</td>
<td>12.5±1.72*</td>
<td>10.1±3.5*</td>
<td>85.6±19.1*</td>
<td>92±14.2*</td>
</tr>
<tr>
<td>Estrogen treated group</td>
<td>40.5±10.3#</td>
<td>50.9±10.2 #</td>
<td>40.1±9.2 #</td>
<td>38.2±5.1#</td>
</tr>
<tr>
<td>BM-MSCs treated group</td>
<td>50.1±10.2 #</td>
<td>53.2±1.4#</td>
<td>45.3±11.7#</td>
<td>43.3±6.2#</td>
</tr>
<tr>
<td>intravaginal BM-MSCs treated group</td>
<td>55.7±19.2#</td>
<td>60.1±11.8</td>
<td>68.6±13.9#</td>
<td>72.1±6.5</td>
</tr>
</tbody>
</table>

#significant p value < 0.05 versus ovariectomized group.
*significant p value < 0.05 versus control group.
#significant p value < 0.05 versus ovariectomized group.

Table 1: Serum E2 and FSH levels two week and one month after MSCs injection in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Means and (±SD) of the vaginal epithelium thickness (mm)</th>
<th>Mean area percent of collagen fiber content (±SD)</th>
<th>mean area percent of estrogen immuno-expression (±SD)</th>
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<tbody>
<tr>
<td>control group</td>
<td>189.10±10.26</td>
<td>84.2±1.3</td>
<td>24.6±3.9</td>
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<tr>
<td>ovariectomized group</td>
<td>27.8±2.14 *</td>
<td>19.9±2.5*</td>
<td>10.9±1.8*</td>
</tr>
<tr>
<td>Estrogen treated group</td>
<td>128.19±5.98 #</td>
<td>69.8±5.2 #</td>
<td>17.1±6.2 #</td>
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<tr>
<td>BM-MSCs treated group</td>
<td>165.74±1.30 #</td>
<td>73.8±1.9#</td>
<td>20.3±10.7#</td>
</tr>
<tr>
<td>intravaginal BM-MSCs treated group</td>
<td>180.79±3.90#</td>
<td>81.1±10.2</td>
<td>22.5±11.9#</td>
</tr>
</tbody>
</table>

#significant p value < 0.05 versus ovariectomized group.
*significant p value < 0.05 versus control group.
#significant p value < 0.05 versus ovariectomized group. (Group I exhibited a significantly greater epithelial thickness than Groups II, III and IV (po0.0001) and no difference was observed between Groups II, III and IV (p=0.0809).

Table 2: 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 (Group I), the ovariectomized rats in Groups II, III, IV and V (experimental groups) treated with estrogen, with MSCs and intravaginal MSCs.

**Histological results**

**Hematoxylin & 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 (Figure 2A). The underlying connective tissue appeared thick with regular packed bundles of collagen fibers (Figure 2B). In ovariectomized group the epithelium appeared thin desquamated with apparent many dilated blood vessels and inflammatory cells infiltration in underlying connective tissue and (Figure 2C). The connective tissue was irregular arrangement and decrease in amount with dilated blood vessels (Figure 2D).

In estrogen treated group the covering epithelium appeared normal with well recognized basal layer with multiple dilated blood vessels (Figure 2E), while the connective tissue appeared normal with regular bundles of collagen fibers with moderate in amount (Figure 2F). In BM-MSCs, treated group showed many protrusions in the basal surface of the epithelium (Figure 2G), also connective tissue increase in amount with regularly appearance (Figure 2H). 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 (Figure 2I), also their connective tissue differentiated into thick regular arrangement bundles of collagen fibers (Figure 2J).

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 (Table 2). 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 and IV (p<0.0001) and 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 (Table 2).

![Figure 2](image1)

**Figure 2:** A) A photomicrograph of a section of the vagina of a control group (group I) showing normal stratified squamous epithelium with multiple papilla (E). Notice that the cells are densely packed, vacuolated with dark nuclei (arrow). H&E, ×400. B) The connective tissue is intensely stained with Sirius (arrow). Sirius, ×400. C) The epithelium of group II showing desquamation (arrow) with many dilated blood vessels in underlying dispersed connective tissue (V). Notice inflammatory cells under epithelium (I). D) The connective tissue is faintly stained by Sirius (arrow) with regular arrangement of collagen fibers. Sirius, ×400. E) The epithelium of group III showing normal epithelial cells with well recognized basal layer (arrow) with multiple dilated blood vessels (V). H&E, ×400. F) The connective tissue is moderate staining with Sirius red (arrow). Notice that the regular arrangement of collagen fibers (C). Sirius, ×400. G) The epithelium of group IV showing many protrusions in the basal surface (arrow). H&E, ×400. H) The connective tissue is moderate to strong staining by Sirius red (arrow). Sirius, ×400. I) The epithelium of group V showing many protrusions in the basal surface (arrow). H&E, ×400. J) The connective tissue is strongly staining by Sirius red (arrow). Sirius, ×400.

**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 (Figure 3A), while the expression of estrogen was minimal in ovariectomized group (Figure 3B), also moderate expression in the epithelium of estrogen treated group (Figure 3C), mild to moderate in group BM-MSCs treated group (Figure 3D) and severe expression in group treated with BM-MSCs intravaginal (Figure 3E).

The mean area percentage of estrogen for all groups was represented in Tables 2. 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 (Tables 3).

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.

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.

![Figure 3](image2)

**Figure 3:** A) A photomicrograph of a section of the vagina of a control group (group I) showing strong positive estrogen receptor in epithelium (arrow). B) The epithelium of group II showing negative estrogen receptor (arrow). C) The epithelium of group III showing mild to moderate estrogen expression (arrow). D) The epithelium of group IV showing moderate estrogen expression (arrow). E) The epithelium of group V showing moderate to severe estrogen expression (arrow). (estrogen immunostaining X400).
Triplicate of TGF-β, Ions and GAPDH genes expression for all groups.

### Table 3: Mean and SD of TGF-β, Ions and GAPDH genes expression for all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Means ±SD of the TGF-β gene expression</th>
<th>Means ±SD of Ions gene expression</th>
<th>Means ±SD of GAPDH gene expression</th>
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<td>control group</td>
<td>11.7±1.26</td>
<td>13.2±1.3</td>
<td>14.3±1.9</td>
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<tr>
<td>ovariectomized group</td>
<td>22.14±2.14 *</td>
<td>29.3±2.9*</td>
<td>28.6±2.21*</td>
</tr>
<tr>
<td>Estrogen treated group</td>
<td>12.19±3.28 #</td>
<td>17.2±1.2 #</td>
<td>18.1±4.2 #</td>
</tr>
<tr>
<td>BM-MSCs treated group</td>
<td>14.2±2.52 #</td>
<td>15.8±2.8#</td>
<td>15.3±9.2 #</td>
</tr>
<tr>
<td>intravaginal BM-MSCs treated group</td>
<td>12.17±1.70#</td>
<td>14.5±2.5</td>
<td>14.9±17.1#</td>
</tr>
</tbody>
</table>

*significant p value < 0.05 versus control group.

#significant p value < 0.05 versus ovariectomized group.

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 [22].

Ovary is the main organ for production of estrogen so ovariectomy causes decrease of this hormone [23]. 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 effects due to decrease of ovarian hormones [24,25]. Estrogen defects begin 15 days after ovariectomy [26].

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 compared to 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. As regard FSH levels 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 [27].

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 [28]. The studies suggest that BM-MSCs can recover the function and structure of injured tissues [29].

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 [30]. So, BM-MSCs used to restore levels of ovarian hormone and could reactivate folliculogenesis in animal model of premature ovarian failure (POF) due to use of chemotherapy [31].

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

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 with no folds. Its epithelium becomes flat decrease [32,33].

Estrogen hormone is vital in maintaining of vaginal structures and functions. As vaginal wall thickness and rugae are depending on estrogen [23,34].

This 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 and. The connective tissue was irregular arrangement and decrease in amount with dilated blood vessels.

Ovariectomy is the cause of the significant decrease of vaginal epithelium thickness and its glycogen content. This decrease in vaginal epithelium, its layers and defects of stratification makes it liable to abrasion [35].

And this leads to decrease the power of protection against bacterial infection [36]. The effects of estrogen on vaginal mucosa are due to that estrogen increase the blood supply of vagina and its vascularization [23,36].

This study showed that there was increase 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 [37]. Estrogen produces their effects (cellular proliferations) through receptors which are present in various tissues as, uterus, breast, and vagina [38].

In this study assessment of estrogen receptors showed that there are strong estrogen receptors expressions 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 was severe expression 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 important role in connective tissue maintenance, as the receptors for estrogen are identified in the connective tissue nuclei in vaginal wall [39].

The estrogen decrease causes defects in collagen fibers. This leads to change of vaginal pH which is normally low [40].

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 [41].

The decrease of collagen also is the cause of weakening of vaginal wall and may predispose prolapse in postmenopausal women [42].

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 height and collagen and connective tissues in O VX 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 [43]. Recent studies showed transplantation of these cells leading to new tissue growth and deposition of collagen [44].

These cells can help in repair of tissues as they are able to differentiate to many types of cells as connective tissues cell types [45].

In case of decrease ovarian hormones specially, 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 [46]. But WHO study published in 2002, reported that increase risk of many diseases in Egypt. Cancer as cancer breast and endometrial carcinoma [47].

So, more safe lines of control hormone deficient are needed. BM-MSCs can be used to treat and avoid these decrease hormone symptoms and complications. These stem cells can be producing their effects on vagina through elevation of estrogen hormones or through direct effect on vaginal epithelium and connective tissues.

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