Comparative Study of Bone Marrow and Adipose Tissue-Mesenchymal Stem Cells In The Treatment of Cyclophosphamide Induced Ovarian Damage In Adult Female Albino rats.

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Abstract: Background: Cyclophosphamide (CLP), used as an anticancer therapy , One of its side effects in women is through inducing ovarian toxicity and consequently infertility **Aim of work:** to evaluate the impact of BM-MSCs&AT-MSCs in the overcome of cyclophosphamide-induced ovarian damage in rats. **Materials&Methods:** Forty female adult Albino rats were randomly divided into four groups. **Group I** (control group). **Group II** (cyclophosphamide treated group) were subjected to intra-peritoneal injection of 150 mg/kg- B.W, followed by a dose of 8 mg/kg every week, the rats were scarified after 3 weeks. **Group III** (BM-MSCs treated group) were treated by cyclophosphamide in the same manner as in group II and after five weeks from the second CLP injection 100 μl of BM-MSCs mixture ($2.5x10^6$cells) in 100 μl PBS was injected intra-venous. **Group IV**(AT-MSCs treated group) were treated by CLP as in group II and after 5 weeks from the second CP injection, 100 μl of AT-MSCs mixture ($2.5x10^6$cells) in 100μl PBS was injected intra-venous, Then 30 days after cell therapy the rats were scarified, Ovarian sample sections were stained with different stains, H&E, Masson´s Trichrome and Immunohistochemical stains for anti-PCNA antibody. The average number of primordial germ follicles, % of collagen fibers, area % of +ve immune-reactivity area for PCNA were determined by histomorphometric tests and analyzed statistically. **Results:** Histological examination of the ovaries of CLP treated group revealed severe degeneration of ovarian follicles with sloughing of granulosa cells. The ovarian follicles treated with BM-MSCs showed a normal appearance of oogenesis in comparison to the cyclophosphamide-group. The AT-MSCs treated group rats showed relative improvement of oogenesis but no graafian follicle was detected. **Conclusion**: BM-MSCs and AT-MSCs were impressive in the therapy of ovarian failure in albino rat model where they repair the ovarian structure and protected ovary against CLP-induced toxicity. However BM-MSCs more effective

1. Introduction

Cyclophosphamide is a chemotherapeutic anticancer agent widely used in the treatment of many types of malignant tumors, however it has a potent ovarian toxic effect in the form of: gonadal damage, ovarian follicular fibrosis associated with insufficiency in the ovarian cortex that leads to a significant reduction in the total number of ovarian follicles (**1**) of the most serious side effects of cyclophosphamide is premature ovarian failure (**2**).

Hypergonadotropic secretion from the gonads is the main cause of premature ovarian failure (POF) accompanied with cessation of menses early before 40years old(**3**).About 0.01 % of women experience POF before age of 30, while 1% - 3% of women practice POF before 40years old (**4**).

Infertility remains a significant issue for most women undergoing POF (**5**) 25% of all POF forms nowadays, can be divided as iatrogenic and connected to tumor therapy such as radiotherapy or chemotherapy (**6**).

Furthermore, metabolic diseases represented in classic galactosemia, mumps, toxins, virus infections[e.g. Human Immunodeficiency Virus(HIV)], and lifestyle factors(e.g. cigarette smoking)which are accompanied with the initiation of POF (**7**) Though, the maximal of cases still idiopathic (**8**).

A suitable therapy for some women suffering from menopausal symptoms is the hormonal replacement therapy (**9**) (**Tan et al., 2010**), however its risks that include cardiovascular disease, stroke, breast and endometrial cancer (**10**).

Introduction of stem cell therapy is a promising tool for overcoming of sever disordered particularly, mesenchymal stem cells (MSCs) which play an important role in the field of regenerative medicine where it characterized by their capability for multiline age differentiation and self-renewal (**11**). MSCs are characterized as undifferentiated cells, with high proliferative capacity, and possess a mesodermal differentiation potential (**12**).

Another alternative source of stem cell is the adipose tissue (AT), which can be harvested easily , non-invasive technique and with sufficient amounts than bone marrow (BM) (**13**). Some authors

established that AT encloses stem cells comparable to BM-MSCs **(14).**

The most generally applied origins of MSC is from BM and AT , due to ease of harvest and potential autologous application **(15),** which type of cells is more preferable and appropriate for stem cell treatment is still a remarkable question which waiting for answered **(16).**

Several researches have demonstrated that AT-MSCs and BM-MSCs possessing the same characters , involving the expression and the morphologic pictures of cell surface antigens **(17),** while some researchers recorded an important differences in the biological activity regarding their differentiation capacities and proliferation rates **(18),** whereas, some investigators contradict results, such as AT-MSCs is more efficient concerning possibility of the clinical application than BM-MSCs, however other authors established that BM-MSCs are preferable greatly than AT-MSCs **(19).**

2. Materials and Methods
A-Material:
1) Albino rats:

The experimental study was conducted on 40 adult female albino rats 10 weeks old, their average weight was 200-220 grams, they were divided into four groups. The rats were kept in experimental animal unit, Kasr El Einy University and they were maintained in a controlled environment with access to food and water. The rats were obtained from Holding company for biological products and vaccines (Vacsera), Handling of the animals will be approved by the care of experimental animal committee of Benha medical university and is in compliance with the national research council's guideline. Ethical protocols for animal treatment will be followed**.**

2) Drug and chemicals:

Cyclophosphamide (Endoxane; Baxter; Deerfield, Illinois, USA):

Used as 1 gram vial (dry powder), the required dose for each rat weighed with the drug being dissolved in 0.9% saline; the female rats were injected intraperitoneally with cyclophosphamide, each rat was held in one hand in the dorsal position, cyclophosphamide was injected into the caudal abdominal cavity using a sterile needle **(20)** Each rat was injected with single dose of cyclophosphamide intraperitoneal in a dose of 150 mg/kg -b.w, followed by a dose of 8 mg/kg every week, for 3 weeks **(21).**

3) Isolation and culture of rat bone marrow derived mesenchymal stem cell:

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine medium **(**GIBCO/BRL**).** Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin streptomycin **(GIBCO/BRL) (22)**.

Cells were incubated at 37°C in 5% humidified CO_2 for 12-14 days as primary culture or until formation of large colonies. The resulting cultures were referred to as first-passage cultures. MSCs in culture are characterized by their adhesiveness and fusiform shape. On day 14, the adherent colonies of cells were identified as being MSCs by their morphology and adherence **(23).**

4) Isolation and culture of rat adipose-tissue derived mesenchymal stem cells:

Rats were anesthetized by injection of Xylazine and Ketamine; 1-2cm³ of preperitoneal adipose tissue was removed. The yellowish white tissue was minced and enzymatically digested in DMEM (Dulbecco's modified Eagle's medium) containing 0.075% collagenase2 at 37°c for 60 min. The cell suspension was filtered with 70 μm sieve. The cells were resuspended in MEM medium supplemented with 1% penicillin/streptomycin and 15% FBS (fetal bovine serum). Erythrocytes and other non –adhesive cells were removed from the culture **(24).**

The cells were collected by centrifugation, and subcultured at 1:3- 1:4 ratio and were counted by trypan blue by their shape (a typical fibroblast-like spindle shape). The adherent AD-MSC were expanded and either assessed for gene expression and flow cytometry, or induced to differentiate toward the chondrogenic, adipogenic and osteogenic lineages. The blue staining of the cells after mixing (1:1) will be used as indicator for cell death **(25).**

5)Stem cell preparation and examination were done at Unit of Biochemistry and Molecular Biology, Biochemistry Department, Faculty of Medicine, Cairo University.

(B) Methods:

Forty adult albino female rats were divided into equal four groups, each group include ten rats as following:

1) Group I (Control Group) included 10 rats that were subdivided into**:**

- **Group Ia**(**negative control group**)**:**included of 5 rats that were chosen randomly, and not received any drugs.

- **Group Ib(positive control group):** included of 5 rats that were injected with PBS for sacrificed after 3 weeks.

2) **Group II (Cyclophosphamide group):** included 10 rats were injected with single dose of cyclophosphamide intraperitoneal in a dose of 150 mg/kg b.w of rats., followed by a dose of 8 mg/kg every week for 3 weeks., the rats were sacrificed to obtain their ovaries for histopathological examination **(21).**

3) **Group III (BM-MSCs group) bone marrow mesenchymal stem cells group:** included 10 rats were injected with cyclophosphamide as group II. After 3 weeks, these rats were injected with BM-MSCs intravenous through the tail veins in a single dose $(5 \times 10^6$ cells/ml) in a volume of 0.3 ml of 0.1mol/l PBS (pH 7.4).30 days after injection of stem cells, **(26)** the rats were sacrificed after 30 days from cell therapy.

4) **Group IV (AT-MSCs group) adipose tissue mesenchymal stem cells group:** included 10 rats were injected with cyclophosphamide as group II. After 3 weeks, these rats were injected with AT-MSCs intravenous through the tail veins in a single dose $(5 \times 10^6$ cells/ml)in a volume of 0.3 ml of 0.1mol/l PBS (pH 7.4).30 days after injection of stem cells, the rats were sacrificed **(24).**

At end of the experiment, The rats were anesthetized with 50mg/kg ketamine and 10mg/kg Xylazine before scarification by oblique incision in lower abdominal wall to reach both ovaries by removal of broad ligament of the uterus,. In each rat, the right ovary was removed in its entirety, weighed, fixed in 10% formaldehyde, for histomorphometrical evaluation and the whole left ovary was preserved for the biochemical studies. Paraffin sections were prepared by dehydrating ovaries in ascending grades of alcohol (50%, 70%, 90% and 100%). Three samples of follicular fluid (5 ml each) were mixed, pooled and centrifuged at 4000 g for 5 min. The supernatant was filtered (0.22 μm pore size) to eliminate contaminating cells potentially present in follicular fluid, aliquoted and stored at −20°C until use **(27).**

♦**Histopathological examination:**

After the slides deparaffinized, and both ovaries were obtained and prepared for:

1- **Light microscope examination**: by using hematoxylin and eosin stain **(H&E)** was used for routine histological examination **(28)** Masson's Trichrome stain for detection of collagen fibers **(29).**

2- **Immunohistochemical stain**: staining for **PCNA (Proliferating cell nuclear antigen)** using the avidin–biotin peroxidase complex technique for detection of specific nuclear proteins **(29).**

3- **CD44:** the marker for mesenchymal stem cells **(20) The (Avidin-Biotin Complex)** ABC **technique was carried out as follows for the expression of CD44**. Paraffin sections were deparaffinized and hydrated. After blocking the endogenous activity of peroxidase using10% hydrogen peroxide, the sections were incubated with primary antibodies. CD44 antibody is a mouse monoclonal antibody clone 2A4 **(30).**

CD44-positive cells (20) showed brown cytoplasmic deposits (Lab Vision Corporation, Neomarkers Laboratories, Westing House, and Thurmont, California, USA).

IV- Morphometric study (By Image analysis)

Slides were examined using computerized image analyzer (Lecia Imaging System Ltd., Cambridge, England). Images were captured live on the screen from sections under a light microscope (Olympus BX-40, Olympus Optical Co. Ltd., Japan) with a fixed video camera. From H &E. sections of different experimental groups the number of different types of ovarian follicles was counted **(24).**

1) **The Primordial follicles:** were counted in each slide using the interactive measuring menu with an objective lens of magnification 20 i.e. of total magnification 200. Ten readings were obtained for each specimen of all subgroups&the mean values were obtained.**(26).**

2) **The other types of follicles (primary, secondary, graafian and atretic follicles):** were counted in each slide using the interactive measuring menu with an objective lens of magnification 10**(26).**

3) **Mean area% of collagen fibers:** was measured in the Masson's Trichrome-stained sections using the color detect menu. The image analyzer was used to measure the area of collagen fiber content and was expressed in an area in relation to a standard measuring frame. This was done in 10 microscopic fields for each rat and their mean was obtained. i.e. of total magnification 100**(24).**

4) **Mean area % of PCNA:** was measured in the immunostained sections were counted in each slide using the interactive measuring menu with an objective lens of magnification 20**(24).**

V-Statistical analysis:

The mean values of the data obtained from the image Analyzer were calculated and compared statistically using the statistical package for social sciences (Windows, version 9; SPSS Inc., Chicago, Illinois, USA). Difference between groups under study were examined for statistical significance for the various parameters using the analysis of variance test. This test is used to find a significant difference between more than two groups. A P value less than 0.05 was considered significant. Data were tabulated and represented graphically **(31).**

3. Results:

I- Histological results:

Haematoxyline and Eosin (Hx. &E.) stain:

Group I (control group):

Examination of rat ovarian specimens of the subgroups control female rats showed normal histological structures .The surface of the ovary was covered with germinal epithelium consisting of single layer of simple squamous epithelium; dense connective tissue layer and tunica albuginea beneath the epithelium. The cortex that was full of growing

follicles and corpus luteum. Stroma of ovary between the follicles had dense appearance consisting of loose connective tissue which contained fibroblast like cells **(Fig. 1)**. Primordial follicles appeared under tunica albuginea, the oocytes are enclosed by one layer of flattened squamous follicular cells **(Fig. 1)**. Primary follicles consisted of an enlarged oocyte with zona pellucida surrounding it. Granulosa cells which are polyhedral cells surrounding the oocyte. These cells were arranged in several layers forming multilaminar primary follicle. Flattened cells of theca externa appeared surrounding the follicle **(Fig.1,3)**. Secondary (Antral) follicles appeared consisting of an oocyte surrounded with well-defined zona pellucida. Several layers of polyhedral cells surrounding the oocyte, forming granulosa cell layer. These follicles had wide cavities full of liquor folliculi **(Fig. 2,3)**.

Mature Graafian follicles appeared consisting of an enlarged oocyte surrounded by clear zona pellucida present at one side of the follicle. Single layer of columnar cells was surrounding the ovum forming corona radiata. Wide cavity full of liquor folliculi appeared within the follicle. The wall was lined by multilayer of polyhedral cells of granulosa cells. The ovum was connected to the wall of the follicle by cumulus oophorus.The flattened cells of theca externa were seen surrounding the follicle **(Fig. 4)** . **Corpus Luteum** appeared polygonal shaped granulosa lutein cells which contain eosinophilic cytoplasm surrounded by Theca lutein cells (smaller in size), the vascularity is normal **(Fig. 1,3)**, the medulla contained loose connective tissue with fibroblasts and few smooth muscle cells. No congestion or dilatation of blood vessels and lymphatics were found in the medulla.

Group II (cyclophosphamide treated rats):
Ovarian sections of these rats revealed marked degenerative changes. They revealed multiple markedly degenerated follicles within the cortex, and medulla appeared degenerated having multiple vacuoles with markedly congested blood vessels. Deposition of hyaline material in the interstitial tissue was noticed **(Figs.5,6)**. The degenerated follicles had degenerated oocytes with Pyknotic nuclei and vacuolated cytoplasm. Granulosa cells of these degenerated follicles appeared vacuolated, disorganized with pyknotic nuclei. Also stroma cells appeared vacuolated **(fig.5,6)**. Some of these granulosa cells were exfoliated within the center of the follicles. Interstitial hemorrhage **(Fig.6)**. The cortex showed disfigurement of follicles which are markedly degenerated primordial follicles with faint nuclei and vacuolated cytoplasm were seen, they were covered by normal germinal epithelium (fig. 6). Sections showed also degenerated unilaminar primary follicle. It had

degenerated oocyte with pyknotic nucleus and vacuolated cytoplasm (Fig. 5).

Group III (BM-MSCs group):
Ovarian sections of these rats revealed good improvement as indicated by relatively normal ovarian structure. Sections showed multiple relatively normal growing ovarian follicles, mature graafian follicle and corpora lutea within the cortex of ovary. In the central zone of ovary, medulla appeared consisting of loose connective tissue containing blood vessels **(Figs. 7,8)**. Some degenerated ovarian follicles still appear **(Fig. 7)**. Mature Graafian follicle consisted of normal oocyte surrounded by corona radiata cells. The follicle was surrounded by zona granulosa cells. The oocyte was attached to the wall of the follicle by cumulus oophorus. The cavity within the follicle was full of liquer folliculi. Flattened cells of theca externa surrounded the follicle **(Fig.7)**. Sections also showed relatively normal multilayered primary follicle. It consisted of an enlarged oocyte surrounded by well-defined zona pellucida, follicular granulosa cells which were formed of multilayer of polyhedral cells and peripheral fusiform theca folliculi cells **(Fig. 8)**.

Group IV (AT-MSCs group):
Ovarian sections of this group revealed mild improvement, and the degenerative changes were still evident. Sections showed single relatively normal growing follicle and corpus luteum **(fig.9)**. Sections also showed relatively normal primordial germ vesicle each composed of an oocyte enclosed by one layer of flattened follicular cells **(fig. 9)**. Other section showed relatively normal secondary follicle. It consisted of relatively normal oocyte surrounded by clear zona pellucida and relatively normal granulosa cells. Some granulosa cells were still vacuolated. Cavities appeared within the follicle **(fig. 10)**.

Masson's Trichrome stain (M.T):
Group I (Control group) showed
Ovarian sections of both subgroups control rats revealed very low collagen fibers appearing green in color surrounding the ovarian follicles and within the ovarian stroma **(Figs.11, 12)**. Group II (cyclophosphamide treated group): ovarian sections of the control subgroups revealed massive increase of collagen fibers surrounding the ovarian follicles and around congested blood vessel. Some vacuoles (V) appeared within ovarian stroma **(Fig.13)**. Ovarian sections of Group III (BM-MSCs) revealed marked decrease of collagen fibers surrounding ovarian follicle and within the ovarian stroma **(Fig.14)**. Ovarian sections of Group IV (AT-MSCs) of this group revealed mild to moderate decrease of collagen fibers surrounding ovarian follicles and within the ovarian stroma **(Fig.15)**.

Proliferating cell nuclear antigen (PCNA) immunostaining:

Ovarian sections of the control subgroups showed positive expression of PCNA which appeared brown to black color in mostly all nuclei of granulosa cells **(Figs.16,17).**Ovarian sections of Group II (cyclophosphamide treated group) revealed negative expression for PCNA in some nuclei of granulosa cells which appeared blue in color massive increase of collagen fibers surrounding the ovarian follicles and around congested blood vessel. Some vacuoles (V) appeared within ovarian stroma **(Fig.18).** Ovarian sections of Group III (BM-MSCs group) revealed positive expression for PCNA in most of nuclei of granulosa cells which appeared brown to black color and negative expression for PCNA in some nuclei of granulosa cells which appeared blue in color **(Fig.19).** Ovarian sections of Group IV (AT-MSCs group) showed positive expression of PCNA in most of nuclei of granulosa cells. Some nuclei showed negative expression of PCNA **(Fig.20).**

CD 44immunostaining:

Sections of BM-MSC treated ovarian tissue have shown positive cell membranous reaction for CD 44 in the form of brown pigmentation of cell membrane and

cytoplasm of granulosa cells **(Fig.21).** Sections of AT-MSC treated ovarian tissue have shown positive cell membranous reaction for CD 44 in the form of brown pigmentation of cell membrane and cytoplasm of granulosa cells **(Fig.22).**

Histomorphometrical results:
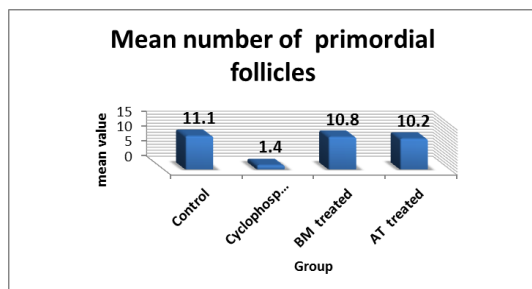**Mean values of number of primordial follicles (Table 1&Graph 1):**

The mean value of the number of primordial follicles has highly significantly decreased (P< 0.01) in cyclophosphamide treated group as compared to control group. In BM stem cell treated group the mean value of the **number of primordial follicles** has highly improved with highly significant difference when compared to cyclophosphamide treated group(P< 0.01).and it has significantly decreased(P < 0.05) in comparison with control ovary. In addition, the average value of number of primordial follicles has highly significantly increased in AT stem cell treated group as compared to cyclophosphamide group (P< 0.01) but it has significantly decreased when compared to control treated gro

up (P < 0.05) and insignificantly decreased when compared to BM stem cell treated group (P>0.05)

Table 1: Comparison between all groups as regarding **number of primordial follicles**.

Group	Mean±SD	Comparison		Sig (p)
Control	11.1±3.73	Control	Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.837**
			AT stem cells treated	**0.548**
Cyclophosphamide treated	1.4±1.07	Cyclophosphamide treated	Control	**0.000****
			BM stem cells treated	**0.000****
			AT stem cells treated	**0.000****
BM mesenchymal stem cells	10.8±2.62	BM stem cells treated	Control	**0.837**
			Cyclophosphamide treated	**0.000****
			AT stem cells treated	**0.625**
AT mesenchymal stem cells	10.2±2.78	AT stem cells treated	Control	**0.548**
			Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.625**

P value > 0.05 means "non significant". ☐ P value < 0.05 means "significant". ☐ P value < 0.01 means "highly significant".


Graph (1): Comparison between all groups as regarding number of primordial follicles.

Mean values of number of primary follicles(Table 2, Graph 2): The mean value of the number of **primary** follicles has highly significantly decreased (P< 0.01) in cyclophosphamide treated

group as compared to control group. In BM stem cell treated group the mean value of the **number of primary follicles** has highly improved with highly significant difference when compared to cyclophosphamide treated group(P< 0.01).and it has significantly decreased (P < 0.05) in comparison with control ovary Also, the average value of **number of primary follicles** has highly significantly increased in AT stem cell treated group as compared to cyclophosphamide group (P< 0.01) but it has significantly decreased when compared to control treated group (P < 0.05) and insignificantly decreased when compared to BM stem cell treated group (P>0.05).

Table 2: Mean values of number of primary follicles: in the studied groups

Group	Mean±SD	Comparison		sig
Control	4.2±1.033	Control	Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.245**
			AT stem cells treated	**0.029***
Cyclophosphamide treated	1.2±0.63	Cyclophosphamide treated	Control	**0.000****
			BM stem cells treated	**0.000****
			AT stem cells treated	**0.000****
BM mesenchymal stem cells	3.7±0.82	BM stem cells treated	Control	**0.000****
			Cyclophosphamide treated	**0.000****
			AT stem cells treated	**0.237**
AT mesenchymal stem cells	3.3±0.63	AT stem cells treated	Control	**0.000****
			Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.237**
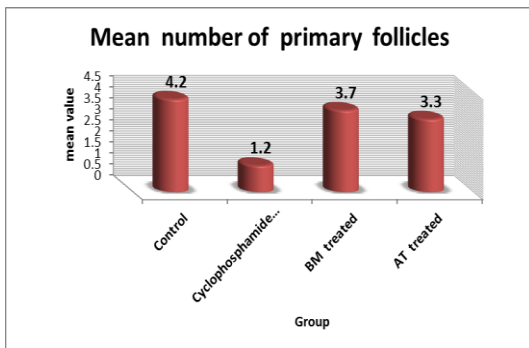
Graph 2: Mean values of number of primary follicles**:** in the studied groups

Mean values of number of secondary follicles (Table 3&Graph 3): The mean value of the number of **secondary** follicles has highly significantly decreased (P< 0.01) in cyclophosphamide treated group as compared to control group. In BM stem cell treated group the mean value of the **number of secondary follicles** has highly improved with highly significant difference when compared to Cyclophosphamide treated group (P< 0.01).and it has significantly decreased(P > 0.05) when matched with control ovaries. Too, the average value of **number of secondary follicles** has highly significantly increased in AT stem cell treated group as compared to Cyclophosphamide group (P< 0.01) but it has significantly decreased when compared to control treated group (P > 0.05) and insignificantly decreased when compared to BM stem cell treated group (P>0.05).

Table 3: Mean values of number of secondary follicles in the studied groups

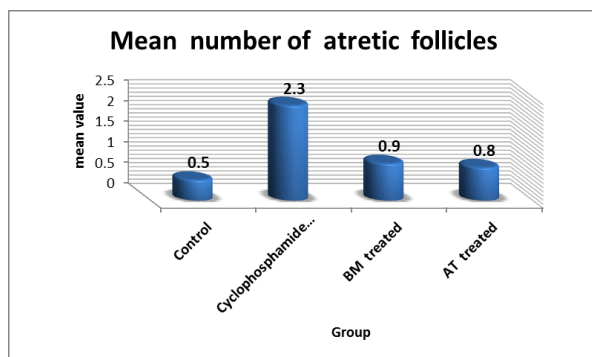| Group	Mean±SD	Comparison		sig
Control	4.0±0.67	Control	Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.425**
			AT stem cells treated	**0.065**
Cyclophosphamide treated	1.1±0.73	Cyclophosphamide treated	Control	**0.000****
			BM stem cells treated	**0.000****
			AT stem cells treated	**0.000****
BM mesenchymal stem cells	3.7±0.95	BM stem cells treated	Control	**0.425**
			Cyclophosphamide treated	**0.000****
			AT stem cells treated	**0.431**
AT mesenchymal stem cells	3.4±0.7	AT stem cells treated	Control	**0.065**
			Cyclophosphamide treated	**0.000****
			BM stem cells treated	**0.431**
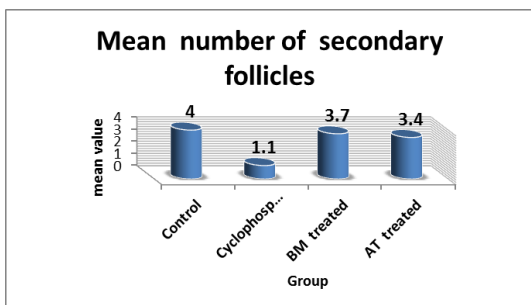
Graph 3: Mean values of number of secondary follicles in the studied groups

Mean values of number of graafian follicles (Table 4&Graph 4): The mean value of the number of primordial follicles has highly significantly

decreased (P< 0.01) in Cyclophosphamide treated group as compared to control group. In BM stem cell treated group the mean value of the **number of graafian follicles** has highly improved with highly significant difference when compared to Cyclophosphamide treated group(P< 0.05).and it has significantly decreased (P > 0.05) when matched with control ovaries. In addition, the average value of **number of Graafian follicles** has highly significantly increased in AT stem cell treated group as compared to Cyclophosphamide group (P< 0.01) but it has significantly decreased when compared to control treated group (P < 0.05) and highly significantly decreased when compared to BM stem cell treated group (P<0.01).

Table 4: Mean values of number of graafian follicles in the studied groups.

Group	Mean±SD	Comparison		sig
Control	1.4±0.52	Control	Cyclophosphamide treated	**0.009****
			BM stem cells treated	**0.131**
			AT stem cells treated	**0.000****
Cyclophosphamide treated	0.6±0.69	Cyclophosphamide treated	Control	**0.009****
			BM stem cells treated	**0.049***
			AT stem cells treated	**0.013***
BM mesenchymal stem cells	1.1±.30	BM stem cells treated	Control	**0.131**
			Cyclophosphamide treated	**0.049***
			AT stem cells treated	**0.000****
AT mesenchymal stem cells	0.0±0.0	AT stem cells treated	Control	**0.000****
			Cyclophosphamide treated	**0.013***
			BM stem cells treated	**0.000****
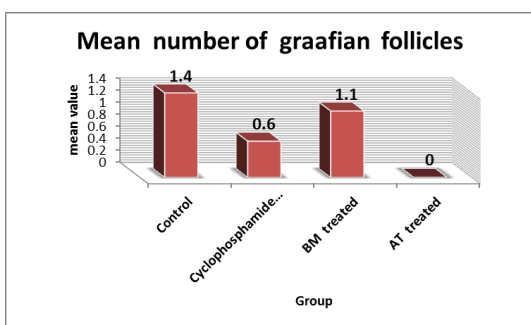
Graph 4: Mean values of number of graafian follicles in the ovarian sections of the studied groups

The **number of atretic follicles (Table 5&Graph 5):** has highly significantly increased (P< 0.01) in cyclophosphamide treated group as compared to control group. In BM stem cell treated group, the **number of atretic follicles** has increased significantly (P> 0.05)in case of when matching with control group it has highly significantly decreased when compared to cyclophosphamide treated group (P<0.01). Also, the **number of atretic follicles** AT has highly

significantly decreased when compared to cyclophosphamide treated group (P<0.01) and significantly increased when compared to control group (P> 0.05) and it has insignificantly decreased when compared to BM stem cell treated group (P>0.05).

Graph5: Mean values of number of atretic follicles in the ovarian sections of the studied groups.

**Table 5: Mean values of number of atretic follicles** in the studied groups.

Group	Mean±SD	Comparison		Sig
Control	0.50±0.53	Control	Cyclophosphamide treated	0.000**
			BM stem cells treated	0. 230
			AT stem cells treated	0. 264
Cyclophosphamide treated	2.30±0.95	Cyclophosphamide treated	Control	0.000**
			BM stem cells treated	0.002**
			AT stem cells treated	0. 000**
BM mesenchymal stem cells	0.90±0.87	BM stem cells treated	Control	0. 230
			Cyclophosphamide treated	0.002**
			AT stem cells treated	0.771
AT mesenchymal stem cells	0.80±0.63	AT stem cells treated	Control	0. 264
			Cyclophosphamide treated	0. 000**
			BM stem cells treated	0.771

The **Mean area% of collagen fiber**s **(Table 6&Graph 6):** it was increased high significantly (P< 0.01) in cyclophosphamide exposed group as compared to control one. In BM stem cell treated group. The **Mean area% of collagen fiber** has markedly improved with highly significant increase when compared to control group (P< 0.01) and it has highly significantly decreased when compared to cyclophosphamide treated group (P<0.01) no

significantly decreased when compared to AT stem cell treated group (P>0.05). Also, the **Mean area% of collagen fiber** has highly significantly decreased in AT stem cell treated group as compared to cyclophosphamide treated group (P< 0.01) and non significantly increased when compared to BM stem cell treated group (P> 0.05) but it has increased high significantly (P< 0.01)when matched with control treated rats .

Table 6: Mean values of Mean±SD of collagen fiber content in the studied groups.

Group	Mean±SD	Comparison		Sig
Control	5.1 ±0.4	Control	Cyclophosphamide treated	0.000**
			BM stem cells treated	0.000**
			AT stem cells treated	0.000**
Cyclophosphamide treated	16.2 ±4.5	Cyclophosphamide treated	Control	0.000**
			BM stem cells treated	0.000**
			AT stem cells treated	0.002**
BM mesenchymal stem cells	9.6 ±1.4	BM stem cells treated	Control	0.000**
			Cyclophosphamide treated	0.000**
			AT stem cells treated	0.125
AT mesenchymal stem cells	10.80±1.9	AT stem cells treated	Control	0.051**
			Cyclophosphamide treated	0.002**
			BM stem cells treated	0.125
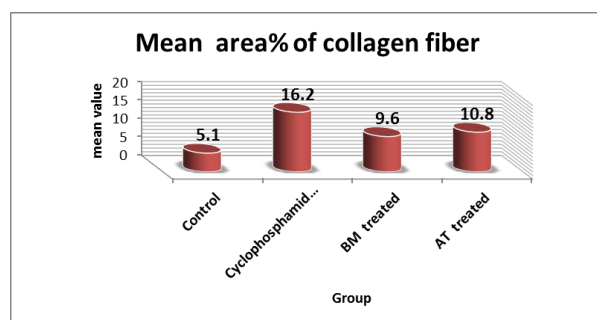
Graph 6: Mean area percent of collagen fiber content in the ovarian sections of the studied groups

Mean values of Mean area% of proliferating cell nuclear antigen immunoreactivity(Table 7&Graph 7):

The **Mean area% of proliferating cell nuclear antigen immunoreactivity** highly significantly decreased (P< 0.01) in cyclophosphamide treated rats in comparison with control un-treated rat group. In BM stem cell treated group The **Mean area% of proliferating cell nuclear antigen immunoreactivity** showed marked improved with significant decrease when compared to control group and non significant increased when compared with AT stem cell treated group (P>0.05) and it has highly significantly

increased when compared to cyclophosphamide treated group (P>0.01).Also, the **mean area% of proliferating cell nuclear antigen immunoreactivity** has highly significantly increased in AT 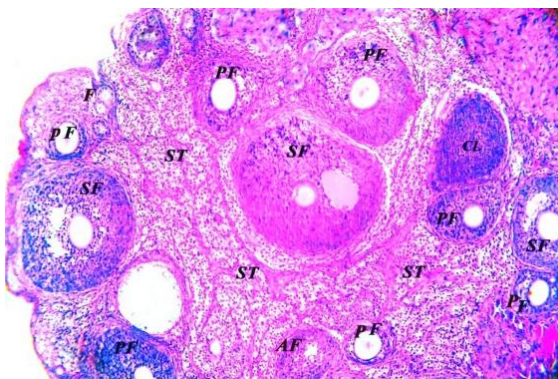stem cell treated group as compared to cyclophosphamide treated group (P< 0.01) and non significantly decreased when compared to BM stem cell treated group (P> 0.05) but it has significantly decreased(P<0.05) when parallel with control treated group.

Table 7: showed Mean values of Mean area% of proliferating cell nuclear antigen immunoreactivity in all groups.

| Group	Mean±SD	Comparison		sig
Control	5.1±0.8	Control	Cyclophosphamide treated	0.000**
			BM stem cells treated	.038
			AT stem cells treated	.032*
Cyclophosphamide treated	0.5±0.3	Cyclophosphamide treated	Control	0.000**
			BM stem cells treated	0.000**
			AT stem cells treated	0.000**
BM mesenchymal stem cells	4.3±.8	BM stem cells treated	Control	.038
			Cyclophosphamide treated	0.000**
			AT stem cells treated	.647
AT mesenchymal stem cells	4.1±1.1	AT stem cells treated	Control	0.051**
			Cyclophosphamide treated	0.000**
			BM stem cells treated	.647
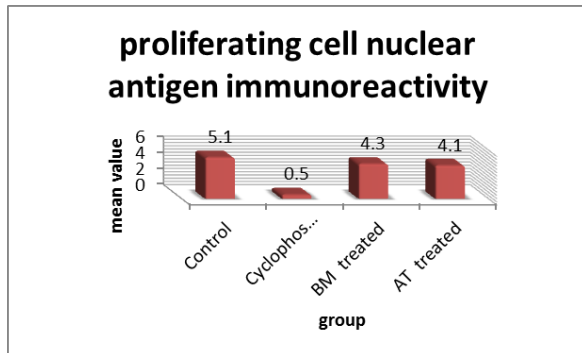
Graph7: Mean area percent of proliferating cell nuclear antigen (PCNA) immunoreactivity in the ovarian sections of the studied groups
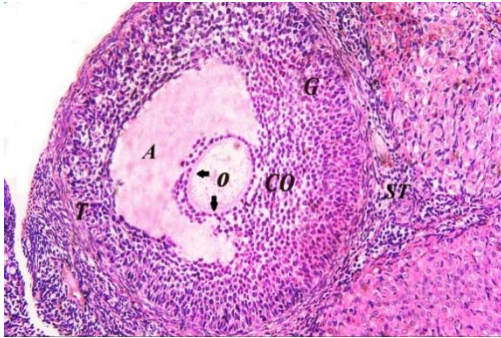

Fig. (2): A Photomicrograph of subgroup Ia(negative control group): rat ovary showing a secondary follicle. It consists of an enlarged oocyte (O) with well-defined zona pellucida (arrow) and surrounded by follicular granulosa cells (G) formed of multilayer of polyhrdral cells and peripheral fusiform theca folliculi cells (T). Two cavities (A) full of liquor folliculi appear within the follicle. **(Hx.&E. X400).**

Fig. (1): A photomicrograph of subgroup Ia(negative control group): adult rat ovary showing wide cortex which is full of primordial follicles (F), primary follicles of different stages (PF), secondary follicles (SF)a, corpus luteum (CL) and single atretic follicle (AF). Stroma cells (ST) in between the follicles are clearly seen.**(Hx.&E. X100).**
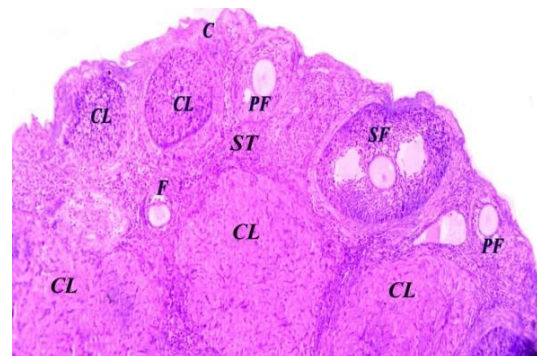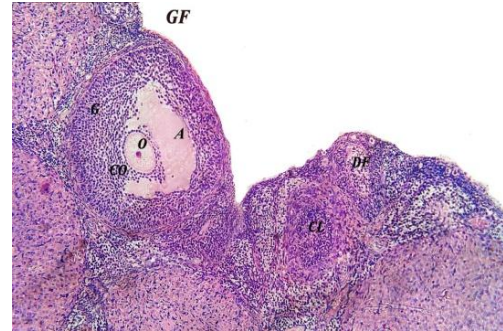

Fig. (3): A photomicrograph of an adult rats of subgroup Ib(positive control group): showing cortex (C) which is full of different growing follicles, primordial follicle (F), primary follicles (PF) and secondary follicles (SC) and multiple corpora lutea (CL) with stroma cells (ST) in between.**(Hx.&E. X100).**
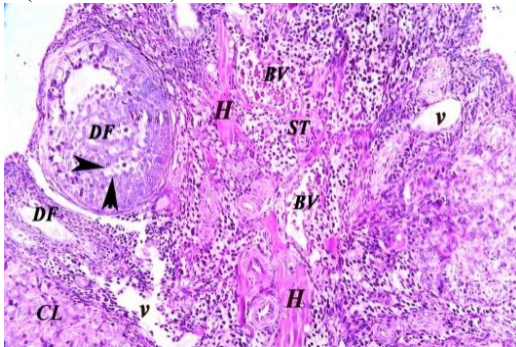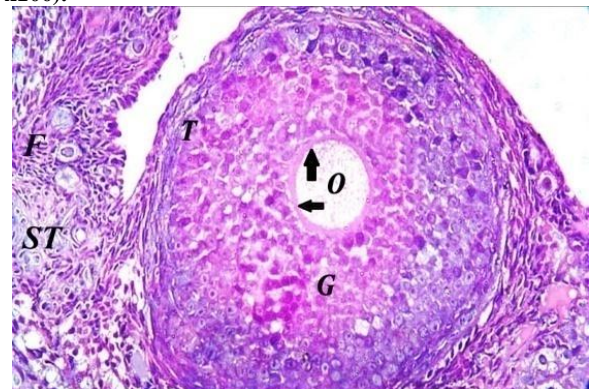
Fig. (4): A photomicrograph of an adult rats of subgroup Ib(positive control group): showing relatively normal mature graafian follicle. It consists of normal oocyte (O) surrounded by normal zona pellucida (arrows). The oocyte is attached to the wall of the follicle by cumulus oophorus (CO). Normal cells of zona granulosa (G) and theca folliculi (T) form the wall of the follicle. Liquor folliculi appears within the antrum (A) of the follicle. Notice, Stroma (ST) cells are seen. (Hx.&E. X400).
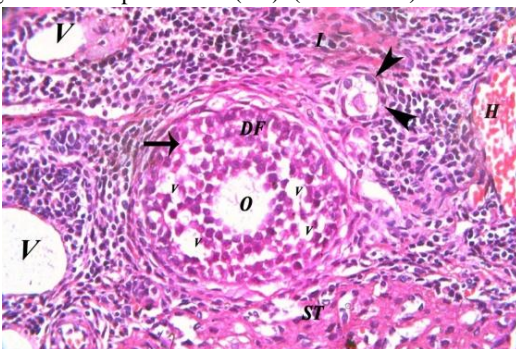

Fig. (5): A photomicrograph of an adult rat ovary of CLP treated group II (CLP group) showing cortex containing multiple markedly degenerated follicles (DF) devoid of oocytes which contain exfoliated granulosa,pyknotic granulosa cells (arrow head). Notice, hyaline material deposition (H), congested blood vessels (BV), presence of vacuoles (V) due to degeneration of stroma cells (ST). notice degenerated corpus luteum (CL). (Hx&E 100).

Fig. (6): A Photomicrograph of an adult rat ovary of CLP treated group II (CLP group) showing a degenerated multilayered follicle (DF) with degenerated oocyte (O). The granulosa cells appear disorganized with pyknotic nuclei (arrow) and vacuolated cytoplasm (V). Interstitial hemorrhage (H) inside congested blood vessels, cellular infilteration (I) and multiple different size vacuoles (V) found inbetween stroma cells (ST). Notice, another primordial follicle contain degenerated oocyte (arrow head). (H&E 400)

Fig. (7): A photomicrograph of an adult rat ovary of group III (BM-MSCs group) showing relatively normal mature graafian follicle (GF) and normal corpus luteum (CL). Mature graafian follicle consists of an oocyte (O) present at one side, its wall is formed of relatively normal cells of granulosa cells (G) and liquor folliculi appears within the cavity of the follicle (A), The oocyte was attached to the wall of the follicle by cumulus oophorus. (CO). Notice, so degenerated follicles (DF) still present in the cortex. (Hx&E x100).
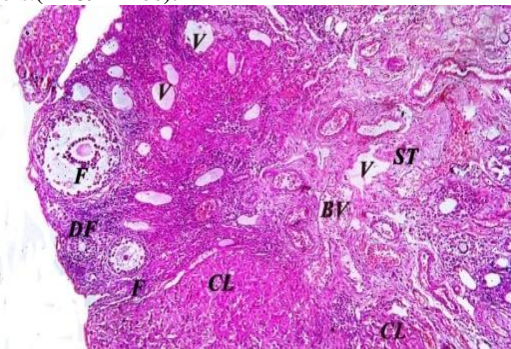

Fig. (8): A photomicrograph of an adult rat ovary of group III (BM-MSCs group): showing relatively normal multilayered primary follicle surrounded by multiple growing primordial follicles (F). It consists of an enlarged oocyte (O) surrounded by well-defined zona pellucida (arrows), follicular granulosa cells (G) which is formed of multilayer of polyhedral cells and peripheral fusiform theca folliculi cells (T). Notice stroma (ST) cells are arranged in clusters.(Hx&E x400).
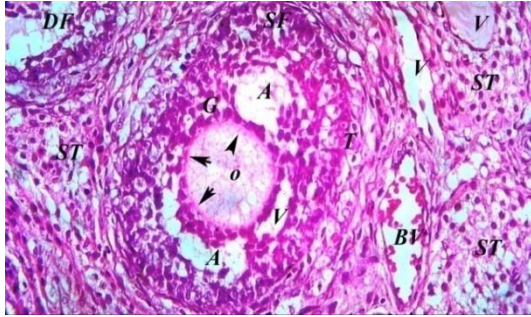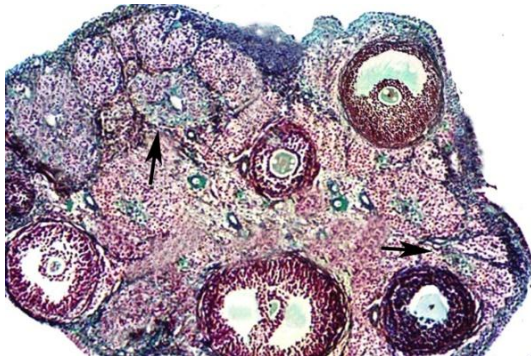

Fig. (9): A photomicrograph of an adult rat ovary of group IV (AT-MSCs group): showing relatively normal growing ovarian follicles (F). Two relatively normal corpora lutea (CL) also appear. Notice: degenerated follicle (DF) and multiple different size vacuoles (V) inbetween stroma cells (ST) with congested blood vessels (BV). (Hx&E x 100).
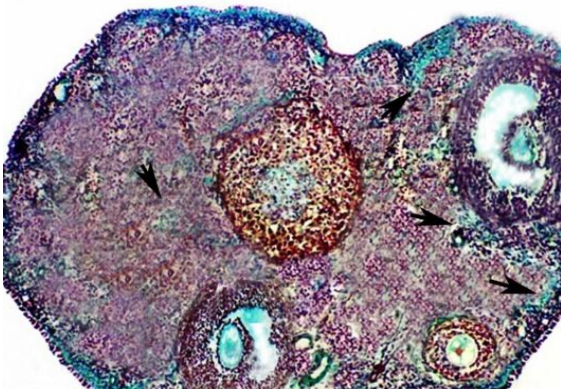
Fig. (10): A photomicrograph of an adult rat ovary of group IV (AT-MSCs group)**:** showing relatively normal growing secondary follicle. It consists of relatively normal oocyte (O) surrounded by clear zona pellucida (arrow head), relatively normal granulosa cells (G), theca folliculi (T)&two cavities (A) appear within the follicle. But still some granulosa cells are vacuolated (V)Notice, multiple different size vacuoles (V) inbetween stroma cells (ST) with dilated blood vessels (BV), degenerated follicle (DF).**(Hx&E. x400).**
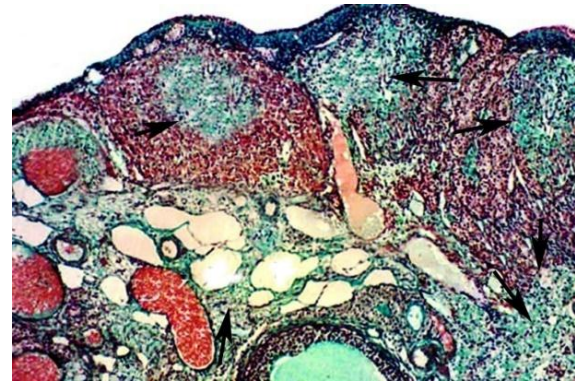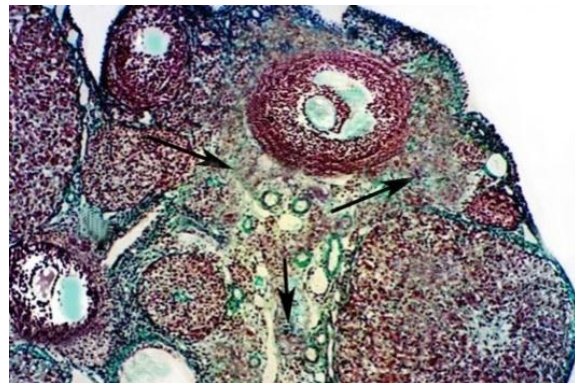
Masson's Trichrome Groups

Fig. (11): A photomicrograph of an adult rat ovary of subgroup Ia(negative control group): showing minimal collagen fibers (arrow) surrounding ovarian follicle. Also few collagen fibers inside stroma (arrow) **(Masson's TrichromeX100).**
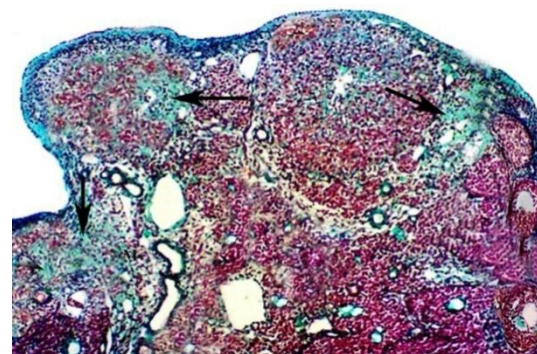

Fig. (12): A photomicrograph of an adult rat ovary of subgroup Ib (positive control group)showing: minimal collagen fibers (arrow) surrounding ovarian follicle. Also few collagen fibers (arrow) appear within the ovarian stroma similar to the control group.**(Masson's TrichromeX100).**

Fig. (13): A photomicrograph of an adult rat ovary of group II (CLP group) showing marked increase in the amount of collagen fibers surrounding degenerated ovarian follicles and inbetween stroma cells (arrows).**(Masson's TrichromeX100).**

Fig. (14): A photomicrograph of an adult rat ovary of group III (BM-MSCs group): showing minimal amount of collagen fibers (arrow) surrounding ovarian follicle and within the ovarian stroma indicating marked improvement similar to control group.**(Masson's TrichromeX100).**
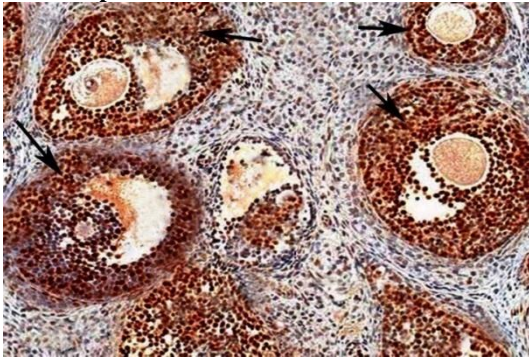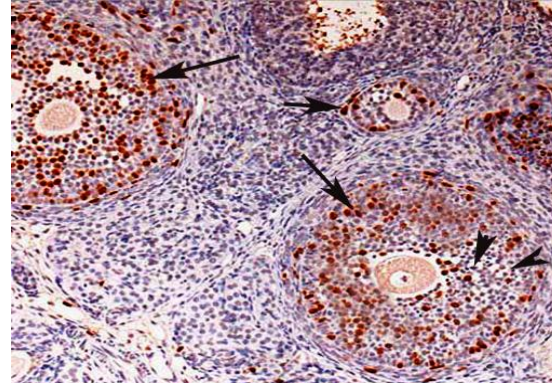

Fig. (15): A photomicrograph of an adult group IV (AT-MSCs group): showing moderate amount of collagen fibers (arrow) surrounding ovarian follicle and within the ovarian stroma, indicating moderate improvement of this group.**(Masson's TrichromeX100)**
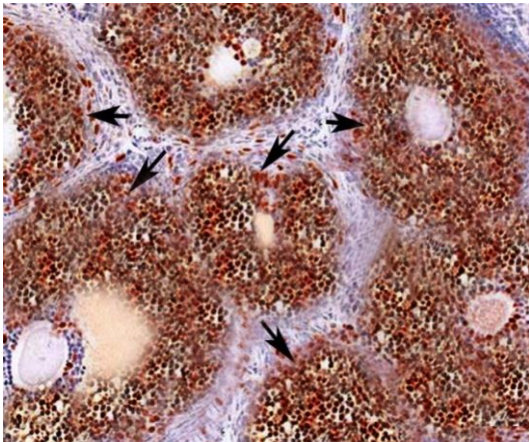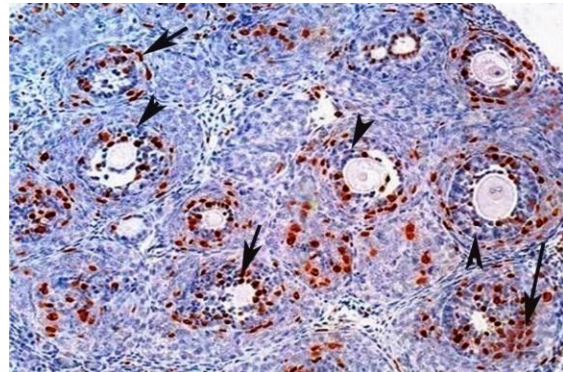
PCNA Groups

Fig. (16): A Photomicrograph of subgroup Ia (control group) adult rat ovary showing different types of ovarian follicles with positive expression of PCNA (arrow) in mostly all nuclei of granulosa cells(brown to black color of nuclei). **(PCNAX200).**

Fig. (17): A Photomicrograph of an adult rat ovary of subgroup Ib (positive control group)showing :different types of ovarian follicles with positive expression of PCNA (arrows) in mostly all nuclei of granulosa cells**(PCNAX200)**

Fig. (18): A photomicrograph of an adult rat ovary group II (CLP group) showing multiple degenerative follicles with negative expression for PCNA in mostly all nuclei of granulosa cells(blue colored nuclei). **(PCNAX200).**
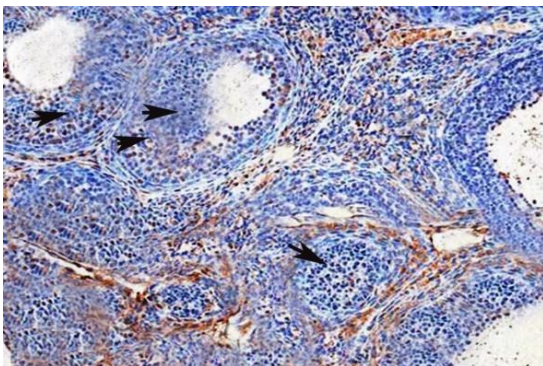

Fig. (19): A photomicrograph of an adult rat ovary group III (BM-MSCs group): showing positive expression for PCNA in most of nuclei (brown to black color - arrow) and negative expression for PCNA in some nuclei (blue color) of granulosa cells (arrow head). **(PCNAX200).**

Fig. (20): A Photomicrograph of an adult rat ovary of g*roup* IV (AT-MSCs group): showing multiple growing ovarian follicles with positive expression of PCNA in nuclei of granulosa cells which appear brown to black colored nuclei (arrow), some nuclei (blue nuclei) show negative expression of PCNA (arrow head) **(PCNAX200).**

Figure (21): A photomicrograph of a section in the rat ovary group III (BM-MSCs group): showing positive cell membranous reaction for CD44 antibody expressed as brown pigmentation of cell membrane and cytoplasm of granulosa cells(black arrow). **(CD44** expressed as **X400).**
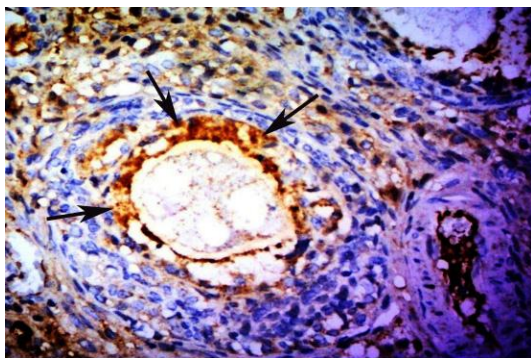
Figure (22): A photomicrograph of a section in the rat ovaries injected by g*roup* IV (Ad-MSCs group): showing positive cell membranous reaction for CD 44 antibody expressed as brown pigmentation of cell membrane and cytoplasm of granulosa cells (black arrow). **(CD 44X400).**

4. Discussion

Cyclophosphamide (CLP) is widely used as a potent alkylating antitumor drug in patients suffering from tumor. CP induces DNA damage in tumor cells, yet, it possessing some adverse side effects through affecting on the patient fertility, where it causing permanent or temporary amenorrhea, reduction of the follicles in ovary makes reduced fertility, or infertility (32,33)

In the present study**,** examination of the CLP treated ovaries has shown massive loss of follicles, markedly shrunken, extensively distorted with the presence of many atretic follicles. Some follicles showed disorganization, as granulosa cells undergo apoptosis and slough from their attachment, the ovum, is anatomically surrounded by the zona pellucida, swimming in the follicular fluid. The zona pellucida and ovum also are subjected for degeneration and necrosis during the growth of ovum. The germinal epithelium has shown dispersed areas of degeneration. These results run with that published by (34)**,**who stated that histological examinations of ovaries in CLP treated group after two months revealed degenerative changes such as tertiary follicles atrophy, degenerative and necrotic changes of the zona pellucida and ovum, these results run with that published by **(35)** who stated decreased thickness of germinal epithelium of the ovarian cortex and oogenesis was completely destroyed, and (24),2016who administrates 2 types of chemotherapy (CLP and busulfan) to rats on ovary and stated substantial degeneration of follicles, marked contracted with irregular shape of the ovary .

The mode of action of chemotherapeutic that leads to degeneration of follicles until now not known well. Some investigators postulated that this loss of follicles may be due to hastening of the natural ovarian aging manner, which attributed to a direct cytotoxic impact on oocytes integrity and apoptosis of a part of primordial follicles, damaging the processes of ovarian folliculogenesis (33).Meirowet al., 2010, postulated that the toxic influence of CLP can be attributed to the releasing of active metabolites as acrolein and phosphoramide mustard from CLP. They proposed that these metabolites can bind to DNA, subsequently leading to failure in synthesis of DNA and cell apoptosis (36).In addition to the previous hypothesis, metabolites of CLP released reactive oxygen species (ROS) via ligation with glutathione , which consequently leads to suppression of the antioxidant defense mechanism of the ovaries (37).

It is found that some chemical substances may interfere with interaction amide the oocyte and granulosa cells(GCs), where GCs are considered one of the most important constituents of ovary as folliculogenesis; and playing an important vital roles inside the follicles such as enhancing the processes of differentiation ,proliferation and apoptotic process.It is proposed that the impact of degeneration of GC on oocytes is in form of liberation of reactive oxygen species (ROS) [38].

It has been clearly observed in the current study extensive interstitial degeneration of ovarian stroma in the form of vacuoles with mononuclear cellular infiltration. marked congested dilated of the blood vessels of the CP-treated group, some dispersed areas of interstitial hemorrhage and these vascular changes in accordance with study of [24](Omar et al.,2016), and the study of [39](Hamzeh et al,2018) in his study received a single dose of 150 mg/kg of CP, IP, for 5days .

It is postulated that vascular changes and local ischemia lead to destruction parts of the normal ovarian cortex which consequently leads to degeneration cortical follicles [33].

Our results confirmed by the morphometric and statistical results which have proved that the mean value of the number of the ovarian follicles (primordial, primary, secondary and graafian) has highly significantly decreased($P< 0.01$)in group treated with CLP as matched with control rat group, The average level of the collagen area % in the ovary between the cortical follicles has highly significantly increased ($P < 0.01$) in CLP treated group as compared to control group. The graafian follicles diameter has highly significantly decreased in CLP treated group relative to control group ($P < 0.01$), These statistical results agreed with (38) who reported that the average number of the ovarian follicles response (primordial, primary, secondary and Graafian) of the control group were higher than that in the CLP testes ($P<0.05$), also the value of the collagen area % of the cyclophosphamide group was high significantly ($P<0.05$) in comparison with the control testes and (35) who found that the Graafian follicles was less than control ($P<0.05$).

The Proliferative cell nuclear antigen (PCNA) functions as an important factor in several necessary cellular procedures, like DNA repair, DNA replication, DNA damage avoidance, sister-chromatid cohesion, cell survival and cell cycle control, in addition to regulating function in the development of follicles in the ovary (40) .

Immunhistochemical study of ovarian section by PCNA stain in the present study stated that CLP treated ovaries, showed degeneration of the germinal epithelium in most ovarian follicles with negative reaction in the nuclei of their oogonia. However, few oogonia in the remaining follicles revealed positive nuclear reaction and the area percent of PCNA immunoreactivity has highly significantly decreased (P< 0.01)in group treated with cyclophosphamide as paralleled with control group. These findings in accordance with (41) and with that published by (9) who demonstrated that starting from one week following CLP therapy,the apoptotic–positive follicles percentage elevated, while at 4 weeks , there are a suppression in   proliferation of cell nuclear antigen expression.

In this work, BM-MSCs transplantation in rat ovaries treated by CLP has shown restoration of architecture and outline of ovarian follicles nearly similar to normal. The ovarian follicles are densely packed and lined with stratified granulosa cells. The adherence of granulosa cells to zona pellucida and theca interna has been also restored, vacuoles have nearly disappeared, however the congestion of the blood vessels has mild improved. These findings agreed with (42) who reported that BM-MSCs, transplanted into ovaries of a cyclophosphamide-treated female mouse model, appeared to differentiate into germ cells, granulosa cells and stroma cells, the study of (17) showed that transplanted BM-SCs could differentiate into germinal cells (immature oocyte) in ovarian follicles of rats and other germ cells of primary and secondary oocyte, in some ovarian follicles of recipient rats. Also reported that after treatment of cyclophosphamide treated infertile group with BM-SCs, the structure of the ovarian follicles restored.

The findings of this study have been supported by the morphometric and statistical results which have proved that in BM stem cell treated group the number of the ovarian follicles (primordial, primary, secondary and graafian) significantly increased in BM stem cell treated group as compared to CLP group (P < 0.05) and the **number of atretic follicles** has significantly increased when compared to control group (P> 0.05), and highly significantly decreased when compared to CLP treated group (P<0.01).

These findings were in agreement with (44) who proved that the number of the ovarian follicles in the BM-MSCs treated and control groups were larger than in the CLP treated testes (P<0.05), also The **number of atretic follicles** has highly significantly increased (P<0.01) in CLP treated group as compared to control group.

In BM stem cell treated group, The mean value of the collagen area %in the ovary between the cortical follicles was markedly improved and it has highly significantly decreased when compared to cyclophosphamide treated group (P<0.01) and this agreed with result of **Boa et al. (2016) (44)**

The BM-MSC-treated ovarian tissue have shown positive cell membranous reaction for CD 44 which means that transplanted BM-MSCs have differentiated into these oogonia as BM-MSCs are CD44 positive. These findings agreed with **(45)** who found that BM-MSCs treated testes were positive for CD 44.

In the current study**,** Histological analysis of the ovaries of rats after AT-MSCs transplantation has shown mild restoration of architecture and outline of ovarian follicles but still less than normal. The primary and secondary follicles have shown early stages of oogenesis but no Graafian follicles were detected, these results are in agreement with those of other investigators who showed that AD-MSC transplanted groups demonstrated an affinity to an elevation in the numbers of early growing ovarian follicles, not reached to differentiate into Graafian follicles directly, Similar findings were recognized by **(40)** who reported the presence of oogonia in the primary and secondary follicles with AT-MSCs transplantation. They demonstrated that injected AT-MSCs differentiated to ovarian germinal cells.

In the present study post ADSCs treatment a vascular alterations was noticed in blood vessel supply of the cortex in ovarian sections which reported also in the work of some authors **(25).** The scientists try to illustrate the increase in blood vessels by the hypothesis that stem cells are responsible for releasing of special mediators which work as a paracrine gland to augment angiogenesis this was illustrated as a side effect of stem cell therapy as they may lead to hemorrhage and veno-occlusive disorders (25, 46).

This has been supported by the morphometric and statistical results which have proved that the mean value of the number of the ovarian follicles (primordial, primary, secondary) was decreased significantly(P<0.05) in contrast with control untreated group and highly significantly increased relative to cyclophosphamide treated group (P< 0.01)., Similar findings were reported by (47) who found that mean number of the primordial, primary and secondary ovarian follicles in rats with AT-MSCs transplantation did not have difference with normal rats and both groups were more than the treated rats with CLP, Another study conflicted by (24) proved

that a significant elevation in the average counts of primordial follicles after ADSCs treatment, as matched with chemotherapy-treated rat group. Comparable data was recorded in other investigations in which the total ovarian response (follicles and corpora lutea) was elevated post ADSCs treatment (26).

The Mean area% of collagen fiber has highly significantly decreased in AT stem cell treated group as compared to cyclophosphamide treated group (P< 0.01) and non-significantly increased when compared to BM stem cell treated group (P> 0.05) and these finding was recorded in the study of (40)

In the current work Immunohistochemical examination of AT-MSC treated ovarian sections stained by PCNA has shown mild positive nuclear reaction in most oogonia in the form of dark brown pigmentation of the nucleus, which has indicated more regeneration and proliferation of cells and moderate reaction in the nuclei of other granulosa cells. This agreed with (24). These results have been supported by the morphometric and statistical results which have proved that the Mean area% of proliferating cell nuclear antigen immunoreactivity has highly significantly increased in AT stem cell treated group as compared to cyclophosphamide treated group (P< 0.01) and non-significantly decreased when compared to BM stem cell treated group (P> 0.05) but it has significantly decreased when compared to control treated group (P< 0.05). results are in agreement with those of some authors(15) who showed that AT-MSC transplanted groups established an affinity for elevation in the numbers of early growing ovarian follicles, which didn`t reach to transformed to Graafian follicles, yet it may continue in the interstitium, playing significant secondary characters in the microenvironment nearby the ovarian oocytes.

But another conflict by (24) who reported that the follicular populations of at different stages of development and ovulation significantly elevated following adipose tissue-derived stem cells , this study not agreed with 26) who reported that human amniotic membrane stem cells (HAMsc) have higher efficacy than AT-SC in treatment of cyclophosphamide–induced ovarian dysfunction .

AT-MSC treated ovarian tissue have shown positive cell membranous reaction for CD44 in the form of brown pigmentation of cell membrane and cytoplasm of oogenic cells which means that transplanted AT-MSCs have differentiated into these oogonia as CD44 characterizes MSCs. This agreed with (24) who reported expression of MSC surface markers CD44 in ATMSCs.

These current finding and with respect to other data, may illustrate the retrieval procedures of oogenesis post MSCs transplantation. There are two hypotheses try to explaining this, 1)the differentiation of MSCs into the target cells via proper generation conditions (48), and 2)the release of growth factors by MSCs into culture medium to diminish germ cell and stromal cell death and to augment folliculogenesis throughout enhancements in the microenvironment (49)**,** that excite the neighborhood oogonial stem cells to reestablish the utility of the host cells (50)**.** The last mode of action is the integration of MSCs with endogenous follicular cells to improve the damaged tissue function**.**

In this study, we show the BM-MSCs have high potency in improving the ovarian function and morphology in rats with chemotherapy-POF. It appears to be greater than the efficiency of AT-MSCs in the restoring the function of ovary, in agreement with other researchers (44) who used BM-MSCs to restore the ovaries in mice of chemotherapy-POF. The transplanted BM-MSCs continued and transferred to ovary of mice ,then differentiated into granulosa cells occupying all different types of ovarian follicles and also corpora lutea. But our results have been conflicted with (51)who found that AT-SCs were preferable than BM-SCs, regarding their ability for maintenance of proliferating process, however had analogous phenotype and morphology, did not expose differentially expressed adipogenic or osteogenic related genes between BM-SCs and AT-SCs.

A new option to treat female infertility or to restore ovarian function is the application of MSC treatment. Several reports have verified the defensive impact of ovarian function resulting from administration of MSCs harvested from different cell origins in POF animal models .this is the first report depending on our knowledge, dealing with the comparative study of BM-MSCs and AT-MSCs in the remedy of ovarian dysfunction, but several preceding trials using BM-MSCs were performed in the same issu**.**

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