

Stem Cell Research 2018-Does Exosomes Derived Bone Marrow Mesenchymal Stem Cells Restore Ovarian Function by Promoting Stem Cell Survival on Experimentally Induced Polycystic Ovary in Adult Female Albino Rats? (Histological and Immunohistochemical Study)- Eman Mohammed Faruk- Benha University

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Introduction

MSCs are a varied population of multipotent stromal stem cells that could be delivered from multiple tissues. Exosomes are small vesicles as products of endocytosis and can be released physiologically under ordinary circumstances by all types of cells. Due to their ability for transmitting hereditary information, they have a vital role in the treatment of many diseases such as liver fibrosis, acute kidney injury and myocardial infarction. PCOS is one of the famous feminine endocrine diseases in the reproductive age and the most common cause of anovulatory and infertility. Chereau published the first description of the disease in 1844, as the change of ovarian morphology. The European Society for Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) in 2003 were published. The diagnostic criteria of the syndrome, according to many studies established during the last decades, in what is called Rotterdam Consensus Criteria. PCOS is a disease with high genetic incidence, and its clinical manifestations mainly include menstrual disorder, secondary amenorrhea, abnormal hormonal serology, loss of hair, obesity, acne, and infertility.

PCOS has been considered as a chronic systemic disease and it is mostly associated with hyper-androgenemia, Oxidative Stress (OS) and chronic inflammation, though the mechanism of pathogenesis has not been well-defined. Many studies have proved that OS level is significantly increased in patients with PCOS, as when these OS were measured by circulating markers, such as Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx). OS level is also observed to be significantly associated with obesity,

hyper-androgenemia, and chronic inflammation, however, OS is considered as an etiological pathogenesis of PCOS, but it is still controversy whether the abnormal levels of OS levels of patients with PCOS derive from the disease or from its complications. FVE is a Mediterranean aromatic seed plant. It is used commonly as a spice and in traditional medicine and as. It was well known that FVE used as a laxative, diuretic, analgesic, bronchodilator and antipyretic.

Phytoestrogen is an active biological substance content present in FVE which can act similar to estrogen, that, increase breast milk production, elevate libido, increase menstrual flow, relieve indigestion and cough. FVE has a clear protective effect against ethanol-induced gastric lesions, which is related to decreases in lipid peroxidation and antioxidant activity . FVE relieves menopausal symptoms in women . In a study, it was reported that FVE could be used as a safe and effective herbal drug for primary dysmenorrhea as used in comparison with mefenamic acid. In Iranian folk medicine, it has been claimed that FVE improves sexual function and infertility in women, however, there is no documented study to clarify this effect. A study showed that the fennel seeds extract has in vitro antioxidant activity and this is important as agonist the oxidative mechanism of PCOS . So for above condition, the aim of our study is to show the efficiency of using BM-MSCs-EX derived exosome in the restoration of hormonal profile and folliculogenesis of induced PCOS in a rat model and the therapeutic effect of MSCs-EX is comparable to FVE with determining the role of oogenesis, meiotic and apoptotic regulators as OCT4.

Materials and Methods

Materials

Testosterone propionate powder was purchased from NAMAA Pharmaceuticals (El-Monofia, Egypt) (CAS number 231 -82-1). It was administered orally as a daily dose of testosterone propionate (dissolved in propylene glycol) 1 mg/100 g body weight for up to 35 days [20]. FVE seeds were bought from Egypt market. The samples were ground by an electrical mill. The aqueous extract was prepared by cold maceration of 100 g of powdered FVE in 500 ml of distilled water for 24 h. Then, the filtered extract was concentrated, dried in vacuum, and the residue was stored in a refrigerator at 2-8°C for use in subsequent experiments. The gavage extract was prepared by solving the powder in a specific volume of normal saline. Multiple doses of FVE at a dose of 150 mg/kg were administered intra-gastrically.

Isolation and characterization of BM-MSCs

Rat BM-MSCs were purchased from the Biochemistry Department, Faculty of Medicine, Cairo University. MSCs culture was prepared according to the method described by Yamazoe, et al. [22]. All cultures were examined using an inverted microscope: Leica DM IL LED with camera Leica DFC295 (Leica Microsystems CMS GmbH, Wetzlar, Germany). BM-MSCs were injected through intravenous route at a single dose of 3×10^6 cells suspended in 0.5 ml Phosphate Buffer Saline (PBS) [23] in which isolation of BM-MSCs was done by flushing out of BM from tibias using PBS (Grand Island, New York, USA) then centrifuged at 1,000 rpm for 5 min. The BM-MSCs were cultured with an RPMI medium (Gibco BRL, USA), 10% fetal bovine serum (FBS, Gibco BRL, USA), and maintained in a cell culture incubator containing 5% CO₂ at 37°C. At 80-90% BM-MSCs confluence, they were detached with 0.25% trypsin-EDTA (Gibco BRL, USA), then subcultured in new flasks. Characterization of BM-MSCs was showed in culture by the presence of spindle-shaped like cells. The flow cytometry (Beckman Coulter) also were used to phenotype BM-MSCs which were suspended (1×10^6 cells/ml) and stained with FITC conjugated monoclonal antibodies, CD29 (Biolegend), and CD90 (Biolegend)..

Conclusion

This study showed for the first time that BM-MSCs derived exosome (MSCs-EX) infusion in induced PCOS rat model showed structural and functional reparative

properties. Further studies are suggested for understanding the exact mechanisms underlying these actions.

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