Effect of melatonin on oxidative stress of differentiated Dopaminergic cells

Hadeer A.M. Ibrahim[a], Abdelaziz M. Hussein[b], Mahmoud Gabr[a], Abdel –Aziz A.F.[c], Rasha A. El-Saeed[c]

[a]Urology and Nephrology Center, Mansoura University, Mansoura, Egypt, [b]Department of Medical Physiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt, [c]Divsion of Biochemistry, Chemistry Department, Faculty of Sciences, Mansoura University, Mansoura, Egypt

Correspondence to Hadeer Ahmed Mohammed Ibrahim, Research Department, Urology and Nephrology Center, Mansoura University, Mansoura 35516, Egypt. Tel: +01061800749; fax: 050-2202717; e-mail: dr.hadeer.qamh@gmail.com, H.A.M@std.mans.edu.eg

Received: 2 February 2023
Revised: 4 April 2023
Accepted: 26 April 2023
Published: 28 September 2023

Egyptian Pharmaceutical Journal 2023, 22:481–489

Background
Although stem cells therapies provide a great deal in the treatment of several disease, they lack their normal functions after transplantation due to inflammation and oxidative stress. Melatonin has a powerful antioxidant ability and can enhance the effect of stem cells.
Objectives
This work aimed to investigate the melatonin's effect on oxidative stress of differentiated adipose-mesenchymal stem cells (AD-MSCs) to dopaminergic (DAergic) cells.
Material and methods
The AD-MSCs cells were characterized after passage 3 by flow cytometry method and divided into four groups: (a) control group that was nontreated AD-MSCs, (b) MSCs+M group that was AD-MSCs cultured with 1 μM melatonin in expansion media for 12 days, (c) DN group that was MSCs treated with neurobasal A media for 12 days, (d) DN+M group which was MSCs cultured with 1 μM melatonin and neurobasal A media for 12 days. After 12 days, the catalase (CAT) activity and malondialdehyde (MDA) level were measured by using ELISA. Also, the gene expression level of MAP-2 was detected.
Results and conclusion
The current study proved that the isolated cells were MSCs due to high expression percentages for CD73 and CD90 and low expression percentages for CD34 and CD45. The DN+M group showed the highest expression of MAP-2 gene when compared to the other different groups ($P \leq 0.05$). Moreover, there was a significant increase in CAT concentration in groups treated with melatonin than other group ($P \leq 0.05$), while, there was no change in MDA level between all groups. It was concluded that melatonin has an effective antioxidative role throughout the differentiation process of AD-MSCs into DAergic neural cells

Keywords:
dopaminergic cells, melatonin, mesenchymal stem cells, oxidative stress

Egypt Pharmaceut J 22:481–489

1687-4315

Introduction

Currently, mesenchymal stem cells (MSCs) therapy is considered as one of the most dynamically developing types of regenerative medicine for treating several diseases as diabetes mellitus, liver injury, and neurodegenerative diseases. Their effectiveness is mostly dependent on their capacity to renew themselves and differentiate *in vitro* under proper conditions into different cell types, as well as on their ability to colonize, remain viable, and permanently engraft into the appropriate target tissue or injured tissue [1,2]. In addition, they can be extracted from various sources like the umbilical cord, placenta, adipose tissue, and skin. Adipose tissue is one of the richest and most important sources of MSCs because of easily obtained via operative procedures like in liposuction, longevity, and rapid growth [3].

Adipose –MSCs (AD-MSCs) are multipotent stem cells. They have the ability to differentiate into myocytes, chondrocytes, osteoblasts, adipocytes, and neuronal cells like dopaminergic (DAergic) cells. Numerous studies have shown that AD-MSCs have the ability to differentiate into a variety of cell types, including neurons which can be used to treat neurological diseases [4–6].

One of the possible reasons that can cause neuronal ischemia and neuronal damage is oxidative stress. Normally, MSCs generate basal reactive oxygen species (ROS) to keep going cell differentiation and

proliferation but incomplete oxygen oxidation in vitro and in vivo leads to increase generation of ROS and DNA damage in MSCs which inhibits the normal function of MSCs through different apoptosis-related pathways and also reduce the self-renewal, proliferation, and differentiation ability of MSCs. Excessively high ROS levels produced by hydrogen peroxide (H_2O_2), hydroxyl radicals, and superoxide anion will induce oxidative stress in MSCs [7]. As a result, the pharmacological preconditioning of MSCs can be considered as a possible way to induce the effect of MSCs [3].

Melatonin, a pineal gland hormone, is a powerful antioxidant and radical scavenger which reduces the negative effects of free radicals. It also has antiapoptotic and anti-inflammatory functions [8]. Owing to its receptor-independent functions that rely on its potential as a free radical scavenger and the transcriptional regulation of antioxidant and detoxification genes, it could be utilized to effectively eliminate hydroxyl radicals, peroxane radicals and Peroxynitrite radicals. Moreover, it has the ability to co-stimulate with different antioxidants to fight the attack of oxidants due to its crucial role in the secretion of antioxidant enzymes such as catalase, glutathione reductase, superoxide, dismutase and glutathione peroxidase [9]. In this study, we aimed to evaluate the antioxidant and radical scavenger effect of melatonin on the differentiated DAergic cells.

Material and methods

Materials

Phosphate-buffered saline (PBS), DMEM-low glucose, fetal bovine serum (FBS), Pencillin-streptomycin antibiotic, neurobasal A medium and B27 supplement were purchased from Gibco, USA. Also, collagenase type I, trypsin/EDTA, and melatonin were purchased from Sigma Aldrich, USA. Anti-CD45, CD34, CD 90, and CD 73 were purchased from BD Bioscience, USA. Catalase (CAT) and Malondialdehyde (MDA) ELISA kits were purchased from Biospes, China. RNeasy Mini Kit, 2X SYBR green master mix and RT2 First Strand Kit were purchased from Qiagen, Germany.

Methods

Isolation and culturing of MSCs from rat adipose tissue

Six Sprague Dawely rats weighing 80–100 g were used to harvest six white adipose tissues from the epididym under sterile conditions. The Mansoura University Ethical Committee gave its approval to all animal investigations. Depending on the isolation protocol of Hasani *et al.*, [10], the MSCs were isolated from adipose tissues. Briefly, PBS was used to wash the adipose tissues. Following that, 0.075% collagenase type I was used for the digestion stage, which was incubated for 45 min at 37°C in a shaking water bath. The digested samples were then centrifuged for 5 min at 1200 rpm at room temperature. Finally, the cells pellet was then suspended in 10 ml of expansion media which included DMEM-low glucose treated with 10% FBS and 1% Pencillin-Streptomycin antibiotic for growing in a 25 cm^2 tissue culture flask. The cells were cultivated in a 5% CO_2 incubator at 37°C at a density of $1\times10^5/cm^2$. When the cells reached 90% confluence, the medium was changed every three days, and trypsin/EDTA was used to subculture the cells.

Phenotype analysis of AD-MSCs

After passage three, about 1×10^6cells were harvested and stained with FITC- or PE-conjugated anti-CD 73, CD 90, CD45, CD34 antibodies to analyze and evaluate that the isolated cells were MSCs. A FACSCalibur flow cytometer was used to analyze the cells (Becton, Dickinson, United States) [11].

Study groups

The isolated MSCs were divided after characterization into 4 groups: a) Negative control group (MSCs), which consisted of normal MSCs, b) Melatonin group (MSCs+M), which consisted of MSCs treated for 12 days with 1 μM melatonin, c) Differentiated neurons group (DN) which consisted of MSCs expanded for 12 days in neurobasal A media including 2% B27 supplement, d) Combined treatment group (DN+M) which included MSCs expanded for 12 days in neurobasal A media including 2% B27 supplement and 1 μM melatonin.

DAergic cells differentiation

After passage 3 and the cells were divided into 4 groups, the expansion media was exchanged for the MSCs+M group with another expansion media contained1 μM melatonin. For the DN+M group, cells were grown in neurobasal A media/expansion media (1:1) including 2% B27 supplement and 1μM melatonin, whereas the culture media for the DN group was changed to neurobasal A media/expansion media (1:1) including 2% B27 supplement. Finally, the control group received only expansion media. All groups were incubated in 5% CO_2 incubator for 12 days at 37°C and examined every 3 days. This protocol was done according to [12] except in the usage of the standard cocktail.

RT-qPCR analysis

The cells from all groups were digested and total RNA was isolated by using RNeasy Mini Kit depending on the manufacture's instruction. The Nanodrop 2000 spectrophotometer (Thermofisher, Massachusetts, USA) was used to measure the concentration of total isolated RNA. About 1 µg of total RNA was used to synthesize First stand c.DNA by RT^2 First Strand Kit.

The step one plus real-time PCR (Applied biosystem, USA) was used to perform Quantitative Real-Time PCR (RT-qPCR) analysis to study different expressions for the Microtubule-associated protein-2 (MAP-2) gene between all groups, which is a specific marker for neurons (Table 1). Glyceraldehyde-3-phosphate (GAPDH) was used as an internal control in the calculation of the relative gene expression, which was then normalised to that of the AD-MSCs group used as a control. These primers were manually designed online at NCBI website (Vivantis Technologies, Malaysia) (Table 1)

Briefly, 25 µL total reaction volume containing 1 µg of cDNA template, 12.5 µl 2X SYBR green master mix, 10 pmol of each primer and nuclease-free water was used for amplification. The following steps was listed as the program's instructions: initial denaturation for 15 min at 95°C, then 40 cycles of denaturation for 15 s at 94°C, annealing for 1 min at 56°C and extension for 1<td:hsp sp="″0.5″/>min at 72°C.

Determination of the antioxidant activity of melatonin using ELISA

At the end of the experiment, the effect of melatonin as antioxidant activator for the secretion of CAT enzyme and decreasing of MDA level were determined by using ELISA kits on all groups after complete differentiation according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was done using SPSS software (version 20). One-way analyses of variance (ANOVA) with LSD post-hoc test were used to find statistically significant different among the three studied groups. Statistical differences were considered significant at P value ≤ 0.05.

Results

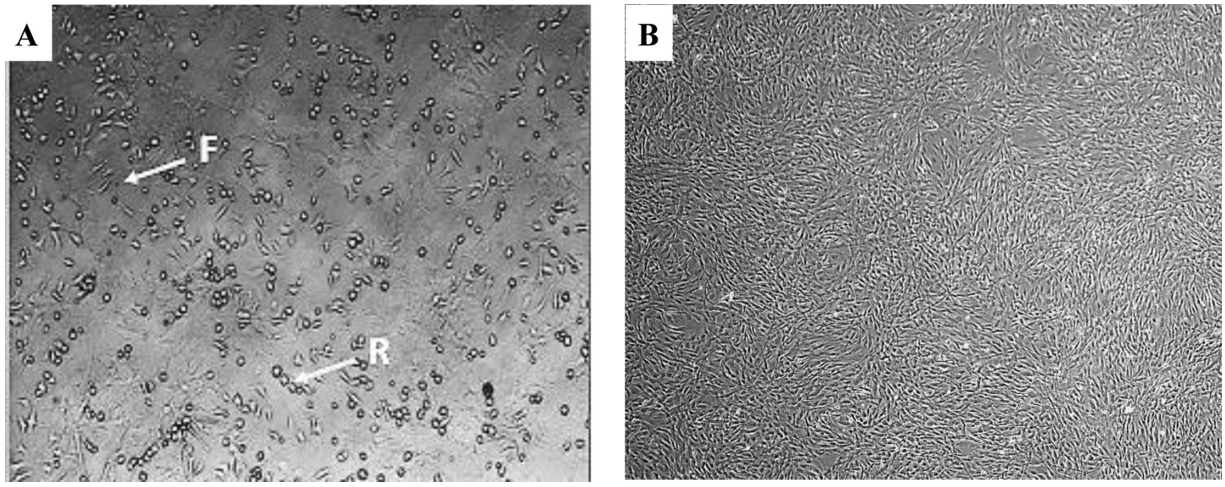
Expansion of AD-MSCs

On the fifth day of isolation, the cells appeared in the form of colonies and clumps with a heterogeneous shape. There was diversity in cellular morphology, including round cells, flat cells and fibroblast-like cells with short and long processes. After 2 weeks,

Table 1 Primer sequence list

Primer	Sequence	Annealing temp.
MAP-2 forward	5'-CAAACGTCATTACTTTACAACTTGA-3'	**56°C**
MAP-2 reverse	5'-CAGCTGCCTCTGTGAGTGAG-3'	
GAPDH forward	5'-TATCGGACGCCTGGTTAC-3'	**56°C**
GAPDH reverse	5'-CTGTGCCGTTGAACTTGC-3'	

Figure 1



Shape of AD-MSCs during the culture. A: at the 5th days from culturing, (R) round cells and (F) fibroblast cells. B: After 2 weeks from isolation.

the fibroblast cells continued to proliferate, multiply, overlap, and become ready to characterize and differentiate while other cells disappeared. (Fig. 1)

Flow cytometry characterization of AD-MSCs

The characterization results by flow cytometery proved that the extracted cells were MSCs because they expressed specific cell surface markers of MSCs as CD90 (90%) and CD73 (64%) and had the negative expression of the unique hematopoietic stem cells surface markers as CD45 (0.65%) and CD34 (0.07%). (Fig. 2).

Effect of melatonin on DAergic differentiation process

At complete differentiation, the cells of MSCs+M group appeared as more elongated spindle-shaped cells with neural-like processes connecting them (Fig. 3a) while the cells in DN group were showed as thinner with shorter extension as illustrated in (Fig. 3b). On the other hand, the cells in DN+ M group showed the best bipolar spindle shape with elongated and branched cell extensions that proved a neuronal appearance (Fig. 3c).

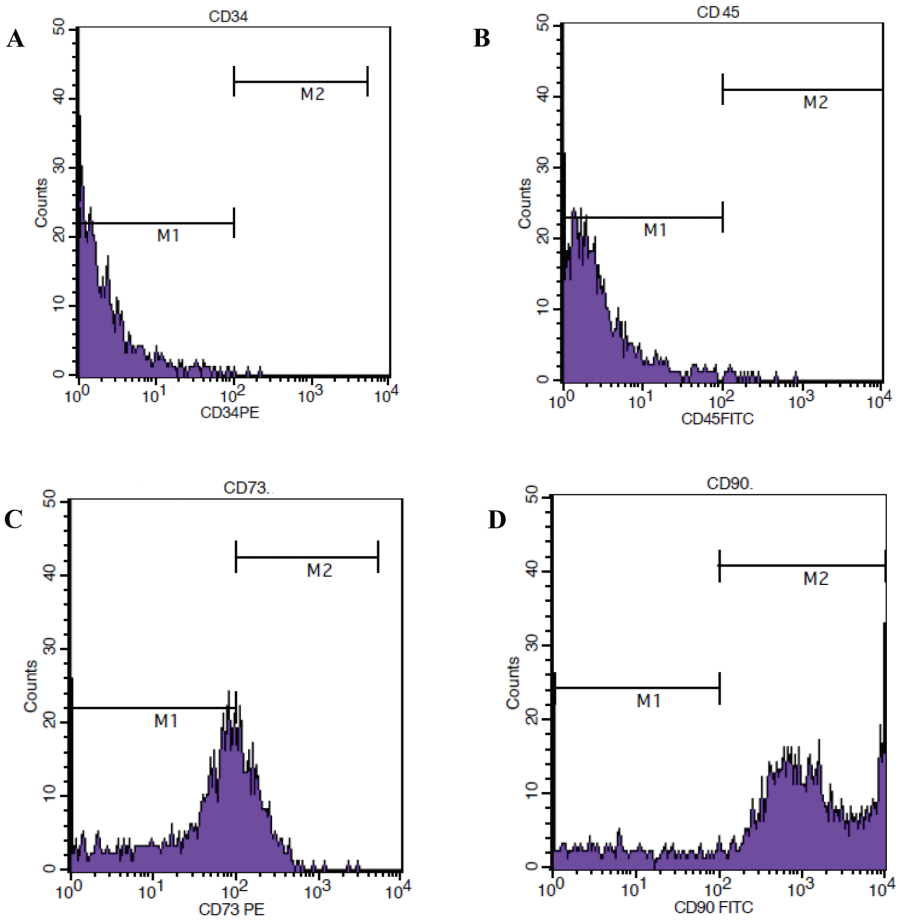
Characterization of differentiated cells by gene expression

There was a marked increase in the expression of the specific neural gene, MAP-2, in the MSCs+M group, DN group and DN+M group when compared to the MSCs group ($P \leq 0.05$). The MSCs+M group showed an increase in the expression level of MAP-2 in contrast to the MSCs group ($P \leq 0.05$). Moreover, the expression level of MAP-2 in the DN group significantly increased than that in the MSCs+M group and MSCs group ($P \leq 0.05$). Furthermore, the highest expression level was showed in DN+M group by comparison to all other groups ($P \leq 0.05$) (Table 2 & Fig. 4).

Melatonin's protective effect on lipid peroxidation

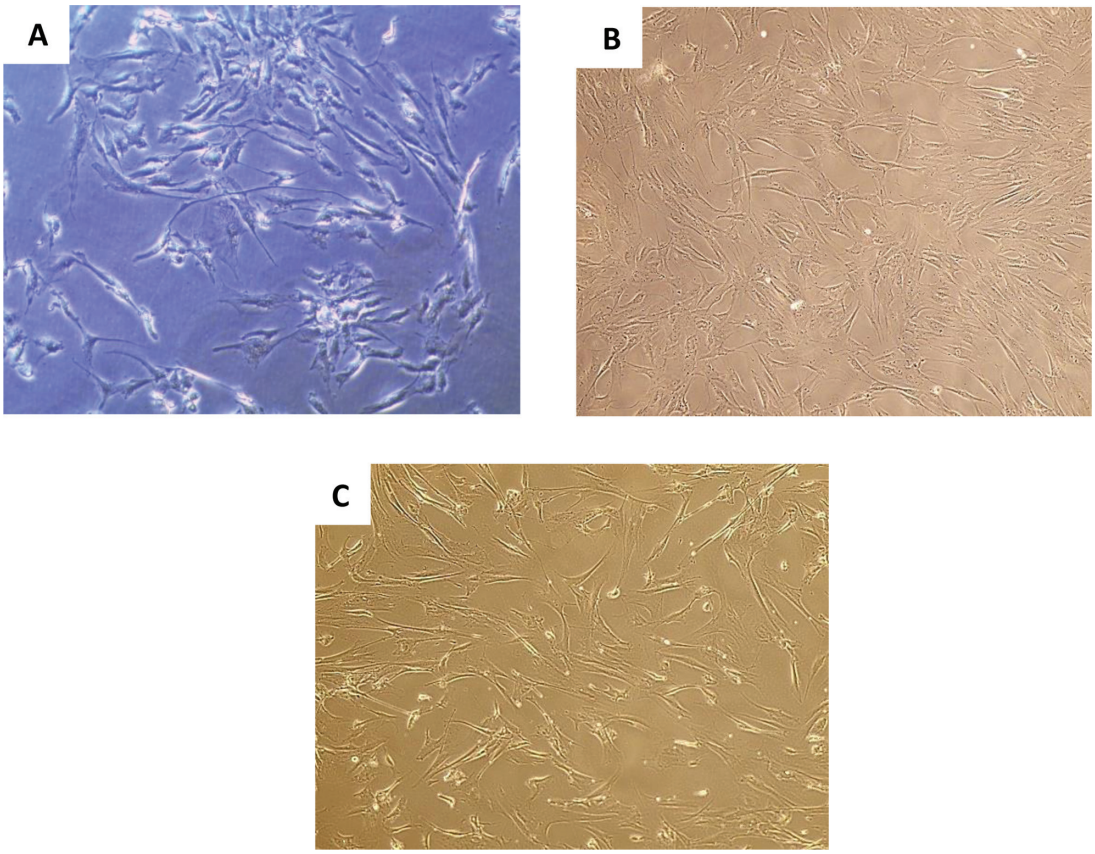
At the end of the experiment, the level of extracellular MDA was measured as a byproduct of lipid peroxidation. The results obtained from the MDA

Figure 2



Flow cytometry results. (A,B) negative expression for CD45 and CD34 as hematopoietic markers, and (C,D) positive expression for MSCs markers like CD90 and CD73. MSC, Mesenchymal stem cells; CD, cluster of differentiation.

Figure 3



Melatonin effect on differentiated DAergic cells. **a** The cells of M+MSCs group which were overlapped and connected with neural-like processes. **b** The cells of DN group that were interconnected by shorter extensions. **c** The cells of DN+M group which were more elongated with branched cell processes.

Table 2 Melatonin effect on MAP-2 expression

Genes	MSCs group	MSCs+M group	DN group	DN+M group
MAP2 (mean±SD)	1[1]	2.25±0.49[a,c,d]	4.95±0.41[a,b,d]	10.99±0.15[a,b,c]

Significant difference compared to corresponding [a]MSCs group, [b]MSCs+M group, [c] DN group and [d] DN+M group by one-way analysis of variance (ANOVA) followed by *posthoc* multiple comparisons (LSD) at $P \leq 0.05$. 1: The SD of this group is zero because it is the control group and we calculate the relative gene expression for the other groups dependent on it.

assay showed no significant difference in the level of MDA between DN+M group, MSCs+M group, MSCs group, and DN group. The DN group, DN +M group and MSCs+M group showed exactly the same MDA level which it was slightly increase by comparison with the MSCs group (Table 3, Fig. 5).

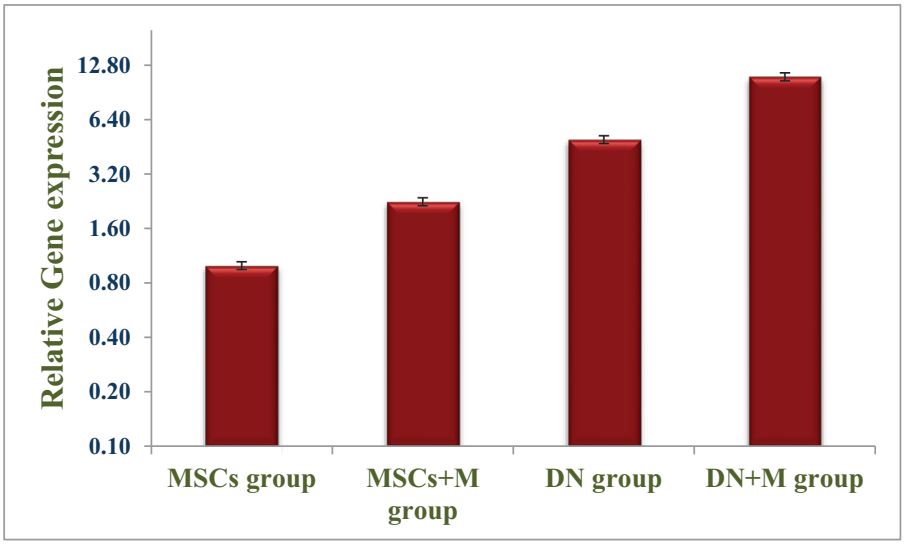
Antioxidant effect of melatonin on CAT activity

No significant difference appeared in CAT activity between the DN group and MSCs group. Also, the MSCs+M group and DN+M group exhibited nearly the same CAT activity. While there was a marked increase in CAT activity in the MSCs+M group when compared with the MSCs group and DN group ($P \leq 0.05$). Moreover, the DN+M group showed higher CAT activity than that in the MSCs group and DN group ($P \leq 0.05$). (Table 4, Fig. 6).

Discussion

Adipose tissue is an important and the highest source of MSCs, which have the capability for differentiating into adipogenic, chondrogenic and osteogenic cell lineages [13]. Different studies documented the potential ability of AD-MSCs to differentiate into non-mesodermal cells like neural cells, hepatocytes and pancreatic beta cells [13]. Due to the minimal neural regeneration after injury, recent research have documented the effectiveness and safety usage of MSCs to provide a great solution in the treatment

Figure 4



Melatonin effect on MAP-2 gene expression. There was a marked increase in the expression of MAP-2 in the treated groups in contrast to MSCs group $P \leq 0.05$. The DN+M group showed the highest expression of MAP-2 compared to other groups $P \leq 0.05$.

Table 3 Melatonin effect on MDA level

Groups	MDA Conc. (nmol/L)
MSCs group (mean±SD)	0.25±0.01
MSCs+M group (mean±SD)	0.26±0.01
DN group (mean±SD)	0.26±0.01
DN+M group (mean±SD)	0.26±0.01

There was no significant difference compared to corresponding [a]MSCs group, [b]MSCs+M group, [c] DN group and [d] DN+M group by one-way analysis of variance (ANOVA) followed by *posthoc* multiple comparisons (LSD).

of several neurological disorders like amyotrophic lateral sclerosis, Parkinson's disease and multiple sclerosis [14,15].

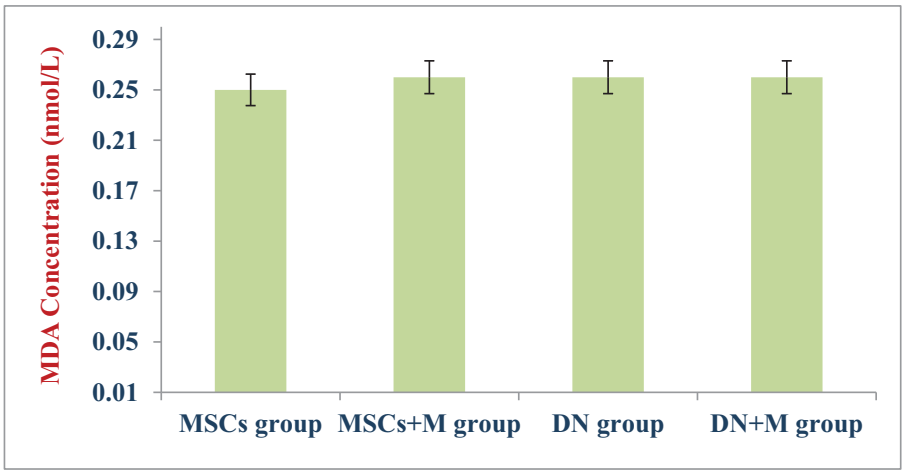
In the present study, the isolated cells showed the specific fibroblast shape of MSCs with high expression for MSCs markers, CD90 (90%) and CD73 (64%), and negative expression for hematopoietic stem cells markers, CD45 (0.65%) and CD34 (0.07%), (Figs. 1 and 2) which agreed with Hasani *et al.*, [10] and the international society for cellular therapy who confirmed the general properties of MSCs involving: plastic adherence together with a specific cell surface markers as CD90, CD73, CD105 and lack of CD45, CD14, CD34, and human leucocyte antigen-DR [16].

Several reports have attempted to induce MSCs differentiation into DAergic neurons to show their benefits in cell therapies for neurodegenerative diseases like Parkinson's disease. Various small molecules, cytokines and neurotrophic factors have been used in different differentiation protocols. Also, growth factors such as NGF, PDGF, BDNF and bFGF can be used in the induction of stable neural differentiation of MSCs [17]. Moreover, Equbal and his colleagues proved the ability of human Wharton's jelly-derived MSCs to differentiate into mature DAerigic cells by two-stage differentiation protocol using cocktail of ascorbic acid, FGF2, EGF, cAMP, NGF, and BDNF in the neurobasal A-medium for 10 days in the first stage until the neurospheres formed which were then dissociated in the second stage into single cells in the presence of induction medium and incubated for 28 days under the hypoxic conditions with 50% media change on every third day. [18].

Recently, it has been found that melatonin neurohormone can be used as an excellent promoter agent to improve the immunomodulatory, regenerative, angiogenic and antiapoptotic capacities of MSCs [19]. Its main functions are regulation of the circadian rhythm, clearance of free radicals, immunity improvement and prevention of biomolecules oxidation [20]. Further, different studies showed that it improved the function of bone marrow-MSCs (BM-MSCs) in sepsis-induced kidney injury and pulmonary ischemia–reperfusion injury cases [21]. Also, it has healing properties by significantly increasing angiogenesis, collagen synthesis, and scar quality [21].

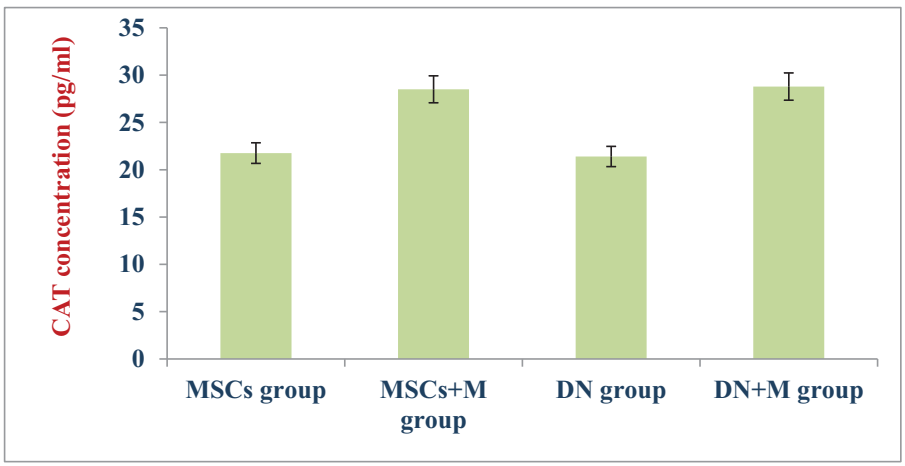
In our study, the differentiation protocol documented the melatonin effect in MSCs+M group to differentiate AD-MSCs into cells with neural-like processes

Figure 5



Melatonin effect on MDA level. There was no significant difference in MDA level between MSCs+M group, DN group and DN+M group but it slightly increase in these groups when compared to the negative control group.

Figure 6



Melatonin effect on CAT concentration. The treated groups with melatonin (DN+M and MSCs+M groups) had a marked increase in CAT concentration when compared to the negative control group and DN group, which nearly produced the same concentration of CAT $P \leq 0.05$.

Table 4 CAT activity in different groups

Groups	CAT Conc. (pg/ml)
MSCs group (mean±SD)	21.76±0.03[bd]
MSCs+M group (mean±SD)	28.50±0.03[ac]
DN group (mean±SD)	21.40±0.02 [bd]
DN+M group (mean±SD)	28.79±0.04 [ac]

Significant difference compared to corresponding [a]MSCs group, [b]MSCs+M group, [c] DN group and [d] DN+M group by one-way analysis of variance (ANOVA) followed by *posthoc* multiple comparisons (LSD) at $P \leq 0.05$.

connecting them (Fig. 3a). Moreover, the DN+M group cells appeared as a perfect shape for differentiated DAergic cells after 12 days (Fig. 3c). In contrast, the cells of DN group were not completely differentiated (Fig. 3b) that proved the ability of melatonin to induce the differentiation of DAergic neurons from AD-MSCs.

Also, the qPCR results confirmed the above result by which there was a significant expression of specific marker for neurons which is MAP-2 in DN+M group. This marker is essential in the synthesis of microtubules and stabilization of the dendritic shape by crosslinking with intermediate filaments during neural development [22]. But the expression level of this gene was decreased in the other treated groups (Table 1& Fig. 4).

Moreover, melatonin acts as a protective agent through different mechanisms like the reduction of oxidative process, blocking the mitochondrial permeability

transition pore and activating uncoupling proteins. Also, it modulates the pro- and anti-inflammatory cytokines to regulate the inflammatory reaction and prevents the cyclooxygenase (COX) expression and inducible nitric oxide synthase (iNOS) [23]. Strong antioxidant melatonin lowers ROS levels by quickly scavenging free radicals, activating antioxidant enzymes, including glutathione peroxidase (GPX) and superoxide dismutase (SOD), as well as by modifying the activity of the complex I and III, which decrease ROS formation [24].

For MSCs to acquire the ideal structure, adapt, and respond to environmental stimuli which affect their self-renewal, differentiation, and metabolism, mitochondrial dynamics are crucial. On mitochondrial dysfunctions and dynamics, melatonin has significant effects. Melatonin can improve mitochondrial dynamics and functions in an experimental model of obesity and related diabetes, regulate mitochondrial dynamics and minimize neuronal damage in prion disorders and improve mitochondrial biogenesis in ischemia/reperfusion-induced cardiac infarction. [25–29].

In 2016, Shuai *et al.*, proved that the pretreatment of BM-MSCs with melatonin can efficiently improve the BM-MSCs function during invitro prolonged passaging by activating antioxidant defense, suppressing cell senescence mechanism, and maintaining the gene expression that regulate cell stemness [30]. Also, Al-Otaibi and his colleagues documented the ability of melatonin to enhance wound healing by cell apoptosis reduction and stimulation of antioxidant enzyme secretion. They found a significant increase in the secretion of CAT, SOD, and other antioxidant enzymes with a marked decrease in the MDA, nitric oxide, and protein carbonyl levels in all groups received BM-MSCs treated with melatonin [21].

In 2019, Hu and Li suggested that melatonin can improve the motility, viability, paracrine secretion and proliferation of MSCs [7]. Melatonin can improve the function of mitochondria of mouse MSCs and so, increase their stress tolerance [31]. Lee and his colleagues documented that melatonin can ameliorate the regenerative ability of MSCs in chronic kidney disease and ischemia by upregulating the cellular prion protein (PrPC) that is included in self-renewal, angiogenesis, and differentiation of stem and progenitor cells [32].

Moreover, different studies documented the high expression of melatonin1 and melatonin 2 receptors on the surface of MSCs. The effect of melatonin on MSCs was performed via receptor-dependent and receptor-independent pathways by which the antioxidant enzymes like CAT and SOD-1 were highly expressed, which lead to increase the MSCs resistance to H_2O_2-dependent apoptosis and increasing their therapeutic values [33,34].

These findings support our results which there was a marked increase in CAT activity in the two groups treated with melatonin (MSCs+M and DN+M) than that in the other groups without melatonin (Fig. 5), while, there was no difference in MDA levels between all groups (Fig. 6). This indicated the protective role of melatonin to increase the activity of antioxidant enzymes and it can be considered as a powerful tool in regulating the antioxidant and ROS secretion to improve the function of MSCs and differentiated MSCs following in vitro transplantation.

Conclusion

From the above results, we concluded that adipose tissue is considered as one of the richest source of MSCs and melatonin can induce the complete differentiation of AD-MSCs into DAergic cells. Moreover, melatonin can induce the secretion of antioxidant enzymes as CAT to fight the attack of oxidants and proved its effect as a powerful antioxidant and radical scavenger. So, the differentiation of AD-MSCs into DAergic cells with melatonin can provide a great tool to treat Parkinson's disease.

Acknowledgements

Not applicable.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare there is no conflict of interest.

References

1 Rodriguez-Fuentes DE, Fernàndez-Garza E, Samia-Meza JA, Barrera-Barrera SA, Caplan AI, Barrera-Saldaña HA. Mesenchymal stem cells current clinical applications: a systematic review. Arch Med Res 2021; 52:93–101.

2 Aierken A, Li B, Liu P, Cheng X, Kou Z, Tan N, *et al.* Melatonin treatment improves human umbilical cord mesenchymal stem cell therapy in a mouse model of type II diabetes mellitus via the PI3K/AKT signaling pathway. Stem Cell Res Ther 2022; 13:164.

3 Zhang Z, Zhang M, Zhang Z, Sun Y, Wang J, Chang C, *et al.* ADSCs combined with melatonin promote peripheral nerve regeneration through autophagy. Int J Endocrin 2022; 2022:5861553.

4 Moon MY, Kim HJ, Choi BY, Sohn M, Chung TN, Suh SW. Zinc promotes adipose-derived mesenchymal stem cell proliferation and differentiation towards a neuronal fate. Stem Cells Int 2018; 2018:5736535.

5 Chi K, Fu RH, Huang YC, Chen SY, Hsu CJ, Lin SZ, *et al.* Adipose-derived stem cells stimulated with n-butylidenephthalide exhibit therapeutic effects in a mouse model of Parkinson's Disease. Cell Transp 2018; 27:456–470.

6 Muñoz MF, Argüelles S, Medina R, Cano M, Ayala A. Adipose-derived stem cells decreased microglia activation and protected dopaminergic loss in rat lipopolysaccharide model. J Cell Physiol 2018; 234(8):13762–13772.

7 Hu C, Li L. Melatonin plays critical role in mesenchymal stem cell-based regenerative medicine in vitro and in vivo. Stem Cell Res Ther 2019; 10:13.

8 Chen YT, Chuang FC, Yang CC, Chiang JY, Sung PH, Chu YC, *et al.* Combined melatonin-adipose derived mesenchymal stem cells therapy effectively protected the testis from testicular torsioninduced ischemia-reperfusion injury. Stem Cell Res Ther 2021; 12:370.

9 Wang B, Wen H, Smith W, Hao D, He B, Kong L. Regulation effects of melatonin on bone marrow mesenchymal stem cell differentiation. J Cell Physiol 2019; 234:1008–1015.

10 Hasani MHMM, Nabiuni M, Parivar K, Yari S, Sahebi A, Miyan J. the effects of embryonic cerebrospinal fluid on the viability and neuronal differentiation of adipose tissue-derived stem cells in wistar rats. Cell J 2020; 22:245–252.

11 Hasani S, Boroujeni ME, Aliaghaei A, Baghai K, Rostami A. Dopaminergic induction of human adipose-derived mesenchymal stem cells is accompanied by transcriptional activation of autophagy. Cell Biol Int 2018; 42:1688–1694

12 Phonchai R, Phermthai T, Kitiyanant N, Suwanjang W, Kotchabhakdi N, Chetsawang B. Potential effects and molecular mechanisms of melatonin on the dopaminergic neuronal differentiation of human amniotic fluid mesenchymal stem cells. Neurochem Int 2019; 124:82–93.

13 Teshima T, Okamoto K, Dairaku K, Nagashima T, Michishita M, Suzuki R, *et al.* Generation of insulin-producing cells from canine adipose tissue-derived mesenchymal stem cells. Stem Cells Int 2020; 8841865.

14 Tirpáková M, VašÍÌ**c**ek J, Svoradová A, Baláži A, Tomka M, Bauer M, *et al.* Phenotypical characterization and neurogenic differentiationof rabbit adipose tissue-derived mesenchymal stem cells. Gen 2021; 12:431.

15 Ababneh NA, Al-Kurdi B, Jamali F, Awidi A. A comparative study of the capability of MSCs isolated from different human tissue sources to differentiate into neuronal stem cells and dopaminergic-like cells. Peer J 2022; 10:e13003.

16 Hernández R, Jiménez-Luna C, Perales-Adán J, Perazzoli G, Melguizo C, Prados J. Differentiation of human mesenchymal stem cells towards neuronal lineage: clinical trials in nervous system disorders. Biomol Ther 2020; 28:34–44.

17 Javanmard F, Koruji M, Moradi F, Kochakian MR, Moosavi SA, Asgari HR. Human umbilical cord mesenchymal stem cells differentiated into neuron-like cells via laminin and schwann cells. Arch Iran Med 2021; 24:548–555

18 Equbal Z, Baligar PN, Srivastava M, Mukhopadhyay A. A two-stage process for differentiation of Wharton's jellyderived mesenchymal stem cells into neuronal-like cells. Stem Cells Int 2021; 6631651.

19 Chan YH, Ho KN, Lee YC, Chou MJ, Lew WZ, Huang HM, *et al.* Melatonin enhances osteogenic differentiation of dental pulp mesenchymal stem cells by regulating MAPK pathways and promotes the efficiency of bone regeneration in calvarial bone defects. Stem Cell Res Ther 2022; 13:73.

20 Roy J, Tsui KC, Ng J, Fung ML, Lim LW. Regulation of Melatonin and Neurotransmission in Alzheimer's Disease. Int J Mol Sci 2021; 22:6841.

21 Al-Otaibi AM, Al-Gebaly AS, Almeer R, Albasher G, Al-Qahtani WD, Abdel Moneim AE. Melatonin pre-treated bone marrow derived-mesenchymal stem cells prompt wound healing in rat models. Biomed Pharmaco 2022; 145:112473.

22 Yousefia B, Davood S, Faghihia F, Joghataeia TM, Latif N. Evaluation of motor neuron differentiation potential of human umbilical cord blood-derived mesenchymal stem cells, *invitro*. J Chem Neuroanat 2017; 81:18–26

23 Galano A, Guzman-Lopez EG, Reiter RJ. Potentiating the benefits of melatonin through chemical functionalization: possible impact on multifactorial neurodegenerative disorders. Int J Mol Sci 2021; 22:11584.

24 Luchetti F, Carloni S, Nasoni MG, Reiter RJ, Balduini W. Tunneling nanotubes and mesenchymal stem cells: New insights into the role of melatonin in neuronal recovery. J Pineal Res 2022; 73:e12800.

25 Lv J, Yi Y, Qi Y, Yan C, Yin W, Meng L, *et al.* Mitochondrial homeostasis regulates definitive endoderm differentiation of human pluripotent stem cells. Cell Death Discov 2022; 8:69.

26 Ren L, Chen X, Chen X, Li J, Cheng B, Xia J. Mitochondrial dynamics: fission and fusion in fate determination of mesenchymal stem cells. Front Cell Dev Biol 2020; 8:580070.

27 Chang JY, Yu F, Shi L, Ko ML, Ko GY. Melatonin affects mitochondrial fission/fusion dynamics in the diabetic retina. J Diabetes Res 2019; 2019:8463125.

28 Zhang X, Zhao D, Wu W, Shah SZA, Lai M, Yang D, *et al.* Melatonin regulates mitochondrial dynamics and alleviates neuron damage in prion diseases. Aging 2020; 12:11139–11151.

29 Agil A, Chayah M, Visiedo L, Navarro-Alarcon M, Ferrer JMR, Tassi M, *et al.* Melatonin improves mitochondrial dynamics and function in the kidney of zucker diabetic fatty rats. J Clin Med 2020; 9:9.

30 Shuai Y, Liao L, Su X, Yu Y, Shao B, Jing H, *et al.* Melatonin treatment improves mesenchymal stem cells therapy by preserving stemness during long-term in vitro expansion. Therano 2016; 6:1899.

31 Go G, Yoon YM, Yoon S, Lee G, Lim JH, Han SY, *et al.* Melatonin protects chronic kidney disease mesenchymal stem/stromal cells against accumulation of methylglyoxal via modulation of hexokinase-2 expression. Biomol Ther 2022; 30:28–37.

32 Lee JH, Yoon YM, Song KH, Noh H, Lee SH. Melatonin suppresses senescence-derived mitochondrial dysfunction in mesenchymal stem cells via the HSPA1L-mitophagy pathway. Aging Cel 2020; 19: 1–14.

33 Mortezaee K, Khanlarkhani N, Sabbaghziarani F, Nekoonam S, Majidpoor J, Hosseini A, *et al.* Preconditioning with melatonin improves therapeutic outcomes of bone marrow-derived mesenchymal stem cells in targeting liver fibrosis induced by CCl4. Cell Tissue Res 2017; 369:303–312.

34 Zhao L, Hu C, Zhang P, Jiang H, Chen J. Melatonin preconditioning is an effective strategy for mesenchymal stem cell-based therapy for kidney disease. J Cell Mol Med 2020; 24:25–33.