بسم الله الرحمن الرحيم

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شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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Effect of Adipose Tissue Derived Mesenchymal Stem Cells versus Metformin on Age-Related Changes of the Cerebellum of Albino Rat

(Histological and Immune-Histochemical Study)

Thesis

Submitted for Partial Fulfillment of the MD Degree
in **Histology and Cell Biology**

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2021

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, I feel always indebted to ***Allah*** *the Most Beneficent and Merciful.*

I wish to express my deepest thanks, gratitude and appreciation to ***Prof. Dr. Soheir Kamal Ahmed****, Professor of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, for her meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to ***Prof. Dr. Hanan Alaa Eldin Amin Saleh****, Professor of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, for her sincere efforts, fruitful encouragement.*

I am deeply thankful to ***Prof. Dr. Ghada Galal Hamam****, Professor of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, for her great help, outstanding support, active participation and guidance.*

I would like to express my hearty thanks to my ***husband****, his* ***mother*** *and my* ***family*** *for their support till this work was completed.*

Doaa Karem Metwaly Mostafa

Words cannot express my thanks, gratefulness respect and love to my parents

I dedicate my work to the spirit of my parents

Also I dedicate my work to my husband and my little angles Omar, Youssef and Salma

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List of Abbreviations

Abb.	Full term
ADSCs	*Adipose tissue derived stem cells*
ADMSCs	*Adipose tissue derived mesenchymal stem cells*
AMPK	*Adenosine monophosphate activated protein kinase*
ANOVA	*One-way analysis of variance*
Anti-GFAP	*Anti-cow glial fibrillary acidic protein*
BBB	*Blood brain barrier*
BM	*Bone marrow*
BM-MSCs	*Bone marrow-derived MSCs*
CNS	*Central nervous system*
DAB	*Diaminobenzidine tetrahydrochloride*
DCN	*Deep cerebellar nuclei*
DM	*Diabetes mellitus*
GFAP	*Glial fibrillary acidic protein*
H&E	*Haematoxylin and eosin*
HPF	*High power field*
HRP	*Horse-radish peroxidase*
IL-6	*Interleukin 6*
MDA	*Malondialdehyde*
MSCs	*Mesenchymal stem cells*
PBS	*Phosphate buffered saline*
PS	*Pial surface*
SD	*Standard deviation*
SNAREs	*Soluble NSF- attachment receptors*
SPSS	*Statistical Package for the Social Sciences*
sybII	*Synaptobrevin II*
TEM	*Transmission electron microscopic*
TNF-α	*Tumor necrosis factor-α*
VN	*Ventricular neuroepithelium*

Abstract

Introduction: Aging is a normal physiological process that affects all organs in the body including the cerebellum. Metformin is an anti-diabetic drug that is used in some age-related diseases. Regenerative medicine using adipose tissue derived mesenchymal stem cells (ADMSCs) is an emerging promising strategy.

Aim: to compare between the role of ADMSCs and metformin on the age-related structural changes of the cerebellum in female albino rats.

Materials and methods: The study included 55 female rats of different ages. They were divided into three groups according to their ages: Group I (Adult rats, from 4-6 months), Group II (Old rats, from 12-18 months) and Group III (Senile rats, from 24-28 months). Group II and Group III were further subdivided into three subgroups, *Subgroup a*: rats were left without treatment. *Subgroup b*: rats were given a single dose of $1X10^6$ ADMSCs via tail vein. *Subgroup c*: Rats received 300mg/kg metformin/day orally. Rats were sacrificed after four weeks. The cerebella were collected and processed for H&E, toluidine blue, immuno-histochemical stains using glial fibrillary acidic protein (GFAP) and synaptophysin. Transmission electron microscopic examination and histomorphometric studies were also performed.

Results: Light and electron microscopic examination of the cerebellum of non-treated old and senile rats (subgroups IIa and IIIa) revealed age-related structural changes compared to group I. Purkinje cells showed degenerative changes with a significant decrease in their mean number. Significant decrease in the mean optical density of Nissl granules was also noticed. The granular layer showed small widely separated granule cells with an apparent decrease in their number. A significant decrease in the mean number of astrocytes in GFAP sections and the mean optical density of synaptophysin reaction was also noticed in the non-treated subgroups (IIa and IIIa) compared to adult group I. Degeneration of axons and atrophy of myelin sheath were also noticed in the medulla of non-treated rats. The structural changes were more obvious in subgroup IIIa compared to subgroup IIa. In ADMSCs treated subgroups (IIb and IIIb), significant improvement of these changes was more noticeable compared to the corresponding metformin treated subgroups (IIc and IIIc) which was confirmed by statistical analysis.

Conclusion: ADMSCs were more effective than metformin in ameliorating some age-related structural changes of the cerebellum in female albino rats.

Keywords: Aging, Cerebellum, Stem Cells, Metformin, GFAP, synaptophysin, TEM.

INTRODUCTION

The cerebellum is one of the vital organs that are affected by aging. The cerebellum has several functions not only related to equilibrium, postural control, and motor coordination, but it is also involved in cognitive functions as learning and memory ***(Sokolov et al., 2017 and Schmahmann, 2019).***

Aging is a gradual process of natural developmental changes that begins in early adulthood. It occurs as result of the continuous accumulation of various deleterious changes in tissues and cells, which increases the susceptibility to diseases and death ***(Harman, 2006).*** The aging process results in deterioration of learning abilities and memory skills resulting in progressive decline in the cognitive functions ***(Cristofol et al., 2012).***

Recently, many studies have focused on the complex etiology, molecular pathways and mechanisms of the normal aging process and the resulting neuronal cell death. The aim has been to discover new drug targets that could be used as anti-aging agents ***(Rao et al., 2017).***

Adipose tissue-derived Mesenchymal stem cells (ADMSCs) have received attention as a promising source of cells for regenerative medicine. Adipose tissue contains hundreds of thousands of MSCs in each gram of fat ***(Park et al., 2013).*** Some researchers have reported that cell therapy might be able to restore the lost cells and enhances the neuronal

regeneration. However, the exact mechanisms involved in determining therapeutic capacity remain unknown ***(Chan et al., 2014).*** Recently, new authors suggest that MSCs show therapeutic efficacy in different age-related degenerative diseases ***(Fabian et al., 2017).***

Metformin is a widely used anti-diabetic drug. It improves glucose utilization, reduces hyperglycemia, inhibits gluconeogenesis, and decreases the utilization of the free fatty acids and serum lipids. Metformin also contributes to the prevention of some forms of human cancer. It has also cardio-protective effects ***(Anisimov et al., 2008).*** This therapeutic profile of metformin supports its use for the diseases related to the age and longevity ***(Novelle et al., 2016).***