Contents

List of Abbreviations

Abb.	Meaning
ABC	Avidin-biotin complex
AD	Alzheimer’s disease
ADSCs	Adipose derived mesenchymal stem cells
ALCAM	activated leukocyte cell adhesion molecule
Al	Aluminium
$AlCl_3$	Aluminium chloride
ANOVA	One-way analysis of variance test
ApoE4	Apolipoprotein E4
APP	Amyloid precursor protein
Aβ	Amyloid beta
AVs	autophagic vacuoles
BM-MSCs	Bone marrow–derived mesenchymal stem cells
CA	Cornu Ammon's
Ca	Calcium
CC26	Clara cell marker 26
CCR	Chemokine receptor
CD	Clusters of differentiation
CFU-Fs	Colony-forming unit fibroblasts
DAB	Diaminobenzidine
DG	Dentate gyrus
DMEM	Dulbecco's modified Eagles medium
DW	Distilled water
ESC	Embryonic stem cells
FBS	Fetal bovine serum
Fig	Figure

Abb.	Meaning
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and eosin
HPF	High power field
ICAM	Intercellular adhesion molecule
IGF	Insulin growth factor
IL	Interleukin
iPSCs	Induced pluripotent stem cells
LFA	Leukocyte function-associated antigen
MAP	Microtubule-associated protein
MCP	Monocyte chemoattractant protein
MSC-CM	Mesenchymal stem cells -conditioned medium
MSCs	Mesenchymal stem cells
MWM	Morris water maze
NFT	neurofibrillary tangles
N.S	Non-significant
NK	Natural Killer
PBS	Phosphate buffer saline
PDGF	platelet derived growth factor
PECAM	Platelet/endothelial cell adhesion molecule
rER	Rough endoplasmic reticulum
S	Significant
SD	Standard deviation
Sec	Seconds
Sox2	Sex determining region Y
TCs	Telocytes
TEM	Transmission electron microscopic
TNF	Tumor necrotic factor
VCAM	Vascular cell adhesion molecule

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and other cognitive abilities. Unfortunately, curative measures are not yet available. Adipose-derived stem cells (ADSCs) have a high proliferation capacity *in vitro* and could differentiate into cells with several neuronal and glial characteristics. Thus, their therapeutic potential could be applicable.

Aim of the work: To evaluate the histological changes of the hippocampus in an Alzheimer's disease model in albino rat and to highlight on the possible therapeutic role of ADSCs.

Materials and Methods: Seventy adult male albino rats were divided into four groups: Group I (control group) included 30 rats. Group II (Alzheimer's group) included ten rats which were subjected to induction of Alzheimer's disease by receiving aluminium chloride orally in a daily dose of 17mg/kg body weight for 75 days. Group III (ADSCs-treated group) included ten rats which were subjected to AD induction, then intravenously injected with a single dose of ADSCs ($1x10^6$) suspended in 0.5 ml PBS and sacrificed after another four weeks. Group IV (Recovery group) included ten rats which were subjected to AD induction and left untreated for four weeks then sacrificed. At the end of the experiment, the Morris water maze test was done for 6 days. Brains were excised and processed for light microscopic and transmission electron microscopic examination. Morphometrical measurements and statistical analysis were performed.

Results: Rats of groups II and IV showed fluctuation in the time required to reach the platform in the Morris water maze test. Loss and degeneration of the pyramidal cells were detected in areas of

Cornu Ammon's (CA1, CA3) of the hippocampus proper and of granule cells in the dentate gyrus (DG). Deposition of amyloid plaques and neurofibrillary tangles were evident. Treatment by ADSCs showed improvement of the histological appearance in all areas examined.

Conclusion: The ADSCs showed a high therapeutic efficiency in treating AD experimental model in rats and could be a challenging therapeutic measure.

Keywords: Alzheimer's disease, Adipose tissue-derived stem cells, aluminium chloride, rat.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder manifested by a progressive decline in cognitive abilities. It is characterized by a progressive decrease in memory, comprehension, language expression, learning capacity, calculation, abstraction, judgment, spatial orientation and recognition of familiar people or places **(Lakshmi et al., 2015).**

Alzheimer's disease has been called "the disease of the century" with significant clinical and socioeconomic impacts. Epidemiological studies pointed out that AD affects 5% of the population over 65 years of age. Parallel with increasing lifespan, the incidence of disease will rise dramatically (**Alcyr et al., 2005**). The precise causes of AD are believed to involve a complex interaction of genetic and environmental factors. However, no particular etiology has been identified that would explain all cases **(Abdel Aziz et al., 2013).**

Since there is no recognized cure for AD, conventional medical treatments have consisted primarily of drug therapy with the hope of managing symptoms. However, such medication falling into a class of drugs known as cholinesterase inhibitors doesn't alter the progression of the disease itself **(Xiaotang et al., 2014)**.

It was previously thought that the adult mammalian brain was devoid of stem cells that could regenerate after injury. However, recent studies have shown that neurogenesis occurs throughout the lifespan of adult mammals **(Kim and Sun, 2012)**. Studies in the adult rodent brain showed advances in the development of therapeutic strategies to replace the lost neurons **(Jiang et al., 2012).**

It is widely accepted now that neurogenesis primarily occurs within two restricted regions in the adult central nervous system: the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone adjacent to the lateral ventricles (**Zhao et al., 2008).** In experimental models of AD, neurogenesis has been thought to be impaired in the subgranular and subventricular zones **(Faure et al., 2011)**. Accumulating evidences indicated that newborn neurons actively participated in cognitive functions involving olfaction and hippocampus-dependent learning and memory processes **(Yan et al., 2014)**.

An effective treatment for AD would be one which would prevent and reverse its histopathological picture and regenerate lost neuronal connections. Accordingly, the only therapy that could offer the possibility of accomplishing these objectives would be stem cell therapy **(Lie et al., 2004; Danilov et al., 2006; Ke et al., 2006).** It has been postulated that stem cell

therapies may replace lost cells by differentiating into functional neural tissue by providing a source of trophic support for the diseased nervous system **(Wilkins et al., 2009).**

Mesenchymal stem cells (MSCs) are present in adult tissues including bone marrow and adipose tissue. However, the clinical use of bone marrow–derived mesenchymal stem cells (BM-MSCs) has presented problems including pain, morbidity, and low numbers of harvested cells. In contrast, adipose derived mesenchymal stem cells (ADSCs) are one of the most advantageous resources due to easier access to adipose tissue and its abundance, with high proliferation and differentiation potential **(Parker and Katz 2006).**

It has been reported that ADSCs had a high proliferation capacity *in vitro* and could differentiate into cells with several neuronal and glial characteristics, including expression of neuronal and glial proteins **(Deng et al., 2006).**

Aim of the work

This study aimed to identify the histological changes of the hippocampus in an Alzheimer's disease model and to highlight on the possible therapeutic role of adipose-derived mesenchymal stem cells in albino rat.

Limbic system

The limbic system was defined as a set of brain structures on both sides of the thalamus, immediately beneath the cerebellum **(Schacter et al., 2011).** It includes the limbic lobe (which consists of hippocampus, cingulate gyrus and parahippocampal gyrus), septal area, insula, amygdala, hypothalamus, mammillary bodies and anterior nuclei of thalamus (Fig.1). The hippocampal formation consists of the hippocampus proper, the dentate gyrus and the parahippocampal gyrus **(Snell, 2010)**. The limbic system plays a vital role in elaboration of emotional behavior and memory (**Singh, 2010)**.

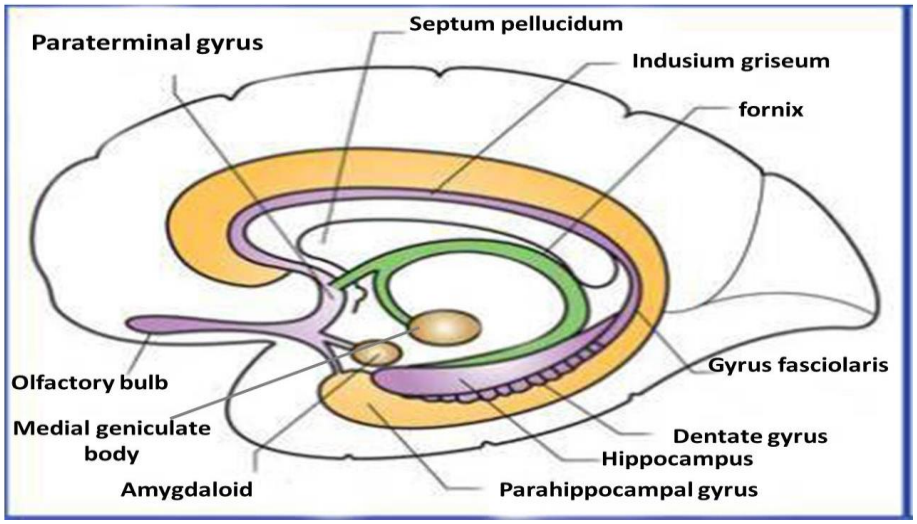

Fig.1: Medial aspect of the right cerebral hemisphere showing structures that form the limbic system **(Singh, 2010).**

Hippocampal anatomy

The hippocampus is located within the brain's medial temporal lobe, underneath the cortical surface **(Singh, 2010)**. It appears as a curved elevation of gray matter that extends throughout the entire length of the floor of inferior horn of lateral ventricle. It is named hippocampus because it resembles a sea horse in coronal section. In the frontal section, the hippocampus is 'C-shaped' hence the name "ram's horn". The convex ventricular surface is covered with ependyma, beneath which lies a thin layer of white matter called the alveus. The alveus consists of axons of the pyramidal cells of the hippocampus and converge medially to form the fimbria which becomes continuous with the crus of the fornix (Fig. 2) (**Snell, 2010).**

The hippocampus contains two main interlocking parts: the hippocampus proper (also called Ammon's horn or cornu Ammon's "CA") and the dentate gyrus**.** Most anatomists use the term "hippocampus proper" to refer to the three CA fields, and the term "hippocampal formation" to refer to the hippocampus proper plus dentate gyrus and subiculum (**Amaral and Lavenex, 2006).** There are three identified cytoarchitectural areas of the hippocampus proper (CA1– CA2 and CA3) **(Fix, 2008).**

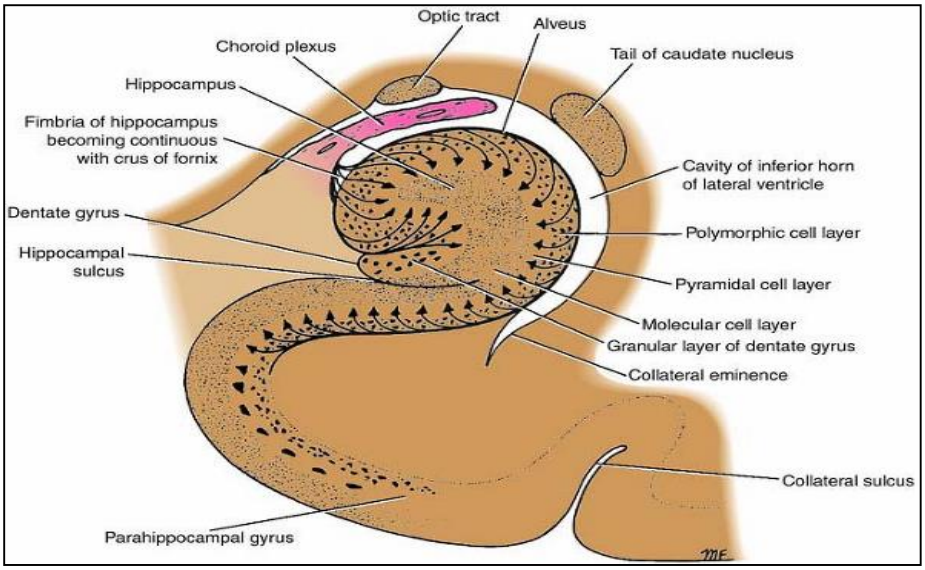

Fig. 2: Coronal section of the hippocampus and related structures (**Snell, 2010).**

In fetal brain, the dentate gyrus develops as a further extension of the hippocampus and occupies the interval between the hippocampus and the parahippocampal gyri. Its surface is toothed hence the name dentate gyrus. The subiculum is a transition zone between parahippocampal gyrus and hippocampues proper (Fig. 3) **(Singh, 2010).**

Hippocampus Histology

The cortical structure of the parahippocampal gyrus is six-layered. As the cortex is traced into the hippocampus, there is a gradual transition from a six- to a three-layered arrangement.

These three layers are the superficial molecular layer (consisting of nerve fibers and scattered small neurons), the pyramidal layer (consisting of many large pyramidal-shaped neurons) and the inner polymorphic layer, which is similar in structure to the polymorphic layer of the cortex seen elsewhere. The dentate gyrus consists of three layers; molecular and polymorphic layers look like that of hippocampus proper and granular layer which replace pyramidal cell layer. The granular layer is composed of densely arranged rounded or oval neurons that give rise to fibers that terminate on the dendrites of the pyramidal cells of CA3 in the hippocampus (Figs. 2 and 3) **(Snell, 2010).**

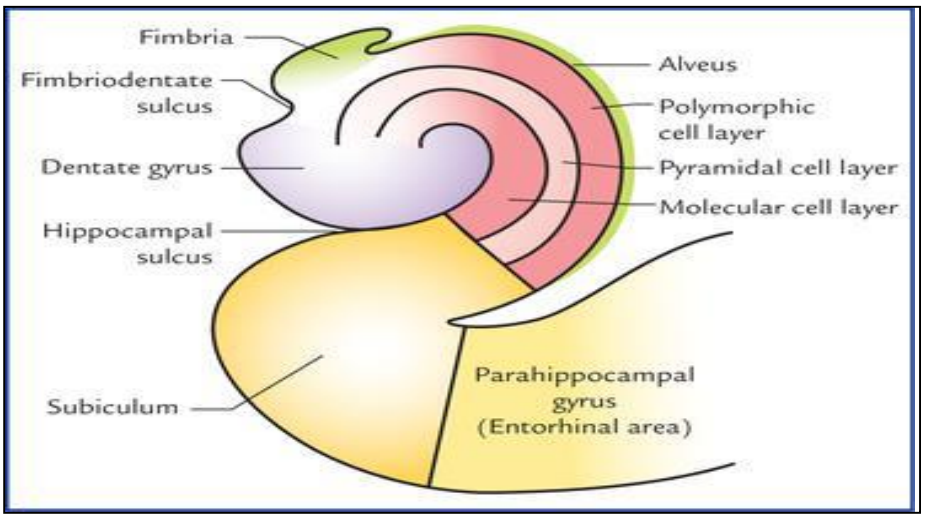

Fig. 3: Coronal section of the hippocampus and its layers **(Singh, 2010).**