Research Article

Histological changes in the substantia nigra in Parkinsonism model in male albino rat

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Abstract

The substantia nigra is a part of the basal ganglia that is present in the midbrain. It is characterized by the presence of dopaminergic neurons in the substantia nigra pars compacta. Parkinsonism disease is one of the most common neurodegenerative diseases affecting elderly patients. It occurs due to progressive degeneration of dopaminergic neurons in the substantia nigra. In this work, Parkinsonism was induced in rats by daily subcutaneous injection of 0.5mg/Kg of rotenone for 28 days. Twenty rats were divided randomly in to 2 groups; control and Parkinsonism groups. Brain specimens were obtained for H&E stain. In Parkinsonism group, shrunken dopaminergic neurons with small dense nuclei and were surrounded with wide pericellular hallows were frequently observed in the substantia nigra. Acidophilic lewy bodies are very characteristic finding in Parkonsonism group. There was also vacuolation in the neuropil.

Keywords: substantia nigra, ganglia, dopaminergic neurons, Parkinsonism disease

Introduction

The substantia nigra is a part of the basal ganglia that is present in the mid-brain. Humans have two substantiae nigrae, one on each side of the midline of the midbrain. It plays an important role in reward, addiction and movement (Sato & Hikosaka, 2002). Substantia nigra is Latin word for "black substance", as the substantia nigra appear darker than neighboring areas due to very high levels of neuromelanin settle in dopaminergic neurons (Tubbs, Loukas, Shoja, Mortazavi, & Cohen-Gadol, 2011).

(Basso & Sommer, 2011) suggested that the medium sized cells that are found in pars compacta, presumed to be the dopaminergic cells of the nigroneostriatal pathway, their ventral dendrites go down deeply in the pars reticulata and some dendrites remain in the pars compacta.

Parkinsonism is the second most common neurodegenerative disease after Alzheimer disease; it affects eldery persons.
Parkinsonism occurs due to progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (Murrell et al., 2008).

The prevalence of Parkinsonism increases with age (Guimarães et al., 2018). And the incidence of Parkinsonism is higher in men than in women. Suggested explanations for the male predominance include the protective effects of estrogens, the higher frequency of occupational toxin exposure as well as the minor head trauma in men, and the recessive susceptibility genes on the X chromosome (Wirdefeldt, Gatz, Reynolds, Prescott, & Pedersen, 2011).

The substantia nigra neuronal loss in Parkinsonism patients is associated with mitochondrial dysfunction and high level of oxidative damage to the macromolecules including proteins, DNA and lipids (Combs et al., 2015).

There are cardinal motor symptoms of Parkinsonism: tremor at rest, bradykinesia (slow movement), rigidity, walking/gait problems and postural instability (balance problems). Observing one or more of these symptoms is the main way that physicians

can diagnose Parkinsonism (Shahed & Jankovic, 2007).

There may be secondary motor symptoms of Parkinsonism which may be Decrease in the ability to perform unconscious movements, including smiling, blinking or swinging your arms when you walk, Speech problems and hardness in writing (Bonifati et al., 2003; Jankovic, 2008).

Material and methods

Twenty adult male albino rats were used in this current study. The weight of each rat rangs between 200 – 250 gm. The animals were purchased and raised in animal house of growing center of the faculty of agriculture, Minia University. The animals were put in clean plastic cages covered with mesh wire and they were fed on standard diet with free access to diet and water. The study was performed in accordance to the guidelines for use of laboratory animals and by approval of the Minia University institutional Ethics Committee.

Preparation of a rat model of parkinsonism

Parkinsonism was induced in male albino rats by injection of rotenone dissolved in dimethyle sulfoxide (DMSO, sigma-Aldrich, MO, USA). Rats were daily subcutaneous injected with 0.5 mg/Kg for 28 day. This dose was a modified dose from (Sharma, Jamwal, & Kumar, 2016).

Experimental design:

Twenty rats were divided into two groups:
1- Group I (the control group): included 10 rats.
2- Group II (Parkinsonism group): includes 10 rats; injected with 0.5 mg / kg daily SC rotenone for 28 day then the animals were sacrified.

Animals were anesthesized with ether inhalation. Then, fixation was done with 10% buffered formalin through intra cardiac perfusion. The brain specimens were dissected out. Transverse sections were done in the midbrain to expose the substantia nigra.

Histological study:

For examination by light microscopic; the samples were fixed in 10% buffered formalin, and they were processed to obtain paraffin blocks. Serial (4-7) μm sections were stained with hematoxylin and eosin (H&E) stains.

Image capture

H&E sections were photographed by using high-resolution color digital camera mounted on a BX51 microscope (Olympus, Japan). The camera were connected to a computer programmed with LC micro application software. Image capture was carried out in the Histology and Cell Biology Department, Faculty of Medicine, Minia University. Images were saved as jpg and they were processed using adobe photoshop 7 to standardize brightness contrast and background color then they was printed.

Results

Light microscopic study:

Hematoxylin and eosin (H&E) stains results:

1-The control group:

Transverse sections of substantia nigra pars compacta showed that numerous dopaminergic neurons were found in the neuropil. The dopaminergic neurons has a basophilic cytoplasm and rounded pale nuclei with prominent nucleoli. Also, different types of neuroglia were observed among the neuropil with heterochromatic nuclei. (Fig. 1).

2- Parkinsonism group:

Sections in the substantia nigra pars compacta in Parkinsonism group showed frequent shrunken dopaminergic neurons with small dense nuclei and were surrounded with wide pericellular hallows. Acidophilic lewy bodies are prominent characteristic finding in this group. Vacuolation also appeared in the neuropil (Fig. 2).

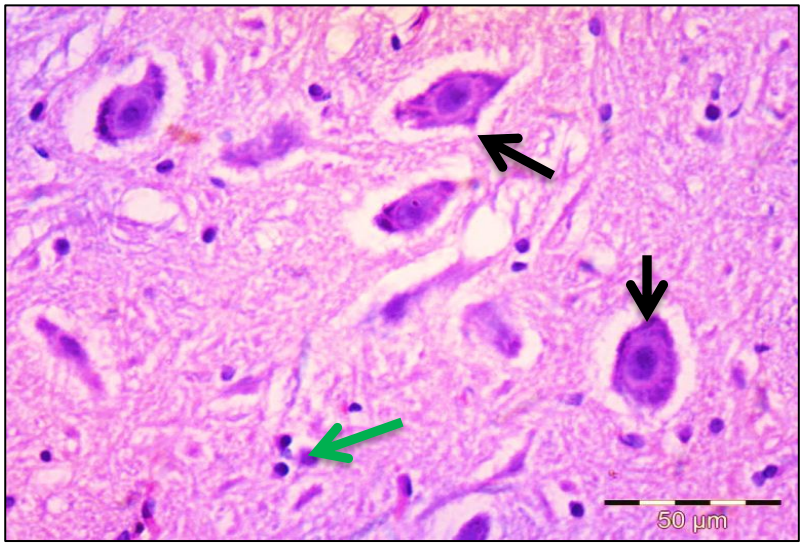

Fig. 1. A photomicrograph of a section in the substantia nigra pars compacta in an adult male albino rat in the control group showing numerous dopaminergic neurons (black arrows); having rounded pale nuclei with prominent nucleoli surrounded by basophilic cytoplasm. Dfferent types of neuroglia (green arrows) with heterochromatic nuclei in the neuropil. H&EX 400

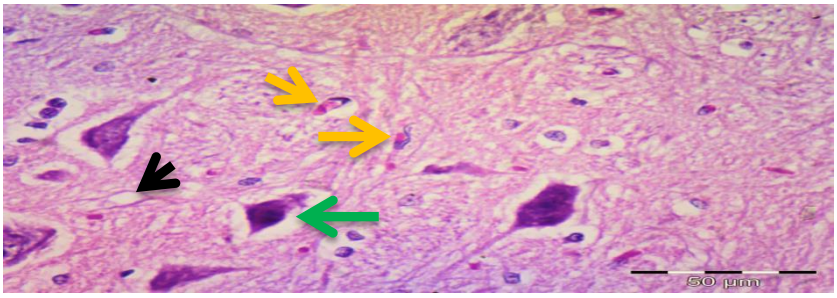

Fig. 2. A photomicrograph of a section in the substantia nigra pars compacta of an adult male albino rat of Parkinsonism group showing shrunken dopaminergic neurons with small dense nuclei (green arrows) and surrounded with wide hallow. Notice vacuolations (black arrow) and the acidophilic Lewy bodies (orange arrow) in the neuropil. H&E x400

Discussion

Parkinsonism is one of the most common neurodegenerative disease affecting eldery persons leading to both motor and non-motor symptoms (Ren et al., 2018)

The aim of the study is to show the histological changes that occur in Parkinsonism in rat substantia nigra.

In Parkinsonism group (group II); it was noticed that there were shrunkening in the dopaminergic cells with small dense nuclei and the cells were surrounded with wide hallow. These finding are in agree with (Teema, Zaitone, & Moustafa, 2016). There was nuclear condensation with intense nuclear refractivity (pyknosis) which indicated the occurrence of apoptosis (Burgoyne, 1999).

These findings are also in agreement with (Singh, Hanson, & Morris, 2017) who reported that Neuronal cell death in Parkinsonism was due to many causes as abnormal accumulation of alpha-synuclein;

oxidative stress and mitochondrial dysfunction.

Dopaminergic neuron death and degeneration were complicated by appearance of vacuolations in the neuropil. Vacuolations were observed in the neuropil in the substantia nigra in our study and this finding was in agreement with (Sekerdag, Solaroglu, & Gursoy-Ozdemir, 2018).

(Langston, Schüle, Rees, Nichols, & Barlow, 2015) revealed the presence of eisinophilic cytoplasmic bodies called lewy bodies which replace nearly all cell component. lewy bodies are due to accumulation of α-synuclein which is an abnormal proteins . This result was in line with the finding in our study.

Mitochondrial dysfunction led to a significant increase in mitochondrial reactive oxygen species (ROS) generation and also led to oxidative stress, which led to decrease of the antioxidant glutathione inside the cell. So, these events lead to apoptotic cell death which is the main cause of Parkinsonism (Lesage et al., 2016).

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