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Original Article

Effects of Ageing and Alloxan- Induced Diabetes Mellitus on the Structure of Inferior Colliculus in male Albino Rats: a Light and Electron Microscopic Study

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ABSTRACT

Background: The inferior colliculus (IC) is an important relay centre in the acoustic pathway that can influence the motor neurons in the brain stem and spinal cord.

Aim of the Work: This research was conducted to study the structural changes in the inferior colliculus of the senile and adult diabetic male albino rats compared to the adult control one using both the light and transmission electron microscopy.

Material and Methods: A total of 30 male albino rats were divided into 3 equal groups including adult (3 - 4 months) and senile (24 months), adult diabetic (3 - 4 months) rats. To induce diabetes, a single dose of alloxan (200mg/kg body weight) dissolved in distilled water was injected intraperitoneally. Two months later, the animals of all groups were anaesthetized with ether and sacrificed, followed by removal of the inferior colliculi, which were processed for microscopic examination.

Results: The histological examination of the inferior colliculus in adult control rats revealed that the nerve processes were relatively abundant than the neuronal somata. The nerve fibers were myelinated, arranged in clusters, and the majority had thick, uniform and regular myelin sheath. The inferior colliculus was constituted of a superficial cortex and a deep central part. The nerve cells in the cortex were few in number and rounded in shape. In the center, many cells of different sizes which exhibited unipolar, bipolar or multipolar processes were observed. In the senile rats, variable degrees of degeneration were observed in both the nerve cells and fibers. The nerve cells were either appeared swollen or showed small size with dense cytoplasm and dark pyknotic nuclei. The myelinated nerve fibers showed thickened myelin sheaths, disrupted sheaths, separation of their lamellae and sometimes showed shrunken degenerated nerve axon. In diabetic rats, most of the neuronal somata appeared small in size with small, dark and pyknotic nuclei. The neuropil showed extensive vacuolation and congested blood vessels with thickened basement membrane. Thin myelinated and unmyelinated nerve fibers were observed with vacuolated cytoplasmic matrix. Silver-stained sections showed irregular-shaped thin nerve fibers.

Conclusions: Diabetic state induced severe degenerative changes in the inferior colliculus, which were worse than those detected in the senile rats. Such changes may contribute in hearing loss of the young diabetics. So, the auditory health and early clinical testing of the diabetics are highly important for early prevention of deleterious complications of diabetes.

Key Words: Inferior colliculus, senility, diabetes, albino rats, light microscopy, transmission electron microscopy

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INTRODUCTION

The inferior colliculus (IC) is an important relay centre in the acoustic pathway. It receives fibers of the lateral lemniscus arising from the brain stem cochlear nuclei and the superior olivary complex en route to the medial geniculate body; and from there to the auditory cortex. Heavily descending output also projects to the inferior colliculus from the auditory cortex. The inferior colliculus can influence the motor neurons in the brain stem and spinal cord through the superior colliculus, tectotegmental and tectospinal tracts. These connections probably mediate reflex turning of

the head and eyes in response to sounds (*Singh, 2002; Crossman & Standring, 2005)*. Electrical and chemical stimulation of the inferior colliculus causes fear and escape behavior revealing that IC is involved in the integration of defensive reactions *(Castilho et al., 1999)*.

Age- related hearing loss (presbycusis) is an increasing communicative disorder in the aging society. It is a major problem since the elderly individuals rely on special senses to compensate for other age-associated disabilities. Difficulty in speech discrimination, a decrease in perception of high frequency tones, decreased ability to localize sounds and a decreased ability to detect signals in noise are characteristic problems encountered in the elderly. Hearing loss involves changes in both the peripheral and central portions of the auditory system. Loss of sensory hair cells especially at the basal turn of the cochlea, atrophy of the stria vascularis, thickening and secondary stiffening of the basilar membrane of the cochlea were observed. Age- associated reduced perfusion of the cochlea may contribute to the formation of reactive oxygen metabolites which may adversely affect the inner ear as well as cause damage to mitochondrial DNA. Damaged mitochondrial DNA may lead to severe affection of the function of the inner ear *(Popelara et al., 2006)*. Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the inferior colliculus. Age-related changes associated with the function of GABA neurotransmitter in the inferior colliculus have been investigated. These studies detected altered function as a result of changes in the synthesis, degradation, uptake, release, and receptor sensitivity of the neurotransmitter, perhaps secondary to cell loss and / or progressive de-afferentation *(Caspary & Milbrandt, 1995)*.

Diabetes mellitus is a genetically determined metabolic disorder associated with vascular and neuropathic complications. There are two major types: Type1 diabetes mellitus (T1DM) and Type2 diabetes mellitus (T2DM). Type 1 (insulin –dependent) is a chronic disease associated with high morbidity and premature mortality since these patients are more prone to its major complications. Type 2DM is an age- related disorder affecting up to 20 % of the population over age 60 *(American Diabetes Association, 2006)*. An association between diabetes mellitus and hearing loss was proved in humans and experimental animals *(Maia & Campos, 2005; Vasilyeva et al., 2009)*. Many of the diabetic complications in older people are associated with natural aging, but they appear earlier in diabetic patients *(Biessels et al., 2002)*.

Reviewing the literature, Age- and /or diabetes –associated changes in the central auditory system have been assessed by studying auditory brainstem responses. Few of them studied the associated structural pathological changes *(Peters, 2007)*. So, the aim of the present work was to study senile changes of the inferior colliculus and to compare the effect of aging and diabetes on the structure of the inferior colliculus using both the light and transmission electron microscopy.

MATERIAL AND METHODS

Thirty male albino rats of Sprague- Dawley strain were used in the present study. The rats were obtained and kept in the Medical Research Center, Faculty of Medicine, Ain Shams University. The rats were housed in separate galvanized iron cages in a thermostatically controlled room and maintained on a standard pellet diet and were allowed free access to water..

The animals were randomly divided into three groups, ten rats each:

- **Group I (control rats):** included adult rats 3-4 months old weighing 150-200 grams.
- **Group II (senile rats):** comprised of 2 years old rats weighing 250-300 grams.
- **Group III (diabetic rats):** included adult rates aged 3-4 months weighing 150-200. The fasting blood glucose level was examined in the rats before the induction of diabetes. The blood samples were withdrawn from tail vein. A drop of blood was placed in glucometer to assess blood glucose level. Fasting blood glucose level in the chosen rats was 85-100 mg/dl. The rats were fasted for 12 hours and then received a single intraperitoneal injection of alloxan (Alloxan Hydrate, SD Fine-chem. limited) in a dose of 200 mg/kg dissolved in distilled water *(Federiuk, et al. 2004)*. The rats were maintained on free access to tasty oral sugar containing fluid for the first two days after alloxan treatment to overcome the marked initial hypoglycemia

due to release of preformed insulin from damaged beta cells. Blood glucose level was examined in the rats (non- fasting) after 48 hours of induction of diabetes. Animals whose blood glucose level exceeded 200 mg/dl were considered diabetic. The rats were left in a diabetic state for two month prior to sacrification.

The animals of each group were anaesthetized with ether. The brains were extracted and the inferior colliculus was carefully removed, the specimens were then processed for light and electron microscopic study. Some specimens were fixed immediately in Bouin´s solution for 7 days, the specimens were washed in distilled water and processed for preparation of paraffin block. Serial sections 5 µm thick were cut and stained with Hematoxylin and Eosin stain *(Drury & Wallington, 1980)* and Glees silver stain *(Bancroft & Gamble, 2002)*.

Other specimens were cut into small pieces (1mm³) and fixed in 2.5% gluteraldehyde for 3 hours. Tissue samples were washed in phosphate buffer at pH 7.4, post fixed in 1% osmium teraoxide for one hour. After dehydration in ascending grades of alcohol, the specimens were cleared in three changes of propylene oxide and finally embedded in gelatin capsules filled with fresh Epon. The capsules were kept in oven at 60ºc for 48 hours for polymerization. Semithin sections 1µm thick were cut using a glass knife (L.K.B. ultramicrotome) stained with 1% aqueous solution of toluidine blue, examined and photographed by Olympus 286 microscope. Ultrathin sections of 50-80 nm thickness were cut and stained with uranyl acetate *(Watson, 1958)* and lead citrate *(Reynolds, 1963)*. The grids were examined and photographed using a Joel S100 electron microscope.

RESULTS

Group I (control adult rats):

Light microscopic examination of the inferior colliculus (IC) of adult rats showed that the nerve processes were relatively abundant than the neuronal somata. The inferior colliculus was constituted of a dorsal superficial part and a deep central part (Fig. 1). The superficial part was composed mainly of nerve fibers with few nerve cells in-between. Most of the somata in the superficial zone appeared small in size with large rounded nucleus. Few of them were apparently medium- sized (Fig. 2). The nerve fibers were arranged transversely parallel to the dorsal collicular surface in the superficial part, while in the intermediate zone, the fibers were arranged in different directions (Figs. 2, 3). The nerve fibers of the ventral portion of IC were arranged in thick longitudinal bundles on each side of middle line (Figs. 1, 4). The deep central part of the inferior colliculus showed many nerve cells of different sizes and shapes in addition to the clusters of nerve fibers. The majority of the nerve cells was medium or large- sized; oval or rounded in shape and exhibited unipolar, bipolar or multipolar processes; few of them were small in size and rounded in shape (Figs. 5-A, B). The neuropil space between the somata was occupied by clusters of myelinated nerve fibers with uniform and regular myelin sheaths, blood vessels, small dark neuroglia (Figs. 6, 7, 8). Silver- stained sections showed the arrangement of the nerve fibers lateral and dorsal to the periaqueductal grey matter forming parallel fascicles of thick regular nerve fibers (Figs. 9, 10). The ultrastructural study showed that neuronal somata had oval or rounded euochromatic nucleus with apparent nucleolus and regular nuclear membrane. Cisterns of the rough endoplasmic reticulum, vesicular mitochondria, and numerous free ribosomes were observed scattered in the perikaryal cytoplasm (Figs. 11- A, B). The myelinated nerve fibers were arranged in clusters and were variable in shape and thickness. They had clear cytoplasmic matrix, few mitochondria and neurofilaments and microtubules. The majority of the nerve fibers had thick, uniform and regular myelin sheath (Fig. 12).

Group II (senile rats):

Light microscopic examination of the inferior colliculus of senile rats showed the arrangement of nerve fibers transversely in the dorsal zone with the presence of multiple small degenerated nerve cells in between (Fig. 13). The central zone clarified various degree of degeneration of the nerve cells. Most of them showed dense cytoplasm and small dark pyknotic nuclei (Fig. 14), while the others appeared swollen with pale cytoplasm and small eccentric nuclei. The myelinated nerve fibers also showed variable degree of degeneration. Some were partially demyelinated or unmyelinated that appeared as empty rounded spaces in the neuropil

(Fig. 15). Apparent increase in the number of the glial cells beside nerve bundles was noticed compared to the adult group (Fig. 16-A). The observed signs of degeneration of the myelinated nerve fibers included also increased thickness of the myelin sheaths, separation of their lamellae or showed shrunken axons separated from the surrounding myelin sheath (Fig. 16-B) The blood vessels appeared dilated and surrounded by disrupted or ill-defined basement membrane (Figs. 17,18). Silver- stained sections showed arrangement of the nerve fibers in thick interrupted bundles running in different directions (Fig. 19).

Ultrastructural study clarified variable degree of degeneration of the nerve cells and processes. Some of the neuronal somata showed multiple cytoplasmic vacuoles and few cytoplasmic organelles which appear small, dark and degenerated (Fig. 20). Other cells showed dark pyknotic nuclei with crenated nuclear membrane. Wide perikaryal areas of degeneration were observed and the degenerated cytoplasmic organelles appeared adherent to the cell membrane. The myelinated nerve fibers exhibited different modalities of degeneration. Some of them showed disrupted myelin sheath and vacuolated cytoplasmic matrix and the others showed myelin balloons as the nerve axons appeared degenerated and compressed to one side of sheath and the myelin sheath protrude against the opposite side (Figs. 21, 22). Some areas showed extensive degeneration of both the nerve axons and the myelin sheaths leaving empty spaces and shrunken degenerated axons. Amalgamated sheaths (i.e. fused myelin sheaths) with irregular contour were also noticed (Fig. 23).

Group III (diabetic rats):

Light microscopic examination of the inferior colliculus of adult diabetic rats showed extensive vacuolation of the dorsal zone with loss of the regular arrangement of both the nerve cells and fibers compared to the control adult group (Fig. 24). Most of the neuronal somata in the dorsal and central zones appeared small in size with small, dark and pyknotic nuclei and a very thin rim of cytoplasm. The neuropil in the central zone showed extensive rounded and irregular vacuoles, most probably represented unmyelinated nerve fibers and tissue edema respectively (Figs. 25, 26). The blood vessels appeared dilated, congested and showed thickened basement membrane (fig. 26). Silver- stained sections showed arrangement of nerve fibers in small bundles. The nerve fibers appeared thin in thickness and irregular in shape (Fig. 27).

Ultrastructural study revealed that most of the neuronal somata showed variable degree of degeneration. The cytoplasm in most of the nerve cells showed scanty organelles. Most of the nerve cells had small dark pyknotic nuclei with dark cytoplasm showing dilated cisterns of rough endoplasmic reticulum and degenerated mitochondria with disrupted cristae (Figs. 28, 29, 31). The nerve fibers showed irregular contour. Thin myelinated as well as unmyelinated nerve fibers were observed. Most of them showed degenerated cytoplasmic matrix (Figs. 28, 30). The unmyelinated nerve fibers were variable in size and were either rounded or oval in shape and some axons appeared swollen with ballooned appearance (Figs. 30, 31).

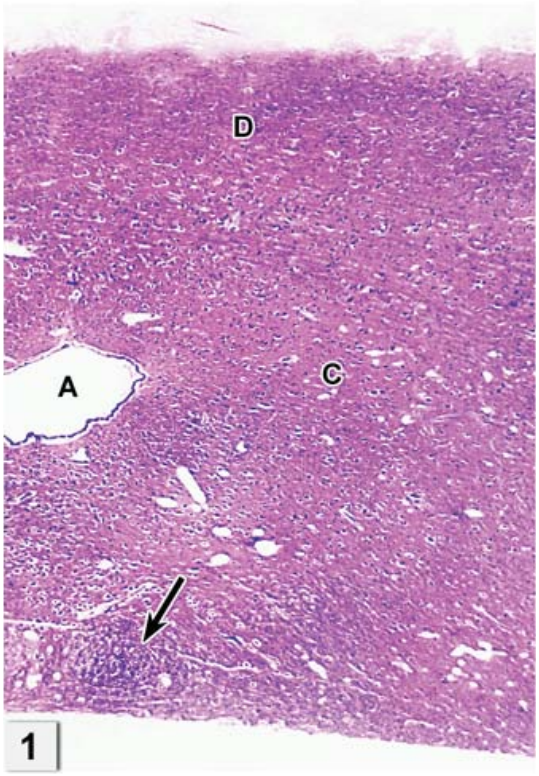

Fig. 1: A photomicrograph of a section of the inferior colliculus of an adult rat showing the dorsal superficial part (D) and a deep (central) part(C). Note the presence of bundles of nerve fibers in the ventral aspect (arrow) of the inferior colliculus. Note also the aqueduct of Sylvius (A). Hx.& E.; 40

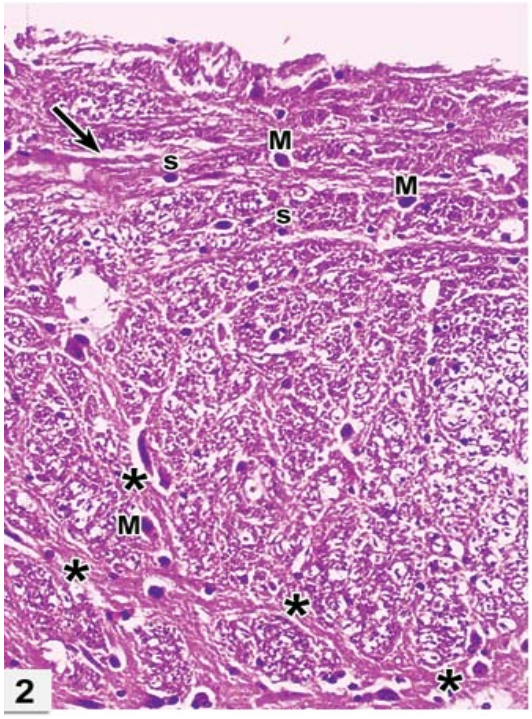

Fig. 2: A photomicrograph of a section of the inferior colliculus of an adult rat showing few nerve cells in between the nerve fibers in the dorsal zone. Note the presence of apparently small (s) and medium-sized (m) nerve cells. Notice also the arrangement of nerve fibers in bundles parallel to the surface in the dorsal zone (arrow) and in different directions (asterisk) in the intermediate zone. Hx.& E.; 200

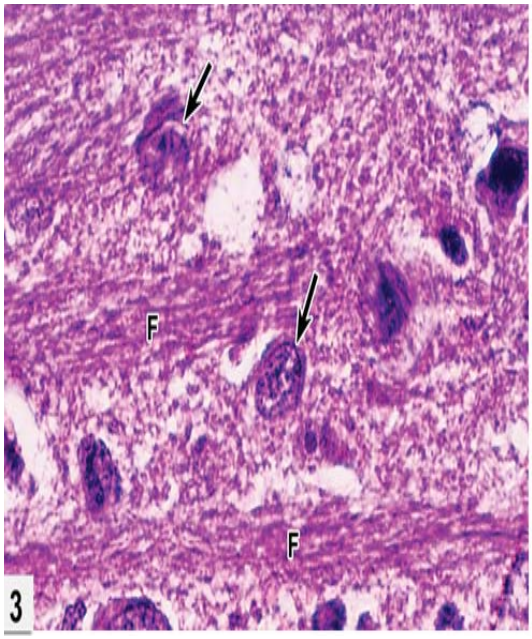

Fig. 3: A photomicrograph of a section of the inferior colliculus of an adult rat showing the transverse arrangement of nerve fibers (F) in the dorsal zone. Note the few nerve cells in between the fibers (arrows). Hx.& E.; 1000

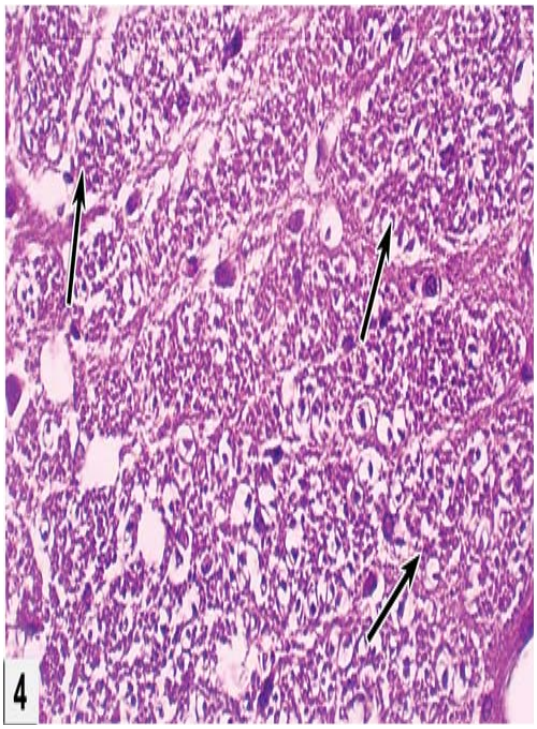

Fig. 4: A photomicrograph of a section of the inferior colliculus of an adult rat showing the nerve fibers in the ventral portion arranged in thick longitudinal bundles (arrows). Hx.& E.; 400

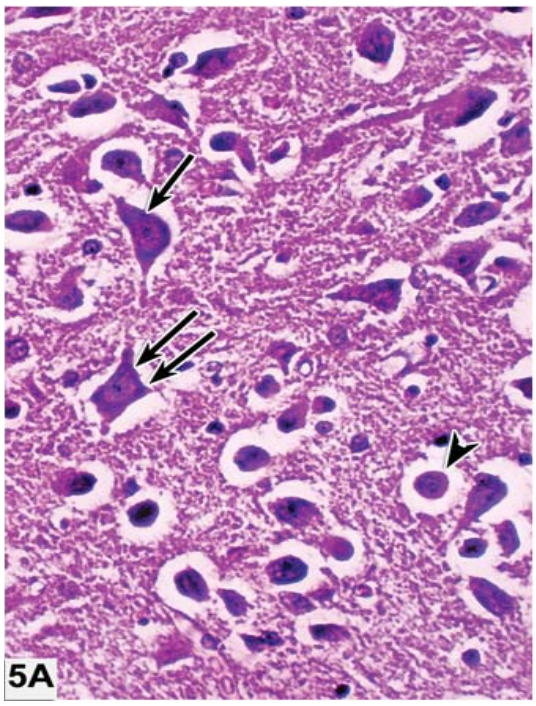

Fig. 5A: A photomicrograph of a section of the inferior colliculus of an adult rat showing the nerve cells in the central part. Note that some of them exhibit bipolar (arrow) or multipolar (double arrows) processes while the others are rounded (arrowhead) in shape. Hx.& E.; 400

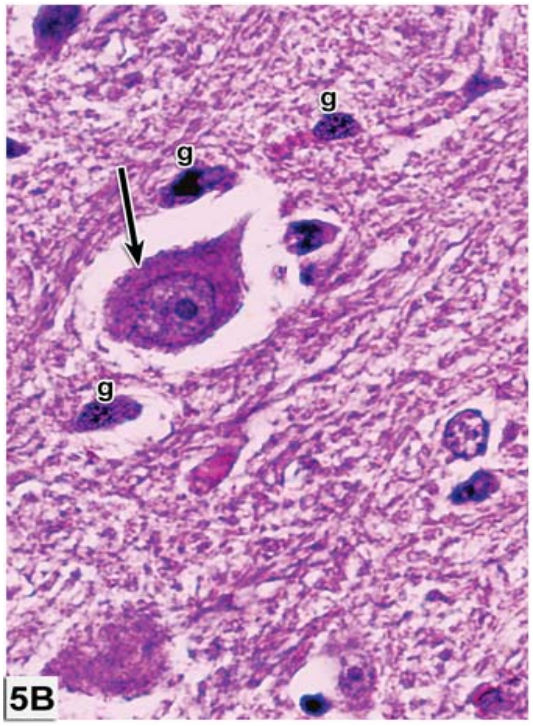

Fig. 5B: A photomicrograph of a section of the inferior colliculus of an adult rat showing a large unipolar nerve cell with eccentric nucleus and abundant cytoplasm (arrow). Note the dark pear- shaped glial cells (g) in the neuropil. Hx.& E.; 1,000

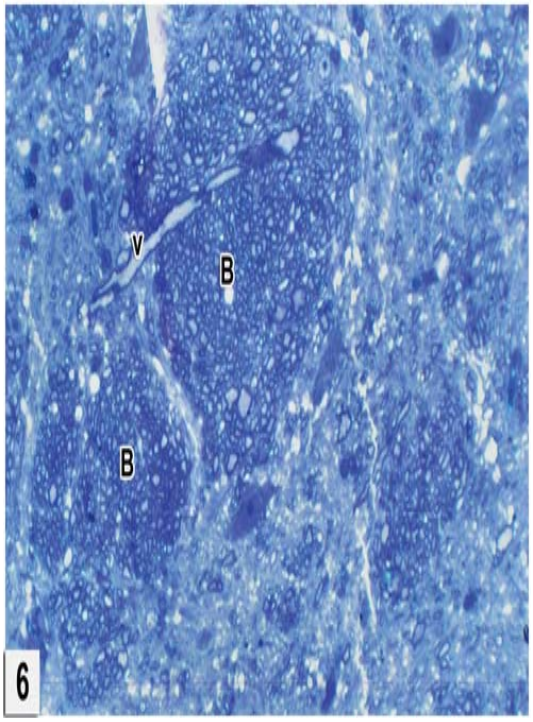

Fig. 6: A photomicrograph of a semithin section of the inferior colliculus of an adult rat showing the myelinated nerve fibers arranged in bundles (B) traversed by a blood vessel (v). Toluidine blue; X 400

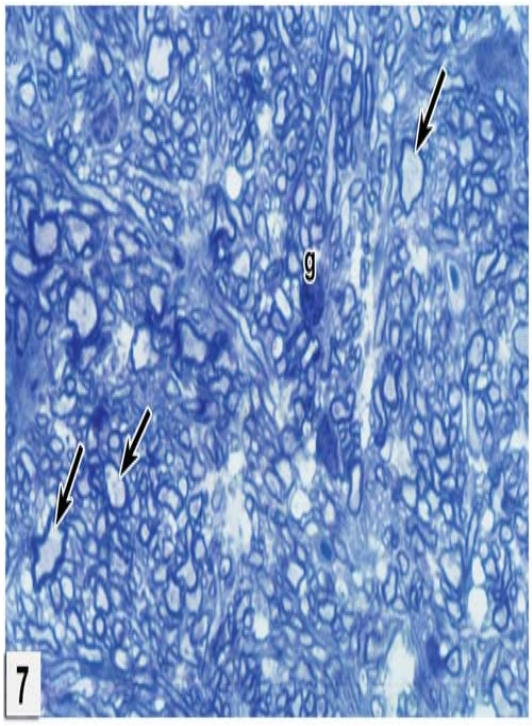

Fig. 7: A photomicrograph of a semithin section of the inferior colliculus of an adult rat showing the myelinated nerve fibers with uniform regular myelin sheaths (arrows). Note the presence of glial cells (g). Toluidine blue; X1,000

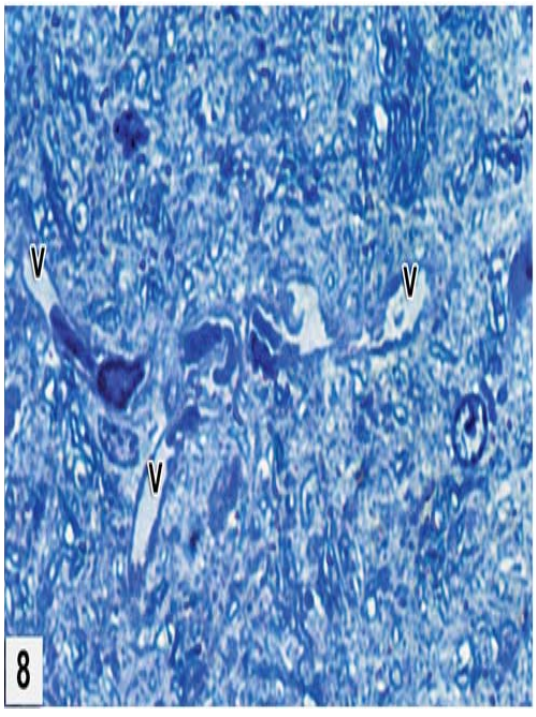

Fig. 8: A photomicrograph of a semithin section of the inferior colliculus of an adult rat showing blood vessels (V). Note the thickness of their walls. Toluidine blue; X1,000

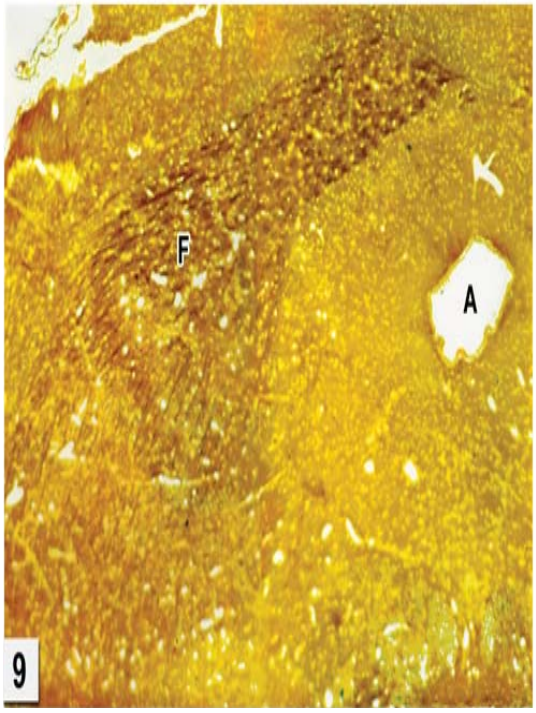

Fig. 9: A photomicrograph of a section of the inferior colliculus of an adult rat showing the arrangement of nerve fibers lateral and dorsal to the periaqueductal grey matter (A) forming bundles of parallel fascicles (F). Silver; X100

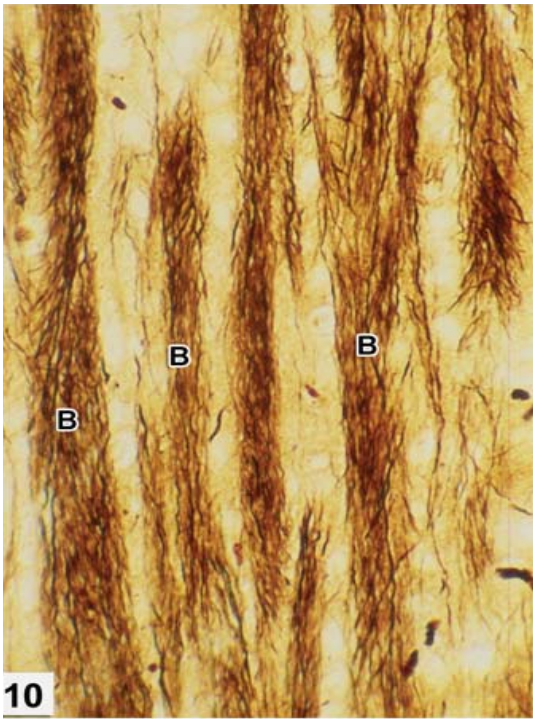

Fig. 10: A photomicrograph of a section of the inferior colliculus of an adult rat showing thick regular bundles (B) of nerve fibers. Silver; X400

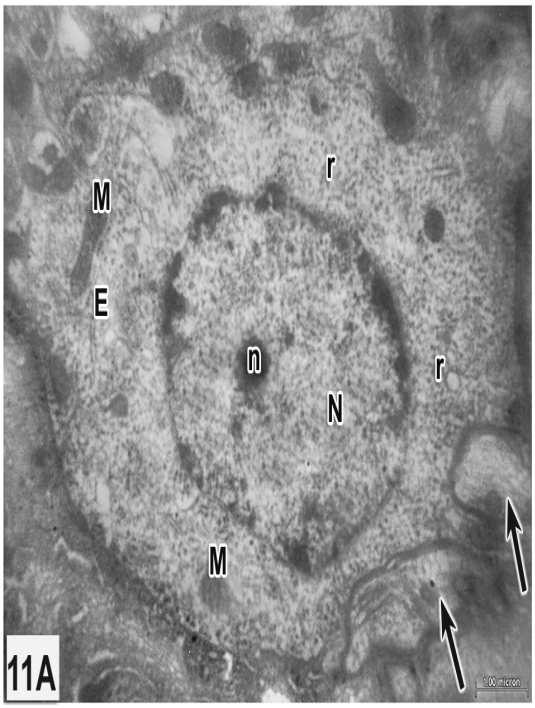

Fig. 11A: An electronmicrograph of the inferior colliculus of an adult rat showing nerve cell with oval euochromatic nucleus (N) and prominent nucleolus (n). Note the cisternae of rough endoplasmic reticulum (E), mitochondria (M) and numerous ribosomes (r) scattered in the cytoplasm. Notice also the adjacent myelinated nerve fibers (arrows). Uranyl acetate and lead citrate; X6,000

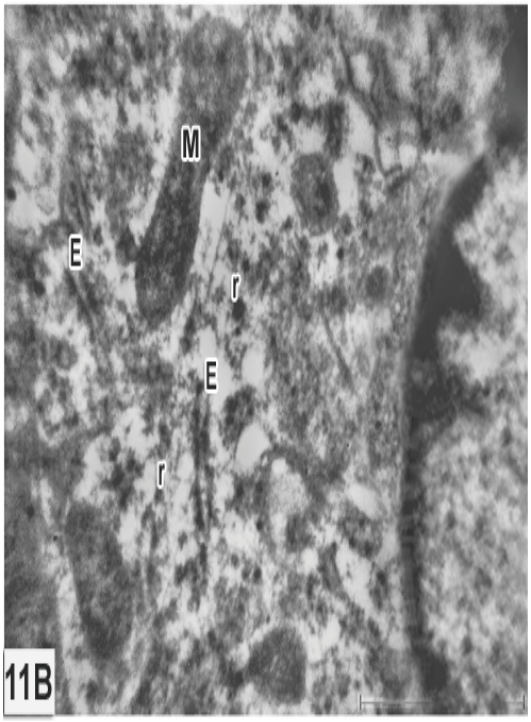

Fig. 11B: A higher magnification of the previous figure showing the cisternae of rough endoplasmic reticulum (E), mitochondria (m) and the scattered ribosomes (r) in the cytoplasm. Uranyl acetate and lead citrate; X20,000

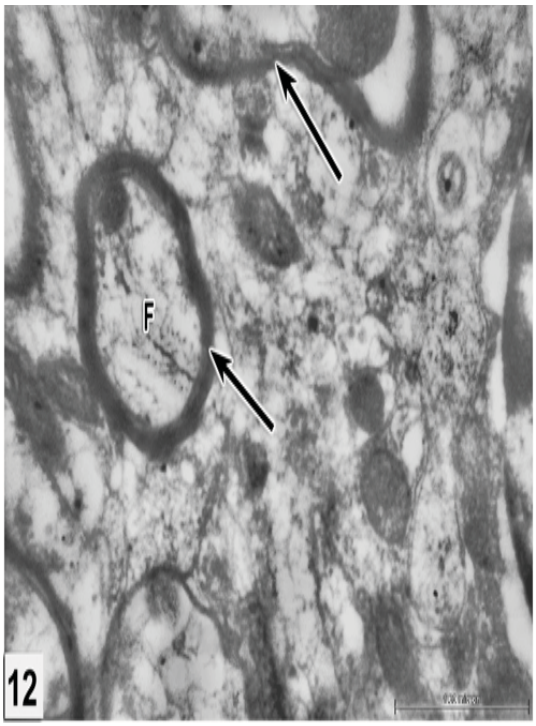

Fig. 12: An electronmicrograph of the inferior colliculus of an adult rat showing thick, uniform and regular myelin sheaths (arrows) surrounding nerve fibers (F). Uranyl acetate and lead citrate; X15,000

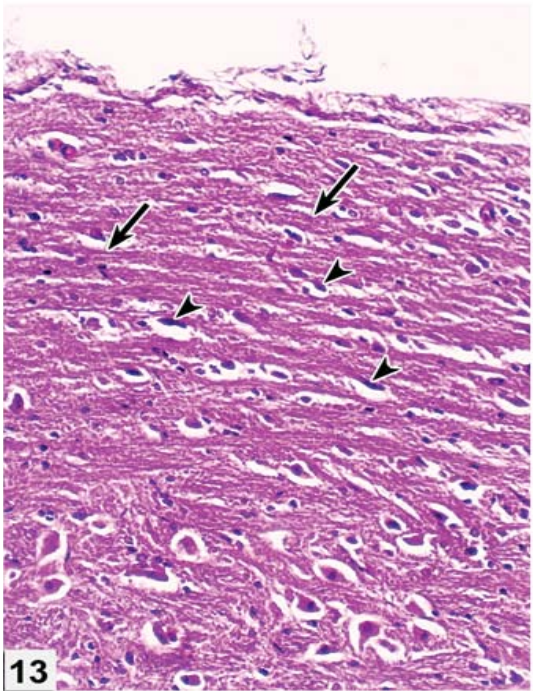

Fig. 13: A photomicrograph of a section of the inferior colliculus of a senile rat showing the transverse arrangement of nerve fibers in thin bundles in the dorsal zone (arrows) parallel to the surface. Note the presence of multiple small degenerated nerve cells (arrowheads) in between. Hx.& E.; 200

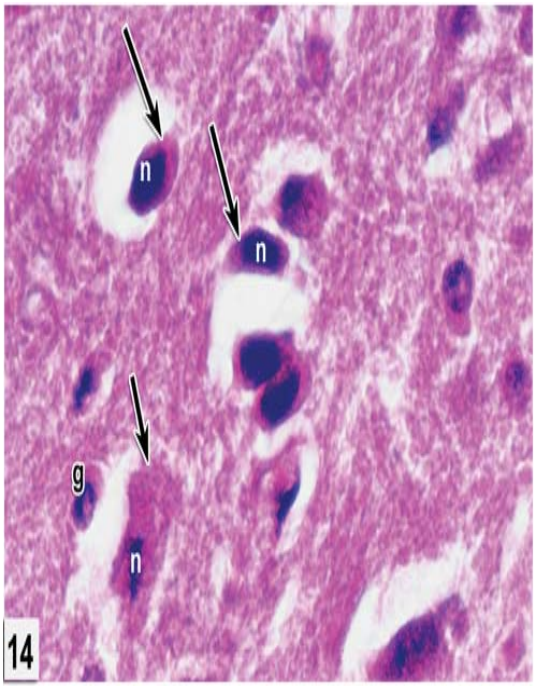

Fig. 14: A photomicrograph of a section of the inferior colliculus of a senile rat showing nerve cells with small dark pyknotic nuclei (n) surrounded with a thin rim of dense cytoplasm. Note the glial cell (g). Hx.& E.; 1,000

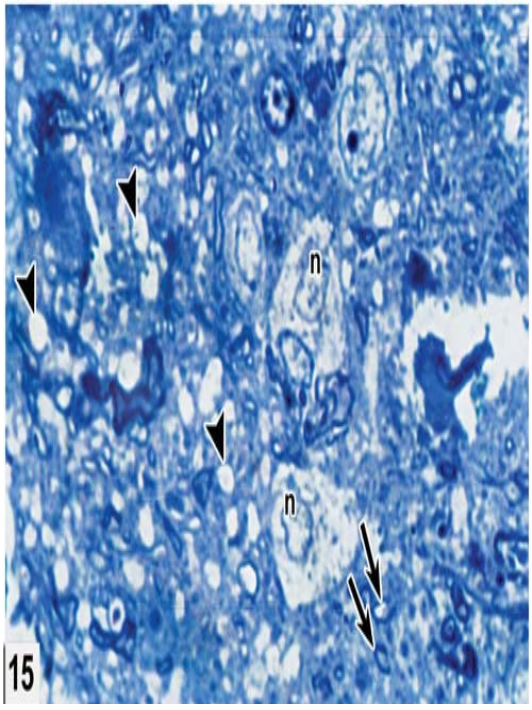

Fig. 15: A photomicrograph of a semithin section of the inferior colliculus of a senile rat showing swollen nerve cells with small eccentric nuclei (n). Note the partially demyelinated nerve fibers (arrows) and the unmyelinated ones (arrowheads) that appear as rounded empty spaces in the neuropil.
Toluidine blue; X1,000

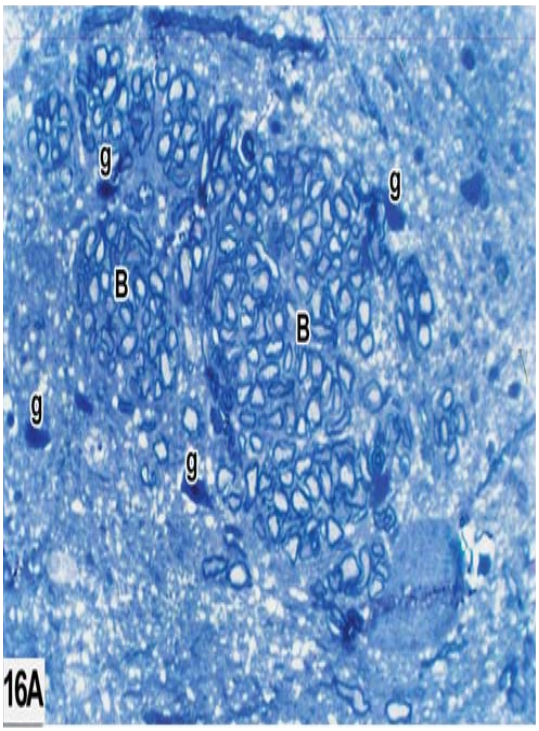

Fig. 16A: A photomicrograph of a semithin section of the inferior colliculus of a senile rat showing bundles (B) of myelinated nerve fibers. Note the dark stained glial cells (g). Toluidine blue; X400

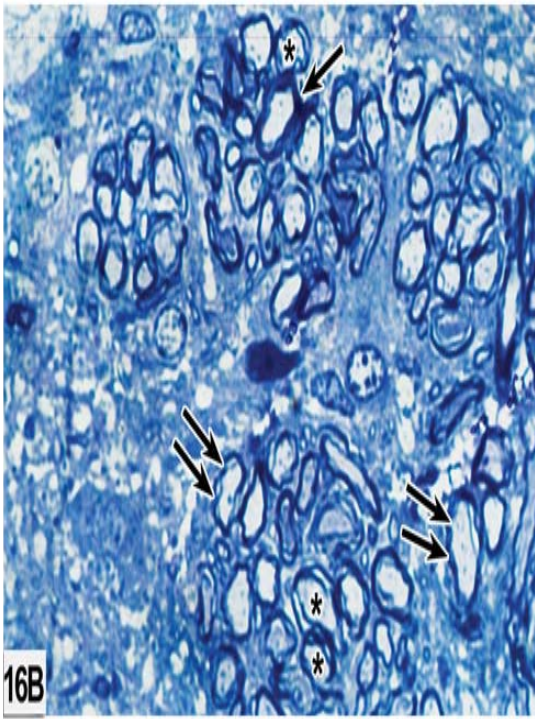

Fig. 16B: A higher magnification of the previous figure showing splitting of lamellae of the myelin sheath (asterisk) of some nerve fibers, thickened myelin sheath (arrow) in the others. Note the nerve fibers with shrunken axons (doublearrows). Toluidine blue; X1,000

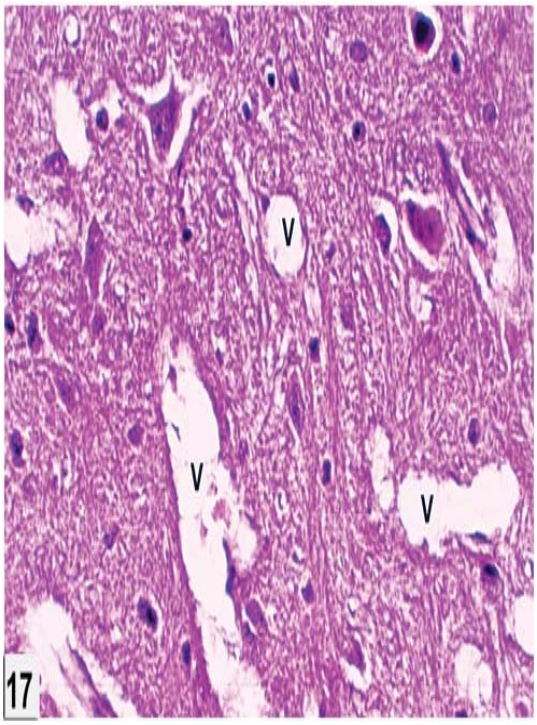

Fig. 17: A photomicrograph of a section of the inferior colliculus of a senile rat showing dilated blood vessels (V) with distorted basement membrane. Hx.& E.; 400

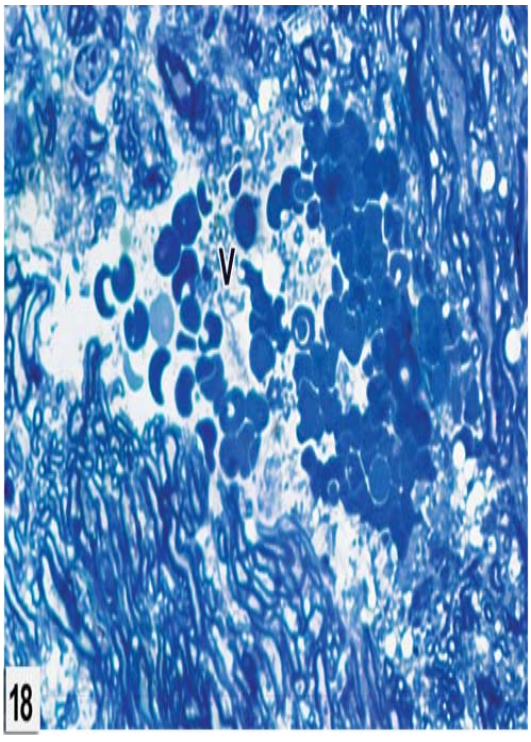

Fig. 18: A photomicrograph of a semithin section of the inferior colliculus of a senile rat showing congested blood vessel (V) with ill-defined basement membrane in between the myelinated nerve fibers. Toluidine blue; X1,000

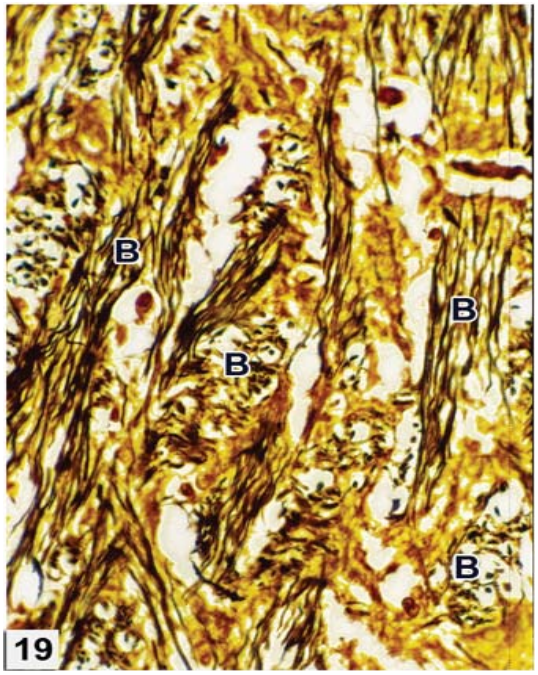

Fig. 19: A photomicrograph of a section of the inferior colliculus in senile rat showing arrangement of nerve fibers in thick interrupted bundles (B) running in different directions. Silver; X400

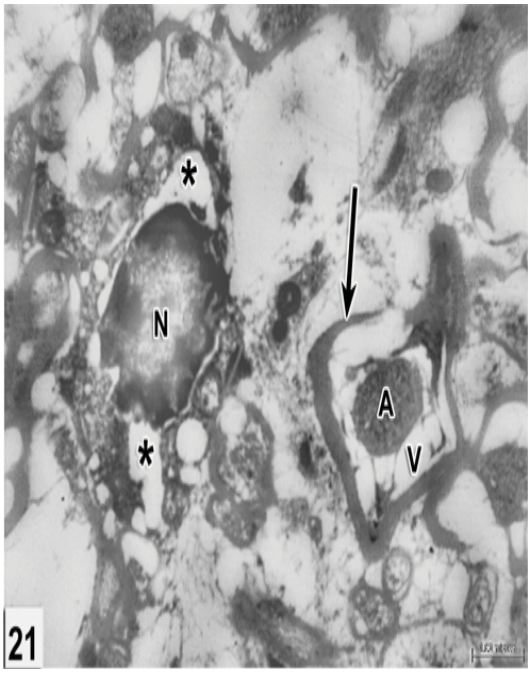

Fig. 21: An electronmicrograph of the inferior colliculus of a senile rat showing a nerve cell with small dark pyknotic nucleus (N) with crenated nuclear membrane. Note the wide perikaryal space (*). Note also the myelinated nerve fiber with disrupted myelin sheath (arrow), vacuolated cytoplasm (v) and shrunken degenerated nerve axon (A). Uranyl acetate and lead citrate; X6,000

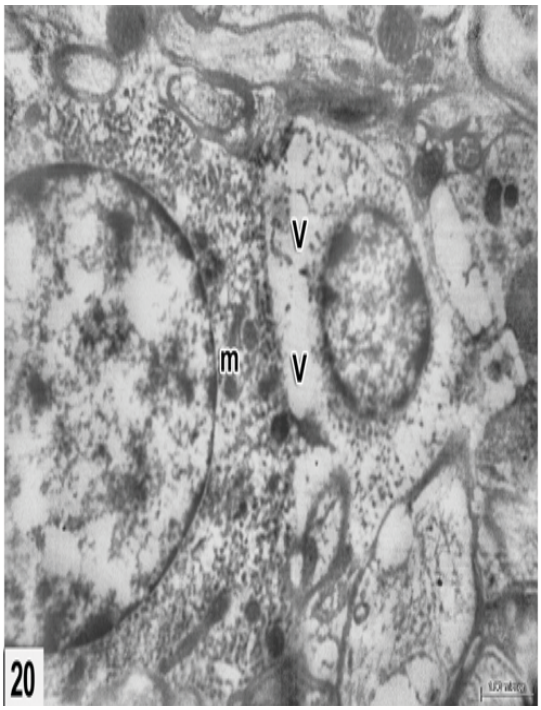

Fig. 20: An electronmicrograph of the inferior colliculus of a senile rat showing a nerve cell with multiple cytoplasmic vacuoles (v) and few cytoplasmic organelles, and another cell with small dark mitochondria (m). Uranyl acetate and lead citrate; X6,000

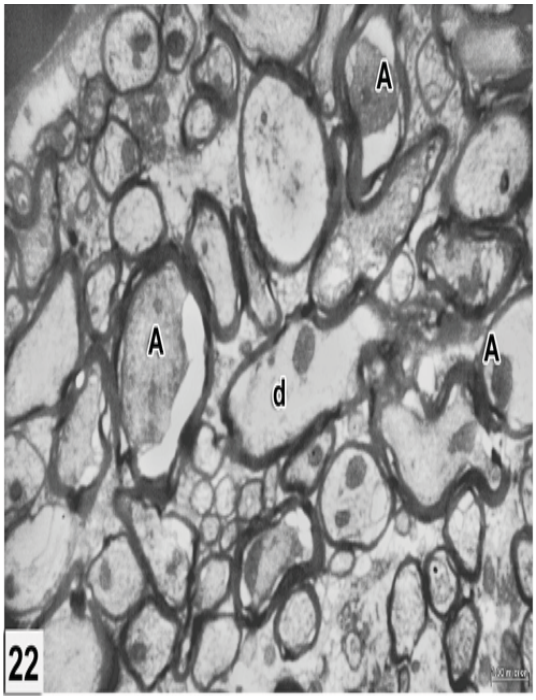

Fig. 22: An electronmicrograph of the inferior colliculus of a senile rat showing myelin balloons with compressed axons (A). Note the degenerated cytoplasmic matrix (d) in other nerve fibers. Uranyl acetate and lead citrate; X4,000

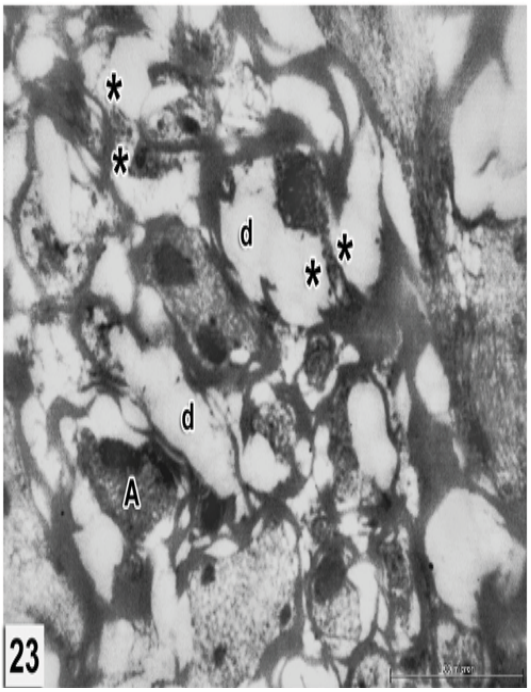

Fig. 23: An electronmicrograph of the inferior colliculus of a senile rat showing extensive degeneration of the myelinated nerve fibers. Note the irregular contour of the myelin sheaths, degenerated cytoplasmic matrix (d), shrunken degenerated axon (A). Notice also that some of the sheaths appear amalgamated (*). Uranyl acetate and lead citrate; X6,000

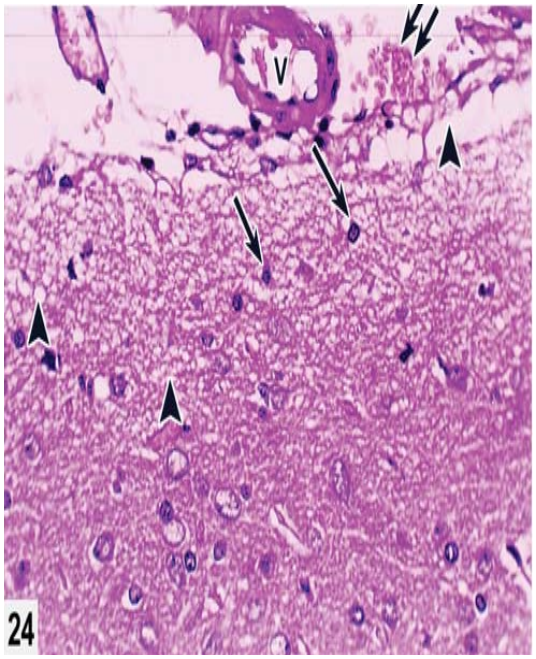

Fig. 24: A photomicrograph of a section of the inferior colliculus of a diabetic rat showing extensive vacuolation (arrowheads) in the dorsal zone with presence of small degenerated irregularly arranged nerve cells (arrows). Note the blood vessel with thick wall (V) on the dorsal surface and the nearby extravasated blood cells (double arrows).
Hx.& E.; 400

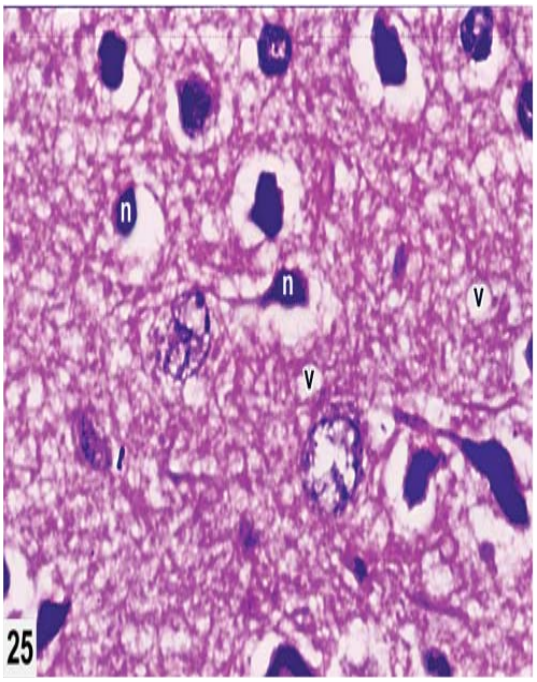

Fig. 25: A photomicrograph of a section of the inferior colliculus of a diabetic rat showing neuronal somata with small, dark and pyknotic nuclei (n) and a thin rim of cytoplasm. Note the extensive vacuolation (v) of the neuropil. Hx.& E.; 1,000

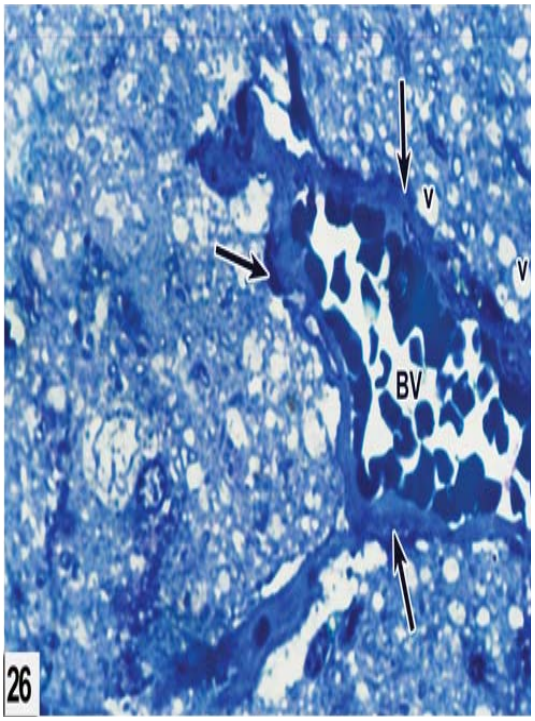

Fig. 26: A photomicrograph of a semithin section of the inferior colliculus of a diabetic rat showing dilated congested blood vessel (BV) with thickened basement membrane (arrows). Note the rounded vacuoles (v) in the neuropil.
Toluidine blue; X1,000

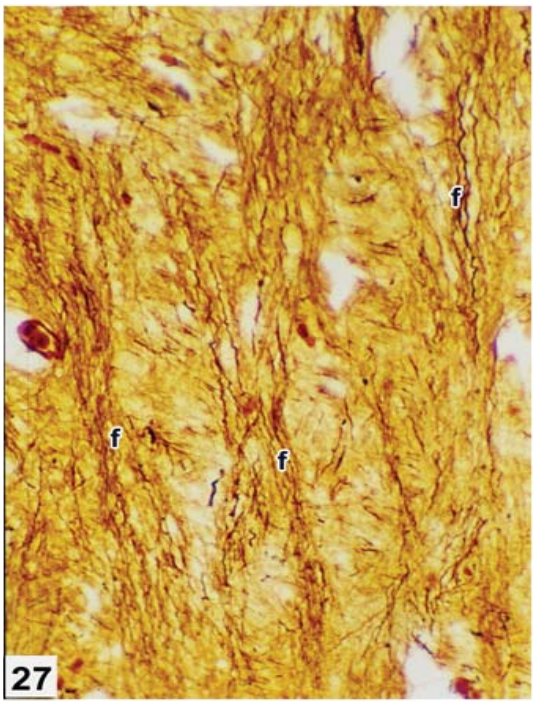

Fig. 27: A photomicrograph of a section of the inferior colliculus of a diabetic rat showing small bundles of thin irregular nerve fibers (f). Silver; X400

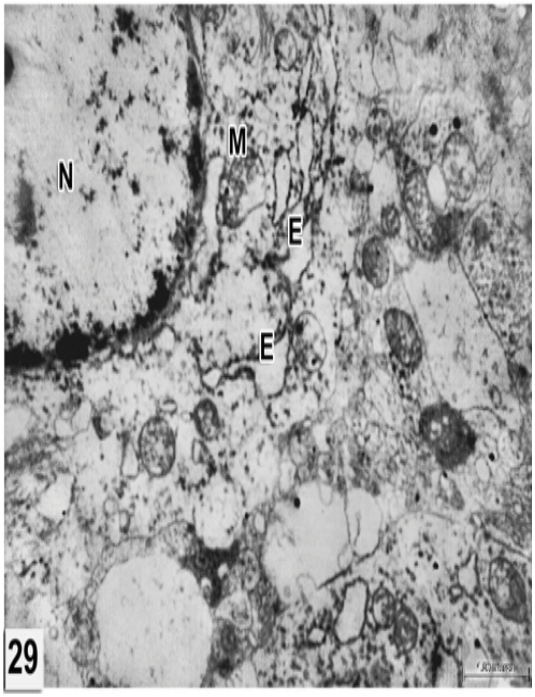

Fig. 29: An electronmicrograph of the inferior colliculus of a diabetic rat showing a nerve cell with dilated rough endoplasmic reticulum (E) and degenerated mitochondria (m) with disrupted cristae in the cytoplasm. Note the nucleus (N). Uranyl acetate and lead citrate; X8,000

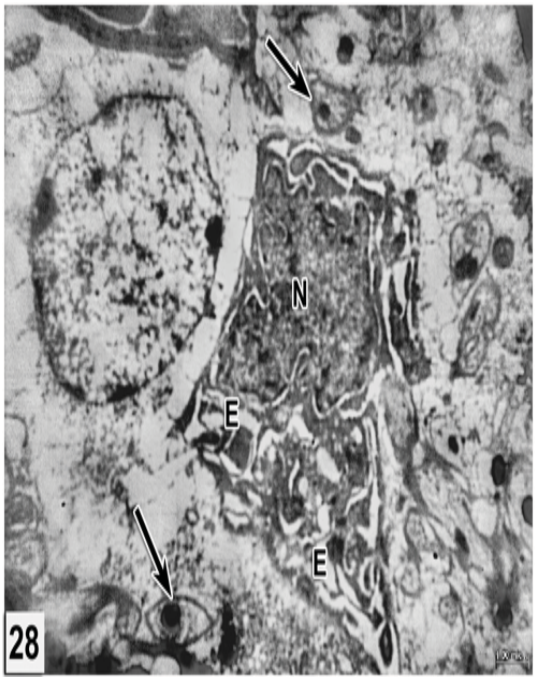

Fig. 28: An electronmicrograph of the inferior colliculus of a diabetic rat showing a nerve cell with small dark pyknotic nucleus (N), dilated rough endoplasmic reticulum (E). Note the nearby thin myelinated nerve fiber (arrow) Uranyl acetate and lead citrate; X3,000

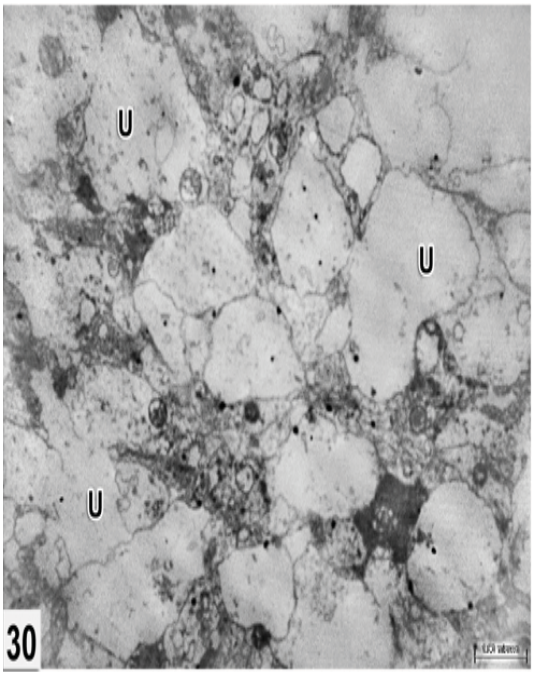

Fig. 30: An electronmicrograph of the inferior colliculus of a diabetic rat showing unmyelinated nerve fibers (U) of variable sizes, irregular contour and degenerated cytoplasm. Note the ballooned appearance of some of them. Uranyl acetate and lead citrate; X8,000

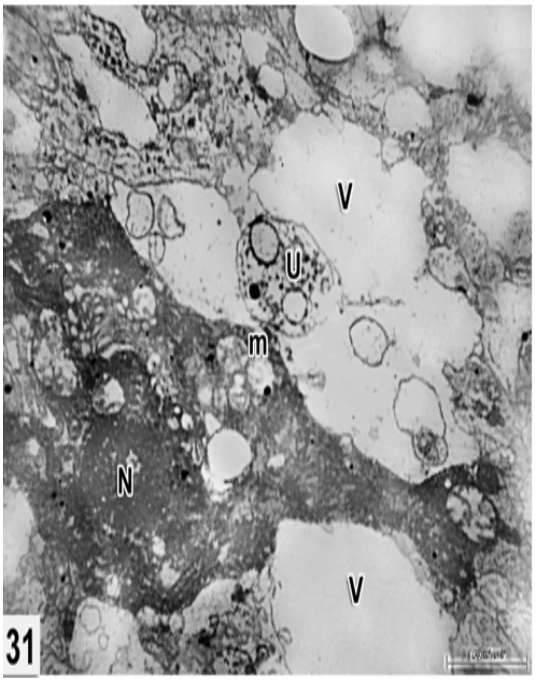

Fig. 31: An electronmicrograph of the inferior colliculus of a diabetic rat showing a nerve cell with small dark eccentric pyknotic nucleus (N), dark cytoplasm and degenerated mitochondria (m) with disrupted cristae. Note the unmyelinated nerve fiber (U) with degenerated cytoplasmic matrix. Notice also the vacuolated neuropil (V). Uranyl acetate and lead citrate; X10,000

DISCUSSION

The inferior colliculus occupies a key position in the auditory pathways. In the present study, the inferior colliculus in adult rats showed that the neuronal processes were more abundant than the neuronal somata. These findings are consistent with those reported by *Andrew and Paterson (1989)* who studied the postnatal development of the inferior colliculus of Sprague Dawley rats between the age of 4 days and 25 days. These authors observed a decrease in the neuronal and glial nuclear profiles per unit area associated with an increase in the neuronal processes, this was attributed to the increase in the volume of neuronal peikarya (i.e. amount of cytoplasm) and proliferation of the neuronal processes as well as increased myelination. *Gabriele et al. (2000)* described the afferent brainstem connections to the inferior colliculus and found that mature projection patterns are in place prior to the onset of hearing. In the current work, the inferior colliculus of the adult rats showed that the colliculus was formed of a dorsal superficial part and a deep central part. The superficial zone was formed mainly of nerve fibers with few nerve cells arranged in-between. These results are in agreement with the previous findings of *Faye-Lund and Osen (1985)*. These authors studied the anatomy of the inferior colliculus in rats using Golgi, Nissl and a combined cell- myelin method and found that it was formed of a central nucleus, an external cortex and a dorsal cortex. The external cortex was located lateral, ventral, ventrocaudal and rostral to the central nucleus. The dorsal cortex was located dorsomedially and dorsocaudally to the nucleus. *Crossman and Standring (2005)* also described the cortex of the inferior colliculus in humans and mentioned that it had four cytoarchitecture layers: layer I contains small neurons with radial dendritic fields; layer II, medium-sized neurons with ovoid dendritic fields parallel with collicular surface; layer III, medium-sized neurons with spherical dendritic fields; layer IV, large neurons with variably shaped dendritic fields. The superficial zone in the present work most probably represented the cortex of the inferior colliculus.

In the current study, the central part of the inferior colliculus was formed of clusters of myelinated nerve fibers and different sizes of oval or rounded nerve cells that exhibited bipolar, multipolar or unipolar nerve processes. Few were rounded in shape exhibiting no processes. These observations are compatible with the results of *Meininger et al. (1986)* who studied the inferior colliculus in mice and observed that the central nucleus contains two major types: the bipolar cells, which are the most abundant and the multipolar cells. *Najdzion et al. (2002)* also described the neuronal structure of the inferior colliculus in the adult bank vole (the rat of the fields) and found that the central nucleus showed a laminated appearance and observed 5 types of neurons, rounded, fusiform cells which constitute the main neuronal type. The other types included the pear-shaped, the large multipolar and triangular. The triangular cells were the least numerous. In the present study, nerve fibers were seen arranged in thick longitudinal bundles on each side of middle line of the ventral portion of IC. The silver- stained sections showed also the arrangement of the nerve fibers in bundles in a dorsomedial direction to periaqueductal grey matter. In accordance with the present results, *Meininger et al. (1986)* found that the fibers of lateral lemniscus which represents the main auditory input to the IC enters it ventrally and divide in two bundles. These two bundles are

directed dorsomedially to the central nucleus; the fibers of the lateral lemniscus are interspersed with other nerve fibers giving the appearance of a dense meshwork of fibers.

The term presbycusis refers to sensorineural hearing impairment in elderly individuals. It affects approximately 30-35% of the population between 65 and 75 years of age and 40-50% of people older than 75 years *(NIDCD Report, 2006)*. In the present study, the inferior colliculus of the senile rats showed extensive degree of degeneration in both the neuronal somata and processes. Some nerve cells appeared swollen with small eccentric nuclei, while the others showed dense cytoplasm and small dark pyknotic nuclei. The myelinated nerve fibers showed thickened, disrupted myelin sheaths, or showed balloons of myelin sheath with the axons compressed against the opposite side of the sheath. Some nerve fibers showed degenerated axons, leaving empty sheaths; while the others were unmyelinated due to lost myelin sheaths. The present findings could be explained by the results found by *Peters (2009)* who mentioned that in normal aging, the myelin sheath of most of the nerve fibers degenerate. Such axons are remyelinated by the oligodendrocytes which remain active during aging and continue to add lamellae to the myelin sheaths, so that the sheaths become thicker. Remyelination occurs by a series of shorter internodes than the original ones. The shorter internodes slow down the conduction velocity along the nerve fibers thus affecting the integrity in neuronal circuits with a subsequent cognitive decline. This author added that some axons degenerate resulting in a reduction in the total number of nerve fibers with subsequent disconnection between the neurons. In the present work, the blood vessels appeared dilated with distorted basement membrane. Since the inferior colliculus has one of the richest blood supply and greatest capillary densities in the nervous system *(Gross et al., 1986)*. So, the observed severely degenerated nerve cells, fibers and disrupted blood vessels in the present study might be contributing factors in the hearing loss in elderly. Moreover, *Popelara et al. (2006)* studied the auditory functions in rats during their lifespan by evaluating cochlear hair cell loss, middle ear compliance and auditory brainstem responses and found that senile rats represented a complex mix of conductive hearing loss and sensorineural hearing loss.

In the present study, alloxan was used to induce diabetes. Alloxan is known to damage pancreatic beta cells by production of hydroxyl radicals, but does not damage the glucagon producing alpha cells. It can be safely used to induce a chronic type 1 DM syndrome in adult rats that closely resemble human type-1 DM in humans *(Aleeva et al., 2002)*. The elevated glucose level in diabetes stiffens the erythrocyte membrane, and increased aggregation of red cells resulting in elevated blood viscosity. Diabetes also accelerates atherosclerosis which may interfere with perfusion of the tissues *(Cho et al., 2008)*. In the current study, the inferior colliculus of the diabetic rats showed congested blood vessels with thickened basement membrane in the extensively vacuolated neuropil in addition to degenerated neuronal somata. The vacuolated neuropil was most probably due to the extensive tissue edema. Similar results were reported by *Cho et al. (2008)* who observed also narrowing of the lumina due to thickening and hyalinization of the vessel walls. Endothelial cell hyperplasia with luminal degenerative material was also detected. *Diniz and Guida (2009)* mentioned that diabetes mellitus was associated with hearing loss in middle aged individuals which was explained by diffuse microangiopathy with consequent malnourishment of the nerves. *Kakarlapudi et al. (2003)* also added that the patients are usually thin and less than 40 years of age and hearing loss is normally progressive and sensorineural. Idiopathic sudden hearing loss was also recorded in patients with type 2 diabetes mellitus *(Fukui et al., 2004)*. *Vasilyeva et al. (2009)* assessed the hearing abilities in middle aged mice with type 1 and type 2 diabetes mellitus for a period of 6 months and observed that induction of diabetes promotes amplification of peripheral hearing loss and affected auditory midbrain resulting in disruption of the central inhibition and enhanced excitation in the inferior colliculus.

In the present work, most of the nerve fibers were unmyelinated and the myelinated ones showed apparently thin myelin sheath. Both types of the nerve fibers showed vacuolated cytoplasm (axoplasm). Some of the unmyelinated nerve fibers appeared swollen. These observations agree with the data reported by *Said (2007)* who mentioned that diabetic neuropathy included axonal degeneration, primary demyelination resulting from Schwann cell dysfunction, secondary segmental demyelination due to impairment of the

axonal control and remyelination. Demyelination results in dysfunction of the affected neurons .This author also added that some axons may appear swollen with ballooned appearance and granular degeneration of the neurotubules and neurofilaments. *Oates (2002)* attributed diabetic neuropathy to increased levels of intracellular glucose in the nerves leading to saturation of the normal glycolytic pathway. Extra glucose is shunted into the polyol pathway and converted to sorbitol and fructose by the enzymes aldose reductase and sorbitol dehydrogenase. Accumulation of sorbitol leads to decreased membrane Na/K-ATPase activity, impaired axonal transport and structural breakdown of nerves. Short-term treatment with aldose reductase inhibitors improves nerve conduction and reduces the neuropathic peripheral pain in subjects with diabetes *(Sima et al., 1988)*. Diabetic neuropathy and angiopathy were also attributed to the increased oxidative stress in diabetes due to prolonged exposure to hyperglycemia with impairment of oxidant/ antioxidant equilibrium. Proteins and lipids are among the prime targets for oxidative stress. Increased protein oxidation, lipid peroxidation and NO levels decrease the levels of enzymatic and nonenzymatic antioxidants and play a major role in diabetic complications *(Ramakrishna & Jailkhani, 2008; Aladag et al., 2009)*. Antioxidant treatment in diabetics was also suggested *(Ceriello & Testa, 2009)*. *Frisina et al. (2006)* utilized the hearing tests to measure both the peripheral (cochlea) and central (brainstem and cortex) auditory functions in aged type 2 diabetes and observed a severe hearing loss in aged diabetics. As mentioned before, deleterious outcomes can occur in the ear and central auditory system with increased duration of diabetes. Therefore, the auditory health and early clinical testing of the diabetics are so important for early prevention of deleterious complications of diabetes.

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تأثير الشيخوخة و داء البول السكرى الناجم عن الحقن بالألوكسان على تركيب البرزة السفلية للمخ الأوسط فى ذكور الجرذان البيضاء: دراسة باستخدام المجهرالضوئى والمجهر الالكترونى

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ملخص البحث

أجري هذا البحث لدراسة التغيرات الباثولوجية فى هيكل البرزة السفلية للمخ الأوسط الناجمة عن الشيخوخة ومرض داء البول السكري فى ذكور الجرذان البيضاء. تم فى هذا البحث إستخدام ثلاثين من ذكور الجرزان البيضاء تم تقسيمهم الى ثلاث مجموعات تتكون كل منها من عشرة جرزان كالتالى:

المجموعة الأولى هى الضابطة وتشمل الجرزان البالغة، والمجموعة الثانية فتضم الجرزان المسنة، اما المجموعة الثالثة فهى مجموعة الجرزان البالغة المصابة بداء البول السكرى. وقد تم إحداث المرض بالحقن بجرعة واحدة من الألوكسان داخل التجويف البريتونى بجرعة ٢٠٠ مج/كجم من وزن الجسم مذاب في الماء المقطر. بعد شهرين تم تخدير الجرزان بإستنشاق الأثير. تم إستخراج البرزة السفلية للمخ الأوسط وأعدت العينات للفحص بواسطة كل من المجهرالضوئى والمجهر الالكترونى.

وقد أظهر فحص الجرزان البالغة إحتواء البرزة السفلية على زوائد عصبية أكثر من الخلايا العصبية وكانت الألياف العصبية مرتبة في مجموعات و مغطاه بغشاء المايلين السميك، والمنتظم الشكل. كما كانت البرزة السفلية مكونه من قشرة سطحية وجزء مركزى غائر. وقد ظهرت الخلايا العصبية قليلة فى العدد ومستديرة فى الشكل فى منطقة القشرة ،بينما كانت فى مركز البرزة كثيرة ومختلفة الأشكال و الأحجام كما كان لها زوائد أحادية، ثنائية أو متعددة الأقطاب. ولوحظ فى فحص عينات الجرزان المسنة درجات متفاوتة من التحلل فى كل من الخلايا والألياف العصبية . فكانت الخلايا العصبية أما منتفخة أو صغيرة فى الحجم مع ستيوبلازم داكن وأنوية صغيرة وداكنة. أماغشاء المايلين للألياف العصبية فظهر أما سميكا،أو متقطع ، أوكانت طبقاته متباعدة أو ظهر فارغا مع تحلل أوانكماش الليفة العصبية. أما فحص عينات الجرزان المصابة بداء البول السكرى فقد أظهر أن معظم الخلايا العصبية صغيرة فى الحجم وبها أنوية صغيرة وداكنة. اما المساحة بين الخلايا والالياف العصبية فلوحظ بها فجوات كثيرة مع زيادة سمك جدر الأوعية الدموية مقارنة بالمجموعة الضابطة. وبدت الألياف العصبية رفيعة وغير منتظمة الشكل،كما لوحظ وجود كل من الألياف العصبية المحاطة وغير المحاطة بغشاء المايلين مع ظهور فجوات بالسيتوبلازم. مما سبق يتضح أن التغيرات الباثولوجية فى الجرزان المصابة بالسكر كانت أسوأ من تلك التي لوحظت في الفئران المسنة وبالتالى قد يؤدى ظهورها فى صغار السن المصابين بداء البول السكرى إلى الإصابة بفقدان السمع و بالتالى ينصح بالأهتمام بالصحة السمعية والكشف الطبى المبكر لمرضى السكر للوقاية من المضاعفات الضارة المحتملة.