Original Article

Effect of Dehydroepiandrosterone (DHEA) Therapy on The Age-Related Changes of the Primary Auditory Cortex in Senile Male Albino Rats. A Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Dehydroepiandrosterone (DHEA) is a metabolic intermediate in the biosynthesis of estrogens and androgens. It is proven to have central and anti-aging effects.

Aim of the Work: To investigate the effect of administration of DHEA on the age-related changes of primary auditory cortex in senile male albino rats.

Materials and Methods: 10 adult male rats aged 4-6 months (200-250 gm) and 30 senile male albino rats aged 20-24 months (350-500 gm) were used in this study. Group I (adult group): 10 rats received no medications for 8 weeks. Group II (senile control group): 20 senile rats and were further subdivided into two subgroups:

- Subgroup IIA (Negative senile control): included 10 rats that were not given any treatment for 8 weeks.
- Subgroup IIB (Vehicle senile control): included 10 rats that rats received 1ml/kg/B.W. of Dimethyl Sulfoxide (DMSO) once daily by oral gavage for 8 weeks.

Group III (senile treated with DHEA):10 rats received 20mg/kg/B.W. of DHEA dissolved in 1ml of DMSO once daily by oral gavage for 8 weeks.

Results: Examination of cerebral cortex of group II showed significant decline in cortical thickness. There were marked degenerative changes and decreased Nissl granules in pyramidal cells. The neuropil showed vacuolation with pericellular spaces. Immunohistochemical study showed significant increase in GFAP positive astrocytes. Administration of DHEA improved the altered cerebral morphology with significant statistical improvement in pyramidal cells and astrocytes count.

Conclusion: DHEA ameliorate age associated changes in the primary auditory area of the rat cerebral cortex. Hence, it is recommended to include the DHEA as a powerful therapeutic strategy for age related hearing loss in aged individuals**.**

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Key Words: AGE related hearing loss, aging, cerebral cortex, DHEA, primary auditory area

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INTRODUCTION

Dehydroepiandrosterone (DHEA) acts as a metabolic intermediate in the synthesis of estrogens and androgens. It was once known as a wonder drug, a fountain of youth that could treat all diseases[1]. It is produced from the adrenal glands and the gonads. It can also be synthesized in the brain and nerves, either by metabolism of circulating hormones or by de novo synthesis from cholesterol. They were called “neurosteroids”[2].

DHEA and its sulphate easter (DHEAS) assist in multiple functions in the nervous system, including plasticity and survival of neurons, behavior and cognition. This suggests that they may have both preventive and therapeutic benefits for a variety of neurodegenerative and neuropsychiatric disorders. They have potential benefit to prevent and treat Alzheimer’s disease[3].

DHEA and DHEAS levels decrease steadily with age. Studying the biological effects of DHEA, gave rise to a theory that raising DHEA to youthful levels could protect the brain, slow the consequences of aging and improve sex function. These benefits could include enhancing wellbeing and optimistically extending life[4].

Age related hearing loss (ARHL), is considered one of the most prevalent sensory deficits in the elderly. Around 25–40% of people over 65 and 80% of people over 85 are affected by it[5]. Unfortunately, many studies have linked ARHL to social isolation, depression, cognitive deterioration and gait difficulties[6].

Aging has multiple effects on both central (brainstem, thalamus and cortex) and peripheral (cochlea) auditory systems. Several studies have studied the effects of aging on the peripheral auditory system. However, few studies have studied the effects.

of aging in the central auditory system, including the primary auditory cortex [7]. Therefore, the aim of the present study was to further describe the age-related changes of the primary auditory cortex and examine the possible effects of DHEA.

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MATERIAL AND METHODS

Drugs

DHEA 50mg tablets were dissolved in dimethyl sulfoxide (DMSO) (both will be purchased from Sigma, St. Louis, MO, USA), DMSO was in the form of colorless liquid. It was used as a vehicle for DHEA at a concentration of 20mg/ml. The dose of DHEA given to rats was 20mg/kg body weight dissolved in 1ml of DMSO by oral gavage once/day for 8 weeks[8].

Experimental animals

40 male albino rats were used for the study. Rats were bought from Animal house of the Medical Research Centre (MRC), Ain Shams University. Rats were housed in wire mesh cages in a regulated room temperature about 21 ±10˚C, humidity 45-50%, and light/dark cycles. They were fed on standard rat diet and had free access to water. They were left to acclimatize to experimental conditions by housing them for 10 days before the experiment. Animal procedures in this study followed the rules of the institutional Animal Research and Ethics Committee.

Study design

10 adult male rats aged 4-6 months (200- 250 gm) and 30 senile male albino rats aged 20-24 months (350-500 gm) were used.

Group I (adult control group): It was composed of 10 adult male rats received no medications for 8 weeks.

Group II (senile control group): It was consisted of 20 senile male albino rats and was subdivided into two subgroups:

- Subgroup IIA (Negative senile control): included 10 rats that were not given any treatment for 8 weeks.
- Subgroup IIB (Vehicle senile control): included 10 rats that rats received 1ml/kg/B.W. of DMSO once daily by oral gavage for 8 weeks.

Group III (senile rats treated with DHEA): 10 Rats received 20mg/kg/B.W. of DHEA dissolved in 1ml of DMSO once daily by oral gavage for 8 weeks[8].

Sample size of the experimental groups was calculated using the resource equation method described by Arifin and Zahiruddin[9]. Accordingly, the required animal number for comparing the three groups was 10 rats per group.

Specimen Collection

At the end of experiment, rats were fasted overnight, anesthetized and sacrificed. The skulls were opened using bone forceps to expose the brain of the rats. The brains were dissected out and the caudal third of both cerebral hemispheres were cut by coronal section.

Tissue Processing for Light microscopy: Paraffin Sections

Specimens of cerebral hemispheres were fixed in Bouin's solution for 24 hours and processed to prepare paraffin blocks. Sections of 5µ thick were cut, stained with hematoxylin and eosin for general histological picture and silver stain for nerve fibers[10].

Semithin Sections

Specimens were fixed in 3% phosphate buffered glutaraldehyde, washed in phosphate buffer saline and fixed in 1% osmium tetraoxide, then dehydrated in alcohol and embedded in epon blocks. Semithin sections were cut at 1µm thickness with a glass knife and stained with 1% toluidine blue stain[10].

Immunohistochemistry

Sections were incubated with a monoclonal antibody glial fibrillary acidic protein (GFAP); (Sigma, St Louis, Missouri, USA) used for detection of astrocytes. Detection of the antibody was carried out using a biotin–streptavidin detection system with 0.05% diaminobenzidine as a chromogen for GFAP[11]. GFAP positive astrocytes appeared brownish in color.

All stained sections were examined with the light microscope and photographed with Olympus E330 camera in the anatomy department of Ain Shams University.

Computer Image Analysis and Statistical Analysis

Computer image analysis was performed to measure the auditory cortical thickness in µm, the count of pyramidal cells and the count of GFAP positive astrocytes, utilizing image analysis software in five non overlapping fields in five different H&E- stained sections and from five different animals in each group. Results will be subjected to statistical analysis by the Statistical package for Social Studies (SPSS) program version 17. Comparison of means was done using one way analysis of variance (ANOVA) followed by post-hoc two-tailed Student's test to compare between groups[12]. Data were represented in tables.

RESULTS

Behavioral and Gross changes

It was observed that senile rats of group II looked more irritable than other groups.

Brain specimens of this group were apparently shrunken and congested compared to adult group.

Histological and immunohistochemical results

H & E staining

Examination of control adult group showed the six layers of the primary auditory cortex: I the molecular layer, II the external granular layer, III the external pyramidal layer, IV the internal granular layer, V the internal pyramidal layer and VI the polymorphic layer. These layers could be easily identified with vertical arrangement of cells. Blood vessels were seen within the cortical layers with narrow perivascular spaces (Figures 1 A,B).

Pyramidal cells were characterized by large, circular nuclei with evident nucleoli. The cytoplasm was basophilic extending along the apical dendrites which were thick and directed towards the surface. Granular cells had pale vesicular rounded nuclei and an outer rim of basophilic cytoplasm. Few neuroglia were also seen in the neuropil. Oligodendroglia were small cells adjacent to neurons or free in the neuropil having oval lightly stained or dark nuclei. Astrocytes had larger nuclei and pale vesicular nuclei, some of them appeared in pairs or observed adjoining the blood capillaries (Figure 1 C).

Examination of sections of the auditory cortex of the senile control subgroups (IIA&IIB) revealed almost similar structure of the primary auditory area of the cerebral cortex.

Examination of senile control groups showed decreased thickness of the cortical layers confirmed by the morphometric study. Areas of vacuolations in the neuropil were seen. There was apparent increased number of oligodendroglia and microglia. Microglia had elongated darkly stained nuclei. Moreover, some areas showed acidophilic masses containing dark nuclei and surrounded by clear areas. Apparent increased thickness of pia matter is noticed (Figure 2 A).

There was reduction in the number of pyramidal cells confirmed by the morphometric study. Many pyramidal cells appeared shrunken, with deeply stained nuclei surrounded by large unstained peri- cellular spaces. Few large pyramidal cells were noticed with dark basophilic cytoplasm, large vesicular nuclei. Some granular cells appeared dark, shrunken with pyknotic nuclei and large peri-cellular spaces. karyolitic nuclei were also seen (Figure.2-B). A neurofibrillary tangle (NFT) that appeared as a long filament within the apical dendrite of the pyramidal cells were also seen (Figure 2 C).

However, examination of senile male albino rats receiving DHEA revealed that the cortical layers preserved their normal proportions and architectures.Compact

neuropil with no vacuolations were seen in this group. Apparent decreased number of neuroglia cells were also seen. Pia matter was still thickened with presence of cellular infiltration (most probably inflammatory cells) in congested blood vessels (Figure 3A). Apparently normal densely aggregated granular cells that looked rounded with pale vesicular rounded nuclei and an outer rim of basophilic cytoplasm were observed. (Figure 3 B). Most of the pyramidal, granular and neuroglia cells were nearly similar to those of the control adult group. Occasional pyramidal cells were still affected which appeared shrunken and nuclei were darkly stained and surrounded by pericellular space. Few microglia cells with rod shaped nuclei were seen. Some fields showed karyolitic nuclei (Figure 3 C).

Sliver staining

Examination of control adult group showed well-organized cell arrangement and high density of nerve fibers in the cortical layers. Apical dendrites of pyramidal cells were clearly defined, being long, thick, and directed towards the surface of the cortex. Collaterals arising from the stem of the apical dendrite were seen (bifurcated apical dendrite). The basal dendrites were short, tapering and branching (Figure 4 A).

Examination of control senile groups revealed apparent decrease in nerve fibers density. Apical dendrites of pyramidal cells were thin, shrunken and broken (Figure 4 A). While examination of senile male albino rats receiving DHEA showed apparent increased density of nerve fibers. The apical process of pyramidal cells appeared relatively long (Figure 4 C).

Semithin results

Semithin sections of the adult group showed large pyramidal cells in the internal pyramidal layer. They had large vesicular nuclei with prominent nucleoli surrounded by faint cytoplasm that contains basophilic Nissl's bodies within it. Oligodendroglia were also seen (Figure 5 A).

Examination of control senile groups showed multiple darkly stained neurons with irregular boundaries. Minute pearly white droplets most probably lipid droplets were also seen (Figure .5 B). Pyramidal cells had very long apical dendrite with neurofibrillary tangle within it (Figure 5 C). The neuropil showed multiple vacuoles. Inflammatory cells were seen in some fields. There was partial depletion of Nissl bodies in the cytoplasm and apical dendrites of pyramidal cells (Figure 5 D).

While semithin sections of DHEA showed restoration of the Nissl granules inside the cell bodies and dendrites of pyramidal cells (Figure 5 E). Dividing granular cells were seen. Scanty vacuolations were noticed in the neuropil (Figure 5 F).

GFAP staining

Control adult group showed mild positive GFAP immunohistochemical staining in the cytoplasm of astrocytes and their processes. Astrocytes appeared small with few short, thin processes (Figure 6 A).

Control senile groups showed strong positive GFAP staining in the cytoplasm of abundant astrocytes with multiple thickened processes (Figure 6 B).

DHEA group showed GFAP moderate positive staining in the cytoplasm of astrocytes and their processes (Figure 6 C).

Statistical results

Cortical Thickness

Meancorticalthicknesswas(1401.47±79.22)(Mean±Sd) in control group, (1227.04 ± 90.29) in senile group and (1366.04 ± 56.12) in DHEA group. There was statistically significant decline in the mean cortical thickness in the senile group compared to the adult group (P=0.001) and

significant rise in the DHEA group compared to the senile group (*P*=0.002) (Table 1).

The Count of pyramidal cells

Mean count of pyramidal cells was (3.5 ± 0.92) (Mean ± Sd) in adult group, (1.12 ± 0.46) in senile group and (2.12 ± 0.64) in DHEA group. There was *highly* statistically significant decline in the mean count of pyramidal in the senile group compared to the adult group (*P*=3.45E-05) and significant rise in the DHEA group compared to the senile group (*P*=0.007516) (Table 2).

The Count of GFAP Positive Astrocytes

Mean count of GFAP positive astrocytes was (6.5 ± 1.19) (Mean ± Sd) in adult group, (14.37 ± 2.66) in senile group and (6.5 ± 1.6) in DHEA group. There was *highly* statistically significant rise in the mean count of GFAP positive astrocytes in the senile group compared to the adult group (P=2.42E- 06) and significant decline in the DHEA group compared to the senile group (P=4.91E-06) (Table 3).

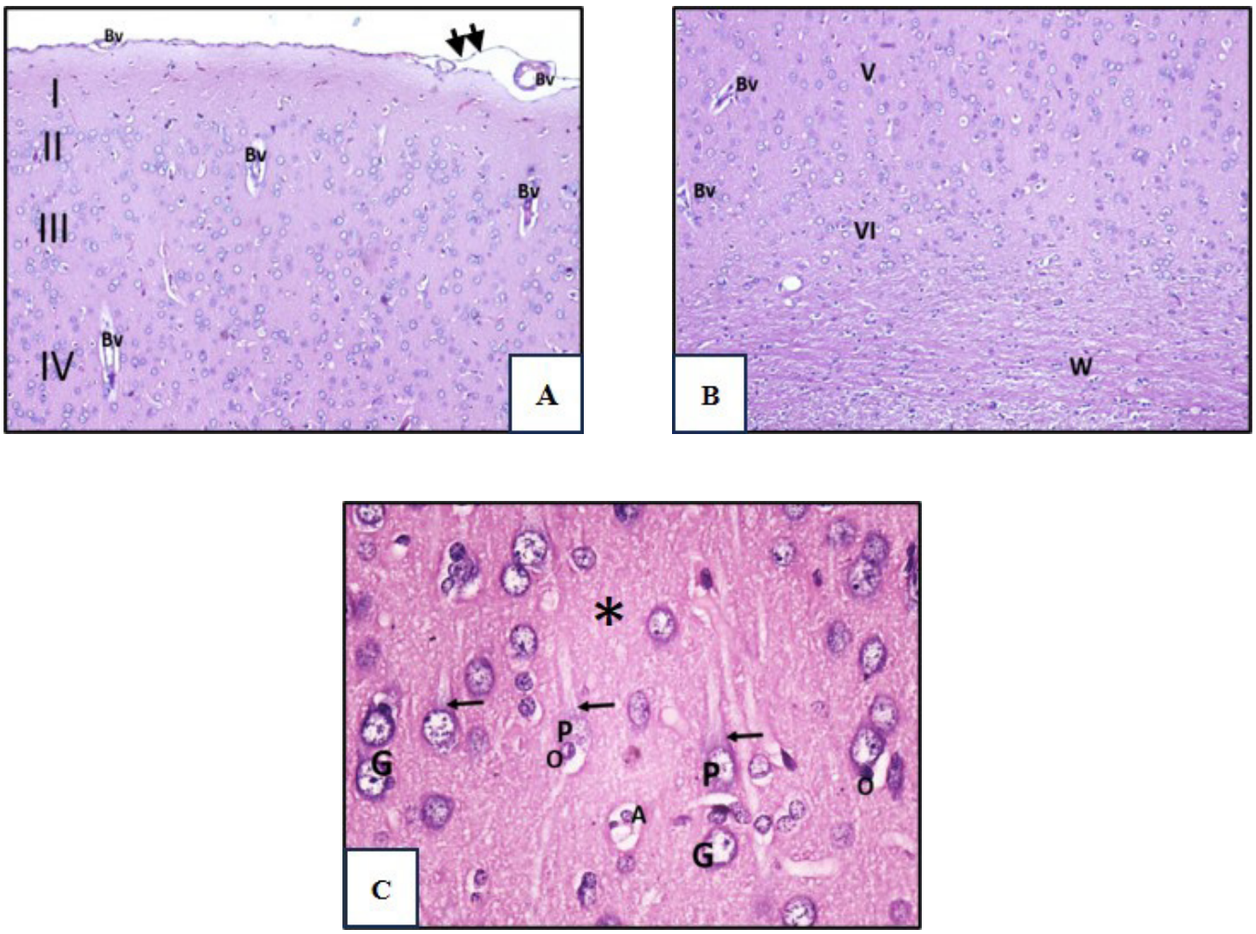

Fig. 1: Photomicrograph of sections in the primary auditory area of adult albino rats (Group I) (A) showing the outer layers: molecular layer (I), external granular layer (II), external pyramidal layer (III) and internal granular layer (IV). The pia matter (arrows). blood vessels (Bv). X100 (B) showing the inner layers: internal pyramidal layer (V) and polymorphic layer (VI). The white matter (W) and blood vessels (Bv). X100 (C) showing the external pyramidal layer. Medium sized pyramidal cells (P) with large rounded vesicular nuclei and long apical dendrites (arrow). Granular cells (G) with pale vesicular rounded nuclei and an outer rim of basophilic cytoplasm. Oligodendroglia (O) and astrocyte (A). Eosinophilic neuropil (*) forming the background for the cells. X400 H&E.

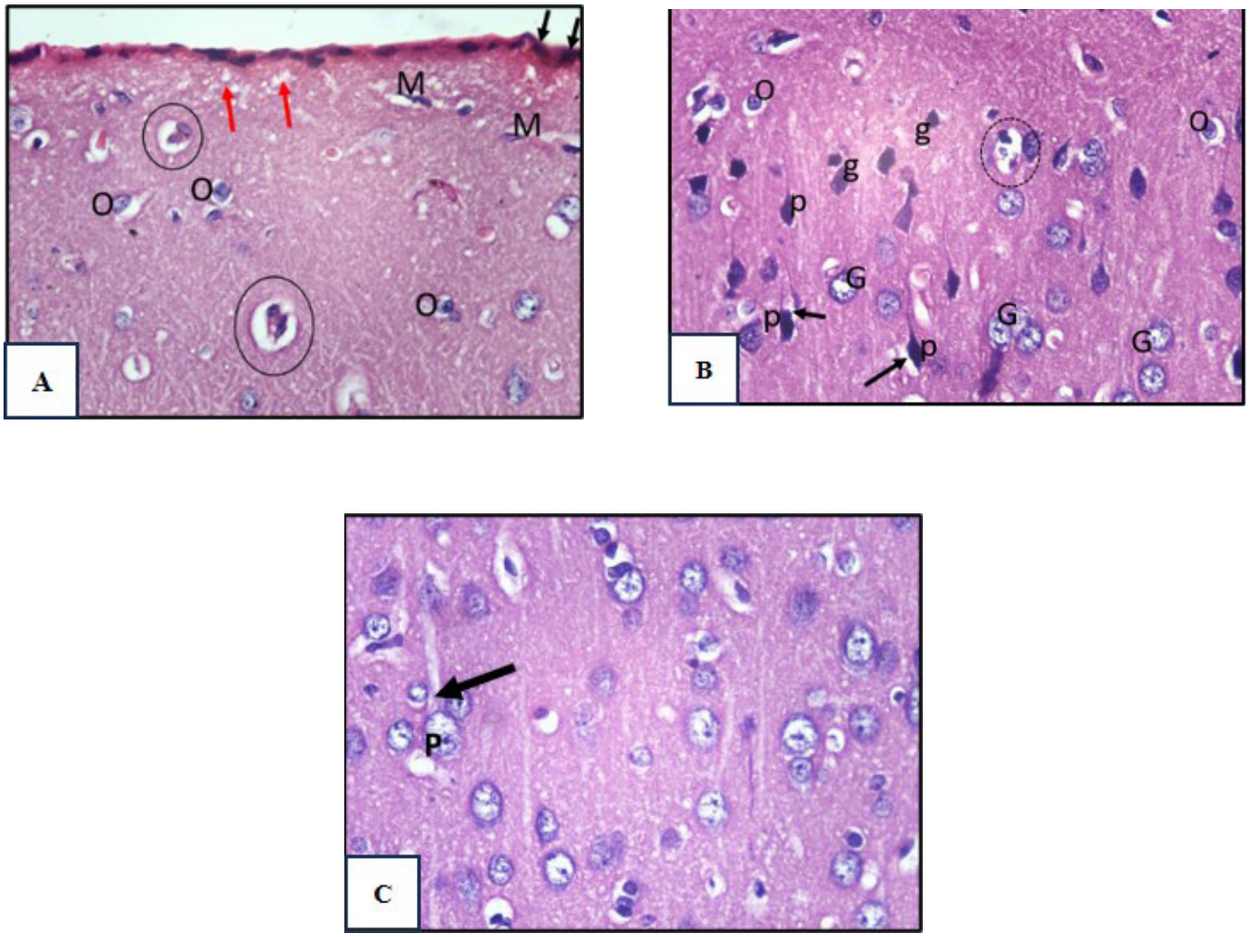

Fig. 2: Photomicrograph of sections in the primary auditory area of senile albino rats (Group II) (A) showing the molecular layer. Wide areas of vacuolated neuropil (red arrows) are seen. Multiple microglia (M) and oligodendroglia (O). Acidophilic masses surrounded by clear areas and containing dark nuclei (circle). Apparent increased thickness of pia matter (black arrow). (B) showing the external granular layer and external pyramidal layer with no clear demarcation between them. Shrunken pyramidal cells (p) with deeply stained nuclei. Dark, shrunken granular cells (g) with pyknotic nuclei. Other granular cells (G) appear rounded with vesicular nuclei. Oligodendroglia (O). karyolitic nuclei (dotted circle) of degenerated neurons.(C) showing the internal pyramidal layer. A large pyramidal cell (P) with large vesicular nucleus and long apical dendrite. The long filament NFT within the apical dendrite (arrow). H&E X400

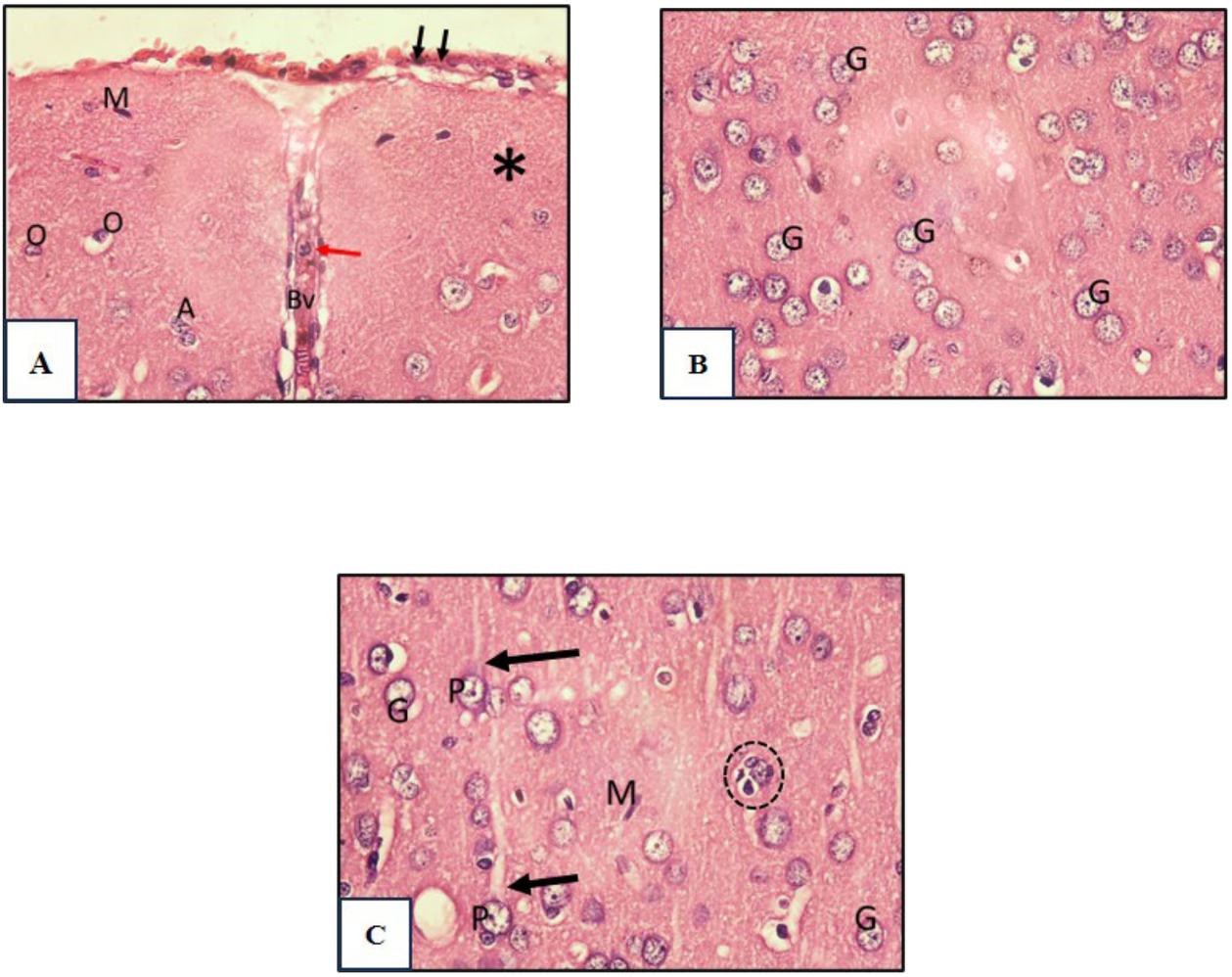

Fig. 3: Photomicrograph of sections in the primary auditory area of senile albino rats treated with DHEA (Group III) (A) showing the molecular layer. Compact neuropil (*) with no vacuolations. Thickened pia matter (arrows). Inflammatory cell (red arrow) in the congested blood vessel (Bv). Astrocyte (A), oligodendroglia (O) and microglia (M). (B) showing the external granular layer. Densely aggregated granular cells (G) consisting of pale vesicular rounded nuclei and outer rim of basophilic cytoplasm. (C) showing the external pyramidal layer. Apparently normal pyramidal cells (P) containing large rounded nuclei with prominent nucleoli. The cytoplasm is basophilic extending along the apical dendrites (arrows) which are thick and directed towards the surface. Notice karyolitic nuclei (dotted circle). Few granular cells (G). M = Microglia. X400 H&E

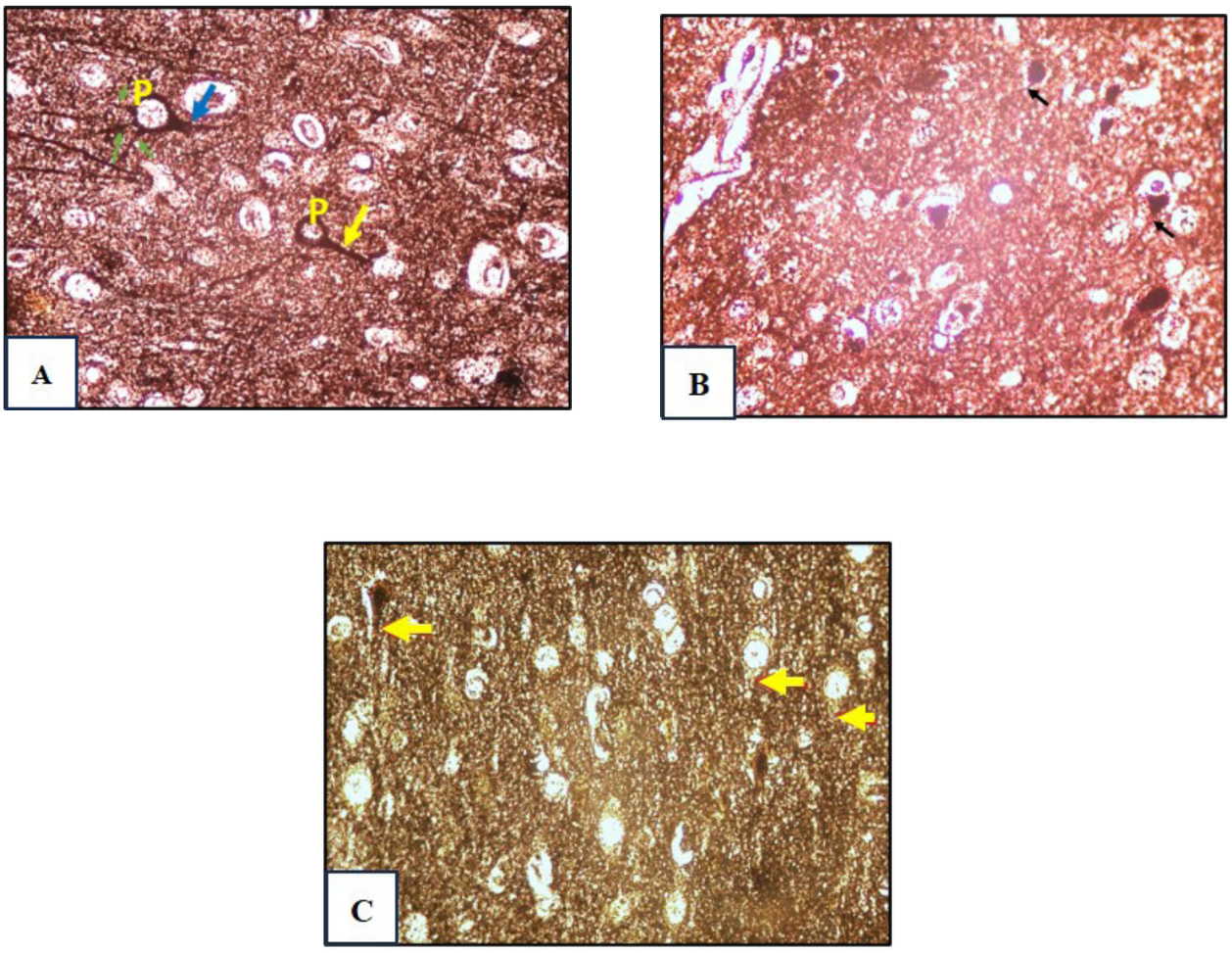

Fig. 4: Photomicrograph of sections in the primary auditory cortex of (A) Group I, showing pyramidal cells (P) with long clearly defined apical dendrite (yellow arrow) and short basal dendrites (green arrows). Branched apical dendrites (blue arrow). (B) Group II, showing apparent decrease in nerve fibers density. Thin, shrunken and broken apical dendrites of pyramidal cells (arrows). (C) Group III, showing apparent increased density of nerve fibers. The apical process of pyramidal cells is relatively long (arrows). X400 Silver (Glees Method).

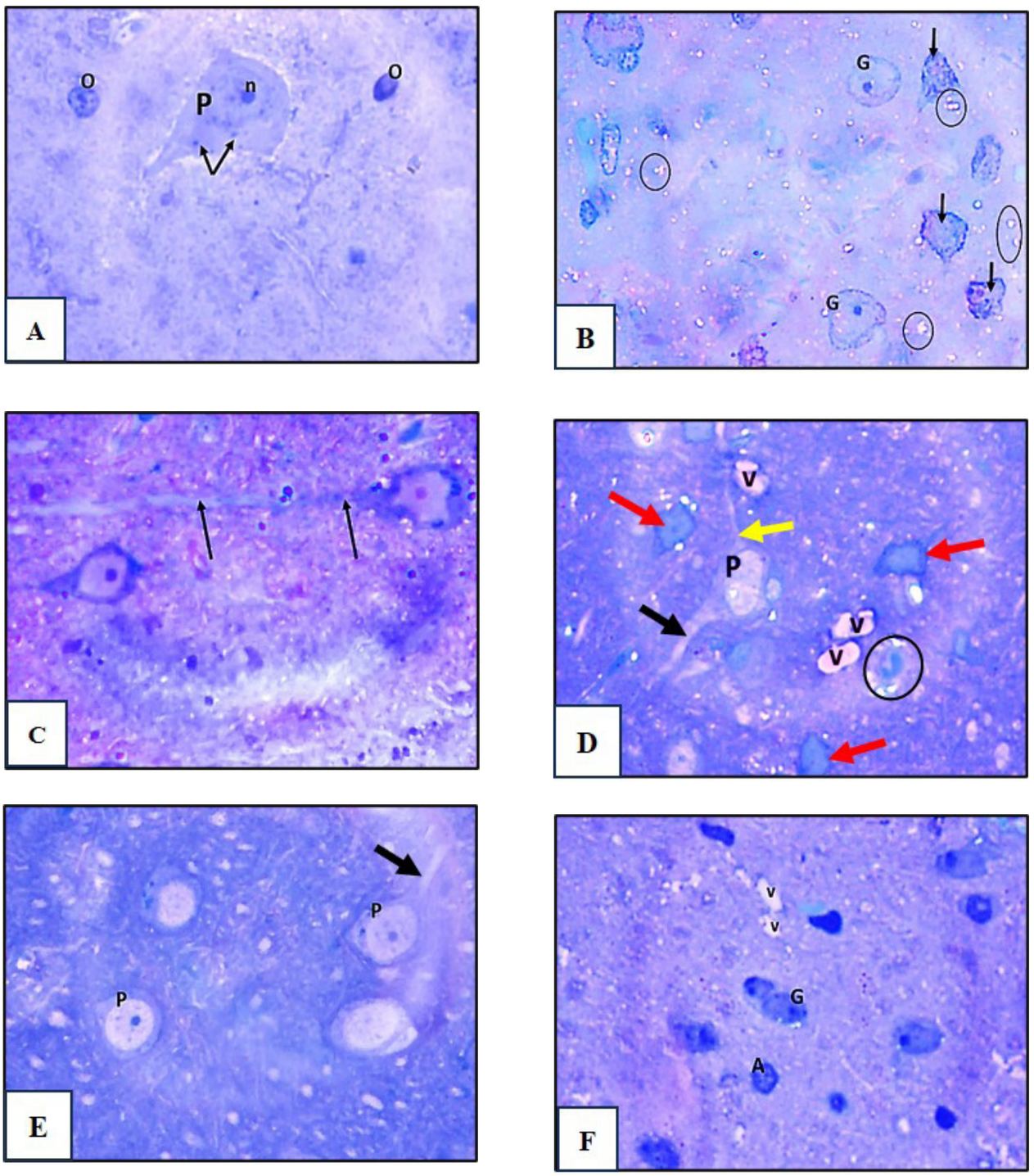

Fig. 5: Photomicrograph of a semithin sections in the primary auditory cortex of (A) Group I, showing large pyramidal cell (P) with large vesicular nucleus with prominent nucleolus (n). Pale cytoplasm containing basophilic Nissl's bodies (arrows). Oligodendroglia (O). (B) Group II, showing multiple darkly stained cells (arrow). Some granular cells (G) with nuclei having prominent nucleoli. Minute lipid droplets with irregular boundaries appearing as pearly white droplets (circle) (C) Group II, showing a pyramidal cell with neurofibrillary tangle (arrow). (D) Group II, showing a pyramidal cell (P) with depleted nissil granules in the cytoplasm, long apical dendrite (black arrow) and short basal dendrite (yellow arrow). Multiple vacuoles (v) in the neuropil. Darkly stained cells with irregular outlines (red arrow) and an inflammatory cell (circle). (E) Group III, showing pyramidal cells (P) having vesicular nuclei and long apical process (arrow). Nissil bodies within the cytoplasm. (F) Group III, showing dividing granular cell (G). Scanty vacuolations (V) in the neuropil. A= Astrocyte. X1000 Toluidine blue.

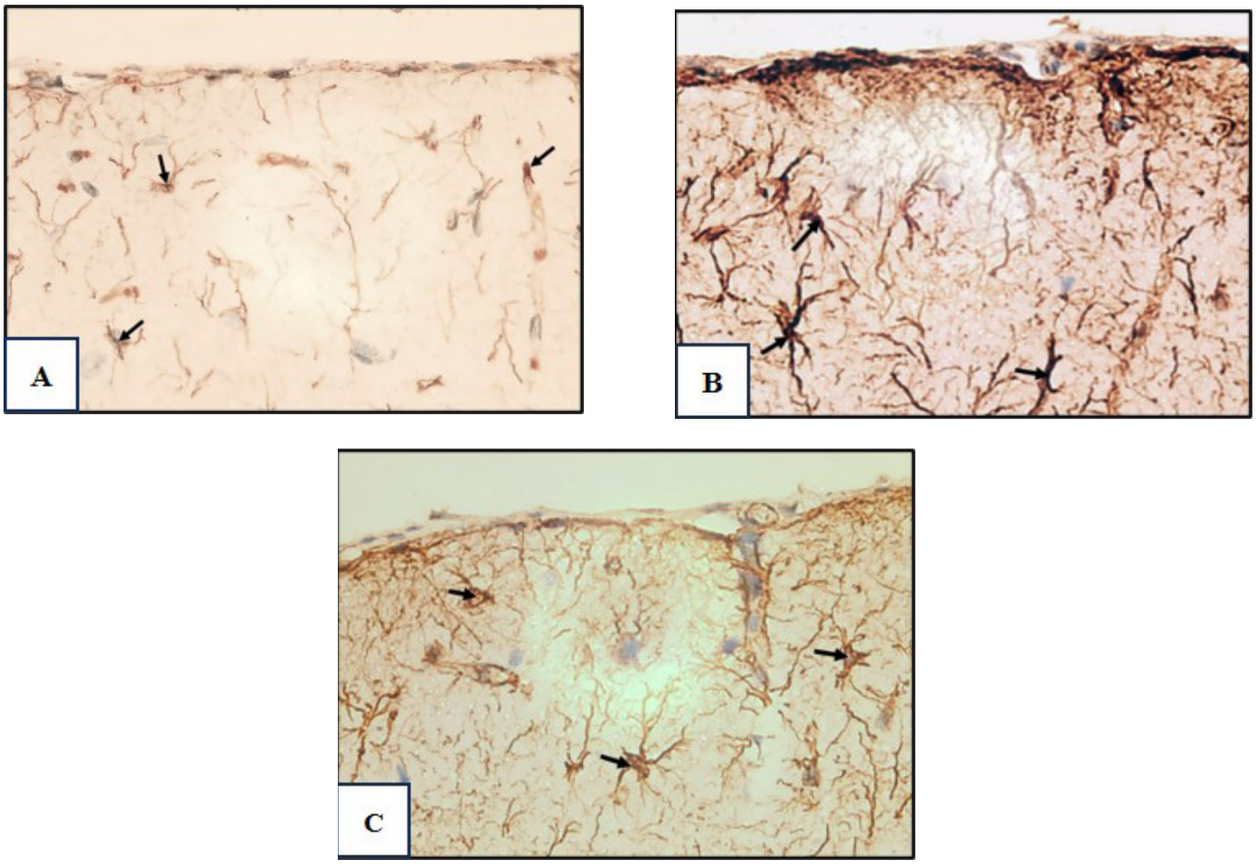

Fig. 6: Photomicrograph of the primary auditory cortex of (A) group I, showing mild positive staining in the cytoplasm of astrocytes and their processes (arrows). They appeared small with short and thin processes. (B) Group II, showing strong GFAP immunoreactivity, with apparent increased number and thickness of dendritic processes (arrows) of astrocytes. (C) Group III, showing moderate positive staining in the cytoplasm of astrocytes and their processes (arrows). Immunohistochemical stain for GFAP X400

Table 1: Mean thickness of auditory cortex in µm

Group	Cortical thickness (Mean ± Sd)
Adult	1401.47± 79.22
Senile	1227.04 ± 90.29
	(P=0.001)*a
DHEA	1366.04 ± 56.12
	(*P*= 0.002)*b

*Significant difference $P \leq 0.0\ 5$ **Highly significant difference $P \leq 0.001$ **a:** compared to adult group **b:** compared to senile

Table 2: Mean count of pyramidal cells

Group	Pyramidal cells (Mean ± Sd)
Adult	3.5 ± 0.92
Senile	1.12 ± 0.46
	(P=3.45E-05)**a
DHEA	2.12 ± 0.64
	(P= 0.007516)*b

*Significant difference $P \leq 0.0\ 5$ **Highly significant difference $P \leq 0.001$ **a:** compared to adult group **b:** compared to senile

Table 3: Mean count of GFAP positive astrocytes

Group	GFAP positive astrocytes (Mean ± Sd)
Adult	6.5 ± 1.19
Senile	14.37 ± 2.66
	(*P*=2.42E-06)**a
DHEA	6.5 ± 1.6
	(*P*=4.91E-06)*b

*Significant difference $P \leq 0.05$ **Highly significant difference $P \leq 0.001$ **a:** compared to adult group **b:** compared to senile

DISCUSSION

Age-related hearing loss (ARHL) is a common sensory impairment and includes genetic and environmental causes. The pathologic picture of ARHL involves degeneration of hair cells, stria vascularis and spiral ganglion. Both the peripheral and central auditory system are affected in ARHL[13]. Therefore, the present research was designated to study the effect of dehydroepiandrosterone (DHEA) on the age-related changes of the primary auditory cortex in senile male albino rats.

Adult and senile albino rats were used in the present study according to Cai *et al*.[14] who noted that rats have become one of the most prevalently used model animals for aging-related studies due to their high similarity to humans in terms of genetic background and physiological structure, as well as their short lifespan and ease of reproduction.

In the current work, it was observed that brain specimens of the senile group were apparently shrunken and congested compared to the adult group. These findings agreed with Banks *et al*[15]. who described that brain aging is characterized by macroscopic changes including decreased brain weight and volume, and microscopic alterations such as changes in vasculature and blood brain barrier permeability.

The current study revealed statistically significant decline in the cortical thickness in the senile group compared to the adult group. These findings were clarified by Giroud *et al*[16]. who confirmed the associations between hearing loss and cortical thickness of superior temporal gyrus as well as hippocampal volume in older individuals. It was reported that the poorer hearing is related to decreased grey matter thickness in the primary auditory cortex. In addition, cortical thinning and a general loss in grey matter volume are neurological signs of ageing. Di Paolo *et al*.,[17] also stated that age-associated atrophy of grey matter is due to neuronal shrinkage and reductions in synaptic density, rather than neuronal loss, especially in the temporal and frontal lobes.

In present study, H&E-stained sections and semithin sections of the cerebral cortex of the senile group showed decreased number of pyramidal cells confirmed by the morphometric study. This was consistent with the results of Zaydi *et al*.[18], who found that senile rats' brains contained less pyramidal cells than adult rats. Additionally, Chi *et al*[19]. demonstrated an association between neuronal loss in neurodegenerative diseases and neuronal cell death during the normal aging.

The senile group of the current study also showed many shrunken pyramidal cells with deeply stained nuclei. karyolitic nuclei were also seen with partial depletion of Nissl granules in the apical dendrites of pyramidal cells. Granular cells looked dark, decreased in size, with pyknotic nuclei and large peri-cellular spaces. These findings agreed with Zhang and Lin[20] who reported that light microscopic examination of aged mice cerebral cortex revealed that the degenerating neurons had smaller cell bodies, lacking Nissl granules, eosinophilic cytoplasm, and deeply stained, pyknotic karyolitic nuclei.

Another observation was the presence of acidophilic masses containing dark nuclei and surrounded by clear areas. This was explained by Garman[21] who reported that those masses could be residual microglial nodules that could remain after activated microglia have cleared any degenerated neurons.

Neurofibrillary tangles (NFTs) of the pyramidal cells observed in sections of the primary auditory cortex of the senile group were declared by Lozupone *et al*.,[22] who confirmed the association of ARHL with temporal cortex neurofibrillary tangles. Mold *et al*[23]. explained the NFTs as hyperphosphorolated form of the tau (tubulin associated unit) protein. It contributes to many neurodegenerative diseases such as Alzheimer's disease and frontotemporal dementia. NFTs have a direct effect on the breakdown of a living neurons and block nerve synapses. Dugger and Dickson[24] reported that Tau protein aggregates were present not only in the cytoplasm of aged neurons, but also in dystrophic neuronal processes (mostly distal axons) in gray matter. The latter have been termed “neuropil threads”.

Furthermore, the current study's observations of areas of vacuolations in the neuropil of the senile group were consistent with Zhang and Lin[20] findings, who reported that swelling of neuronal processes may cause the neuropil near degenerating neurons to be finely vacuolated.

Moreover, the present work showed apparent increased number of oligodendroglia and microglia in the senile group. According to Powrie and Smith[25], oxidative stress and developing inflammation are linked to brain aging. Chowen and Garcia-Segura[26] reported that microglia participate in age-related neuroinflammation through production of inflammatory mediators, particularly in the elderly brain where they have been considered as the CNS's macrophages. Also, Choi *et al*.,[27] reported that many investigations in ARHL have proved that age-related

changes to the auditory system include immunological responses mediated by macrophages.

Cellular infiltrations most probably inflammatory cells were detected in some fields of the senile group in our study. Aoki *et al*.,[28] noted an association between ARHL in the elderly and inflammatory indicators such as increased white blood cell, C-reactive protein (CRP) and interleukin-6 (IL-6). As inflammatory cytokines rise with aging, it is possible that chronic inflammation in older persons will affect ARHL.

In the present study, minute lipid droplets appearing as pearly white droplets were also seen in the cerebral cortex of the senile group. Delage *et al*.,[29] reported that old microglia contain increased lipofuscin granules made of lysosomal lipo-pigments and proteins, fat droplets and phagocytic inclusion bodies. This accumulation increases the lysosomal overload of aged microglia, reduces the capacity for phagocytosis and causes failure of internal degradations. Furthermore, Salas *et al*.,[30] mentioned that electron microscopical studies in aged rat auditory cerebral cortex confirmed the association between aging and the accumulation of membrane-bound inclusions and lipofuscin deposits.

In the present work, silver-stained sections of the primary auditory area of senile rats revealed thin and shrunken apical dendrites of pyramidal cells. These findings agreed with those of Castelli *et al*.,[31] who reported that the most common age-related neuronal changes are reduction of dendrites number and length, with loss of dendritic spines.

In the present research, there was highly statistically significant rise in the mean count of GFAP positive astrocytes in the senile group compared to adult group. These findings agreed with Colmenárez- Raga ***et al***.,[32] who reported that increases in GFAP production in the rat auditory cortex are observed in astroglia in neuronal degeneration. Also, Salas *et al*.,[30] suggested that astrocytes in the dendate gyrus of aged rats change their shape, increase their number and complexity. Similarly, Human studies indicate that astrocytes are more susceptible to aging-induced changes in cerebral cortex[33], while other studies suggest that there is no detectable difference in astrocytes number in aged brain[15].

Administration of DHEA to senile rats, however, showed a marked improvement in cerebral cortex morphological changes. As there was statistically significant rise in the cortical thickness in the DHEA group compared to the senile group. This was agreed by Navarri *et al*.,[34] and Penhale *et al*.,[35] who reported a positive relationship between cortical thickness and DHEA levels.

Examination of H&E stained sections and semithin sections of senile male albino rats receiving DHEA showed almost preserved normal histoarchitecture. Apparently normal granular cells and pyramidal cells were seen besides some dividing granular cells. Compact neuropil with scanty vacuolations were also observed in this group. That was explained by Ahdoot- Levi *et al*.,[36] who proved that treating male rats with DHEA showed its efficacy via start of the neurogenesis by altering the activity of astrocytes. Furthermore, many studies suggested that DHEA could be an effective drug to be used in clinical researches to decrease neurodegeneration in human[37].

Also, the results of Hu *et al*[38] suggested that astrocyte-conditioned medium can stimulate in ***vitro*** neuronal differentiation, extension of neurites, and expression of synaptic proteins in the auditory cortex of rats. However, Uchida *et al*[39]. reported that it is well known that after adulthood, neurogenesis occurs in a few selected regions in human brain, such as the hippocampus, subventricular zone, and olfactory bulb.

In the current study, the DHEA group showed apparent decreased number of neuroglia cells with presence of few inflammatory cells in congested blood vessels. Aoki *et al*.,[28] reported that DHEA may decrease the inflammation by the inhibition of tumor necrosis factor (TNF) and IL-6. DHEA is confirmed to have anti-inflammatory properties, antioxidant activity and inhibit apoptosis.

Additionally, Giannini *et al*.,[40] reported that DHEA supplementation can restore brain-derived neurotrophic factors (BDNF) in the CNS. As the decline in the expression of BDNF leads to neuronal shrinkage and degeneration in the cortex and the hippocampus, a process that may be linked to aging which approve that administration of DHEA may lead to slowdown the aging process[8].

However, in DHEA treated group occasional pyramidal cells were still affected which appeared shrunken with darkly stained nuclei and surrounded by pericellular spaces. Few microglia cells with rod shaped nuclei were seen. Some fields showed karyolitic nuclei. So, the preventive effect of DHEA on age related changes was not total.

In the present work, silver-stained sections of DHEA treated group revealed apparent increased density of nerve fibers. The apical process of pyramidal cells appeared relatively long. Cumberland *et al*[41]. reported that DHEA intake leads to increased spine density in the rat hippocampus. Moreover, Prakash *et al*[42]. reported that rats treated with DHEA showed increased dendritic spines density in pyramidal cells of cortex and hippocampus.

In addition, semithin sections of the DHEA group displayed restoration of the Nissl granules inside the cell bodies and dendrites of pyramidal cells. Dividing granular cells were also seen. That was clarified by Mihailoff & Haines[43] who reported that Nissl bodies are responsible for protein synthetic activity and increase in number in large active neurons. Cumberland *et al*.,[41] reported that many studies support the role of DHEA; as a neuroactive steroid in many aspects of neuroactivity.

In the present work, significant decline in the mean count of GFAP positive astrocytes in the DHEA group compared to the senile group was observed.

Prakash *et al*.,[42] reported also that GFAP-positive cells was dramatically decreased in the cortex and hippocampus of adult rats treated with DHEA proving the decrease in activated astrocytes in their neurons.

Son *et al*[44]. mentioned that the adverse effects of DHEA are minimal, such as mild acne, seborrhea, facial hair growth, and ankle swelling in women. Larger studies are needed to sufficiently prove the safety of DHEA.

CONCLUSION

Administration of DHEA has beneficial effects as it can ameliorate age associated changes in the primary auditory area of the senile rat cerebral cortex. Hence, it is recommended to include DHEA as a powerful therapeutic strategy for age related hearing loss in aged individuals.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور العلاج بالديهيدروإيبيأندروستيرون على التغيرات المصاحبة لتقدم العمر في القشرة السمعية الأولية في ذكور الجرذان البيضاء المسنة: دراسة نسيجية وهستوكيميائية

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مقدمه: يعد الديهيدروإيبيأندروستيرون وسيطاً أيضياً في التركيب الحيوي للإستروجين والأندروجين، وقد أثبت أن له تأثيرات على الجهاز العصبي المركزي وله القدرة على تأخير آثار الشيخوخة

هدف البحث: تهدف هذه الدراسة إلى دراسة دور العلاج بالديهيدروإيبيأندروستيرون على التغيرات المصاحبة لتقدم العمر في القشرة السمعية الأولية في ذكور الجرذان البيضاء المسنة.

مواد و أساليب العلاج: في هذه الدراسة تم استخدام ١٠جرذان من ذكور الجرذان البيضاء البالغة عمرها ٤-٦ أشهر ويتراوح وزنها بين (٢٠٠-٢٥٠ جرام) ، و٣٠ جرذاً من ذكور الجرذان البيضاء المسنة عمرها ٢٠-٢٤ شهراً، ويتراوح وزنها بين (٢٥٠-٣٠٠جرام) ، وتم تقسيم الجرذان إلى أربع مجموعات كما يلي : المجموعة الأولى (المجموعة البالغة) : ١٠ جرذان لم تتلق أي علاجات لمدة ٨ أسابيع ، المجموعة الثانية (المجموعة المسنة) : ٢٠ جرذ تم تقسيمهم لمجموعتين فرعيتين: (٢أ) ١٠ جرذان لم تتلق أي علاجات لمدة ٨ أسابيع ومجموعة (٢ب) ١٠ جرذان تلقت ١ مل/كجم من وزن الجسم من محلول دايميثيل سلفوكساید عن طريق الفم مرة يومياً لمدة ٨ أسابيع .، المجموعة الثالثة (مجموعة الديهيدروإيبيأندروستيرون) : ١٠ جرذان تلقت ٢٠مجم/كجم من وزن الجسم من عقار الديهيدروإيبيأندروستيرون المذاب في ١ مل/كجم من وزن الجسم من محلول دايميثيل سلفوكساید عن طريق الفم مرة يومياً لمدة ٨ أسابيع

النتائج: في هذه الدراسة أظهر فحص المقاطع للمجموعة المسنة انكماش الخلايا العصبية واحتوائها على أنوية شديدة الاصطباغ، بالإضافة إلى زيادة ظاهرية في عدد الخلايا الدبقية الصغيرة وظهور مساحات واسعة من الفراغات في ألياف الخلايا العصبية كما لوحظ في مقاطع المجموعة الثالثة ظهور العديد من الخلايا العصبية التي بدت طبيعية، مع انخفاض ظاهرية في عدد الخلايا الدبقية بالمقارنة مع المجموعة الثانية

الاستنتاج: يلخص من هذه الدراسة أن للديهيدروإيبيأندروستيرون تأثير علاجي على تأثير التقدم في السن على نسيج القشرة السمعية الأولية، ولذلك ينصح باستخدامه في علاج كبار السن المصابين بفقدان السمع المرتبط بالتقدم بالعمر.