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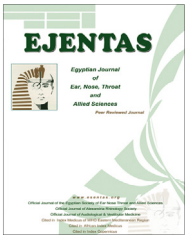

ORIGINAL ARTICLE

The effects of concomitant Ginkgo intake on noise induced Hippocampus injury. Possible auditory clinical correlate

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Abstract This study was conducted to determine the injurious effects of noise on the hippocampus, and to show whether Ginkgo biloba (Gb) has any modulatory effect on hippocampal injury. Fifteen adult male albino rats were divided into three groups; control group, noise group and protected group. The noise group was exposed to 100 dB Sound pressure level (SPL) white noise, six hours/day for four consecutive weeks. The protected group was exposed to the same noise level with the administration of Gb extract to the animals (50 mg/kg daily) for 4 weeks. In the noise exposed group, both pyramidal cell layer and dentate gyrus (DG) granular cell layer showed a decrease in thickness with loss and degeneration of many cells. The protected group showed preservation of many parameters as compared to the noise group i.e. increase in thickness of Cornu Ammonis area3 (CA3) & DG; increase in surface area of cells and increased vascularity. In conclusion, noise had detrimental effects on cells of Cornu Ammonis area1 (CA1), CA3 & DG of the hippocampus. In view of this finding, the clinical auditory hazardous effects in people exposed to harmful noise such as tinnitus, as well as memory disturbances and learning disabilities might have a new dimension. The administration of Gb protected the hippocampus against the injurious effect of noise. The probable mechanism and usefulness of Gb in reducing the previously mentioned effects are discussed.

1. Introduction

In everyday life, cognitive tasks are often performed in the presence of task-irrelevant environmental noise. Accordingly, numerous studies on noise effects showed that it may evoke substantial impairments in cognitive performance.[1] It is estimated in the European Union countries that approximately 40% of the population is exposed to road traffic noise exceeding 55 dB sound pressure level (SPL), while about 20% are exposed to levels more than 65 dB. Traffic noise pollution is

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also severe in cities of developing countries and can reach 75–80 dB each day.[2] It was found that noise exposure induces psychological disorders, impairs learning and memory acquisition, consolidation and recall functions[3], with possible involvement of the hippocampus.[4] Prenatal auditory stimulation of chicks by noise at 110 dB SPL negatively affected the spatial orientation as well as the spatial learning and memory.[5]

It is documented in previous researches that the hippocampus is among sites in the brain that coordinates spatial learning and memory.[6] It is also found to be the first brain region affected in age associated impairments of the brain like Alzheimer's disease.[7] Moreover, hippocampus is also known to respond to sound stimulation[8]and seems to play a role in auditory sensory gating[9]and tinnitus.[10] It has been reported recently that noise might lead to damage of the hippocampus.[3,11]

Ginkgo biloba (Ginkgoaceae) is an important and widely used herb of the Chinese traditional medicine. It was known to have a variety of biological and pharmacological properties that made it useful as a cardioprotective, antiasthmatic, and antidiabetic agent.[12] In addition, it had been shown to possess neuroprotective properties against hypoxia, ischemia, seizure activity and peripheral nerve damage.[13] In accordance, it was shown – in clinical researches – to be effective in treating dementia in Alzheimer's disease and in enhancing memory and cognition[14] and improve the capacity of geriatric patients to cope with the stressful demands of daily life.[15]

There has been no research – up to our knowledge – that proved protective effect of Gb administration against hippocampus involvement due to noise exposure. The aim of this work was to explore the effects of noise exposure on the different regions of the hippocampus with possible auditory clinical correlate and also to investigate the efficacy of Gb extract in protection against these effects.

2. Methodology

2.1. Animals

Fifteen adult male Sprague Dawley albino rats aged from six to eight weeks and weighing 150–200 grams were used in this study. They were housed individually in clean wire mesh cages under standard conditions of temperature 30 ± 2 °C with regular 12 h light/12 h dark cycle and were fed standard diet and water ad libitum. All experimental procedures were performed after approval of ethics committee (FMASU 1554/2013), and in accordance with guidelines of the Institutional Animal Care and Use Committee of the research center – Faculty of Medicine – Ain shams University.

Rats were divided into three groups (5 rats for each group):

Group I (control group): animals were given 1 ml of distilled water (DW) by gastric gavage and were sacrificed after four weeks.

Group II (noise exposed group): exposed to noise for four weeks.

Group III (protected group): exposed to noise as group II and Gb was administered throughout the noise exposure period.

2.2. Noise exposure

The experimental animals were exposed to 100 dB-(SPL) white noise, 6 h/day for 4 weeks. The animals were put in a cage with a loud speaker Star 200 watt, connected to noise generator Beltone model NB 103, installed 30 cm above their cage. The level of the produced noise was checked using Sound Level Meter model Quest 2008. To avoid the influence of handling-stress on evaluation of effects due to noise exposure, control rats were kept in the above-described cage during the corresponding period of time, without noise stimulation.

2.3. Drugs

Ginkgo biloba (Gb): Tanakan tablets contain 40 mg of the pure extract made of leaves of maidenhair tree. Tablets were ground and suspended in DW, then, the suspension was administered to the animals by gastric gavage in a dose of 50 mg/kg daily.[16] It was calculated that an oral dose of 240 mg in humans corresponds roughly to an oral dose of 50 mg/kg in rats.[17]

2.4. Histological study

2.4.1. Light microscopy (LM)

At the end of the experimental period, rats were anaesthetized using ether, then, sacrificed with a lethal dose of ether according to the protocol of Animal care and Use Committee of Ain Shams University. The right hippocampus was extracted, fixed in 10% formal saline for 5 days, dehydrated, cleared and embedded in paraffin. Sagittal sections of the hippocampus (5 μm thick) were cut and stained with H&E.[18]

2.4.2. Transmission electron microscopy (TEM)

The left hippocampus was extracted. Samples were fixed in phosphate buffered formal glutaraldehyde (2.5%) and post-fixed with a 1% osmium. The specimens were then dehydrated, cleared and embedded in epoxy resin. Ultra thin sections (60 nm) were cut and examined using a transmission electron microscope (JEOL 1010 EX II, Japan) at the regional Mycology and Biotechnology center, AL-Azhar University, Cairo, Egypt.[18]

2.4.3. Morphometric and statistical analysis

Measures were obtained from all animals in the group. Five specimens from five different rats of each group were examined ($n = 5$). For each specimen, five different non overlapping high power fields (×400) were captured by a digital camera Olympus (DP 20(. Five different readings from every captured photo were counted and the mean was calculated for each specimen. Image analysis procedures were done using image pro-express program (version 6).

Measurements were counted by an independent observer blinded to the specimens' details to perform an unbiased assessment.

2.4.3.1. The following morphometric data were measured;.

- The thickness of the pyramidal cell layer in CA1 and CA3 regions (lines were drawn vertically – perpendicular to the pyramidal-molecular layers junction – from the upper most pyramidal cell to the lower most one detected in each line drawn. The line is then measured by the software.).
- The thickness of the granular cell layer in the dentate gyrus (DG) (lines were drawn vertically – perpendicular to the line separating the granular cells from the hilus – from the upper most granular cell to the lower most