Annona Muricata L. Leaf Extract Affects the Number of Neurons in the Temporal Lobe of Adult Wistar Rats Exposed to Noise

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Abstract. By producing free radicals, noise is a major environmental stressor that causes oxidative stress. Long-term exposure to high decibel levels can harm the neurological system as well as other extra-auditory organs of the body. Antioxidant chemicals found in *Annona muricata L.*, popularly known as soursop, may have neuroprotective benefits against oxidative damage. The purpose of this study is to assess how the leaf extract of *Annona muricata L.* affects the quantity of neurons in the temporal lobe of adult Wistar rats who have been exposed to noise. Thirty male Wistar rats were split into treatment and control groups after a posttest-only control group design. Over the course of 14 days, both groups were subjected to 95 dB noise for four hours every day. Leaf extract from *Annona muricata L.* was also given to the treatment group. An independent t-test was used to compare the histologically determined neuronal numbers in the temporal lobe. This research show there were much more temporal lobe neurons in the treatment group than in the control group ($p = 0.003$). Because of its antioxidant qualities, *Annona muricata L.* leaf extract may have neuroprotective effects against noise-induced brain damage.

1. Introduction

Because of its pervasiveness and harmful impacts on human health, noise is becoming more widely acknowledged as a public health concern. Noise, which is defined as undesired or upsetting sound, is a powerful environmental stressor that can cause both physiological and psychological problems in addition to being an annoyance. Long-term exposure to noise has been linked to a number of health issues, such as cardiovascular disease, cognitive decline, and disturbed sleep[1].

Through the overproduction of reactive oxygen species (ROS), noise exposure causes oxidative stress at the cellular level by upsetting the equilibrium between oxidants and antioxidants. Particularly in brain areas linked to sensory processing, such the temporal lobe, this imbalance causes lipid peroxidation, DNA damage, and neuronal death[2]. Higher

cognitive processes and auditory perception are impacted by noise-induced neurotoxicity, which is especially dangerous for the temporal lobe, which contains the auditory cortex.

Antioxidant therapy has been suggested as a possible preventative measure to combat oxidative damage. *Annona muricata L.*, sometimes referred to as graviola or soursop, is a natural source of antioxidants. Bioactive substances like flavonoids, phenolics, and acetogenins, which have potent anti-inflammatory, antioxidant, and neuroprotective properties, are abundant in many sections of this plant, but especially in the leaves [3].

In models of neurotoxicity and metabolic diseases, earlier research has shown that *Annona muricata L.* leaf extract can help reduce neuronal damage[4]. Its function in shielding the brain from oxidative stress brought on by noise is still poorly understood. Examining this possibility could help create substitute treatments for neurodegeneration brought on by noise.

The purpose of this study is to assess how the leaf extract of *Annona muricata L.* affects the quantity of neurons in the temporal lobe of adult Wistar rats who have been exposed to noise. Under oxidative stress situations, it is thought that the extract's antioxidant qualities can prevent neuronal death and maintain brain integrity.

2. Materials and Methods

In order to assess the neuroprotective impact of Annona muricata L. leaf extract on temporal lobe neurons in adult Wistar rats exposed to noise, this study used a true experimental design with a randomized posttest-only control group.

2.1. Animals

A qualified laboratory animal supplier provided thirty healthy male Wistar rats (Rattus norvegicus) measuring 200–250 grams and between the ages of two and three months. The rats were given full access to standard feed and water during their seven days of acclimatization, which included standard circumstances (temperature 22–25 °C, humidity 50–60%, and a 12-hour light/dark cycle). Every experimental procedure corresponded with international criteria for the care and use of laboratory animals and was approved by the Institutional Animal Ethics Committee.

2.2. Experimental Groups and Noise Exposure

Two groups of 15 animals each were randomly selected from among the animals:

- Group I (Control): exposed to 95 dB noise for 4 hours per day over 14 consecutive days, without any treatment.
- Group II (Treatment): received the same noise exposure protocol and were orally administered Annona muricata L. leaf extract at a dose of 100 mg/kg body weight once daily for 14 days.

A Real-Time Analyzer (version 5.2.0) with external speakers positioned 30 cm away from the cages was used to provide noise exposure. Throughout the exposure sessions, a calibrated decibel meter was employed to keep the sound pressure level constant.

2.3. **Preparation and Administration of Extract**

Fresh *Annona muricata L.* leaves were cleaned, allowed to air dry, and then powdered after being verified by a botanist. By macerating 70% ethanol for 72 hours at room temperature, the powdered substance was removed. After filtering and using a rotary evaporator to concentrate it, the extract was kept at 4°C for storage. The treatment group received 100 mg/kg body weight of the extract every day by oral gavage after it had been diluted in distilled water.

2.4.**Histological Procedure**

After being anesthetized with a ketamine and xylazine combination on day 15, all of the rats were sacrificed. The brains were promptly removed and preserved for 48 hours in 10% neutral buffered formalin. After being dissected and paraffin-embedded, the temporal lobes were sectioned at a thickness of 5 μm and stained with Hematoxylin-Eosin (H&E).

A 40× magnification light microscope was used to evaluate the neurons. Four non-overlapping high-power fields (HPFs) in the temporal cortex were chosen and captured on camera from each brain region. Each field's neural density was determined by manually counting the neurons and calculating the mean value. To maintain objectivity, an expert histologist who was blind to the group assignments conducted all observations.

2.5.**Statistical Analysis**

The Shapiro-Wilk test was used to determine whether the data were normal. An independent t-test was used to examine the variation in the mean number of neurons between the two groups. SPSS version 18 was used for all statistical analyses, and $p < 0.05$ was chosen as the significance level.

3. Results

The study's findings showed that there was a statistically significant difference between the control and treatment groups' numbers of neurons in the temporal lobe. After receiving leaf extract from *Annona muricata L.* during noise exposure, the treatment group's neuronal count was significantly higher than that of the control group.

The temporal lobe's neuronal cells were quantitatively analyzed using a high-power field (40× magnification), which showed significant group differences. The treatment group, which was given Annona muricata extract after being exposed to noise, had a significantly higher mean ± standard deviation (SD) of 38.80 ± 9.05 neurons than the control group, which had a mean ± SD of 25.17 ± 13.63 neurons. As indicated in Table 1, statistical analysis using an independent t-test revealed that this difference was statistically significant ($p = 0.003$).

Figure 2 provides a visual representation of these results, showing the comparative mean neuron counts between the treatment and control groups in a bar chart. Neuronal density is noticeably higher in the treatment group. Double asterisks (**) indicate a statistically significant difference ($p < 0.01$), while error bars show the standard deviation. The neuroprotective effect of *Annona muricata L.* leaf extract in reducing neuronal death brought on by extended exposure to noise is supported by this finding.

Table 1. Mean number of neurons per high-power field (40× magnification) in the temporal lobe of Wistar rats after 14 days of noise exposure.

Group	Mean±SD (neuron/field)	n	p-value
Control (Noise)	25.17 ± 13.63	15	0.003
Treatment (Noise + *A. muricata*)	38.80 ± 9.05	15	

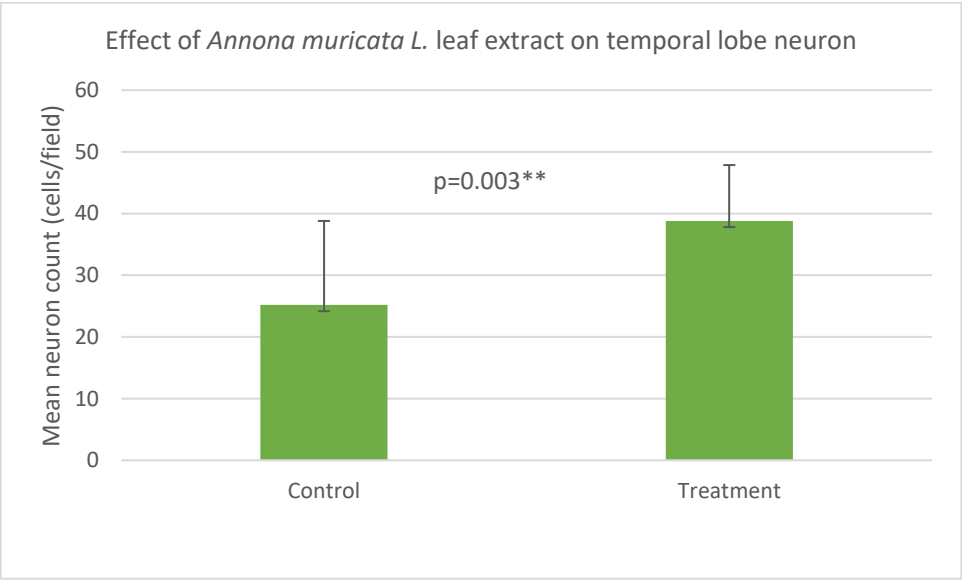

Fig 1. Bar chart comparing mean neuron counts between groups. Error bars represent standard deviation. Double asterisks (**) indicate statistically significant difference ($p < 0.01$).

Hematoxylin and Eosin (H&E) staining was used to histologically analyze the temporal lobe, and the results showed clear changes in neuronal density between the treatment and control groups (Fig. 2). There were fewer neurons per field in the control group, along with indications of cellular integrity loss and degeneration. Rat brain slices treated with Annona muricata extract, on the other hand, showed a noticeable increase in neuronal density together with maintained shape and distinct nuclei. These results imply that *Anonna muricata L.* leaf extract has a neuroprotective effect, which may lessen the harm that noise exposure causes to neurons. The extract's function in preserving neural structure is further supported by the visual portrayal, which also validates quantitative results.

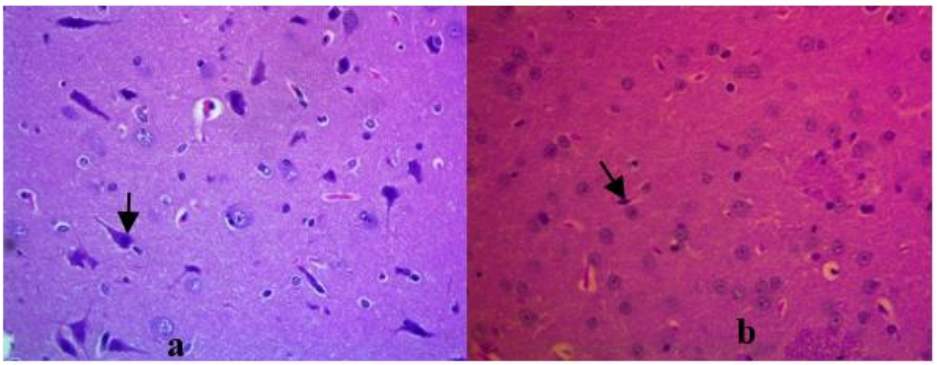

Fig 2. Representative Hematoxylin and Eosin (H&E)-stained sections (40× magnification) of the temporal lobe: (a) Control group showing reduced neuronal density (20–25 cells per field), (b) Treatment group showing increased neuronal density (40–50 cells per field). Arrows indicate preserved neurons.Colour illustrations.

4. Discussion

4.1. Neuroprotection through Antioxidant Mechanisms

High-intensity noise exposure produces reactive oxygen species (ROS), which can cause inflammation, apoptosis, and mitochondrial dysfunction, all of which can negatively impact neuronal populations with high metabolic demands, such those in the auditory cortex. Loud noise activates intrinsic apoptotic pathways (e.g., caspase-dependent cell death) by causing mitochondrial enlargement, DNA damage, and decreased oxidative phosphorylation[5]. Furthermore, ROS stimulates lipid peroxidation in neuronal membranes, particularly those that are high in polyunsaturated fatty acids, which results in ion-channel malfunction, membrane disruption, and synaptic failure[6].

Research conducted on rats exposed to 95–100 dB noise for 4–6 hours per day revealed considerable increases in nitric oxide (NO) and malondialdehyde (MDA), as well as decreases in the antioxidant enzymes glutathione peroxidase (GSH-Px), SOD, and CAT, suggesting systemic oxidative stress[2].

Rich in flavonoids, tannins, phenolic acids, and vitamin C, *Annona muricata L.* leaf extract has demonstrated potent antioxidant qualities in both in vitro and in vivo experiments. Techniques such as DPPH, ABTS, and FRAP tests exhibit strong ROS scavenging capabilities. Additionally, treatment raised SOD, CAT, and GSH-Px activities while lowering MDA levels in liver and brain tissues in rats. Specifically, ethyl acetate and butanol fractions reduced lipid peroxidation and enhanced enzymatic antioxidant activity[7]. According to a prior study, the ethanolic extract of *Annona muricata* leaves demonstrated a moderate level of efficacy in inhibiting free radicals, with an IC_{50} value of 32.6230 ppm, and a very high antioxidant capacity, reaching 24,228.60 mg GAEAC/L. Numerous antioxidant components, such as phenols, flavonoids, tannins, beta-carotene, vitamin C, tocopherols, and saponins, were present in the extract. Through antioxidant-mediated processes, these bioactive ingredients work in concert to counteract oxidative stress, supporting the extract's possible neuroprotective benefits[8].

These antioxidants shield mitochondrial DNA and stop the cascade of lipid peroxidation and apoptotic damage by neutralizing ROS and stabilizing mitochondrial membranes.

Therefore, in high-risk areas like the auditory cortex, A. muricata extract functions as an efficient neuroprotective agent by preserving mitochondrial health and neuronal integrity.

4.2. Cellular and Molecular Implications

The auditory cortex and other neuronal populations with high metabolic demands can be severely harmed by reactive oxygen species (ROS) brought on by noise exposure. These neurons produce a lot of ATP through their mitochondria, which makes them susceptible to inflammation, apoptosis, and mitochondrial malfunction when overloaded with ROS[9]. The activation of intrinsic apoptotic pathways, which are characterized by caspase activation and pro-apoptotic gene expression (e.g., Bcl-2 family)[10], is triggered by mitochondrial damage, which includes mtDNA disruption and reduced oxidative phosphorylation. This results in the loss of neurons in cortical areas that modulate auditory function[11].

The polyunsaturated fatty acid-rich neuronal cell membrane is vulnerable to lipid peroxidation by ROS, which impairs ion channel function, membrane integrity, and synaptic transmission. This cycle exacerbates inflammation and neuronal damage if antioxidant defense is delayed[12].

Leaf extract from *Annona muricata L.* includes a combination of phytochemicals with strong antioxidant properties, including flavonoids, tannins, phenolic acids, and vitamin C. These substances stabilize mitochondrial membranes, shield mtDNA, and prevent lipid peroxidation by scavenging superoxide, hydroxyl radicals, and peroxides[13]. In particular, investigations using ethanol extract in rodents have demonstrated improved cell viability in brain regions, decreased levels of malondialdehyde, and preserved SOD/CAT enzyme activity[14].

Moreover, oxidative stress brought on by noise frequently starts inflammatory cascades in the central nervous system. Neuronal injury is exacerbated by microglial activation, which releases cytokines such TNF-α, IL-1β, and IL-6. *A. muricata's* neuroprotective potential is enhanced by its anti-inflammatory qualities, which are mediated by cytokine downregulation and suppression of NF-κB activation[13]. For neurons in high-risk areas like the auditory cortex, the combination antioxidative and anti-inflammatory effects offer a complete defense[13,14].

Furthermore, noise can exacerbate neuronal injury by upregulating pro-inflammatory cytokines (such as TNF-α, IL-6, and IL-1β)[13]. In peripheral and neural tissues, *A. muricata* has been shown to have anti-inflammatory properties by reducing glial activation and cytokine release[8]. The anti-inflammatory and antioxidant properties work in concert to prevent noise-induced neurodegeneration[8,14].

4.3. Alignment with Related Studies

Our findings support the neuroprotective properties of the Annona muricata L. extract. An ethanol extract of A. muricata leaves was found to significantly reduce neuronal degeneration and preserve cerebellar cellular architecture in adult Wistar rats exposed to noise stress. Recent studies show that oral administration of A. muricata ethanol extract (100 mg/kg body weight) for 14 days significantly decreased Purkinje cell degeneration and maintained the cellular organization of the molecular and granular layers of the cerebellar cortex in rats exposed to noise, which supports these findings. Comparing treated animals to untreated counterparts, histological examinations showed increased neuronal density and decreased structural breakdown[8].

The bioactive phytochemicals in A. muricata leaves, such as phenolic compounds and alkaloids, which have anti-inflammatory and antioxidant qualities, are thought to be responsible for the neuroprotective effects. These substances are known to suppress apoptotic signaling pathways, stop lipid peroxidation, and scavenge reactive oxygen species (ROS).

Additional in vitro data also demonstrates that polysaccharide fractions of A. muricata shield HT22 hippocampus neurons from hydrogen peroxide-induced oxidative stress by lowering levels of ROS and MDA, reviving antioxidant enzymes like SOD, and suppressing pro-apoptotic mediators like caspases and Bax[15].

A. muricata leave extract may be a powerful neuroprotective agent, especially in oxidative and inflammatory stressors like noise-induced neurodegeneration, according to these studies taken together.

4.4. Limitations and Future Directions

Despite confirming structural neuroprotection, this study did not capture mechanistic insights, such as measurement of MDA, SOD, and CAT levels, or neurobehavioral outcomes like cognitive assessment. Future research ought to include biochemical tests (such as antioxidant enzyme activity and TBARS, profiling of inflammatory markers (e.g., TNF-α, IL-6), and behavioral tests to gauge functional recovery, such as the Morris Water Maze.

To clarify specific neuroprotective processes, dose-response studies, long-term monitoring, and molecular analyses (such as western blot for Bcl-2, Bax, and caspase pathways) are also necessary.

5. Conclusion

This study shows that when adult Wistar rats are subjected to high-intensity noise, the leaf extract of *Annona muricata L.* significantly protects the neurons in their temporal lobes. When compared to the control group, the treatment group's mean neuronal count was higher, indicating that the extract may be able to lessen the harm that noise does to neurons. *Annona muricata's* potent anti-inflammatory and antioxidant qualities, which combat oxidative stress and maintain neural integrity, are probably responsible for these effects.

The results provide credence to the possibility of using *Annona muricata L.* as a natural remedy to stop noise-induced neurodegeneration. To clarify the underlying mechanisms and validate functional effects, more investigation involving molecular and behavioral characteristics is advised.

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