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Ameliorative Histological Effects of Aqueous Neem Leaf Extract (*Azadirachta indica*) on Lead Induced Neurotoxicity of Lateral Geniculate Body of Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Lead exposure remains a significant public health burden across the globe, impacting millions of individuals and posing a major threat to nervous system development and function. Among the vulnerable targets of lead's neurotoxic assault is the lateral geniculate body (LGB), a crucial relay station within the visual pathway. This brain region acts as a gateway for visual information, processing signals from the retina and transmitting them to the visual cortex for interpretation.

Aim: This study seeks to investigate the potential neuroprotective effects of aqueous *Azadirachta indica* (Neem leaf) extract against lead-induced neurotoxicity in the LGN of adult rats.

Methods: Twenty (20) adult wistar rats weighing 150-200g were randomly grouped into four(4) (A-D, n=5). Group A Normal control Group B received (70mg/kg)(Orally) Lead only, Group C received (200mg/kg)(Orally) *Azadirachta indica* (Neem leaf), Group D received (70mg/kg Lead and 200mg/k *Azadirachta indica*)(Orally). Alterations in the relative weight of the LGB were determined, and antioxidant status was assessed by measuring Superoxide Dismutase (SOD) and catalase (CAT) and Lactate Dehydrogenase (LDH) levels. The effects of neem leaf extract on lactate dehydrogenase were also evaluated. Additionally, histological changes in the cerebellum due to lead exposure were examined using H&E and Cresyl Violet staining.

Results: Lead exposure led to alterations in the relative weight of the LGB, SOD and CAT level was significantly (*P,*<0.005) reduced compared to the control, while the LDH was significantly increased(*P.>0.005)* and disruptions in lactate dehydrogenase activity. Aqueous neem leaf extract administration showed potential ameliorative effects mitigating these alterations and histological examination revealed ameliorative changes in the LGB associated with lead exposure.

The study highlights the potential neuroprotective effects of aqueous neem leaf extract against lead-induced neurotoxicity, emphasizing its role in mitigating oxidative stress and preserving histological integrity in the LGN. Insights into the effects of neem on carbohydrate metabolism enzymes and cerebellar histology contribute to a comprehensive understanding of its therapeutic potential.

Keywords: Azadirachta indica; lateral geniculate body; Neurotoxicity; lead exposure; lactate dehydrogenase.

1. INTRODUCTION

Lead, a ubiquitous environmental contaminant, presents a significant global health risk, particularly impacting the intricate workings of the nervous system. Its widespread presence in air, water, and dust exposes millions, especially children, to its detrimental effects. According to the World Health Organization, an estimated 800 million children globally face the consequences of lead exposure, jeopardizing their cognitive development and long-term well-being (WHO, 2022). This pervasive threat demands immediate attention due to its detrimental impact on individuals and communities.

The insidious nature of lead poisoning lies in its ubiquity. Common sources of exposure include contaminated water from aging infrastructure, lead-based paint in older homes, and dust particles arising from industrial emissions and mining activities [1]. Children, with their developing bodies and increased hand-to-mouth tendencies, are particularly susceptible to lead dust ingestion, making them a population of heightened concern. This widespread exposure underscores the urgent need for effective interventions to mitigate lead's harmful effects and protect public health.

Among the vulnerable targets of lead's neurotoxic assault is the lateral geniculate body (LGB), a crucial relay station within the visual pathway. This brain region acts as a gateway for visual information, processing signals from the retina and transmitting them to the visual cortex for interpretation. However, lead disrupts this delicate process, causing significant structural and functional alterations within the LGB. Studies have documented lead-induced neuronal atrophy, synaptic loss, and altered neurotransmitter levels, ultimately leading to visual impairments like amblyopia and even blindness [2,3].

Amidst the bleak picture of lead's neurotoxic impact, *Azadirachta indica*, commonly known as Neem, offers a ray of hope. This medicinal plant

boasts a rich history of traditional use and recent scientific investigations have revealed its potent antioxidant and anti-inflammatory properties [4]. Studies suggest that Neem's bioactive compounds, such as azadirachtin and nimbolides, effectively scavenge free radicals, mitigate oxidative stress, and modulate inflammatory pathways [5]. This raises exciting possibilities for Neem's potential in counteracting lead-induced neurotoxicity, offering a natural approach to neuroprotection. Native to the arid parts of Asia and Africa, it has a long history of usage as a pesticide, fungicide, and contraceptive in India and Africa. Almost every component of the tree, including the seeds, leaves, roots, bark, trunk, and branches, has a variety of applications [6]. Neem is known as a "Pharmaceutical Wonder" because it is thought to be a repository of several medicinal compounds. Neem has more than 300 phytochemicals that are diverse chemically and physically. The anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, neuroprotective, and cardioprotective properties of the golden plant have all been demonstrated in various disease models [7]. Neem exerts it potential beneficial effects by modulating different cellular and molecular mechanisms like free radical scavenging [8], xenobiotic detoxification [7].

"Lateral geniculate nucleus (LGN; also called the lateral geniculate body or lateral geniculate complex) is a structure in the thalamus and a key component of the mammalian visual pathway. It is a small, ovoid, ventral projection of the thalamus where the thalamus connects with the optic nerve. There are two LGNs, one on the left and another on the right side of the thalamus. In humans, both LGNs have six layers of neurons (grey matter) alternating with optic fibers (white matter)" [9,10].

"The lateral geniculate nucleus (LGN) belongs to the category of sensory projection nuclei of the thalamus and plays an essential role in normal visual processing. The lateral geniculate nucleus has broadly distributed connectivity projecting to various regions of the extrastriate cortex and receiving input from the same as well as from hindbrain and other midbrain structures. It is located in the posteroventral region of the thalamic nuclei, immediately abutting the pulvinar and posterior to the inferior choroidal point of the choroid plexus. The nucleus' name is from its lateral position relative to the medial geniculate nucleus and the sharp bend (Latin geniculum, "joint") of its laminae" [11].

"The Lateral geniculate body receives information directly from the ascending retinal ganglion cells via the optic tract and from the reticular activating system. Neurons of the LGN send their axons through the optic radiation, a direct pathway to the primary visual cortex. In addition, the LGN receives many strong feedback connections from the primary visual cortex" [9]. "In humans as well as other mammals, the two strongest pathways linking the eye to the brain are those projecting to the dorsal part of the LGN in the thalamus, and to the superior colliculus" [11].

"Both the left and right hemisphere of the brain have a lateral geniculate nucleus, named after its resemblance to a bent knee (*genu* is Latin for "knee"). In humans as well as in many other primates, the LGN has layers of magnocellular cells and parvocellular cells that are interleaved with layers of koniocellular cells. In humans the LGN is normally described as having six distinctive layers. The inner two layers, [1,2] are magnocellular layers, while the outer four layers, [3,4,5,6], are parvocellular layers. An additional set of neurons, known as the koniocellular layers, are found ventral to each of the magnocellular and parvocellular layers" [12]. This layering is variable between primate species, and extra leafleting is variable within species.

"The basis of the structure of the lateral geniculate nucleus is mostly in terms of its three distinct cell types: magnocellular (M), parvocellular (P), and koniocellular (K). P and M cells are arranged in six different layers (four dorsal P layers and two ventral M layers), with retinal ganglion signals from the ipsilateral eye synapsing on layers 2, 3, and 5, and signals from the contralateral eye synapsing on layers 1, 4, and 6. M cells in the LGN receive input from the large-field, motion-sensitive Y-type retinal ganglion cells, while P cells receive input from the small-field, color-sensitive X-type retinal ganglion cells. Koniocellular cells project into regions ventral to each of the P and M laminae" [13].

2. MATERIALS AND METHODS

2.1 Materials

List of materials used for the study: Rats, Dissecting Set, Cages, Gloves, Feeding Trough, Oxidative marker Assay, water Bottle, Saw Dust, Neem Leaf (*Azadirachta indica*), Lead Acetate,

Oral gavage, Chow, weighing Scale and Measuring Cylinder

2.1.1 Procurement and Housing

The Wistar rats used in this study were obtained from Babcock University, Ilisan, and transported to Olabisi Onabanjo University, Obafemi Awolowo College of Health Sciences, Sagamu campus. The rats were housed in the animal facility under controlled conditions with a 12-hour light-dark cycle and provided with standard rat chow and water ad libitum.
Twenty (20) presumably healthy male Wistar rats (average weight 200g) were randomly distributed into four groups, A-D, each group with its matched pair control. The rats were fed with rat pellets from Ladoke feeds in Ibadan and given water *ad libitum*. All rats were carefully assessed, screened and found to be healthy.

2.1.2 Preparation of the extract

Freshly collected Neem leaves 20g were homogenized with 1000ml distilled water in an electric blender (S-742) at room temperature. The homogenate was filtered through a sterilized cheese cloth. A cold extraction method was used to obtain the crude active agents of Neem leaves in a rotator evaporator. This extract was subjected to freeze drying to obtain a powdery substance which was dissolved in distilled water for administration to the animals repeatedly at the predetermined dosages orally.

2.1.3 Experimental groups

The rats were divided into four groups based on treatment: Group A (control), B (70mg/kg) (Orally) C (200mg/kg) (Orally) *Azadirachta indica* (Neem leaf), and D (70mg/kg Lead and 200mg/k *Azadirachta indica*)(Orally). Each group consisted of five (5) male rats.

2.1.4 Drug administration

Group A was administered physiological saline (0.9% w/v NaCl),
Group B was given lead acetate (70 mg/kg b.w/day),
Group C was given *Azadirachta indica* leaf extract (200 mg/kg b./day
Group D was given lead acetate (70 mg/kg b.w/day,) and aqueous extract of *Azadirachta indica* leaf extract (200 mg/kg b./day
The administration was done Orally and lasted for 14 days.

2.2 *In vitro* Antioxidant Assays

2.2.1Determination of superoxide dismutase (SOD) activity

Principle: The assessment of SOD activity was conducted based on the inhibition of the auto-oxidation of epinephrine at pH 10.2. Superoxide dismutase inhibits the oxidation of epinephrine to adrenochrome, a reaction catalyzed by superoxide radicals (O2-), which are generated through the xanthine oxidase reaction. The auto-oxidation of epinephrine involves distinct pathways, with one of them being a free radical chain reaction involving superoxide radicals, thus inhibitable by SOD.

Reagents: 0.05M Carbonate Buffer (pH 10.2): Prepared by dissolving 14.3 g of Na2CO3.10H2O and 4.2 g of NaHCO3 in 900 ml of distilled water and adjusting the pH to 10.2.

0.3mM Adrenaline Solution: Prepared by dissolving 0.0137 g of adrenaline in 200 ml of distilled water and adjusting the volume to 250 ml. Freshly prepared before use.

Procedure: Tissue homogenate (1 ml) was diluted in 9 ml of distilled water to create a 1 in 10 dilution.

An aliquot of 0.2 ml of the diluted enzyme preparation was added to 2.5 ml of 0.05M Carbonate buffer (pH 10.2) and equilibrated in the spectrophotometer.

The reaction started with the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline, mixed quickly, and monitored for absorbance at 480 nm every 30 seconds for 150 seconds.

SOD activity was calculated using the formula:

$$\text{Increase in absorbance per minute} = (A_3 - A_0 / t)$$

Where A_0 = Absorbance after 0 seconds, A_3 = Absorbance after 150 seconds, t = Time of final absorbance (150sec)

$$\% \text{ inhibition} = (\text{Increase in absorbance/min of sample} \times 100 / \text{Increase in absorbance/min of blank})$$

1 Unit of SOD activity is defined as the amount of SOD necessary to cause 50% inhibition of the

oxidation of adrenaline to adrenochrome during an interval of one minute.

Specific activity = (Enzyme activity x dilution factor/ Total protein (mg))

2.2.2 Determination of catalase (CAT) activities

Principle: Catalase exerts a dual function, decomposition of H_2O_2 to give H_2O and O_2, and oxidation of hydrogen donors. In the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a measure of catalase activity (Aebi, 1984).

Procedure: Fifty (50) microliter of each sample (serum, liver or pancreatic tissue homogenates) was added to a cuvette containing 450 µL of phosphate buffer (0.1M, pH 7.4) and 500 µL of 20 mM H_2O_2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to 1 mmol of H_2O_2 consumed / min/ mg of protein and is expressed as units per milligram of protein.

Calculation: Units/ml = (ΔA/min x d x 1/ V x 0.0436)

d = dilution of original sample for Catalase Reaction
V = Sample volume in Catalase Reaction (ml)
0.0436 = εmM for hydrogen peroxide
1 = Total reaction volume.

2.3 Preparation of Tissues for Haematoxylin and Eosin Histological Examination

The lateral geniculate body tissues were carefully dissected and fixed in formal saline solution (0.85% NaCl, 90ml water, 10ml formaldehyde) for 24 hours. After fixation, tissues underwent a dehydration process in a series of alcohol concentrations (60%, 70%, 80%, 90%, 95%, first absolute alcohol, and second absolute alcohol) at one-hour intervals. Subsequently, tissues were cleared using xylene, a hydrophilic clearing agent, changed at hourly intervals initially in the first xylene and then in the second xylene.

The tissue was embedded in paraffin wax by burying the tissue in a metal mold containing blocks, ready for sectioning. The tissue was sectioned at 4micron using rotary microtome and the section were floated on hot water bath to attach the section to relabeled slide. The section was dried on hot plate and ready for staining

2.3.1 staining

2.3.1.1 Haematoxylin stain

Harris Alum Haematoxylin solution was prepared by dissolving alum in hot water and adding hematoxylin powder dissolved in absolute alcohol. The solution was boiled and cooled rapidly. Glacial acetic acid was added for nuclear staining sharpness.

Slides were dewaxed in xylene, hydrated in descending alcohol grades, and stained in Harris Hematoxylin solution for 5 minutes. Differentiation in 1% acid alcohol was followed by bluing, rinsing, and dehydration in alcohol grades.

Nuclei appeared blue-black under the microscope after staining.

2.3.1.2 Eosin stain

2% Eosin solution was prepared in a mixture of 70% acid alcohol and distilled water. Sections were stained with Eosin solution for 30 seconds to 3 minutes.

Stained sections were dehydrated in alcohol grades, cleared in xylene, and mounted with DPX mounting medium.

Nuclei were stained blue-black, and cytoplasm appeared pink, indicating successful Haematoxylin and Eosin staining.

2.4 Preparation of Tissues for Cresyl Violet Examination

2.4.1 Tissue preparation

Fixation: The Cerebellum tissue samples were first fixed in a suitable fixative like formalin to preserve cellular structures.

Dehydration: After fixation, the tissue was dehydrated through a series of alcohol solutions (e.g., 70%, 95%, 100%) to remove water from the tissue.

Clearing: The dehydrated tissue was cleared using a clearing agent (e.g., xylene) to make it transparent, ensuring proper penetration of the staining solution.

2.4.2 Staining process

Rehydration: The cleared tissue was rehydrated through a series of alcohol solutions in reverse order (100%, 95%, 70%) to bring the tissue back to water.

Staining with cresyl violet: Prepare Staining Solution: Cresyl Violet staining solution was prepared by dissolving Cresyl Violet acetate in distilled water. The solution was filtered to remove any impurities.

Staining Time: The tissue sections were immersed in the Cresyl Violet staining solution for a few minutes. Dehydration: The tissue was re-dehydrated through a series of alcohol solutions (70%, 95%, 100%) to remove excess water. Clearing: The tissue was cleared with a clearing agent (e.g., xylene) to remove alcohol and prepare it for mounting. Mounting: The stained tissue section was placed on a glass slide. A mounting medium (e.g., DPX) was applied evenly over the tissue to enhance clarity and preserve the staining. Coverslipping: A coverslip was gently placed over the tissue, ensuring there were no air bubbles. It was pressed down gently to remove excess mounting medium. The edges of the coverslip were sealed with nail polish to prevent drying and preserve the slide.

2.4.3 Image analysis

The stained sections were examined under a bright-field microscope with magnifications ranging from 10x to 40x. Images were captured using a digital camera attached to a computer. Image analysis software (Java Application Software - Image J) was employed to quantify and analyze the staining patterns, allowing for a detailed evaluation of collagen distribution and tissue morphology.

The result of the pictomicography showed the Collagen fiber as blue, the Nuclei as black and the Muscle, cytoplasm, keratin as red

2.4.4 Photomicrography

Image acquisition and analysis: A bright light microscope (10 - 40x magnification objective) was used. Digital camera - OMAX Toup view 3.7 attached to P.C - HP was used. Java Application Software (image J Software) was used.

3. RESULTS

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analyzed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test. Differences were considered significant when $P < 0.05$.

3.1 Morphological Assay

3.1.1 Relative weight in the lateral geniculate bodies of adult male wistar rats

The relative weight of the lateral geniculate bodies in the lead-only group is significantly reduced compared to the control group (indicated by letter 'a'). This reduction suggests that lead exposure has led to pathological changes affecting the weight of the lateral geniculate bodies. Treatment with *Azadirachta indica* extract, either alone or in combination with lead, results in a higher relative weight of the cerebellum compared to the lead-only group. The combination of lead and *Azadirachta indica* extract shows a significant increase compared to the lead-only group (indicated by letter 'b').

3.2 *In vivo* Antioxidant Assays

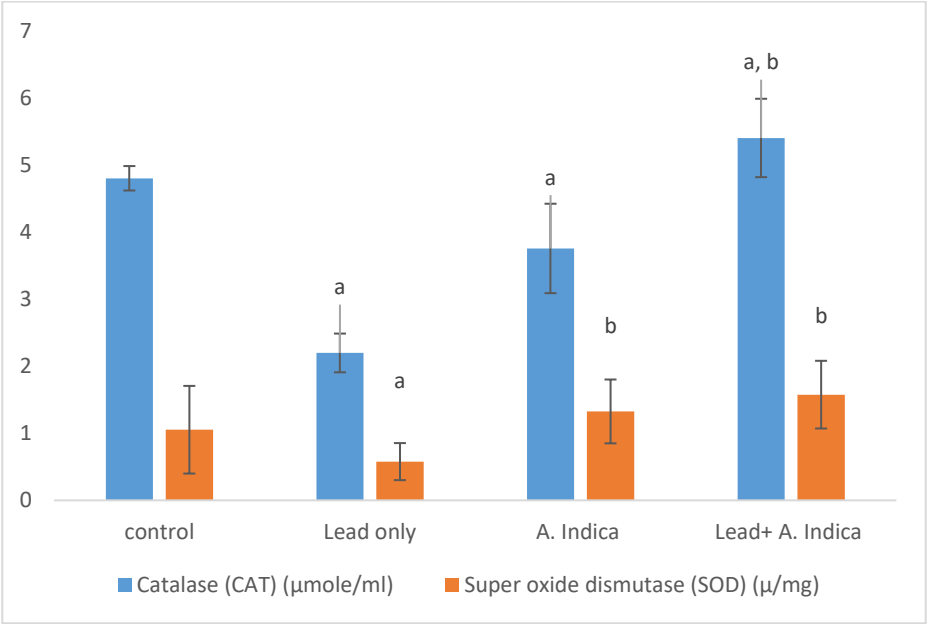
3.2.1 Superoxide dismutase (SOD) analysis and catalase (CAT) analysis

The measurements include catalase (CAT) activity in µmole/ml and superoxide dismutase (SOD) activity in µ/mg.In the lateral geniculate bodies of adult male wistar rats, lead exposure significantly reduced CAT and SOD activities compared to the control group (indicated by letter 'a'). Treatment with *A. Indica* extract alone or in combination with lead restored these activities, with the combination showing a significant increase compared to lead only group (indicated by letter 'b'). Overall, the results suggest that *Azadirachta Indica* extract has ameliorative effects against lead-induced oxidative stress in the examined brain regions.

3.2.2 Lactate dehydeogenase level (LDH)

LDH levels in the lead-only group were significantly elevated compared to the control group, as indicated by the letter 'a'. This suggests that lead acetate exposure led to an increase in LDH activity, indicating pathological changes. Treatment with *Azadirachta Indica* extract alone (*A. Indica*) significantly reduced LDH levels compared to the lead-only group (indicated by letter 'a'). This implies that *A. Indica* has an ameliorative effect, preventing or reversing the lead-induced increase in LDH

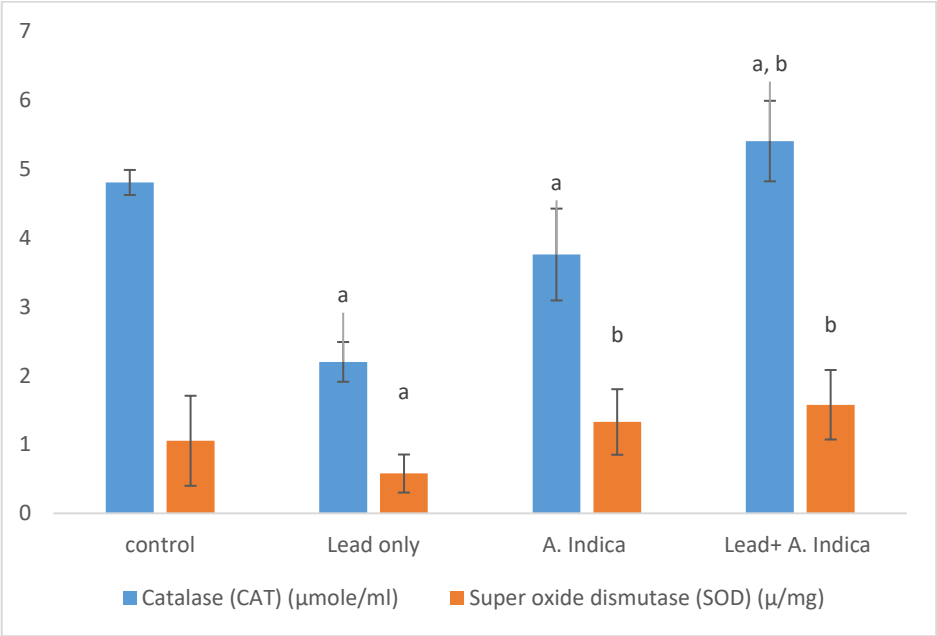
activity. When *Azadirachta Indica* extract was administered in combination with lead (Lead + *A. Indica*), LDH levels were significantly reduced compared to the lead-only group (indicated by letter 'b'). This suggests a synergistic effect, indicating that the extract may have a protective influence against lead-induced pathological changes in LDH levels



Graph 1. Provides information on the relative weight of the lateral geniculate bodies in adult male Wistar rats

Each value is an expression of mean ± SEM. (P <0.05)

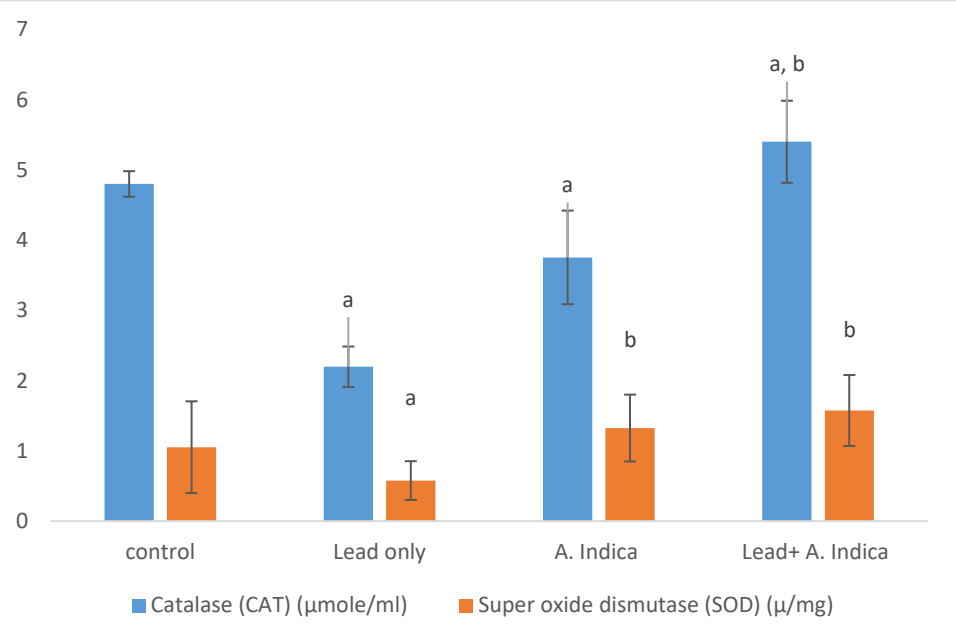
[A] - Values were significant when compared to the control group, [B]-Values were significant when compared to the lead only group, [C]- Values were significant when compared to the A. indica group



Graph 2. Showing the results of the evaluation of the antioxidant

Each value is an expression of mean ± SEM. (P <0.05)

[A] - Values were significant when compared to the control group, [B]-Values were significant when compared to the lead only group, [C]- Values were significant when compared to the A. indica group



Graph 3. Presents information on the lactate dehydrogenase (LDH) levels of adult male Wistar rats

Each value is an expression of mean ± SEM. (P <0.05)

[A] - Values were significant when compared to the control group, [B]-Values were significant when compared to the lead only group, [C]- Values were significant when compared to the A. indica grou

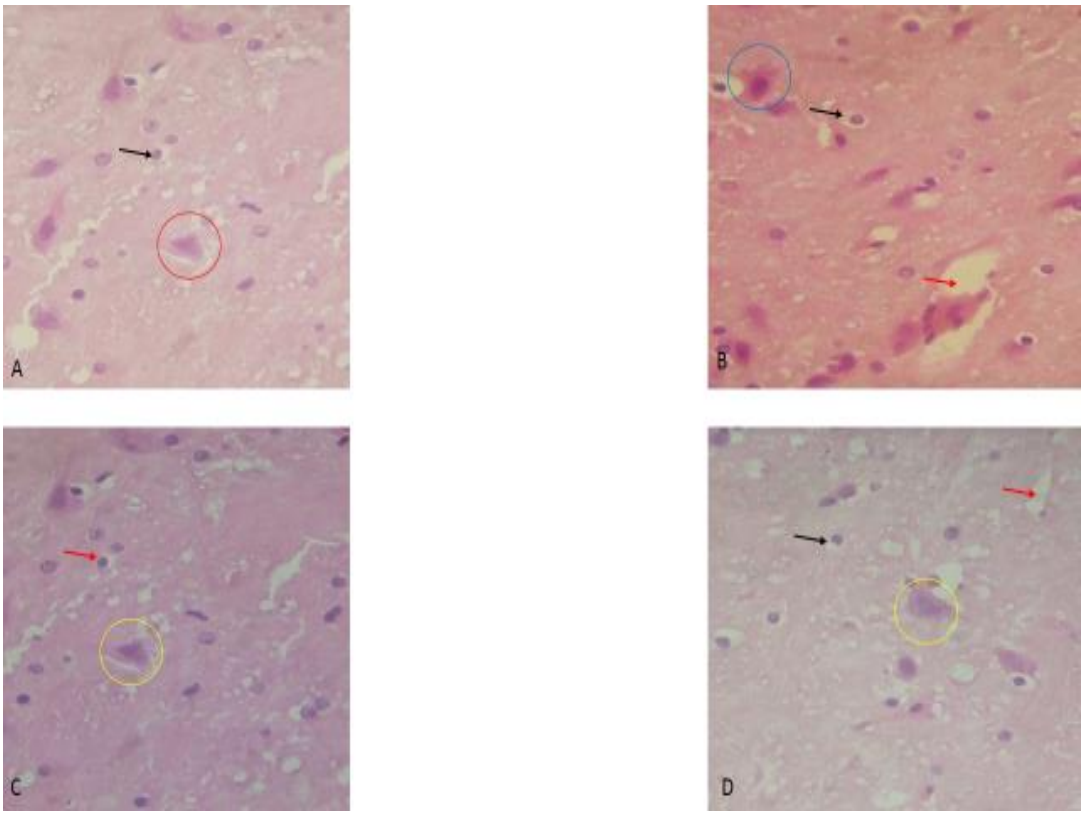


Plate 1. Photomicrograph of lateral geniculate bodies tissue

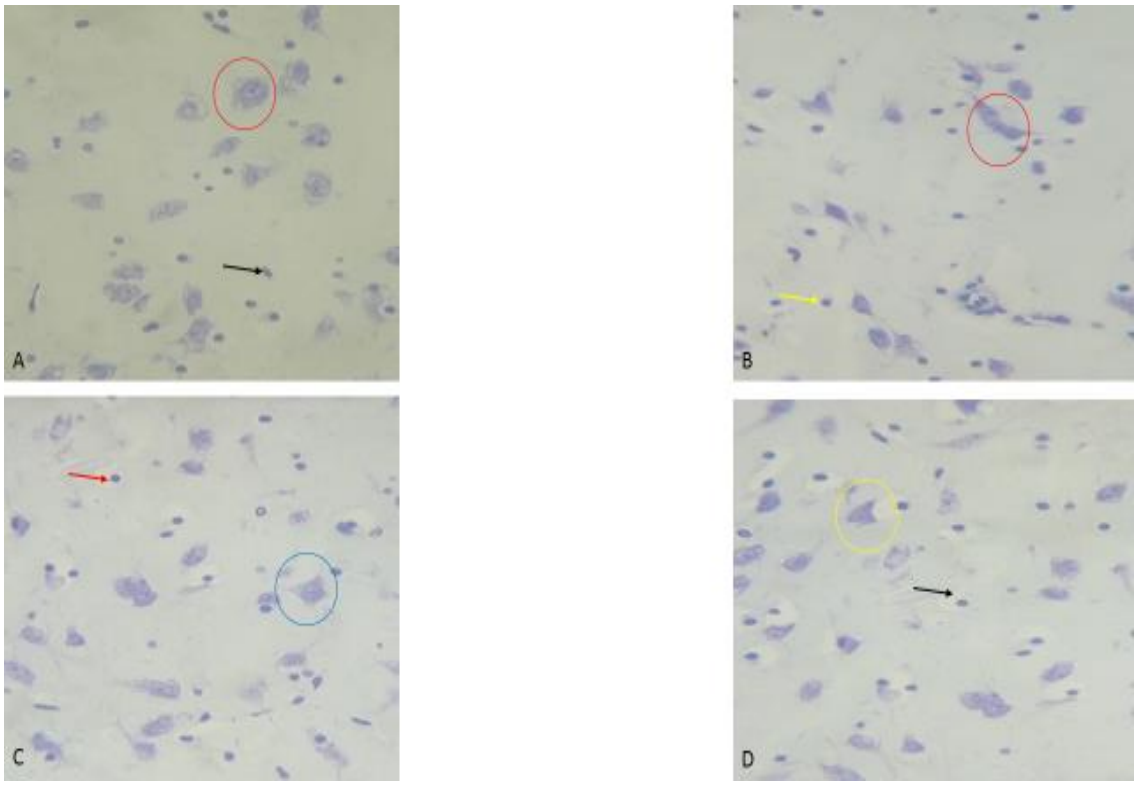


Plate 2. Photomicrograph of lateral geniculate bodies tissue showing

3.3 Histological Assay

3.3.1 Hematoxylin and eosin stain

Photomicrograph of lateral geniculate bodies tissue shows Control group showing well differentiated and organized geniculate neurons cells (red circle) and neuroglia cells (black thin arrow), induced group (b) shows degeneration of the geniculate neurons (blue circle), dilated blood vessels (red thin arrow) with vacuolated neuroglia cells (black thin arrow), induced and treated with 100mg/kg neem extract (c) shows differentiated geniculate neurons (yellow circle) and neuroglia cells (red thin arrow), induced and treated with 200mg/kg neem extract (d) shows regenerated geniculate neurons (yellow circle) neuroglia cells (black thin arrow) and blood vessels (red thin arrow).H/E X400.

3.3.2 Cresyl violet stain

Control group showing well differentiated and organized geniculate neurons cells (red circle) and neuroglia cells (black thin arrow), induced group (b) shows atrophic degenerated geniculate neurons (red circle) and neuroglia cells (yellow thin arrow), induced and treated with 100mg/kg neem extract (c) shows differentiated geniculate neurons (blue circle) and neuroglia cells (red thin arrow), induced and treated with 200mg/kg neem extract (d) shows regenerated geniculate neurons (yellow circle) neuroglia cells (black thin arrow). Cresyl violet stain X400.

4. DISCUSSION

“Lead is a heavy metal that is widely distributed in the environment and can cause various health problems in humans and animals. One of the mechanisms by which lead exerts its toxic effects is through the induction of oxidative stress” [14]. “Oxidative stress is a condition in which there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, leading to cellular damage. Superoxide dismutase (SOD) and catalase (CAT) are two important antioxidant enzymes that play a crucial role in protecting cells from oxidative damage” [15]. “Lead acetate is a toxic compound that can cause oxidative stress in various tissues and organs. Lead acetate can affect the activity of SOD and CAT, leading to an imbalance between ROS production and antioxidant defense as seen in our study. A study conducted on rats showed that lead treatment significantly decreased the activity of SOD, CAT when compared to control groups” [16]. The

decrease in SOD activity may result in the accumulation of O_2^-, which has been shown to inhibit other antioxidant enzymes. SOD, CAT, form the first line of defense against ROS, and a decrease in their activities contributes to oxidative stress in the tissues.

"A study conducted on rats showed that lead acetate increased oxidative stress in the bronchoalveolar lavage fluid (BALF) and lung tissue of rats" [17]. "The study found that lead acetate caused a significant dose-dependent increase in the levels of malondialdehyde (MDA) and nitric oxide (NO) with a decrease in glutathione (GSH) level and SOD activity in the BALF and lung tissue, the results of this formentioned study also corresponds with the result of our study as there was a decrease in SOD and CAT activity in the brain tissue. Another study investigated the effect of lead acetate on oxidative stress in mice" [18]. "The study found that lead acetate significantly increased the levels of ROS and MDA in mice, which is associated with increase in oxidative stress. Meanwhile, severe DNA damage and ultrastructure alterations were observed. SOD and CAT are two important antioxidant enzymes that play a crucial role in protecting cells from oxidative damage. SOD catalyzes the dismutation of superoxide anion (O_2-) to hydrogen peroxide (H_2O_2) and oxygen (O_2). H_2O_2 is then converted to water and oxygen by CAT. SOD and CAT work together to prevent the accumulation of O_2^- and H_2O_2, which can cause oxidative damage to cells" [19].

"Azadirachta Indica, commonly known as neem, has been studied for its antioxidant activity. The neem plant is a rich source of antioxidants and has been widely used in traditional medicine for the treatment and prevention of various diseases. The antioxidant activity of neem is attributed to its various active constituents, including nimbin, nimbidin, nimbolide, limonoids, quercetin, and ß-sitosterol. Studies have shown that different parts of the neem plant, such as the leaves, bark, and seed oil, exhibit significant antioxidant activity" [20]. Research has demonstrated the antioxidant potential of *Azadirachta Indica* through various in vitro and in vivo studies. The plant's leaf and bark extracts have been found to scavenge free radicals and prevent disease pathogenesis [21]. The presence of polyphenolic flavonoids in neem leaves, such as quercetin and ß-sitosterol, contributes to its antioxidant and antibacterial activities [22]. Additionally, the plant's seed oil has been shown to exhibit antioxidant activity, further highlighting its potential as a natural antioxidant [21]. *A. Indica* ability to scavenge free radicals and reduce oxidative stress may contribute to its therapeutic effects.

Azadirachta Indica possesses anti-inflammatory properties that complement its antioxidant mechanism [23,24]. Compounds like nimbidin and nimbin inhibit pro-inflammatory signaling pathways, reducing the production of inflammatory mediators and further mitigating oxidative damage.

Lactate dehydrogenase (LDH) is an enzyme that plays a crucial role in the conversion of glucose to energy in cells. It catalyzes the conversion of pyruvate to lactate, which is important in anaerobic metabolism. LDH is found in many tissues, including the heart, liver, kidneys, and skeletal muscles [25]. Elevated levels of LDH in the blood can indicate tissue damage or disease. LDH has been implicated in various pathologies, including cancer, bone loss, and immune modulation. Several studies have investigated the effect of lead acetate on LDH levels in different tissues. For example, a study on rats found that lead acetate increased LDH levels in the serum [26]. Another study on Wistar rats found that lead acetate caused testicular tissue injury and increased LDH levels in the testicular tissue [26], the results of the above mentioned study correspond with the results of our study, one of the major mechanism in which lead acetate use in affecting LDH level is through the induction of oxidative stress as mentioned above. *Azadirachta Indica* is rich in bioactive compounds, with nimbin, nimbinin, nimbidin, azadirachtin, and quercetin being among the key constituents [27,22]. These compounds contribute to the plant's antioxidant properties by scavenging free radicals and modulating oxidative pathways, the modulating activity exhibited by *A. Indica* leaf extrat had positive effect on the level of LDH activity.

The relative weight of the lateral geniculate bodies in the lead-only group is significantly reduced compared to the control group (indicated by letter 'a'). This reduction suggests that lead exposure has led to pathological changes affecting the weight of the lateral geniculate bodies. Treatment with *A. Indica* extract, either alone or in combination with lead, results in a higher relative weight of the lateral geniculate bodies compared to the lead-only group. The combination of lead and *A. Indica* extract shows

a significant increase compared to the lead-only group (indicated by letter 'b').

Lead acetate exposure triggers a cascade of physiological alterations in the lateral geniculate bodies. Studies have consistently reported an increase in the relative weight of the lateral geniculate bodies in animal models exposed to lead acetate [28]. Lead disrupts calcium homeostasis, interfering with various calcium-dependent processes crucial for neuronal survival and function. Additionally, oxidative stress emerges as a key player, with lead acetate promoting the generation of reactive oxygen species (ROS) and impairing antioxidant defense mechanisms in the lateral geniculate bodies. The disruption of neurotransmitter systems, particularly the glutamatergic and GABAergic pathways, contributes to the pathological changes observed. Lead acetate interferes with the release and uptake of neurotransmitters, leading to imbalances that impact synaptic plasticity and neuronal communication within the cerebellum. Neuro-inflammation is another critical aspect of lead acetate-induced cerebellar pathology. Activation of microglia and the release of pro-inflammatory cytokines contribute to a chronic inflammatory state, further exacerbating neuronal damage and dysfunction. Chronic inflammation is closely linked to oxidative stress, and *Azadirachta Indica* possesses anti-inflammatory properties that complement its antioxidant mechanism [23,24]. Compounds like nimbidin and nimbin inhibit pro-inflammatory signaling pathways, reducing the production of inflammatory mediators and further mitigating oxidative damage, which will also have positive changes on the weight of the lateral geniculate bodies.

The H&E staining reveals distinct changes in lateral geniculate body tissue. In the control group, well-differentiated and organized geniculate neurons and neuroglia cells are observed. However, the induced group shows atrophic degeneration of geniculate neurons and neuroglia cells. Treatment with 100mg/kg extract demonstrates the differentiation of geniculate neurons and improved neuroglia cells, while 200mg/kg extract leads to the regeneration of both geniculate neurons and neuroglia cells. The presence of dilated blood vessels in the induced group suggests potential vascular alterations induced by the neurotoxicity, which are mitigated with extract treatment.

The Cresyl Violet staining further highlights cellular details in the lateral geniculate body tissue. In the induced group, atrophic degeneration of geniculate neurons and neuroglia cells is evident. Treatment with 100mg/kg extract results in the differentiation of geniculate neurons and improved neuroglia cells, while 200mg/kg extract induces regeneration of both cell types. The distinct coloration from Cresyl Violet emphasizes neuronal and glial changes, providing additional insight into the potential ameliorative effects of the neem leaf extract on induced neurotoxicity in the lateral geniculate body of adult Wistar rats.

5. CONCLUSION

Our study provides valuable insights into the effects of *Azadirachta Indica* leaf extract administration on various physiological systems, including the morphology, immune, and nervous systems. The potential neurological impact of *Azadirachta Indica*, especially in the lateral geniculate body, remains an area of ongoing research and concern. Thus, research, particularly in the context of chronic administration, is needed to elucidate the mechanisms and clinical implications of these changes.

The present study aimed to contribute to this growing body of knowledge by comprehensively assessing the effects of chronic *Azadirachta Indica* administration in the lateral geniculate body of Wistar rats. Our study sheds light on the intricate effects of *Azadirachta Indica* on antioxidant defenses, and lateral geniculate body morphology. By utilizing a multi-faceted approach encompassing histology, biochemistry, organ weight, and enzyme activity, we intended to shed light on potential neurological implications.

The results of this study suggest that *Azadirachta Indica* has a number of potential benefits, including effects on the morphology, immune system, and nervous system. The dose-dependent nature of some of these effects suggests that *Azadirachta Indica*'s side effects may be predictable and manageable. However, further studies are needed to better understand the mechanisms underlying these effects and to determine their clinical significance. While our study provides valuable insights, it is essential to conduct further research to unravel the underlying mechanisms driving these changes. This understanding is pivotal for optimizing

patient care and tailoring treatments to individual needs.

In the broader context, our study emphasizes the need for a more nuanced understanding of the effect of *Azadirachta Indica*'s effects on various physiological systems. Continued research in this area is crucial.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures will adhere to ethical guidelines and will be approved by the relevant ethics committee at the Anatomy Department, Olabisi Onabanjo University, Ogun State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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