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Assessment of the Ameliorative Effects of Aqueous Neem (*Azadirachta Indica*) Leaves Extract on Lead Acetate Induced Neurotoxicity in Cerebellum of Adult Wistar Rats

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ABSTRACT

Lead is a heavy metal that can cause oxidative stress, inflammation, and neuronal damage in the central nervous system, especially in the cerebellum, which is responsible for motor coordination and cognitive function. Neem plant also called Azadirachta Indica is a medicinal plant that has antioxidant, anti-inflammatory, and neuroprotective properties according to previous literatures. This study aim to assess the ameliorative effect of aqueous neem leaf extract (Azadirachta Indica) on lead acetate induced neurotoxicity in cerebellum of adult wistar rat evaluating body weight, Histology, Lactate Dehydrogenase level, Catalase (CAT) and Super-Oxide Dismutase (SOD) parameter. After a period of 14 days of acclimatization, 20 adult wistar rats weighing(150-200g) were randomly allocated to four groups, group1(distilled water only), group2 Lead only(70mg/kgPO), group3 A. Indica extract (200mg/kgPO) and group4 (70mg/kg Lead + 200mg/kg extract of A. Indica). The rats were subjected to oral administration of Lead and aqueous seed extract of Azadirachta Indica for fourteen consecutive days before sacrifice, the cerebellum was analysed for histopathological and biochemical changes using standard laboratory procedure. Results were expressed as expressed as mean ± Standard Error of Mean (SEM) and analyzed by one-way ANOVA. Differences were considered significant when P<0.05. The results showed that lead acetate caused significant reduction in the SOD and CAT (P<0.05) activity, but in the Lead+ A. Indica group there was significant increase in the SOD and CAT activity. Also, in the Lactate De-Hydrogenase level (LDH) and the relative weight of the cerebellum, there was a significant increase in the lead only group (P < 0.05), which across the remaining treatment group, there was a significant decrease. The histology of the cerebellum shows severe degeneration and hyperplasia of the glia cells in the lead induced group while in the group induced and treated with Lead+ A. Indica shows regeneration of the cerebellum tissue. The study concluded that aqueous Neem leaf has potential therapeutic benefits against lead acetate-induced neurotoxicity in the cerebellum by enhancing the antioxidant defence system, reducing the inflammatory response.

Key words: Lead, Cerebellum, Neem leaf, Neurotoxicity

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INTRODUCTION

Through the improvement of antioxidant activity, suppression of bacterial growth, and modification of genetic pathways, plant products or natural products demonstrate an essential role in the prevention and treatment of diseases. Due to their low side effects and accessible qualities, several plants are still being eagerly explored for their potential medicinal roles in the management of diseases. It is common knowledge that allopathic medications cost a lot of money and have detrimental effects on healthy tissues and a variety of biological processes. The fact that many pharmacologically effective medications come from natural resources, including medicinal plants, is widely acknowledged [1, 2].

Native to the arid parts of Asia and Africa, *Azadirachta Indica* (Neem) is a subtropical

tree [3, 4]. Neem *(Azadirachta Indica)* 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 [5]. 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. Glycoproteins, triterpenes, limonoids, flavonoids, phenols, tannins, nimbins, saponins, catechins, azadirachtin, and gallic acid are the main phytochemicals found in neem [6, 7]. 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 [8-10]. Neem exerts its potential beneficial effects by modulating different cellular and molecular mechanisms like free radical scavenge [11], xenobiotic detoxification [12], DNA repair [13,14], cell cycle modulation [15] programmed cell death and autophagy[16], immune surveillance, signaling pathways conferring anti-inflammatory [17,18], anti-angiogenic and anti-metastatic activities. Neem also has anti–feedant, insecticide, antimicrobial, larvicidal, antibacterial, antiviral, and spermicidal [19] actions. Neem is known to possess anti–plasmodia activity thereby conferring it anti-malarial effects. It is also used treat diseases like cancer, diabetes, atherosclerosis, small pox, leprosy, urinary tract diseases, infections, hypertension [20].

The posterior cranial fossa is home to the cerebellum, which is a component of the hindbrain. It is a portion of the medulla oblongata and pons and is connected to the brain stem by three cerebellar peduncles. It is separated from two structures by the fourth ventricle's cavity. It is made up of two laterally massive hemispheres connected at the midline by vermis. The only structures that come out of the cerebellum are the deep nuclei of the cerebellum [21].

Cerebellar cortex, a highly tangled sheet of tissue that houses nearly all the neurons in the cerebellum, surrounds these nuclei. The complex arrangement of folds and fissures that defines the cerebellar cortex can be seen in a cross section through the cerebellum. There are three layers in the cerebellar cortex, The molecular layer is the outer- most layer and is largely a synaptic layer, containing the connections of a number of neurons (e.g., basket and stellate cells) with the dense dendritic arborizations, the Purkinje cells, whose cell bodies are the predominant component of the Purkinje cell layer. The innermost layer of the cortex is the granule cell layer containing Golgi cells, Lugaro cells, unipolar brush cells, and the highly abundant granule cells. Almost all of the neurons of the cerebellar cortex use either the excitatory neurotransmitter glutamate or the inhibitory neurotransmitter gamma-aminobutyric acid [22].

The cerebellum receives information from the spinal cord, and other part of the brain and then regulates motor movement. The cerebellum coordinates voluntary movement such as posture, balance, coordination and speech, resulting in smooth and balanced muscular activity. Damage to the cerebellum can lead to loos of coordination of motor movement, inability to judge distance and when to stop (dysmetria), the inability to perform rapid alternating movements (adiadochokinesia), movement tremors, staggering, and wide based walking known as ataxic gait [23].

Many heavy metals, including Pb, are known to induce over production of Reactive Oxygen Species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes [24]. Also, it has been shown to enhance the production of ROS in a variety of cells resulting oxidative stress [25]. ROS are the byproducts of many degenerative reactions in many tissues, which will affect the regular metabolism by damaging the cellular components [26]. Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions can induce the over production of ROS, such as superoxide radical (O+), H2O and hydroxyl radical (OH+) in plant cells. In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage [27].

MATERIALS AND METHODS

The following were used during the time frame of the research

Forty (40) Male Wistar Rats, pelleted rat feed, plastic cages, gloves, water bottles, bowls for

feed, Aqueous *Azadirachta Indica*, capillary tubes, plain bottles, syringe, Cotton wool, weighing scale, titrating pipettes, oral cannulas, beakers, measuring cylinder, spatula, burette, conical flask, light microscope, spectrophotometer, glass slide, dissecting set, dissecting board, gloves, centrifuge, freezer, pipette, micropipettes tips.

Drugs and Chemicals

Lead acetate (Merck Germany), phosphate buffer (pH 7.4, 0.1 M) (Oxford laboratories, Mumbai India), bluing solution, glacial acetic acid, Hydrochloric Acid (HCl), Haematoxylin and eosin staining, ellman reagent, gluthathion acid, thiobarbituric acid, carbonate buffer (Oxford laboratories, Mumbai India), Distilled water, aderenaline, Dichromate/acetic acid solution, Hydrogen peroxide, potassium hepaxodichromate, all other chemical and reagent used in this study was of analytical grade.

Animal Husbandry and Experimental Design

Animal experiments were conducted in animal house of the Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu campus, Ogun State, Nigeria. Forty adult male Wistar rats (150 ± 50 g) were used in this study. Rats were housed under a standard laboratory condition and a 12/12°h light/dark cycle. Rats had free access to taped water and a standard laboratory diet. All the animal experiments and protocol were maintained according to the rules and regulations of National Institute of Health (NIH, (2011) for laboratory animal care and use. All the animal carcasses were buried deep in the ground covered with lime and disinfectant at least two feet beneath the natural surface and covered with soil. The study protocol was conducted for fourteen days. Animals were randomly and equally divided into four groups of ten animals after fourteen day acclimatization as follows

Group A

Control group receive physiological saline (0.9% w/v NaCl, p.o) at the same volume of other groups daily throughout the experiment.

Group B

Received daily administration of lead acetate (70 mg/kg b.w/day, p.o) daily throughout the experiment (Lead only).

Group C

Received daily administration of the aqueous extract of *Azadirachta Indica* leaf extract (200 mg/kg b./day, p.o.) daily throughout the experiment (*A. Indica* only).

Group D

Received daily administration of lead acetate (70 mg/kg b.w/day, p.o) and aqueous extract of *Azadirachta Indica* leaf extract (200 mg/kg b./day, p.o.) Daily throughout the experiment (Lead+*A. Indica*).

Preparation of the Aqueous Leaf Extract of *Azadirachta Indica*

The aqueous extract of *Azadirachta Indica* leaf was prepared by drying the *Azadirachta Indica* leaf at room temperature for two [28] months and was grinded to fine particles, after which 500 g of the powder was macerated in one litre of distilled water for 24 hours. The mixture was then filtered and the filtrate was concentrated using rotary evaporator at 60°C. The concentrated filtrate was collected in a bottle and kept at room temperature before usage. The concentrate was reconstituted in distilled water to prepare the dose of *Azadirachta Indica* used in the study.

Preparation and Administration of Lead Acetate

10 g of lead acetate was dissolved in 100 ml of saline water. 70 mg/kg dose [29] of lead acetate was obtained from the stock solution according to the method described by [30].

Animal Sacrifice and Determination of Organ Weight

The animals were sacrificed by cervical dislocation 6 hours after the expiration of research. The head was detached from the body and the cerebellum weight was determined per 100 gram body weight using a weighing scale

Procedure for Blood Collection

Blood was Collected from the retro orbital sinus under mild ether anesthesia, the rat was restrained, the neck gently scuffed and the eye made to bulge. A capillary tube was inserted dorsally in to the eye and blood was allowed to flow by capillary action through the capillary tube into sample bottle.

Determination of catalase activity

Tissue catalase activity was determined according to the method of Sinha. The principle is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as a un-figure intermediate. The chromic acetate then produced in

measured calorimetrically at 570-610nm. Since dichromate has no absorbance in this region, the presence of this compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction was stopped at a particular time by the addition of dichromate acetic acid mixture and the remaining H_2O_2 is determines by measuring chromic acetate colorimetrically after heating the reaction.

Procedure

0.1 ml of homogenate was mixed with 1.0ml of 0.01M phosphate buffer (pH 7.4), and incubated with 0.4ml of 0.2M H_2O_2 at 37°C accurately for 1.0 min and reaction was stopped with 2.0 ml of 5% potassium dichromate (1:3 with glacial acetic acid). Further the samples were incubated in boiling water bath for 15min and centrifuged at 5000 rpm for 15min and supernatant was used to quantify the amount of H_2O_2 to calculate catalase activity at 570nm. One unit represents 1.0_{μ} mole of H_2O_2 consumed/min/mg protein.

Determination of Super-Oxide Dismutase (SOD) Activity

SOD activities in the brain were determined by the method of [31].

Principle

The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay of this enzyme. Superoxide anion generated by xanthine oxidase reaction caused the oxidation of epinephrine to adenochrome produced per superoxide anion introduced increasing the concentration of epinephrine. The result led to the proposal that auto-oxidation of epinephrine proceeds by at least two distinct pathways where only one of them is a free radical chain reaction involving superoxide radical and hence can be inhibited by superoxide dismutase [32, 33].

The homogenates were diluted with distilled water in ratio 1:9. An aliquot of 0.2 ml of the diluted sample was added to the 2.5 ml of 0.05M carbonate buffer, pH 10.2 to equilibrate in the spectrophotometer cuvette and the reaction started by adding 0.3ml freshly prepared 0.03 mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water [34]. The increase in absorbance at 480 nm was monitored every 30 sec for 150 sec [35, 36].

Calculation

Increase in absorbance per minute = $\frac{A3-A0}{2.5}$

Where A0 = absorbance after 30 sec
A3 = absorbance after 150 sec

$$\%\text{inhibition} = 100 - 100 \times \frac{\text{Increase in absorbance of substrate}}{\text{Increase in absorbance of blank}}$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 min.

Unit of SOD activity = $\frac{\%\text{ inhibition}}{50}$

Histological Studies

After careful removal of organs, they were trimmed of fat. They were weighed and immediately fixed in 10% formal saline [37]. After fixing the tissues, they were put into ascending grades of alcohol and then cleared in xylene. They were embedded in paraffin and serial sections of 5µm were obtained. Sections were stained with hematoxylin and eosin and cresol violet staining technique [38- 40]. The slides were viewed under light microscope (CELESTRON LCD DIGITAL MICROSCOPE, MODEL 44348) and photomicrographs were taken (400×).

Statistical Analysis

All the values are expressed as mean ± Standard Error of Mean (SEM). Analysis of data was done using Graph Pad 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 $P<0.05$ [41].

RESULTS

Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced oxidative stress in the cerebellum of adult male wistar rats.

The figure 1 presents the results of the evaluation of the ameliorative activity of *Azadirachta Indica* (neem) aqueous leaf extract against lead acetate-induced oxidative stress in the cerebellum of adult male Wistar rats. The measurements

include Catalase (CAT) activity in µmole/ml and Super-Oxide Dismutase (SOD) activity in µ/mg. In the cerebellum, 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.

Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced pathological changes in the Lactate De-Hydrogenase level (LDH) of adult male wistar rats

The figure 2 presents data on the ameliorative activity of *Azadirachta Indica* (neem) aqueous leaf extract against lead acetate-induced pathological changes in the Lactate De-Hydrogenase (LDH) levels of adult male Wistar rats. 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.

Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced pathological changes in the relative weight in the cerebellum of adult male wistar rats

Figure 3 provides data on the ameliorative activity of *Azadirachta Indica* (neem) aqueous leaf extract against lead acetate-induced pathological changes in the relative weight of the cerebellum in adult male Wistar rats. The relative weight of the cerebellum in the lead-only group is significantly increase compared to the control group (indicated by letter 'a'). This increase suggests that lead exposure has led to pathological changes affecting the weight of the cerebellum. Treatment with *A. Indica* extract, either alone or in combination with lead, results in a lower relative weight of the

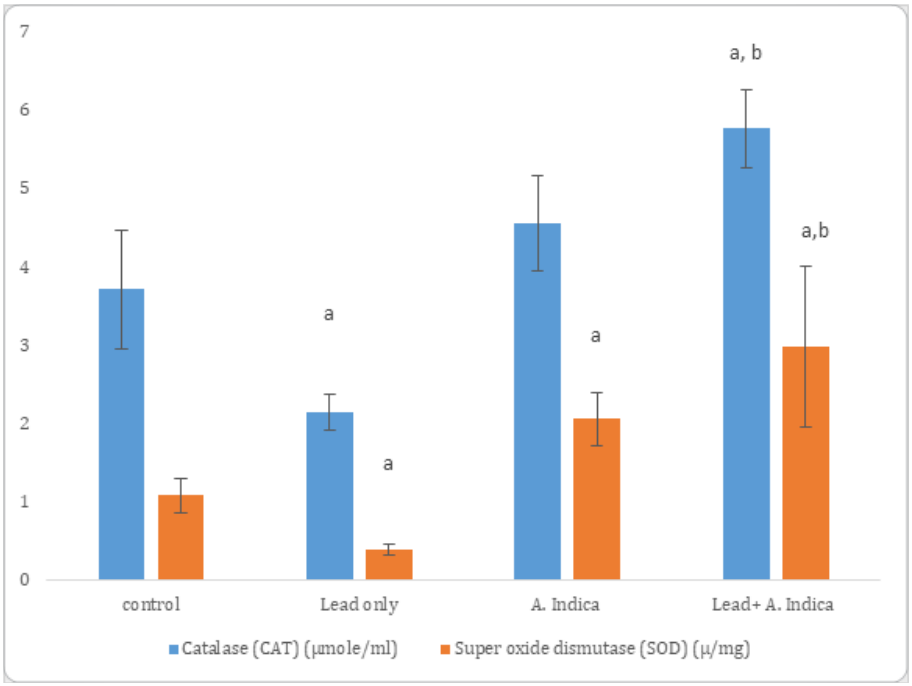

Figure 1: Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced oxidative stress in the cerebellum of adult male wistar rats. Each bar is an expression of mean ± SEM. (P<0.05) A - Values were significant when compared to the control group, B-Values were significant when compared to the lead only group, C- Values were significant when compared to the *A. Indica* group.

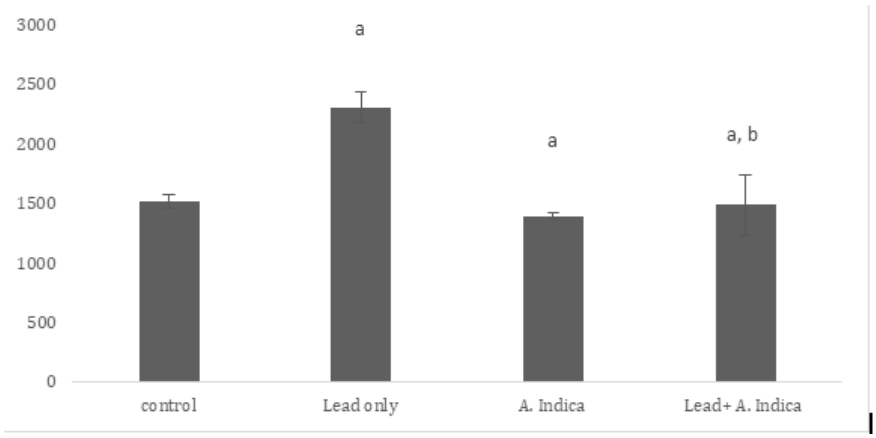

Figure 2: Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced pathological changes in the Lactate De-Hydrogenase level (LDH) of adult male wistar. Each bar is an expression of mean ± SEM. (P <0.05) A - Values were significant when compared to the control group, B-Values were significant when compared to the lead only group, C- Values were significant when compared to the *A. Indica* group.

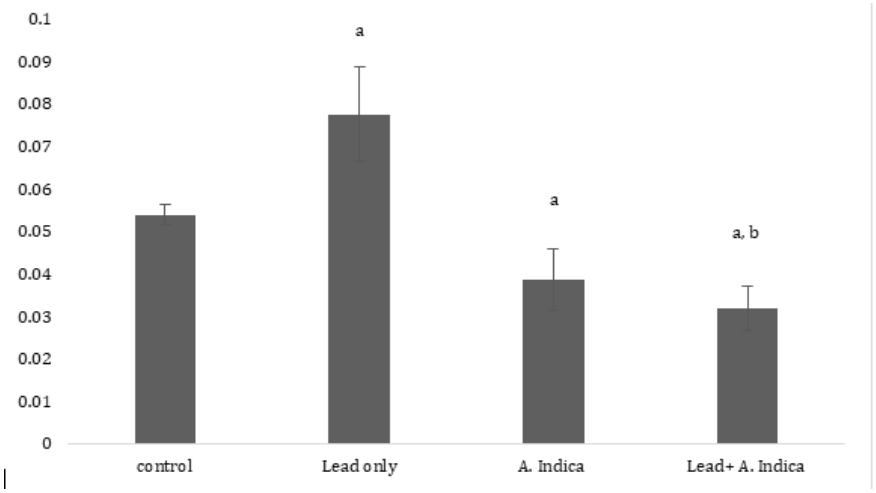

Figure 3: Ameliorative activity of *Azadirachta Indica* aqueous leaf extract against lead acetate induced pathological changes in the relative weight in the cerebellum of adult male wistar rats Each value is an expression of mean ± SEM. (P <0.05) A - Values were significant when compared to the control group, B-Values were significant when compared to the lead only group, C- Values were significant when compared to the *A. Indica* group.

cerebellum compared to the lead-only group. The combination of lead and *A. Indica* extract shows a significant reduction compared to the lead-only group (indicated by letter 'b').

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. 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. Super-Oxide Dismutase (SOD) and Catalase (CAT) are two important antioxidant enzymes that play a crucial role in protecting cells from oxidative damage (16). Lead acetate is a toxic compound that can cause oxidative stress in various tissues and organs. A study conducted on rats showed that lead acetate increased oxidative stress in the Broncho Alveolar Lavage Fluid (BALF) and lung tissue of rats. The study found that lead acetate caused a significant dose-dependent increase in the levels of malondialdehyde and Nitric Oxide (NO) with a decrease in glutathione (GSH) level and SOD activity in the BALF and lung tissue, the results of this for mentioned study also corresponds with the result of our study as there was a decrease in SOD and CAT activity in the brain tissue as in [Figure 1]. 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 this also correspond with the result of our study SOD and CAT are two important antioxidant enzymes that play a crucial role in protecting cells from oxidative damage. SOD catalyzes the disputation 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.

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. Studies have shown that different parts of the neem plant, such as the leaves, bark, and seed oil, exhibit significant antioxidant activity. Research has demonstrated the antioxidant potential of *Azadirachta Indica* through various *in vitro* and *in vivo* studies. *A. Indica* ability to scavenge free radicals and reduce oxidative stress may contribute to its therapeutic effects seen in [figure 1].

Lactate De-hydrogenase (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. Elevated levels of LDH in the blood can indicate tissue damage or disease [as seen in figure 2]. 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. Another study on Wistar rats found that lead acetate caused testicular tissue injury and increased LDH levels in the testicular tissue, the results of the above mentioned study correspond with the results of our study as seen in, 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. 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 extract had positive effect on the level of LDH activity.

Previous study on rats found that lead acetate caused histological alterations in the cerebellum, including neuronal degeneration, vacuolation, and gliosis. Another study on albino rats found that lead acetate caused toxicity in the cerebellum, as evidenced by histological changes such as neuronal degeneration, vacuolation, and gliosis. Glial cells constitute a significant proportion of the central nervous system and are integral for maintaining neural function, providing support, and participating in immune responses. Lead acetate exposure has been linked to glial cell dysfunction, leading to degeneration and hyperplasia (Figure 4 to Figure 7).

Astrocytes, the most abundant glial cells in the brain, are particularly susceptible to the toxic effects of lead acetate. Lead acetate activates microglia, initiating a pro-inflammatory response characterized by the release of cytokines and

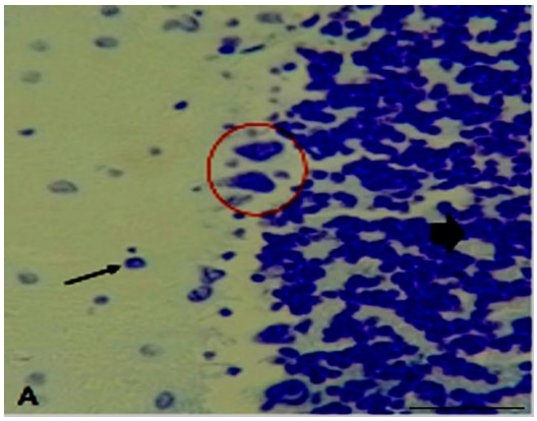

Figure 4: Control group showing well differentiated and organized cerebellum pyramidal cells (red circle) on the purkinje layer, granular layer (black thick arrow) and neuronal cells (black thin arrow) on the molecular layer. (Cresyl Violet) Magnification: ×400, Scale Bar =120µm

other inflammatory mediators. One major mechanism responsible for the changes seen in the histo-architecture of the cerebellum is Lipid peroxidation, a chain reaction that results in the degradation of lipids and the production of reactive aldehydes, which will lead to the disruption of cellular membrane. *Azadirachta Indica's* antioxidant compounds inhibit lipid peroxidation by intercepting and terminating the free radical chain reactions. This protects cellular membranes from damage and helps maintain their structural integrity, as seen in [Figure 8 to Figure 11].

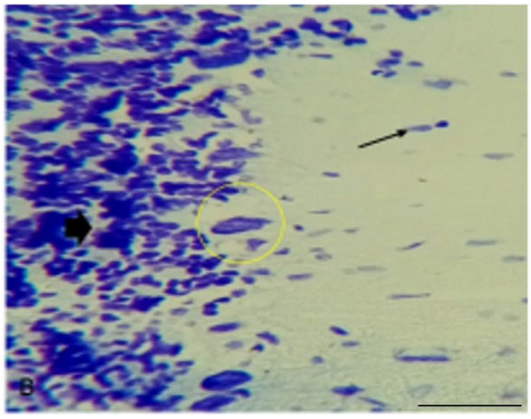

Figure 5: The lead acetate only treatment group cerebellum tissue shows severe degeneration and hyperplasia of the glia cells (black thick arrow) on the granular layer, scanty and pyknotic pyramidal cells (yellow circle) on the purkinje layer, and neuronal cells (black thin arrow) on the molecular layer. (Cresyl Violet) Magnification: ×400, Scale Bar =120µm.

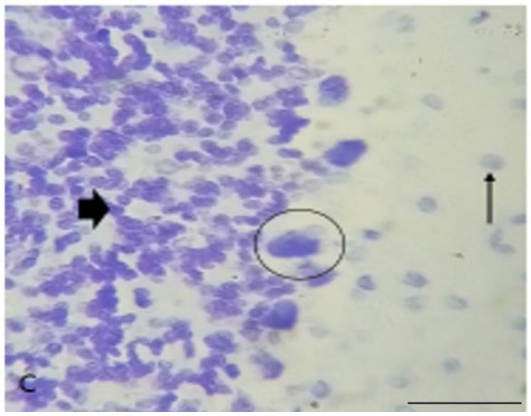

Figure 6: A. Indica only treatment group cerebellum tissue shows differentiated pyramidal cells(black circle) on the purkinje layer, glia cells(black thick arrow) on the granular layer and neuronal cells on the molecular layer(black thin arrow). (Cresyl Violet) Magnification: ×400, Scale Bar =120µm.

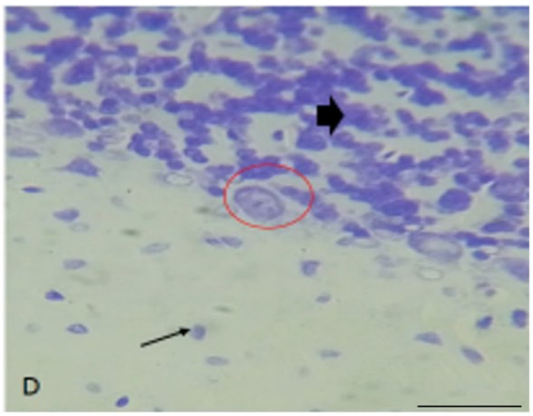

Figure 7: Induced and treated with lead acetate and *A. Indica* shows regenerated cerebellum tissue with scanty pyramidal cells (red circle), well distributed glia cells on the granular layer(black thick arrow), and neuronal cells (black thin arrow). (Cresyl Violet,)Magnification: ×400, Scale Bar =120µm.

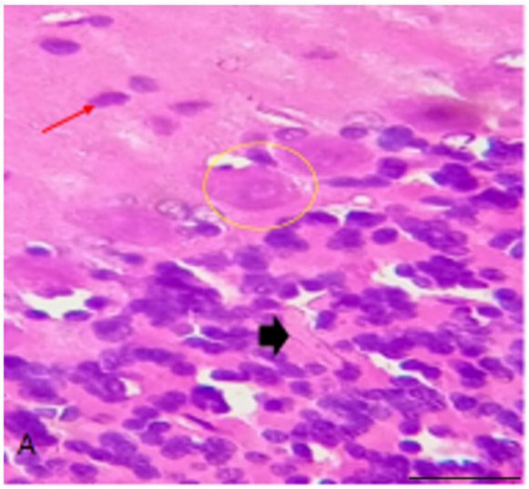

Figure 8: Control group showing well differentiated and organized pyramidal cells (yellow circle) on the purkinje layer, granular layer (black thick arrow) and neuronal cells (red thin arrow) on the molecular layer. Staining techniques: hematoxylin and eosin, Magnification: ×400, Scale Bar =120µm.

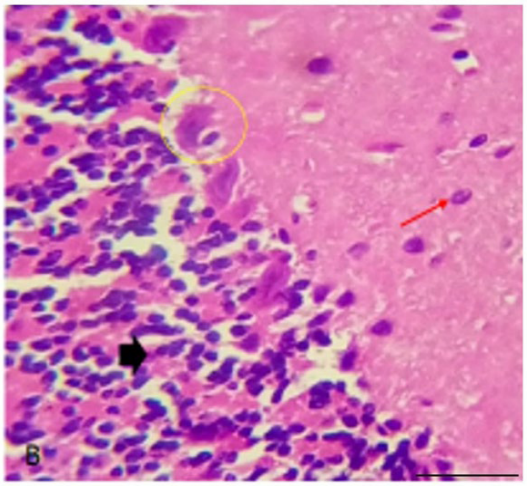

Figure 9: Induced with lead acetate only shows degeneration and hyperplasia of the glia cells (black thick arrow) on the granular layer, vacuolated and pyknotic pyramidal cells (yellow circle) on the purkinje layer, and neuronal cells (red thin arrow) on the molecular layer Staining techniques: hematoxylin and eosin, Magnification: ×400, Scale Bar =120µm.

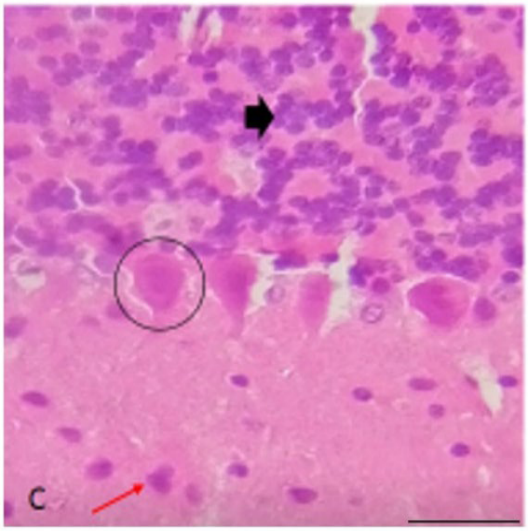

Figure 10: Treated with A. Indica only shows differentiated pyramidal cells (black circle) on the purkinje layer, glia cells(black thick arrow) on the granular layer and neuronal cells on the molecular layer (red thin arrow) Staining techniques: hematoxylin and eosin, Magnification: ×400, Scale Bar =120µm

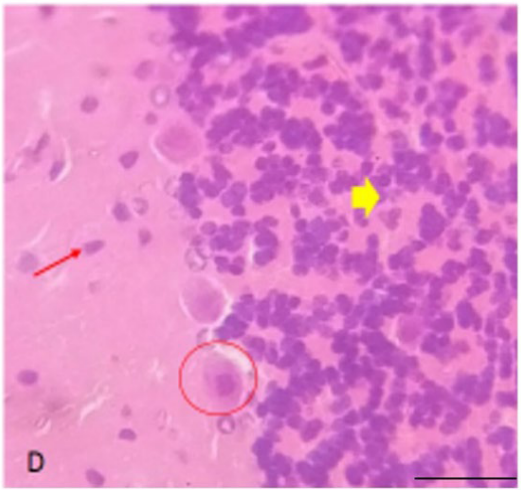

Figure 11: Induced and treated with lead acetate and *A. Indica* shows regenerated cerebellum tissue with scanty pyramidal cells(red circle), well distributed glia cells on the granular layer(yellow thick arrow), and neuronal cells(red thin arrow). Staining techniques: hematoxylin and eosin, Magnification: ×400, Scale Bar =120µm

CONCLUSION

In conclusion, the comprehensive investigation into the ameliorative effects of *Azadirachta Indica* (neem) aqueous leaf extract against lead acetate-induced damage in adult male Wistar rats reveals promising neuroprotective properties. The observed restoration of catalase and superoxide dismutase activities in the cerebellum, particularly in the combined treatment group, underscores the potential of A. Indica in mitigating lead-induced oxidative stress. Furthermore, the reduction in lactate dehydrogenase levels and the increase in cerebellar weight following *A. Indica* treatment suggest not only amelioration of pathological changes but also a potential role in modulating key enzymes and protective effects against alterations in cerebellar morphology. The regeneration of cerebellar tissue and the inhibitory impact on glial degeneration and hyperplasia further highlight the therapeutic potential of *A. Indica* in countering lead-induced cerebellar pathology. These findings support the traditional use of *A. Indica* in herbal medicine and suggest its potential application in neuroprotective strategies against environmental toxin-induced damage. The antioxidant and anti-inflammatory properties attributed to *A. Indica's* active constituents, such as nimbidin and nimbin, present a promising avenue for future research and the development of therapeutic interventions. Overall, this study provides valuable insights into the multifaceted ameliorative effects of *A. Indica* against lead-induced neurotoxicity, laying the groundwork for further exploration of its therapeutic potential in neurological disorders associated with oxidative stress and environmental toxin exposure.

REFERENCES

1. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62:183-93.
2. Alfwuaires M, Algefare AI, Mahmoud O, et al. Protective potential of apigenin against lead acetate induced alterations in cerebellum of rats. Indian J Anim Res 2023; 57:96-101.
3. Arumugam A, Agullo P, Boopalan T, et al. Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. Cancer Biol Ther 2014; 15:26-34.
4. Borst K, Dumas AA, Prinz M. Microglia: Immune and non-immune functions. Immunity 2021; 54:2194-208.
5. Claps G, Faouzi S, Quidville V, et al. The multiple roles of LDH in cancer. Nat Rev Clin Oncol 2022; 19:749-62.
6. Efferth T, Koch E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. Curr Drug Targets 2011; 12:122-32.
7. El-Beltagi HS, Salama ZA, El Hariri DM. Some biochemical markers for evaluation of flax cultivars under salt stress conditions. J Nat Fibers 2008; 5:316-30.
8. Erhirhie EO, Ekene NE, Ajaghaku DL. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. J Nat Sci Res 2014; 4:100-6.
9. Foyer CH. Oxygen processing in photosynthesis. Biochem Soc Trans 1996; 24:427-33.
10. Gupta AK. Use of neem and neem based products in organic farming. Indian Farming 2022; 72.

11. Heyman L, Houri-Haddad Y, Heyman SN, et al. Combined antioxidant effects of Neem extract, bacteria, red blood cells and Lysozyme: possible relation to periodontal disease. BMC Complement Altern Med 2017; 17:1-8.
12. Ibrahim NM, Eweis EA, El-Beltagi HS, et al. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012; 2:41-6.
13. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med 2018; 54:287-93.
14. Iman M, Taheri M, Bahari Z. The anti-cancer properties of neem (*Azadirachta indica*) through its antioxidant activity in the liver: its pharmaceutics and toxic dosage forms. A literature review. JCIM 2022; 19:203-11.
15. Incalza MA, D'Oria R, Natalicchio A, et al. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascular Pharmacol 2018; 100:1-9.
16. Isaac UE. Ameliorative effect of administering avocado (*Persea americana*) leaf extract on lead acetate toxicity in the brain-cerebellum of albino rats. J Complment Altern Med Res 2020; 10:29-37.
17. Islas JF, Acosta E, Zuca G, et al. An overview of Neem (*Azadirachta indica*) and its potential impact on health. J Funct Foods 2020; 74:104171.
18. Wang J, Li J, Cao J, et al. Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. Afr J Microbiol Res 2010; 4:1100-4.
19. Khan AA, Allemailem KS, Alhumaydhi FA, et al. The biochemical and clinical perspectives of lactate dehydrogenase: an enzyme of active metabolismEMID-DT 2020; 20:855-68.
20. Malecka A, Jarmuszkiewicz W, Tomaszewska B. Antioxidative defense to lead stress in subcellular compartments of pea root cells. Acta Biochim Pol 2001; 48:687-98.
21. Mani MS, Joshi MB, Shetty RR, et al. Lead exposure induces metabolic reprogramming in rat models. Toxicol Let 2020; 335:11-27.
22. Matovic V, Buha A, Dukic-Cosic D, et al. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. FCT 2015; 78:130-40.
23. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170-5.
24. Shoja MM, Ramdhan R, Jensen CJ, et al. Embryology of the craniocervical junction and posterior cranial fossa, part II: embryogenesis of the hindbrain. Clin Anat 2018; 31:488-500.
25. Naik MR, Bhattacharya A, Behera R, et al. Study of anti-inflammatory effect of neem seed oil (*Azadirachta indica*) on infected albino rats. J Health Res Rev 2014; 1:66-9.
26. Omobowale TO, Oyagbemi AA, Adejumobi OA, et al. Preconditioning with *Azadirachta indica* ameliorates cardiorenal dysfunction through reduction in oxidative stress and extracellular signal regulated protein kinase signalling. J Ayurveda Integr Med 2016; 7:209-17.
27. Benisheikh AA, Muhammad FM, Kelluri H, et al. *Phytochemical extraction and antimicrobial studies on crude leaf extract of Azadirachta indica* (Neem) in semi-arid region of Borno State, Nigeria. IJRR 2019; 6:516-22.
28. Patel SM, Venkata KC, Bhattacharyya P, et al. Potential of neem (*Azadirachta indica L.*) for prevention and treatment of oncologic diseases. InSemin cancer bio 2016; 40:100-115.
29. Ponnusamy S, Haldar S, Mulani F, et al. Gedunin and azadiradione: human pancreatic alpha-amylase inhibiting limonoids from neem (*Azadirachta indica*) as anti-diabetic agents. PLOS 2015; 10:e0140113.
30. Samarghandian S, Borji A, Afshari R, et al. The effect of lead acetate on oxidative stress and antioxidant status in rat bronchoalveolar lavage fluid and lung tissue. Toxicol Mech Methods 2013; 23:432-6.
31. Sarah R, Tabassum B, Idrees N, et al. Bio-active compounds isolated from neem tree and their applications. NBCs 2019:509-28.
32. Sarkar S, Singh RP, Bhattacharya G. Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. 3 Biotech 2021; 11:178.
33. Shori AB, Baba AS. Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes and hypertension by *Azadirachta indica*-yogurt. J Saudi Chem Soc 2013; 17:295-301.
34. Sgaier SK, Millet S, Villanueva MP, et al. Morphogenetic and cellular movements that shape the mouse cerebellum: insights from genetic fate mapping. Neuron 2005; 45:27-40.
35. Shilpa G, Renjitha J, Saranga R, et al. Epoxyazadiradione purified from the *Azadirachta indica* seed induced mitochondrial apoptosis and inhibition of NFκB nuclear translocation in human cervical cancer cells. Phytotherapy Res 2017; 31:1892-902.
36. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47:389-94.
37. Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents 2005; 5:149-56.
38. Vardhan S, Sahoo SK. In silico ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. Comput Biol Med 2020; 124:103936.
39. Garcia MX, Foote C, Van Es S, et al. Differential developmental expression and cell type specificity of Dictyostelium catalases and their response to oxidative stress and UV-light. Biochim Biophys Acta 2000; 1492:295-310.

40. Al Osman M, Yang F, Massey IY. Exposure routes and health effects of heavy metals on children. Biometals 2019; 32:563-73.

41. Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: A review of recent research. Carbohydrate polymers 2012; 90:1395-410.