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A Study of Some Effects of Aqueous Extract of Neem (Azadirachta Indica) Leaves on the Lead Acetate Induced Neurotoxicity in the Superficial Layers of Superior Colliculus of Adult Wistar Rats (*Rattus norvegicus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author FPB designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author AAJ interpreted the histological findings. Author OEB interpreted and analyzed the biochemical findings while Author AOH collected the plant leaves and prepared the plant extract. All authors read and approved the final manuscript

Research Article

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ABSTRACT

Aim: To investigate some neuroprotective effects of aqueous extract of Neem leaves against damaging actions of lead acetate induced neurotoxicity in the superficial layers of the superior colliculus of adult wistar rats.
Study Design: Histological and Biochemical study.
Place and Duration of study: Department of Anatomy, Faculty of Basic Medical Sciences, LAUTECH, Nigeria between January 2012 and August 2012.
Methodology: 40 adult wistar rats (average weight 200±16.2g) were randomly assigned into 4 groups (N=10) of Control C, Treatments T1, T2 and T3 and treated with distilled water, 1% lead acetate and 200mg/kg b.wt of aqueous extract of neem, 1% lead acetate, and 200 mg/kg b.wt of aqueous extract of neem respectively for14days.They were sacrificed by cervical dislocation and processed for routine histopathological studies,

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bioassay of some antioxidant parameters as well as lipid peroxidation.
Results: Statistically significant (P=0.05) body weight, wet brain weight as well as neuronal cell loss was recorded in T2 while T1 and T3 showed statistically insignificant weight loss compared to control. Oxidative stress enzymes Superoxide dismutase, Glutathione reductase and Glutathione peroxidase levels were significantly (P=0.05) reduced in T2 compared to the control but relatively increased in T1 and T3. T3 and T1 respectively recorded 65% and 55% increments above T2 in Glutathion levels while Lipid peroxidation level was drastically reduced in T3 and to some extent in T1 compared to increased level in T2. Group T2 showed sparsely distributed pyknotic pyramidal neurons with obliterated soma and few glial cells while T1 sections appeared less distorted. Neem offers some ameliorative protection to the pyramidal neuronal and glial cells of the superficial layers of the superior colliculus against lead compared to T2, however the control and T3 sections relatively appeared normal.
Conclusion: Neem offers ameliorative protection to the pyramidal neuronal and glial cells of the superficial layers of the superior colliculus against lead acetate induced neurotoxicity in wistar rats and also further affirms its antioxidative potential.

Keywords: Neem; neurotoxicity; lead acetate; superior colliculus; pyramidal neurons.

1. INTRODUCTION

Lead (Pb) is one of the environmental pollutants that can threaten the life of living creatures in many ways. Various industries producing batteries, paints and pigments make use of lead extensively and as such, the population may get exposed to lead by food and water contamination as well as air pollution caused by industrial emission and gasoline containing lead compounds [1].

Herbal preparations used for the treatment of diseases and its knowledge are handed down from generation to generation and has been used in healthcare delivery in many parts of Africa and the rest of the world. Effective health cannot be achieved in Africa, until orthodox medicine is complemented with traditional medicine [2]. At least 89% of Africans depend on plant medicine for their healthcare [2]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization [3]. Medicinal plants have continued providing valuable therapeutic agents, both in modern and in traditional medicine [4]. With the associated side effects of modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions [5]. *Azadirachta indica*, (*A. Indica*;) also called (Neem) belongs to the family of meliaceae [6] and it is one of the most useful medicinal plants. Understanding the mechanisms responsible for the translation of sensory signals into the commands for movement is one of the fundamental goals of neurobiology and superior colliculus may provide a relatively simple model for studying how sensory systems initiate and guide movement. In the superior colliculus, the juxtaposition of cellular layers with sensory and motor functions is especially useful for studies of these mechanisms. Although behavioral studies have shown that superior colliculus is not needed for object recognition, but plays a critical role in the ability to direct behaviors toward specific objects, and can support this ability even in the absence of the cerebral cortex concerned [7]. The superior colliculus also provides an ample opportunity to investigate how, within a single brain structure, signals from the different senses are combined and used to guide adaptive motor responses [8]. Lead is known to induce a broad range of physiological,

biochemical, and behavioural dysfunctions in laboratory animals and Man [9]. The toxicity of lead (Pb) is closely related to age, sex, route of exposure level of intake, solubility, metal oxidation state, retention percentage, frequency of intake, absorption rate, duration of exposure, mechanisms and efficiency of excretion [9]. Reactive oxygen specie (ROS) are by-products of many degenerative reactions in many tissues which affect regular metabolism by damaging the cellular components [10] and Pb including many heavy metals are known to induce overproduction of reactive oxygen specie which consequently enhance lipid peroxidation, decrease saturated fatty acid and increase unsaturated fatty acid content of the membranes [11], meanwhile overproduction of reactive oxygen specie (ROS) in a variety of cells almost always result in oxidative stress [12]. Lead has been associated with various forms of cancer,nephrotoxicity, neurotoxicity and cardiovascular diseases in Man [13]. The present study was performed to examine the lead induced oxidative stress and neurotoxicity in the superficial layers of superior colliculus as well as the protective effects of neem leaves extract as an antioxidant.

2. MATERIALS AND METHODS

Forty presumably healthy adult wistar rats of both sexes (average weight 200g) were maintained in the Animal Holdings of the Department of Anatomy LAUTECH, Ogbomoso, Nigeria. They were fed with standard laboratory mouse chow (LADOKUN feeds Ibadan) and were given water *ad libitum*. At the end of acclimatization period the animals were randomly assigned into four groups (N=10) of Control C and Treatments T1, T2 and T3. T1 received 1% lead acetate [14] and 200 mg/kg b.wt of aqueous extract of neem [15] while T2 received 1% lead acetate and T3 received 200 mg/kg b.wt of aqueous extract of neem. All the animals were exposed for a period of 14 days and at the end of administration, all the rats were sacrificed by cervical dislocation. The skull was opened with the aid of dissecting set and the wet brains were removed en-mass and weighed on an analytical balance, after which the areas of the superior colliculus (SC) were trimmed out using [16] and processed immediately for histological techniques and bioassay.

2.1 Enzyme Assay

For the measurement of the activities of the enzymes, GSH and Lipid peroxidation, part of the SC specimens were weighed and homogenized in a sucrose buffer (0.25M Sucrose, 10Mm HEPES, 1 mM EDTA, pH 7.4) and the homogenate was centrifuged at 1000 x g for 60min at 4ºC for the assay of superoxide dismutase (SOD). The activity of Superoxide dismutase (SOD) was measured as previously described by [17] which is an assay based on the ability of SOD to inhibit the autoxidation of pyrogallol by 50%. The assay mixture of 1 ml contained in final concentration, 50 mM Sodium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.48 mM Pyrogallol and appropriate amount of tissue extract containing 7-10 µg of protein. The change in absorbance of assay mixture was monitored spectrophotometrically at 420 nm for 3 min at 25ºC against blank. One unit of enzyme activity is defined as the amount of enzyme that causes 50% maximal inhibition of pyrogallol autoxidation. The remaining brain tissues were homogenized in cold phosphate buffered saline (PBS) with 1mM EDTA and the homogenate was centrifuged at 1200 x g for 15 minutes at 4ºC and the supernatant analyzed for the activities of the following parameters:

2.1.1 Glutathione reductase (GR)

The Glutathione reductase (GR) activity was measured by the modified method of [18]. The reaction mixture of 1ml contained in final concentration, 4.1 mMTris-HCl (pH 7.5), 15mM $MgCl_2$, and 5.7 mM EDTA, 60 mM KCl, 2.6 mM Glutathione (oxidized) and 0.1 mM NADPH. The reaction was started by the addition of tissue extract containing approximately 100 µg of protein. One unit of enzyme activity is defined as 1 µmol of NADPH oxidized/min/mg protein. The decrease in absorbance was monitored at 25ºC at 340 nm.

2.1.2 Glutathione peroxidase (GPx)

This was determined using modified method as described by [19]. The assay mixture of 1 ml contained in final concentration, 10 mMPotassium phosphate buffer (pH 7.0), 25 mM EDTA, 0.5mM Glutathione (reduced), 2mM Sodium azide, 1.5 IU Glutathione reductase, 0.1 mM NADPH and the cytosolic fraction containing about 50 µg of protein. The reaction was started by the addition of t-butyl hydroperoxide and the decrease in absorbance was monitored at 25ºC at 340 nm. One unit of enzyme activity is defined as 1 µmol of NADPH oxidized/min/mg protein.

2.1.3 Glutathione (GSH)

Using commercially available kit (NWK-GSH01, North West Life science specialities,LLC.,as previously described by [20].

2.1.4 Malonaldehyde (MDA)

The level of lipid peroxidation was assessed in the brain tissue by measuring the formed malonaldehyde (MDA), an end product of fatty acid peroxidation, using thiobarbituric acid reactive substance (TBARS) method [21]. 10% tissue homogenate was centrifuged at 1000xg for 10 min and protein removed with half volume of 20% trichloroacetic acid (TCA). The supernatant in 10 mM Potassium phosphate buffer (pH 7.4) was incubated at 80ºC for 15 min in water bath with 0.53% Thiobarbituric acid in glacial acetic acid and centrifuged. The concentration of MDA-TBA complex was determined spectrophotometrically at 532 nm against blank.

2.1.5 Histological Techniques

The SC specimens were fixed in 10% formol calcium for 72 hours after which they were processed for routine histological techniques, sectioned at 6µ and stained with Cresyl violet as described by [22] for Nissl's substance. Qualitative observations of SC sections were done with every 10th section chosen from each animal. Using brightfield compound Nikon microscope, YS100 (attached with Nikon camera), the slides were examined and photographed under 400X objective Lens. Using Image-Pro Express software, counts of neurons with prominent nucleolus within a measured monolayer of pyramidal cells at several microscopic fields were done. The neuronal transverse diameter for the pyramidal cells was also determined. The absolute neuronal density per unit area of section for each region was estimated [23].

2.1.6 Statistical analysis

The data were analyzed using the computerized statistical package ‘SPSS Version 11’. Mean and standard error of mean (SEM) values for each experiment group was determined.The means were compared by analysis of variance at a level of significance of 95 and 99%.

3. RESULTS AND DISCUSSION

3.1 Body Weight

The results obtained here showed that there was a significant (P=0.05) weight loss (163.60±0.38)g in group T2 and an insignificant weight loss (189.90±0.42)g in group T1when compared to the control group C (224.40±0.14)g while an insignificant weight gain was recorded in treatment group T3 with (216.30±0.51)g as seen in Table I. These changes however revealed a percentage weight loss of -9.91% and -27.38% respectively in groups T1 and T2 as against percentage weight gain of 3.08% and6.20% respectively in the treatment group T3 and Control group C as shown in Table 2.

Table 1. Body weights (g) of the rats before and after treatment of Animals

Groups	N	Initial Weight	Final weight
C	10	210.49±1.64	224.40±0.14
T1	10	208.71±1.32	189.90±0.42††
T2	10	208.40±2.06	163.60±0.38†
T3	10	209.62±1.28	216.30±0.51††

† *Significant (P=0.05).*
††*Insignificant.*

Table 2. Difference and % Difference in the body weights at the end of administration

Groups	N	Difference(G)	% Difference (%)
C	10	13.91	6.20
T1	10	-18.81	-9.91
T2	10	-44.80	-27.38
T3	10	06.68	3.09

3.2 Brain Weight

Significant ($P < 0.01$) loss in brain weight was seen in the treatment group T2 (1.32±0.44) g) compared to the control group with (1.84± 0.3)g while insignificant ($P > 0.01$) brain weight loss was also seen in the treatment groups T1 and T3 with (1.63 ± 0.13 &1.80 ± 0.90)g respectively compared to the control group in Table 3 .

3.3 Neuronal Density and Neuronal Diameter

Treatment group T2 showed significantly (P=.05) reduced neuronal density and neuronal diameter of (306.19±6.11)sq.cm and 0.52±0.39)µm respectively translating into a percentage neuronal loss of 43.41% compared to the control section with neuronal density and neuronal diameter of (541.15±12.26)sq.cm and 1.28±0.41) µm.The neuronal densities and neuronal

diameter in Treatment group T1 and T3 0.96±0.22)µm} and {(501.09±16.02)sq.cm & 1.16±0.51)µm} respectively compared to the control group and these values also translated to percentage neuronal loss of 10.64% and values obtained were insignificantly lower {(483.55±21.44)sq.cm &7.40% as seen in Table 4.

Table 3. Wet brain weights (g) of rats at the end of administration

Group	N	Mean± SEM (G)	F-value	D.O.F	2-TPROB.
C	10	1.84± 0.31	2.81	6	0.001
T1	10	1.63± 0.13††			
T2	10	1.32± 0.44†			
T3	10	1.80± 0.90††			

†P < 0.001, Values are Statistically significant
††P > 0.001, Values are Statistically insignificant

Table 4. Neuronal density and cells diameter of Pyramidal cells and percentage neuronal loss

Groups	Pyramidal cells/SQ.CM	Pyramidal cells diameter (µM)	Neuronal loss (%)
C	541.15±12.26	1.28±0.41	-
T1	483.55±21.44	0.96±0.22	10.64
T2	306.19±6.11	0.52±0.39	43.41
T3	501.09±16.02	1.16±0.51	07.40

3.4 Bioassay

This revealed a slightly decreased values in the specific activities of SOD in the treatment group T2 with (8.14±0.17)*U/mg protein* compared to the control group C with (11.04±2.19)*U/mg protein* while group T1and T3 recorded significantly (P=.05) increased values (14.15±1.30 and 14.61±3.51)*U/mg protein* respectively. The results obtained for GR showed that the specific activity was drastically reduced in group T2 with (21.41±3.16)*mU/mg protein* compared to the control group C with (31.49±2.13)mU/mg protein but on the contrast the groups T1 and T3 recorded significantly (*P*=.05) increased values (36.66±1.18 and 38.40±1.31)*mU/mg protein* respectively while the specific activity of GPx was decreased significantly(*P*=.05) for treatment group T2 with Mean±SEM (0.73±0.27)*mU/100mg protein* compared to the control group C with (2.29±0.85)*mU/100mg protein* while group T1and T3 recorded significantly (*P*=.05) increased values (4.13±1.61and4.76±0.11)*mU/100mg protein* respectively as shown in Table 5.

Table 5. Antioxidant enzyme activities in the superior colliculus (SC)

Groups	SOD (U/MG protein)	GPX MU/100MG protein	GR MU/MG protein
C	11.04±2.19	2.29±0.85	31.49±2.13
T1	14.15±1.30	4.13±1.61	36.66±1.18
T2	8.14±0.17	0.73±0.27	21.41±3.16
T3	14.61±3.51	4.76±0.11	38.40±1.31

Groups T1 and T3 respectively recorded significant ($P<.05$) of 55% and 65% increments above T2 in the GSH levels while MDA level was drastically reduced in T3 with Mean±SEM (1.69±0.21)*µM/mg protein* and to some extent in T1 with Mean±SEM(1.83±0.22)*µM/mg protein* compared to increased level in T2 with (3.58±0.39)*µM/mg protein* as shown in Table 6.

Table 6. GSH and LPO levels (*µM/mg protein*) in the superior colliculus (SC)

Groups	GSH	LPO
C	4.15±1.66	2.28±0.61
T1	4.75±1.14	1.83±0.22
T2	2.12±0.18	3.58±0.39
T3	6.09±1.06	1.69±0.21

3.5 Histological Findings

The histoarchitecture in the treatment group T3 and control C revealed distinct and seemingly normal pyramidal cells in the stratum griseum superficiale (SGS) and the stratum opticum (SO) while glial cells were also evenly distributed. However the pictorial representation in treatment group T2 showed pyknotic pyramidal cells with scantily distributed glial cells. These findings were similar to treatment group T1 but far less pronounced.

3.6 Discussion

As a pervasive environmental pollutant, Lead has been reported to induce a broad range of physiological, biochemical and behavioral dysfunctions in Man and laboratory animals [24]. Lead (Pb) is recognized as a serious threat to human health because it affects both the CNS and PNS [9].The present study demonstrated some ameliorative effects of *A. Indica* leaves extract on the body weights, wet brain weights, neuronal distribution and the enzymatic activities of some antioxidant parameters after 14 days of Lead exposure. Relative to the control group, body weight observations made here revealed an insignificant weight loss in group T1 compared to a significant (*P=0.05)* weight loss in group T2 while an insignificant weight gain was observed in group T3 when compared to a significant weight loss obtained in group T2. However an insignificant weight loss was obtained in group T1 compared to an insignificant weight gain seen in group T3 as shown in Table 1 and this is in conformation with the earlier reports of [25,26] that rats fed with Pb experience decreases in growth rate as well as the reports of [27] which showed that the weight of the rats treated with 200mg/l Pb for 3 weeks were significantly reduced. The pattern of weight distribution in the wet weight of the brain is also similar to that obtained in the body weights with significant P=0.05 brain weight loss in treatment group T2 compared to control group and slight loss in groups T1 and T3 as shown in Table 2, and this finding is also in an agreement with the earlier reports of [28] that reduced brain weight is associated with the effects of many neurotoxins. This pattern of data distribution thus suggests that *A. Indica* may be responsible for weight recovery with a tendency of inhibiting the bioaccumulation of Pb in the tissue and in the process reducing lead concentration. As a matter of fact, Lead-nutrients interactions with evidence from reported experiments have also positioned nutritional factors to have impact on health outcomes following Pb exposure [29.30]. The Histological findings in the control group as obtained from Fig.1 showed normal evenly distributed supporting neuronal cells with normal pyramidal neuronal cells and this forms a watershed of comparison to fairly

normal picture obtained from treatment group T1 having slightly distorted cells as obtained from Fig. 2 and thus pointing to the fact that *A. indica* offered some ameliorative effects which is in a process of reducing neurotoxicological effects of Pb because from the lead exposed group T2, distorted pyramidal neurons with obliterated soma with broken axons in the SUG (Superficial griesium) and OP(Optic Nerve layer) with scanty glial cells was seen as evident in Fig. 3 whereas the *A. indica* exposed group revealed a histoarchitecture similar to that of control group as seen in Fig. 4. The histological observations noted from the lead exposed group revealed neuronal degeneration which is also in conformity with earlier reports of Engin Deveci [31] that when rats received lead acetate at dose of 500 ppm in their drinking water for 60 days they showed degeneration in some of the neuronal cells, which has almost always been resulting in cell death [32] which has been classified mainly into two types as necrosis and apoptosis [33],while necrosis affects cell population and is often characterized by inflammation and release of intracellular organelles as a result of disruptions of the plasma membrane as well as cytoplasmic swelling [34], apoptosis is a genetically determined, biologically meaningful active process playing a role opposite to mitosis in tissue size regulation, shaping organs and removing cells that are immunologically reactive against self, infected or genetically damaged whose continuous existence pose a danger to the host and is actively involved in physiological and developmental processes [35]. Also [36] reported that in structures with compact volume such as hippocampus, lateral geniculate body and superior colliculus, estimation of total number of neurons is possible just as neuron counting from histological sections has been used to estimate the total number or cell density in the brain region [37].

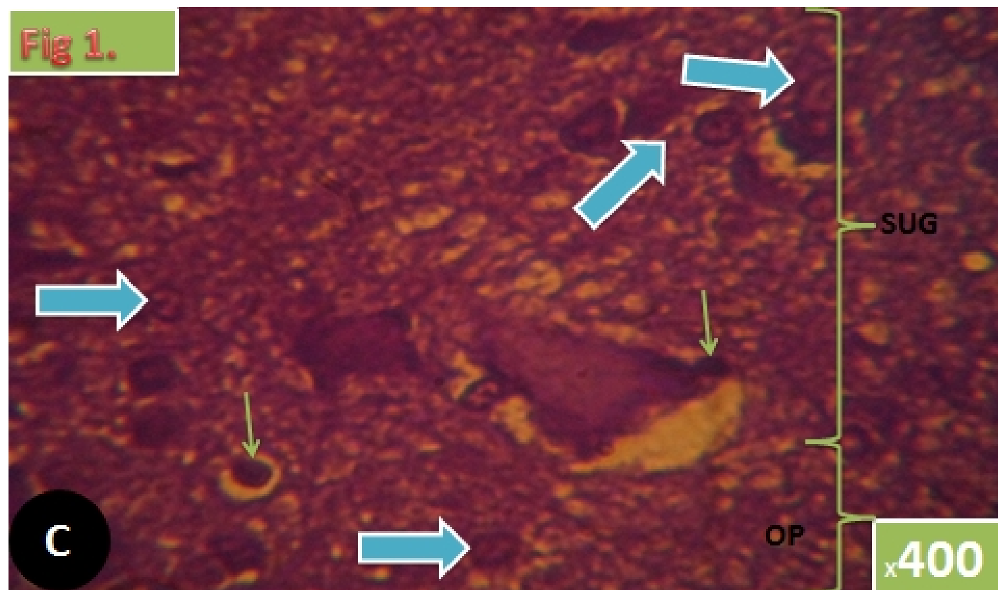

Fig. 1. Photomicrograph of Superior colliculus (control section C) showing the neuronal distributions in the SUG (Superficial griesium) and OP(Optic Nerve layer). Note the distinctly stained normal pyramidal neurons (Blue arrows) with centrally located nuclei as well as well distributed glial cells (Green arrows) *Cresyl violet stain* x400

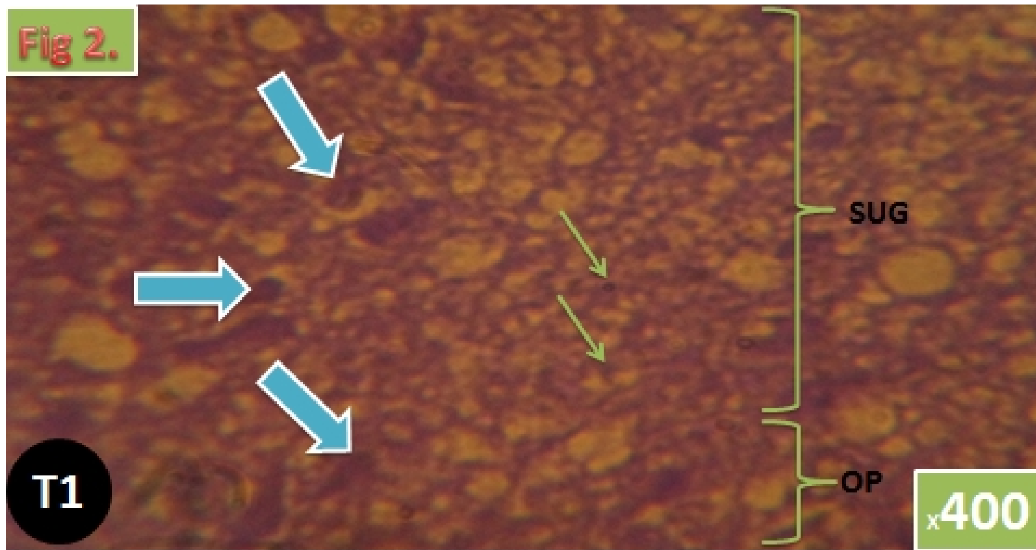

Fig. 2. Photomicrograph of Superior colliculus (Treatment section T1) showing the neuronal distributions in thein the SUG (Superficial griesium) and OP(Optic Nerve layer). Note the distinctly stained and sparcely distributed Pyknotic pyramidal neurons (Blue arrows) with obliterated soma as well as very few glial cells (Green arrows) *Cresyl violet stain* x 400

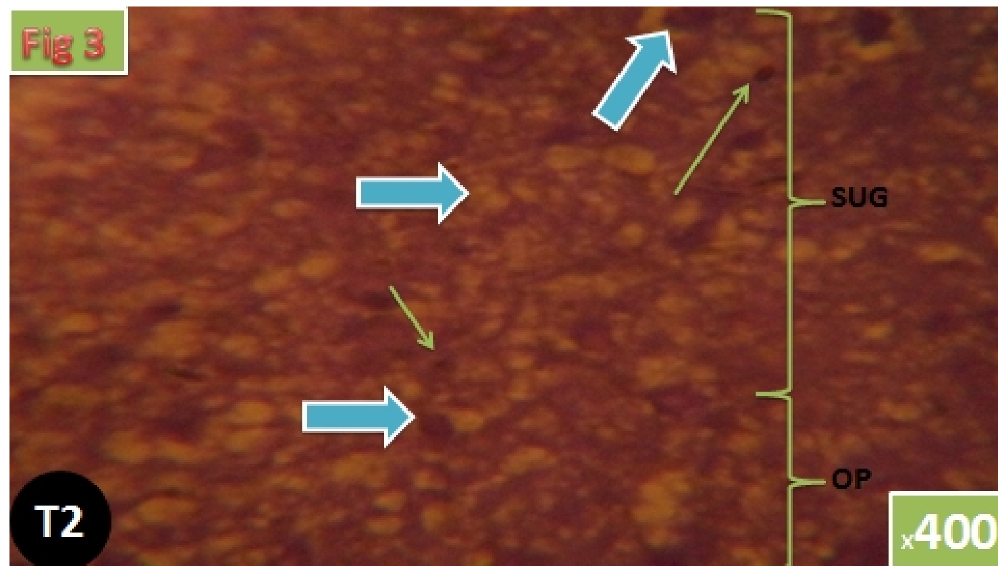

Fig. 3. Photomicrograph of Superior colliculus (Treatment section T2) showing the neuronal distributions in the SUG (Superficial griesium) and OP(Optic Nerve layer). Note the distinctly stained and sparcely distributed Pyknotic pyramidal neurons (Blue arrows) with obliterated soma as well as very few glial cells (Green arrows) Cresyl violet stain x400.

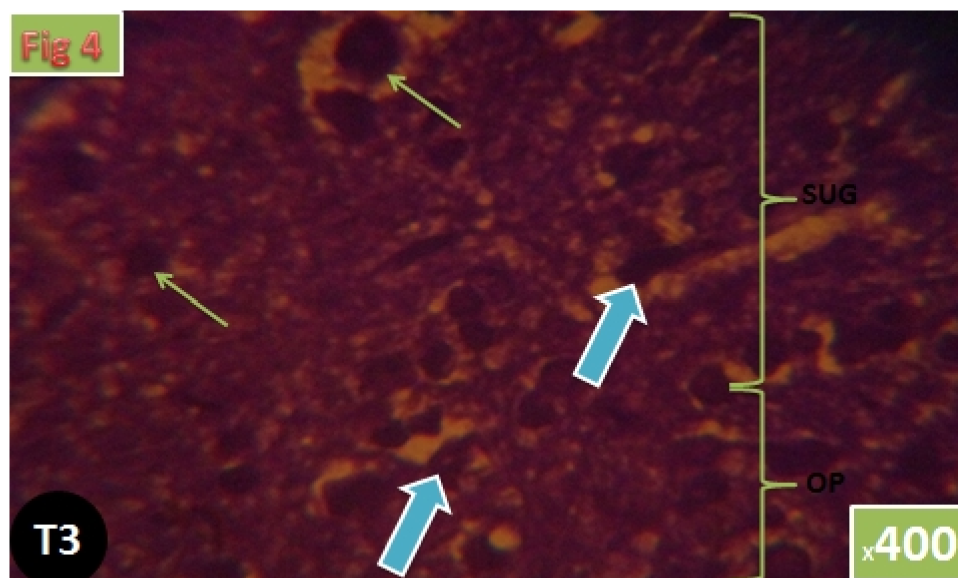

Fig. 4. Photomicrograph of Superior colliculus (Treatment section T3) showing the neuronal distributions in the OP (Optic Nerve layer). Note the distinctly stained normal pyramidal neurons (Blue arrows) as well as well distributed glial cells (Green arrows) *Cresyl violet stain* x400.

The statistically significant *P*=0.05 reduced neuronal diameter recorded in the Pb exposed group compared to the control clearly underlines the neurotoxicity potential of Pb as this also accounted for about 43% neuronal loss while in the treatment groups T1 and T3, reduced neuronal diameter was also noticed but this is far statistically insignificant of about 10% and 7% respectively when compared to the control as obtained from Table 4. Neuronal population and diameter of the neem groups was similar to their number in the healthy control groups. This chemoprevention of cell loss by Neem leaf extract is associated with cell proliferation and this is in an agreement with earlier reports of [38,39]. Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids [40].It is also a known fact that generation of ROS such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and LPO in the presence of heavy metal ions are known to damage various cellular components including proteins, membrane lipids and nucleic acids [41]. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS [42]. In this study, determined activities of the antioxidant defense system like SOD, GR and GPx showed decreased values in the Pb exposed group compared to the control but higher values were recorded in the neem exposed group as seen in Table 5 and this is also in conformity with earlier findings of [43].The decreased activity of SOD may be a response to increased production of H_2O_2 and O_2^- as well as decreased protein expression levels as reported by [44]. The decreased activity of enzymes could also be due to their decreased protein expression levels from Pb toxicity condition as reported earlier [45]. Since an antagonistic effect between selenium (as a cofactor) and Pb according to [45] affects GPx activity, it can thus render GPx a potential target for Pb toxicity because a lead-associated reduction in selenium

uptake may increase the susceptability of cell to oxidative stress. It is clear that GPx needs GSH to decompose H_2O_2 or other peroxides with the simultaneous oxidation of GSH into GSSG, however, GR which is another component of the antioxidant defense system will reduce GSSG back to GSH and thereby will support the antioxidant defense mechanism indirectly. The presence of disulfide at the active site of GR as earlier reported by [46], has been suggested to be a target for Pb and will result in inhibition of GR activity. Glutathione (GSH) is a cysteine-containing peptide found in most forms of aerobic life [47], it is synthesized in the cytoplasm of the liver cells and then transported to other body organs via the circulatory system [48]. GSH has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced [49]. Due to its high concentration and central role in maintaining the cell's redox [49] state, GSH is one of the most important cellular antioxidants. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. In this study as seen from Table 6, since Pb has a strong affinity for thiol (SH) group, hence, significantly reduced level of GSH was recorded in the Pb exposed group T2 compared to the control and this is also in conformity with the earlier reports of [50] that GSH level in the Pb exposed brains were lower than that of the control. While the *A. indica* exposed groups T1 and T3 recorded values at par with the control and this clearly portrayed *A. indica* as an ameliorative oxidant. Lipid peroxidation is a free radical process involving a source of secondary free radical, which further can act as second messenger or can directly react with other biomolecules, enhancing biochemical lesions. Lipid peroxidation occurs on polysaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction. Hydroxyl radical initiates ROS and removes hydrogen atom, thus producing lipid radical and further converted into diene conjugate. Due to lipid peroxidation, a number of compounds are formed, for example, alkanes, malonaldehyde (MDA), and isoprotanes. Lead toxicity leads to free radical damage by two separate, although related, pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of Lipid peroxidation, and (2) the direct depletion of antioxidant reserves [51]. When compared to the control and other groups in this study, significantly $P<0.05$ high level of MDA was recorded with extremely low values obtained in the Neem exposed groups,a finding that is in agreement with the earlier reports of [52] that antioxidants reduce MDA production level. Moreover, recent studies have proposed that oxidative damage due to an impaired oxidant/antioxidant balance is a mechanism of lead toxicity [53, 54]. Resistance to oxidative stress depends on the status of operative antioxidant systems in cells and tissues. It prevents the uncontrolled formation of free radicals and the activation of oxygen species or inhibits their reactions with the biological structures [55].

4. CONCLUSION

This study demonstrated that exposure to lead could have generated oxidative stress which resulted in cytoarchitectural alteration in the superficial layers of the superior colliculus as well as lipid peroxidation associated with reduction in the antioxidant status in the superior colliculus of the brain. *A. Indica* co-treatment resulted in the prevention of the lead induced damage. The protective effects of *A. Indica* are due to the radical scavenging activity of its components. Consequently, *A. Indica* is useful in the preventive treatment of lead toxicity.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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