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Neuro-Protective Attenuating Efficacy of *Niazimicin* on Neonate Prefrontal Cortex in Second Week Lead-Induced Toxicity; Wister Rat Model

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ABSTRACT

The activities of Lead, a quite extensively studied environmental and occupational heavy metal, is been desired by several as a means of income, creating boundless possibility for it neurotoxic effect which is more pronounce in children neuropsychological development. *Niazimicin (Nz)*, an isolate of *Moringa Oleifera* (*MO*), has neuro-protective property. Thus we explored if *Nz* can protect against prenatalPb-induced toxicity as well as PFC damage, across various days in 2nd. A total of 70 adultWistarrats (180 - 200g) comprising of 50 female and 20 male, were randomly assigned into five (5) groups, each group comprised of 10 female with 4 male, housed in plastic cages under natural light and dark cycles at room temperature with access to standard rat chow and water *ad libitum.* Group A received distilled water, group B was given tween 80 vehicle, group C received *Nz*, group D received Lead (Pb) while group E were given Pb+*Nz*. Pregnancy was confirmed by vaginal smear, and all administrations were through oral route for 3 days (day 8-10), afterward two female each from various group were sacrificed in order to harvest pops in day 1, 7, 14, 21 and 35. One-way ANOVA was used to analyze data, followed by Tukey's multiple comparisons test, using Graph pad Prism 6. Approval was obtained from the University of Ilorin Research Ethics Committee with no: UERC/ASN/2016/359. Pb significantly reduced the body weight (day 7, 21 and 35) and the relative brain weight changes (day1) with obvious reduction across other days. MDA and Calcium ion with SOD, GPx, LDH and G6PDH levels showed a significant increase with decrease respectively.Pb promote pyknotic and chromatolytic neurons, reducing deeply stained neurons, and the density of glial fibrillary acidic protein (GFAP) positive reactive astrocytes expression was increased. However, *Nz* ameliorated those Pb toxic effects, with mild toxic outcome on *Nz* alone treated groups. *Nz* was able to ameliorate neonatal Pb-induced neurotoxicity, which account for morphological damages, lipid peroxidation, oxidative stress, metabolic stress and astrogliosis. However, this finding has been able to elucidate the potentials of *Nz* as a neuro-protective agent against Pb-induced neurotoxicity.

Key word: *Moringa Oleifera* (*MO*), *Niazimicin* (*Nz*), Neurotoxicity, prefrontal cortex (PFC),

INTRODUCTION

Among heavy metals, Lead (Pb) is referred to as a strong occupational toxin, and it is one of the oldest environmental burden which has remain a serious health challenge in the world today[1]. The general public is exposed to Pb through industrial materials, air from the immediate surroundings (ambient air), food, drinking water and consumer products[2]. However, the rate of Pb poisoning has become common in Nigeria and the world at large in recent times, up to 70% of young Nigerian children have been reported to have blood Pb concentrations ⩾10 μg/dl as a result of Pb poisoning[3,4] and is a problem of great public health concern in Zamfara state in Nigeria, due to its high occurrence, morbidity and mortality rate. Lead poisoning remain one of the most significant and prevalent diseases of

environmental origin accounting for approximately 0.6% of the global burden of disease globally[5,6]. Lead contamination to human/animals occur through Pb absorption via the respiratory and gastrointestinal systems, it has the potential of causing irreversible health effects that are known to interfere with a number of body functions (primarily affecting the central nervous, haematopoietic, hepatic and renal system resulting to serious disorders)[1,7,8,]. About 30-40% of inhaled Pb enters the bloodstream, once it is absorbed, 99% is retained in the blood for about 30-35 days, dispersed and accumulated in other tissues such as the brain, liver, renal cortex, aorta, spleen, lungs, teeth and bones[9]. In the central nervous system (CNS), Pb toxicity is more common in children than adults due to the high percentage of circulating Pb gaining access to the brain of children, and may produce obvious symptoms of acute brain disease, damage or malfunction (encephalopathy) such as headache, ataxia, convulsions as well as coma or lesser deficits which include learning disorders likewise hyperactive behaviour[10]. Hence, foetuses and young children are at greater risk to the neurological effects of Pb toxicity because they have higher intestinal Pb absorption as well as more susceptible nervous systems which are still undergoing development[11]. Children with greater Pb levels may be affected with delayed growth, decreased intelligence, short-term memory and hearing loss, even at low Pb exposure they may appear inattentive, hyperactive and irritable, but at higher levels, Pb can cause permanent brain damage and even death[12]. Consequences of Pb exposure on the prefrontal cortex have also been observed in the form of depletion in executive functions, mood regulation likewise decision-making, and evidence suggest that; the exposure of the developing central nervous system (CNS) to Pb toxicity and other neurotoxic substances, account for these significant deficits in cognition, executive functions, social behavioural changes and motor abilities in adulthood[13,14]. However, Drumstick plant commonly known as *Moringa oleifera* (MO) is a medicinal plant (rich in nutrient) with reported antioxidant, anti-inflammatory and neuroprotective properties, beneficial in the management/treatment of Pb-induced neurotoxicity and neurodegenerative diseases[15]. Phytochemicals present in the leaves of MO include *Niazimicin (Nz), flavonoids, tannins, steroids, triterpenoids, saponins, anthraquinones, alkaloids, moringin and reducing sugars*[16,17]. Thus we explored if *Nz,* an isolate of *MO*, can protect against prenatalPb-induced toxicity as well as prefrontal cortex (PFC) damage, across various days in 2nd. General increased levels in hormones and reduced levels of enzymes that take part in oxidative stress might be attributed to the reduced development activity taking place in the foetus [18]. Superoxide Dismutase (SOD) a key antioxidant, and administration of 0.5 mls of *annona muricata* leaf extract has a protective effect on tissue against oxidative damage[19].

MATERIALS AND METHODS

Method: A total of 70 adult Wistar rats (180 - 200g) were bought from Department of Zoology, Faculty of Life Sciences, University of Ilorin. The rats comprised of 50 female and 20 male, they were randomly assigned into five (5) groups, each group comprised of 10 female with 4 male, housed in plastic cages under natural light and dark cycles at room temperature with access to standard rat chow and water *ad libitum.* Group A received distilled water, group B were given tween80 as vehicle, group C received *niazimicin (Nz)*, group D received Lead (Pb) while group E were given Pb+*Nz*. Pregnancy was confirmed by vaginal smear, and all administrations were through oral route for 3 days (day 8-10), afterwards two female each from various group were sacrificed in order to harvest pops in day 1, 7, 14, 21 and 35. One-way ANOVA was used to analyze data, followed by Tukey's multiple comparisons test, using Graph pad Prism 6. *All protocol and treatment procedures carried out were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines, and* Approval was obtained from the University of Ilorin Research Ethics Committee with no: UERC/ASN/2016/359

Table 1: Experimental Animal Grouping
Note: Niazimicin (NZ); Lead (Pb); Lead + Niazimicin (Pb+*Nz*); Tween80 (Tw80)

Group	Administration	Dosage (ml)	2 nd	Duration (Days)
Control	Water	*ad libitum*	day 8- day 10	3
Tween 80	TW80 vehicle only	0.5ml	day 8 - day 10	3
Niazimicin	*Nz* only	0.5ml	day 8 - day 10	3
Lead (Pb)	Pb only	0.5ml	day 8- day 10	3
Lead plus	*Nz*Pb+*Nz*only	0.5ml	day 8 - day 10	3

Procurement of *Moringa oleifera (MO)* seed: *Mo* seeds (2.0 kg) were obtained from, the Department of Agriculture, Faculty of Life Sciences, University of Ilorin, and authenticated in the same school Botany department, with specimen voucher number: UILH/001/1217.

Preparation of *Moringa oleifera (MO)* seed: The seeds were air dried at room temperature (28-30℃) for about three weeks and grinded into fine powder using an electronic blender. Subsequently niazimicin extraction and characterization was done at same school Chemistry Department, as described by Eilert, *et al.,*[20] and modified by Murakami, *et al.,*[21].

Tissue Preparations: 24 hours after the last administration of TW80, *Nz* and Pb was noted as day 1, in which two female rats were anaesthetized with ketamine and sacrificed in day 1, 7, 14, 21 and 35 from each group in order to harvest pops. One out of the two rats from each group, were perfused with 10% formol saline and pops brain tissues were harvested and further fixed in 10% formalin for histological and immunohistological examination. The other one was not perfused, but rather stored in 30% sucrose for the purpose of enzymatic and other biochemical investigation. Although, pops brain were excised out of the cranium and brain weights were taken and recorded.

Photomicroscopy: Slides were viewed using LEICA DM 750 microscope connected to a digital camera (LEICA ICC50) and a desktop computer.

Statistical analysis: Results were presented as Mean ± SD and analyzed using descriptive and inferential statistics. One-way ANOVA was used to analyze data, followed by Tukey's test for multiple comparisons. Graph pad Prism 6 was the statistical package for data analysis and P-values $\leq$ 0.05 were considered statistically significant.

RESULTS

Results of body and brain weight change in 2nd

Table 2: Mean Latency of Body Weight Changes in Rats for Day 1, 7, 14, 21 and 35.
Note: Control (CON); Tween80 (TW80); Niazimicin (Nz); Lead (Pb); Niazimicin+Lead (Nz+Pb) group Values

Group	Day 1	Day 7	Day 14	Day 21	Day 35
CON	6.543±0.414	15.73±0.208	24.07±0.473	25.79±1.632	71.20±1.054
TW80	5.150±1.033	17.42±0.875[a]	35.29±1.633[a]	42.28±1.910[a]	80.67±1.622
Nz	7.210±0.989[bc]	15.07±0.379[b]	23.33±1.060[b]	27.12±3.081[b]	72.97±3.147
P	4.677±0.103[c]	10.51±0.374[abc]	19.67±6.355[b]	39.73±1.849[ac]	25.54±19.02[abc]
Pb+*Nz*	4.293±0.768[ac]	8.107±0.488[abc]	17.69±0.908[b]	32.97±3.584[b]	68.57±2.268[d]

are presented in Mean±SD. a = significantly different from control group; b = significantly different from TW80 group; c = significantly different from Nz group; d = significantly different from Pb group ($P \leq 0.05$).

Table 3: The mean Latency of Relative Brain Weight in Rats for Day 1, 7, 14, 21 and 35.
Note: Control (CON); Tween80 (TW80); Niazimicin (NZ); Lead (Pb); Niazimicin+Lead (NZ+Pb) group Values

Group	Day 1	Day 7	Day 14	Day 21	Day 35
CON	0.297±0.015	0.653±0.025	1.133±0.058	1.363±0.099	1.333±0.058
TW80	0.257±0.045	0.897±0.097[a]	1.320±0.040	1.337±0.153	1.643±0.040[a]
Nz	0.340±0.040[bc]	0.690±0.017[b]	1.233±0.058	1.233±0.099	1.433±0.116[b]
Pb	0.190±0.010[ac]	0.617±0.032[b]	0.980±0.125[abc]	1.283±0.055[a]	1.340±0.080[ab]
Pb+*Nz*	0.450±0.050[abc]	0.527±0.051[bc]	1.053±0.055[b]	1.287±0.012	1.447±0.035[b]

are presented in Mean±SD. a = Significantly different from control group; b = Significantly different from Pb group; c = Significantly different from Lead (Pb)+Niacimicin group; d = Significantly different from Nz group ($P \leq 0.05$).

Biochemical Analysis:

Expression of MDA in 2nd

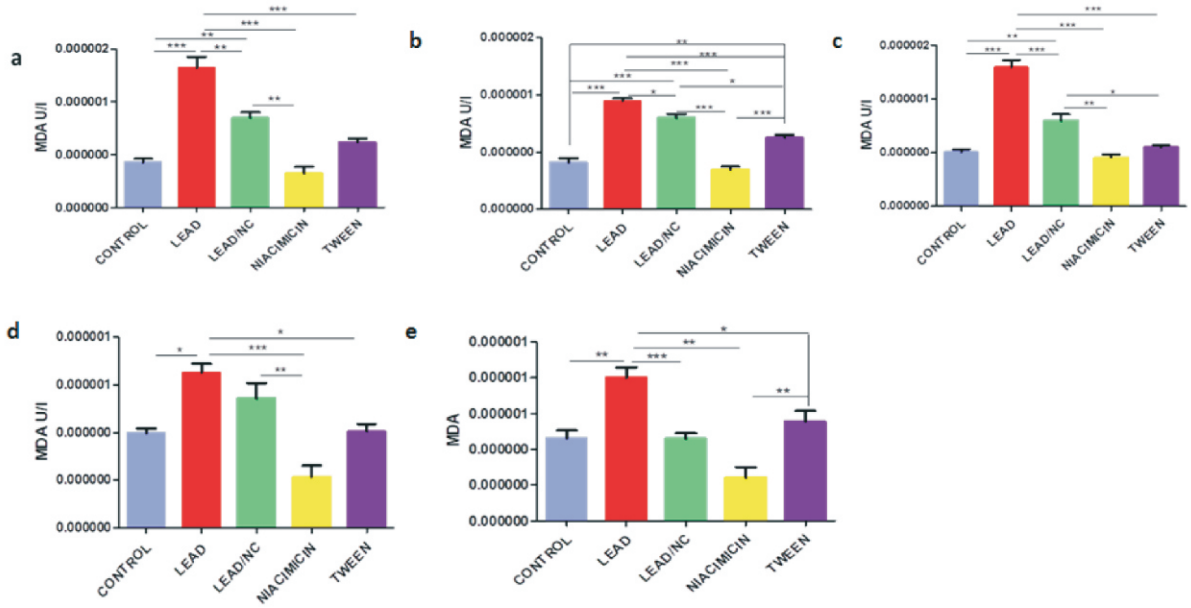

Figure 1 (a-e): Expression of MDA in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 **(a)**, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Expression of SOD in 2nd

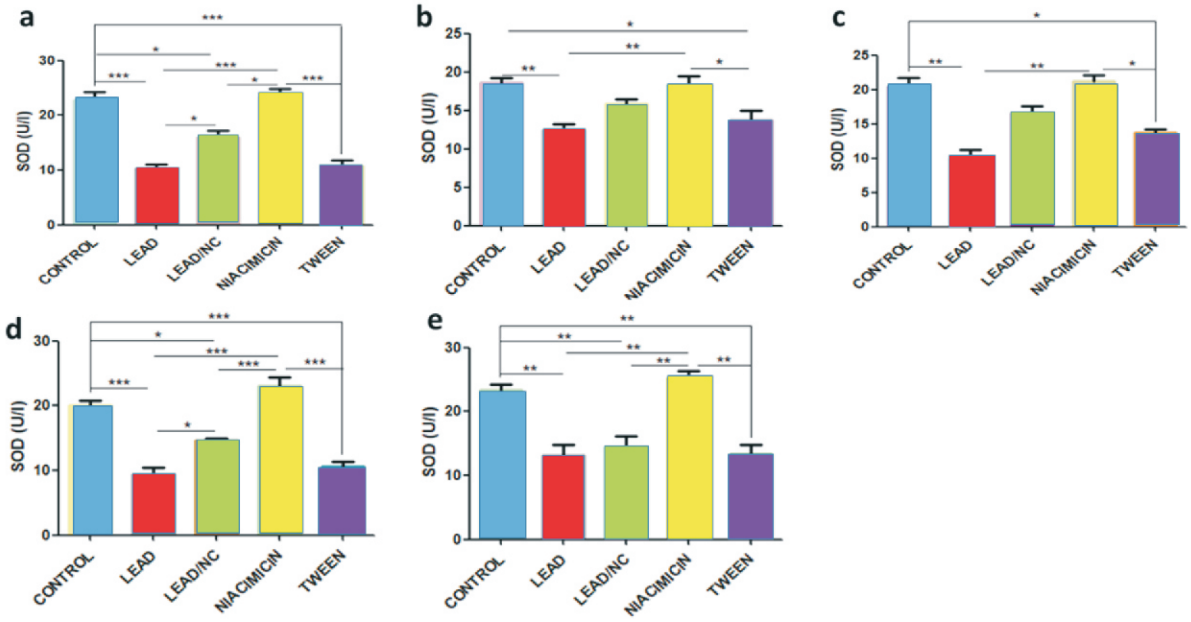

Figure 2 (a-e): Expression of SOD in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 **(a)**, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Expression of GPx in 2nd

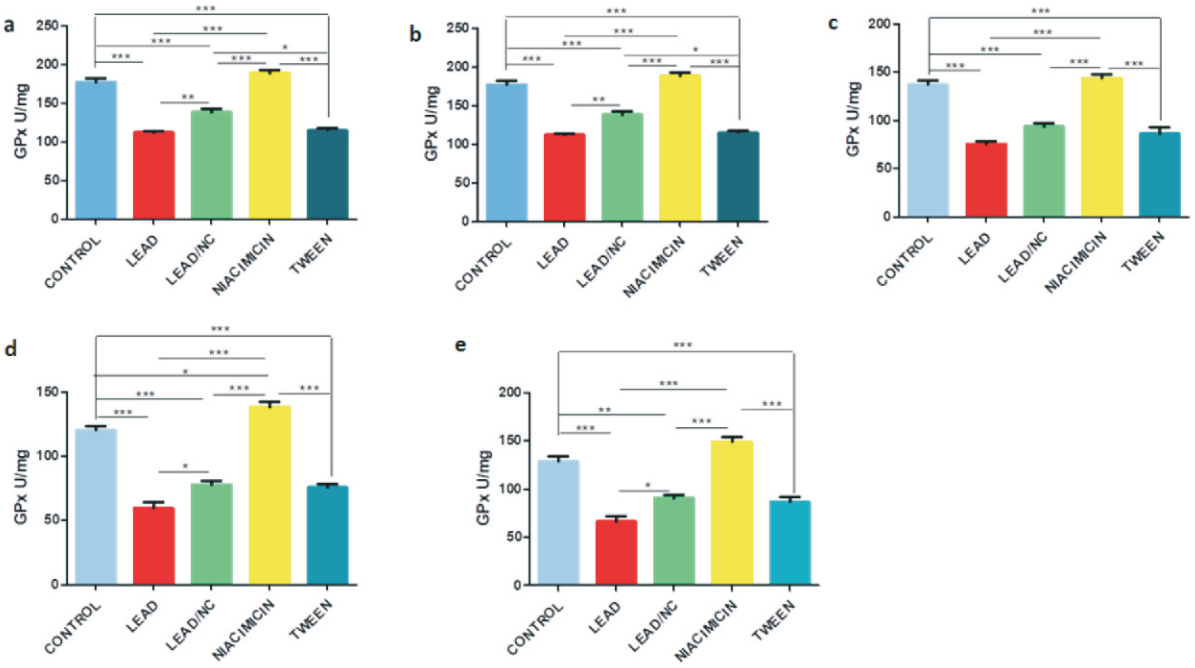

Figure 3 (a-e): Expression of GPx in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 **(a)**, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Expression of LDH in 2nd

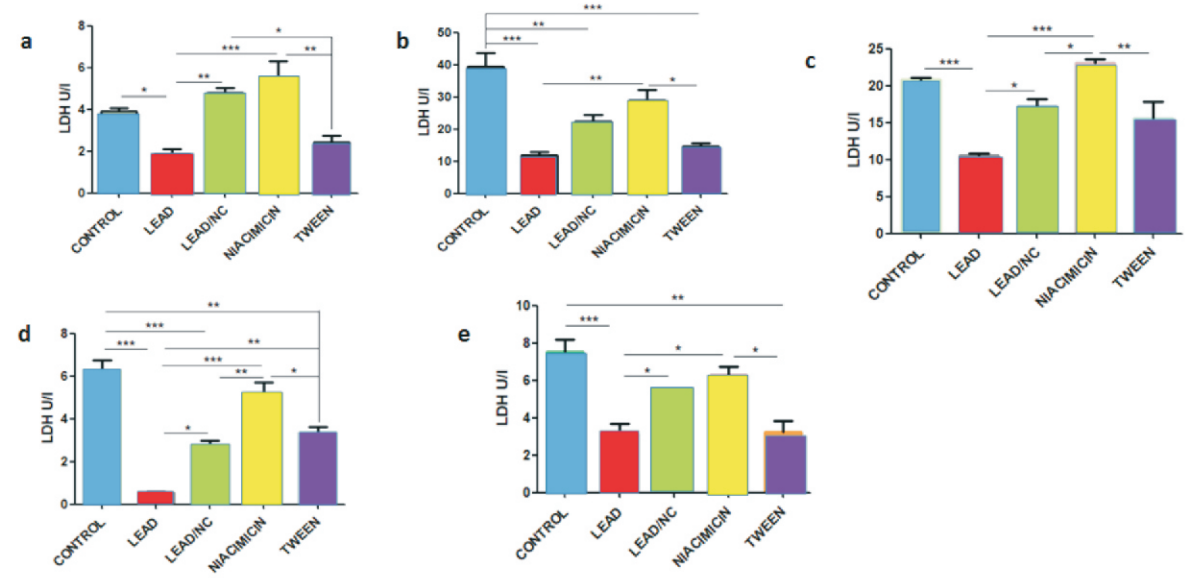

Figure 4 (a-e): LDH (to show Cytotoxicity) Expression in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 **(a)**, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Expression of G6PDH in 2nd

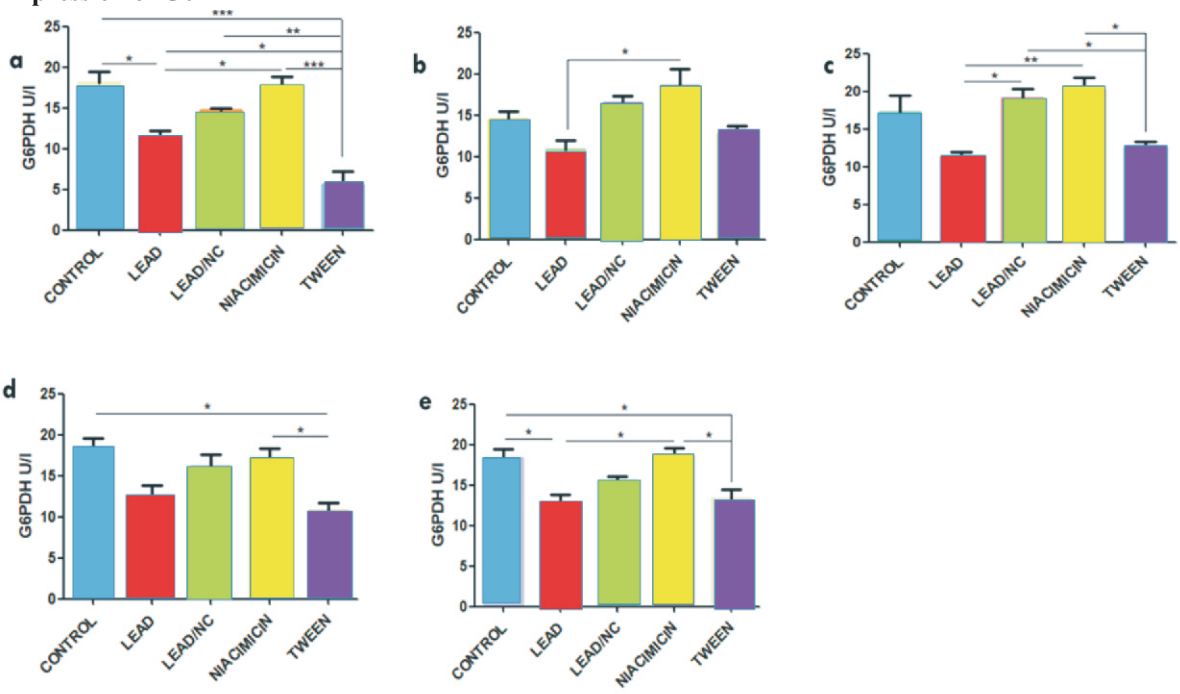

Figure 5 (a-e): Expression of G6PDH in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 **(a)**, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Expression of CALCIUM in 2nd

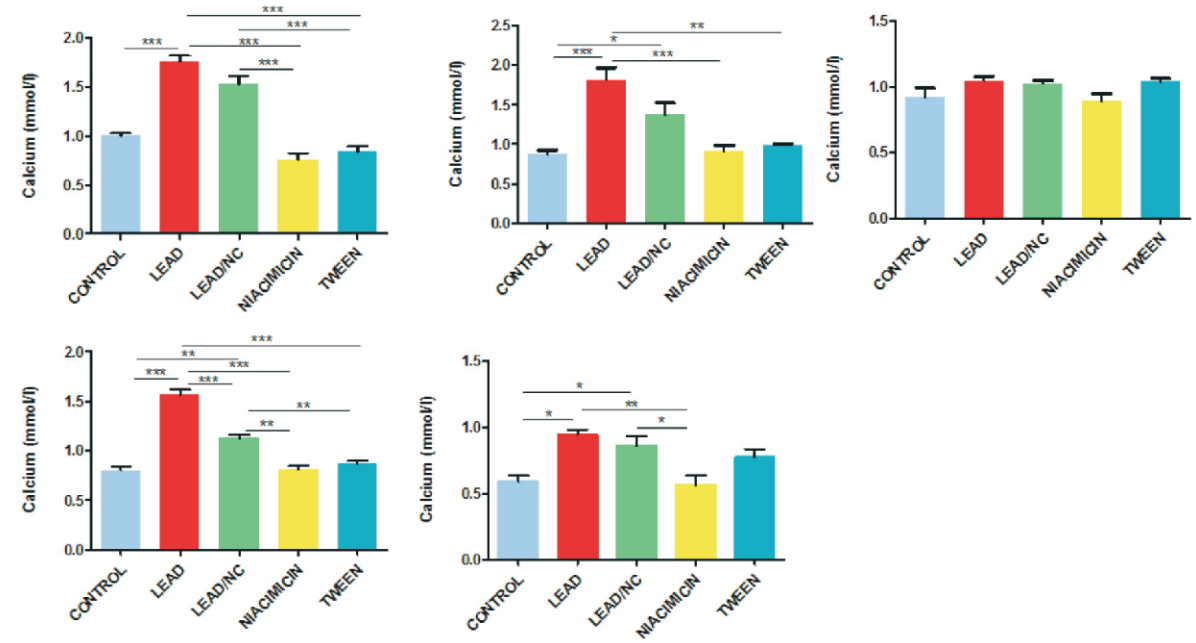

Figure 6 (a-e): Expression of Calcium ion in the Prefrontal Cortex of Pups Exposed to Treatment at 2nd Across all Groups, Sacrificed at Day 1 ***(a)***, 7 **(b)**, 14 **(c)**, 21 **(d)** and 35 **(e).**(***P < 0.05).

Histological Observation:

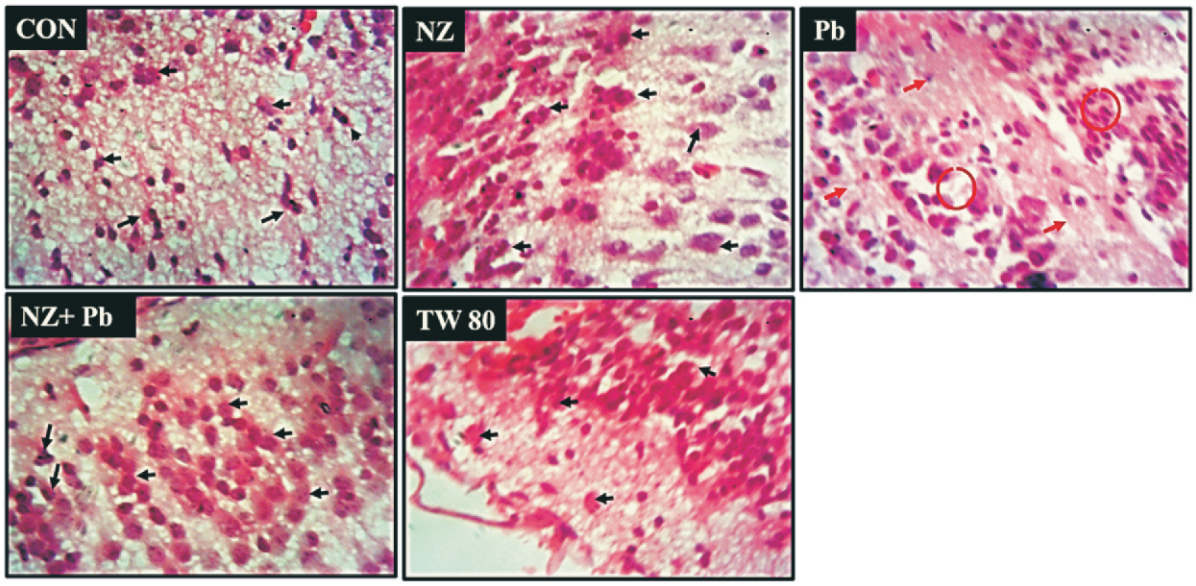

Plate 1: Histoarchitecture of the prefrontal cortex of pups in 2nd , sacrificed at day 1. X400. (CON) and (NZ) group reveals the normal histological features of the PFC characterized by large pyramidal neurons (black arrows); (Pb) the presence of necrotic and/or pyknoticpyramedial neurons (red arrows) likewise hypertrophy and hyperplasia of cells (red circles), where prominently seen in this group; (NZ+Pb) and (TW 80) exhibited pyramidal neurons similar to that of the control groups (black arrows).

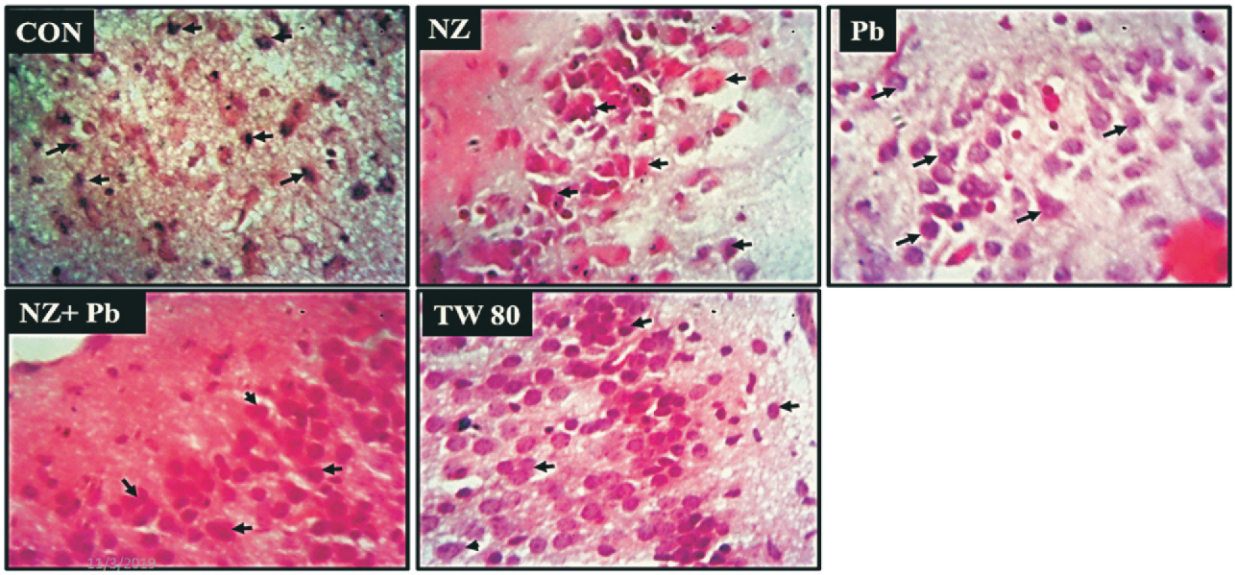

Plate 2: Histoarchitecture of the prefrontal cortex of pups in 2nd , sacrificed at day 7. X400. (CON) and (NZ) group reveals the normal histological features of the PFC characterized by large pyramidal neurons (black arrows); (Pb), (NZ+Pb) and (TW 80) exhibited pyramidal neurons similar to that of the CON (black arrows).

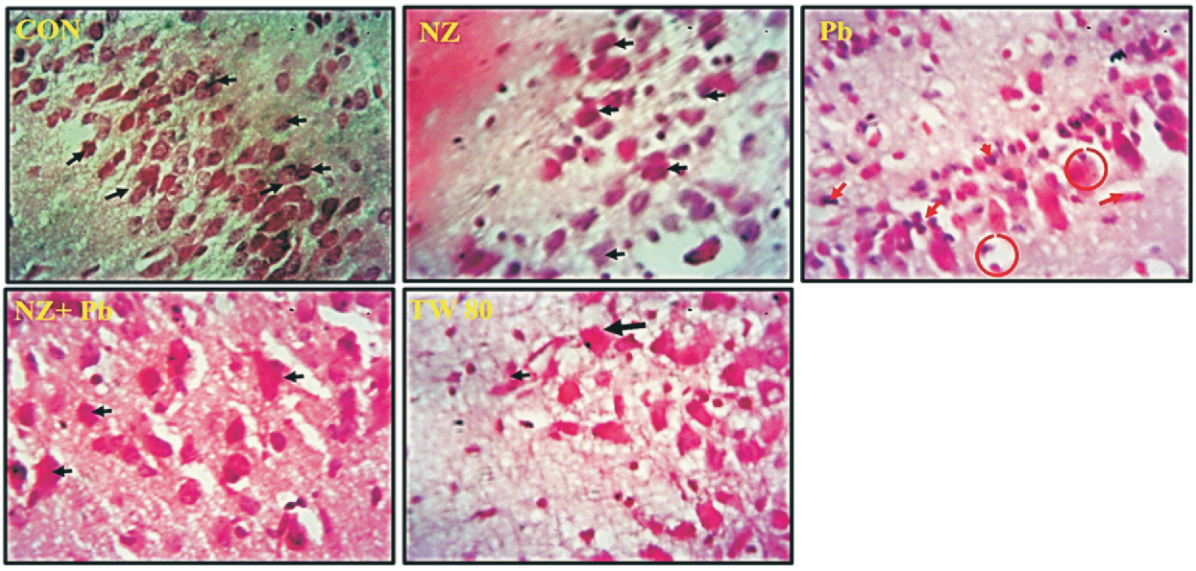

Plate 3: Histoarchitecture of the prefrontal cortex of pups in 2nd , sacrificed at day 14. X400. (CON) and (NZ) group reveals the normal histological features of the PFC characterized by large pyramidal neurons (black arrows); (Pb) the presence of necrotic and/or pyknoticpyramedial neurons (red arrows) likewise hypertrophy and hyperplasia of cells (red circles), where prominently seen in this group; (NZ+Pb) and (TW 80) exhibited pyramidal neurons similar to that of the control groups.

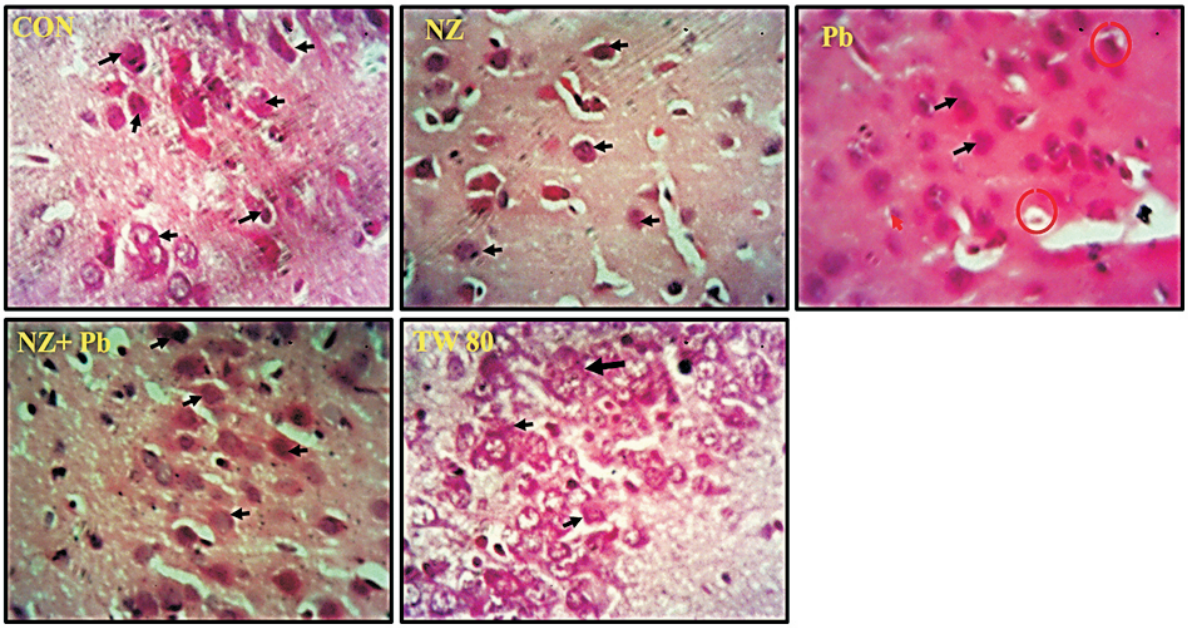

Plate 4: H&E of the prefrontal cortex of pups in 2nd , sacrificed at day 21. H&E; X400. (CON) and (NZ) group reveals the normal histological features of the PFC characterized by large pyramidal neurons (black arrows); (Pb) the presence of normal pyramidal neurons (black arrows), necrotic and/or pyknoticpyramedial neurons (red arrows) likewise hypertrophy and hyperplasia of cells (red circle), where prominently seen in this group; (NZ+Pb) and (TW 80) exhibited pyramidal neurons similar to that of the control groups (black arrows).

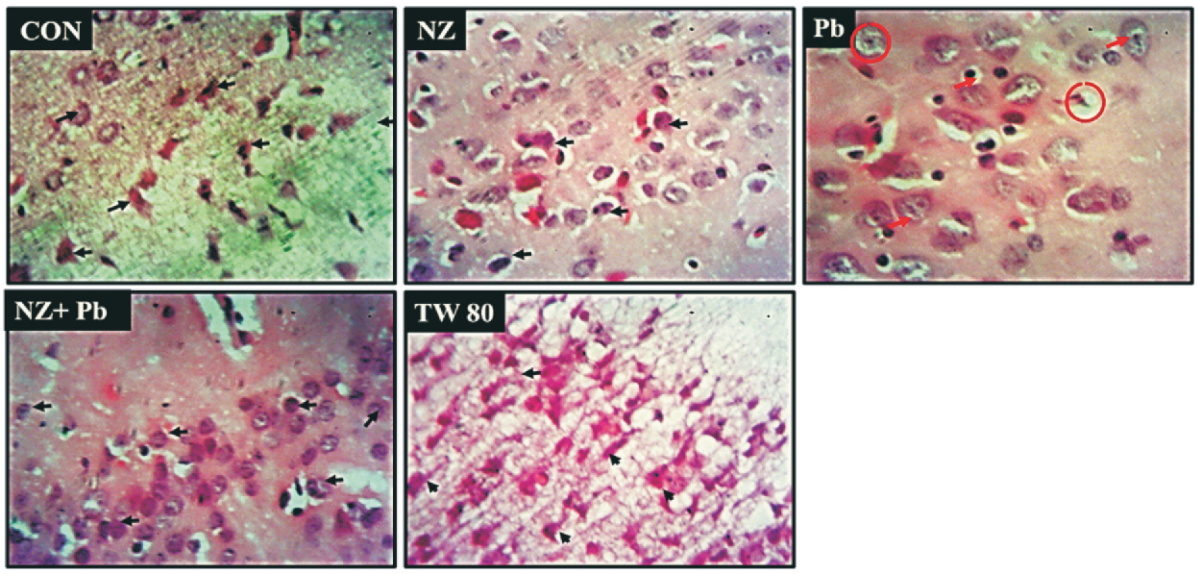

Plate 5: Histoarchitecture of the prefrontal cortex of pups in 2nd , sacrificed at day 35. H&E; X400.(CON) group reveals the normal histological features of the PFC characterized by large pyramidal neurons (black arrows); (NZ) and (NZ+Pb) showed few scattered pyknotic neurons (black arrows); (Pb) the presence of pyknoticpyramedial neurons (red arrows) likewise hypertrophy and hyperplasia of cells (red circle), where prominently seen in this group; (TW 80) exhibited pyramidal neurons similar to that of the control groups (black arrows).

Cresyl Fast Violet (CFV):

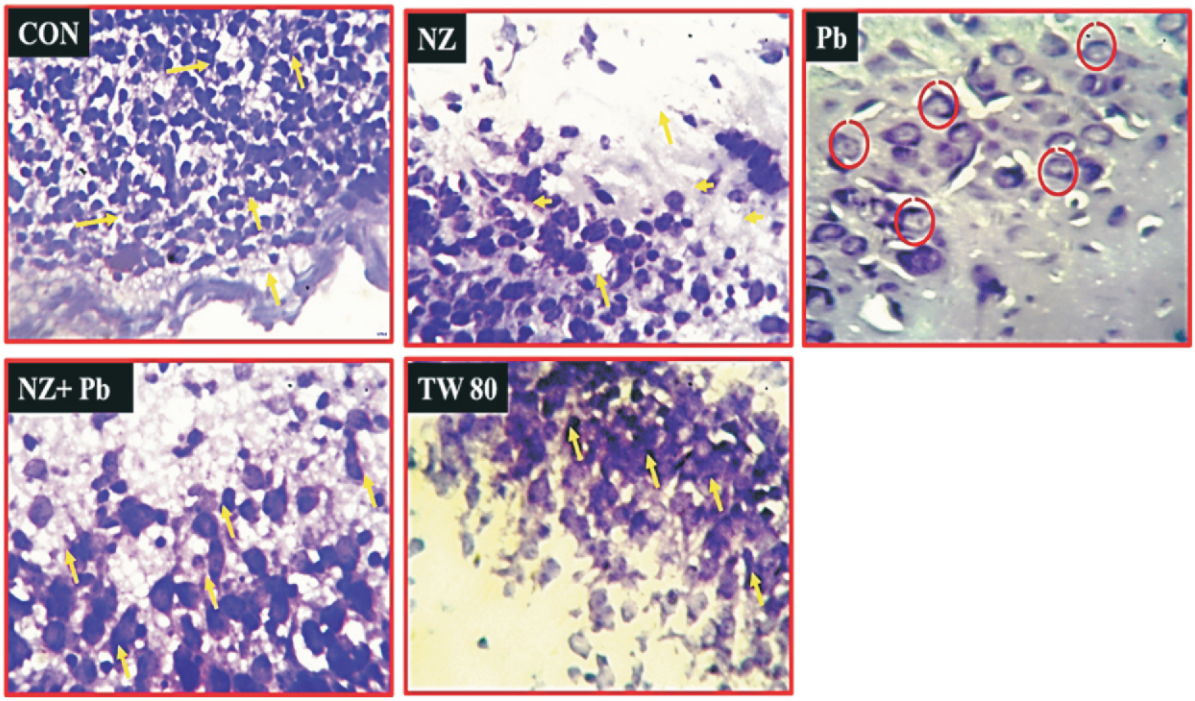

Plate 6: Photomicrograph showing the prefrontal cortex of Pups in 2nd , sacrificed at day 1. CFV; X400.(CON) and (NZ); the prefrontal cortex of control and **N**iazimicin group revealed deeply stained Nissl substance (yellow arrows)throughout the cortical and subcortical layers; (Pb) lead group showed chromatolytic neurons captured in red circles; (NZ+Pb) few dispersed deeply stained neurons where present in Niazimicin+lead treated group identified by the yellow arrows; (TW 80) this group is quite similar to that of the control, but exhibiting less deeply stained Nissl substance (yellow arrows)when compared to control group.

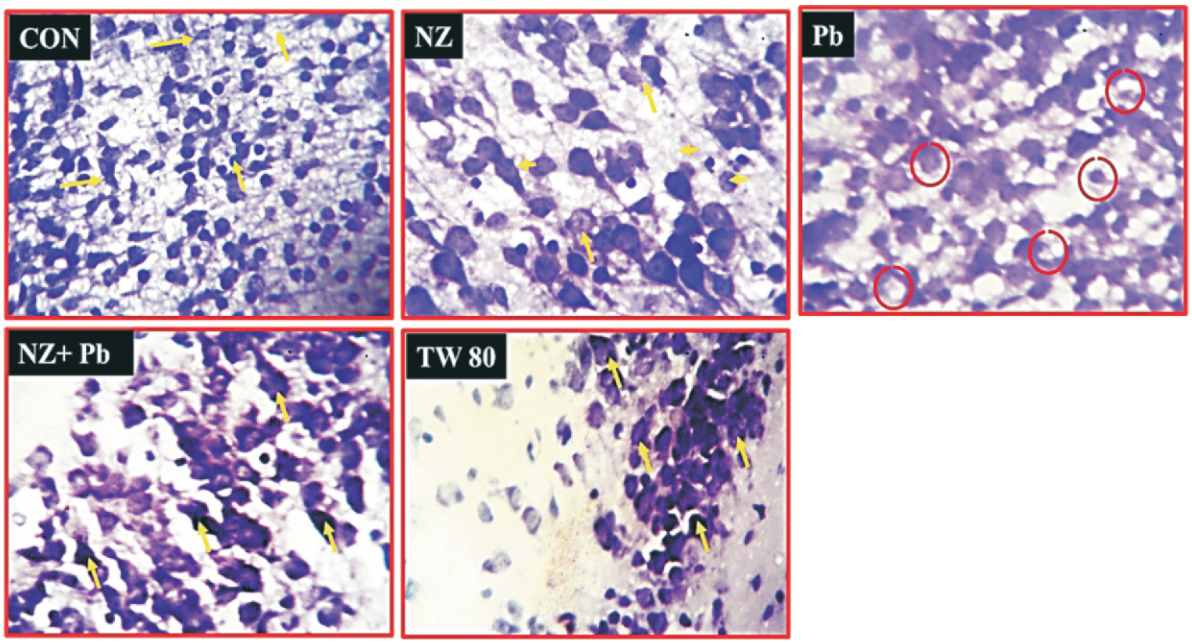

Plate 7: Photomicrograph showing the prefrontal cortex of Pups exposed in 2nd , sacrificed at day 7. CFV; X400.(CON) and (NZ); the prefrontal cortex of control and Niazimicin group showed deeply stained Nissl substance (yellow arrows)throughout the cortical and subcortical layers; (Pb) lead group reveal pyknotic and/or chromatolytic neurons captured in red circles; (NZ+Pb) few scattered deeply stained neurons where present in Niazimicin+lead treated group identified by the yellow arrows; (TW 80) this group is quite similar to that of the control, but exhibiting less deeply stained Nissl substance (yellow arrows)when compared to control group.

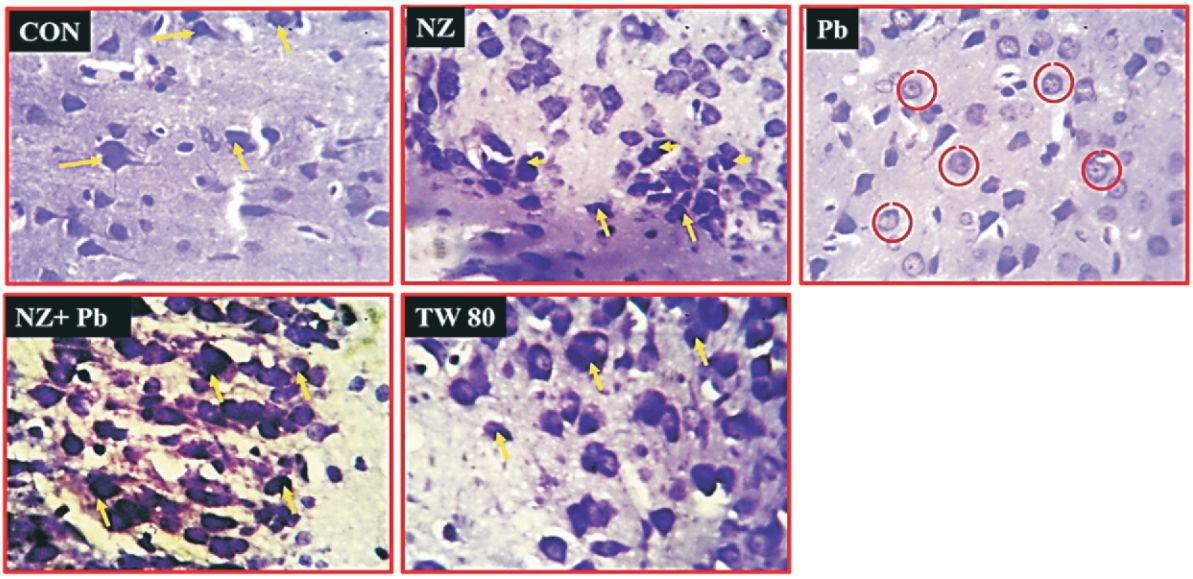

Plate 8: Photomicrograph showing the prefrontal cortex of Pups exposed in 2nd , sacrificed at day 14. CFV; X400. (CON) and (NZ); the prefrontal cortex of control and Niazimicin group showed deeply stained Nissl substance (yellow arrows)throughout the cortical and subcortical layers; (Pb) lead group reveal chromatolytic neurons captured in red circles; (NZ+Pb) evenly distributed deeply stained neurons where present in Niazimicin+lead treated group identified by the yellow arrows; (TW 80) this group is quite similar to that of the control, but exhibiting less deeply stained Nissl substance when compared (yellow arrows)to control group.

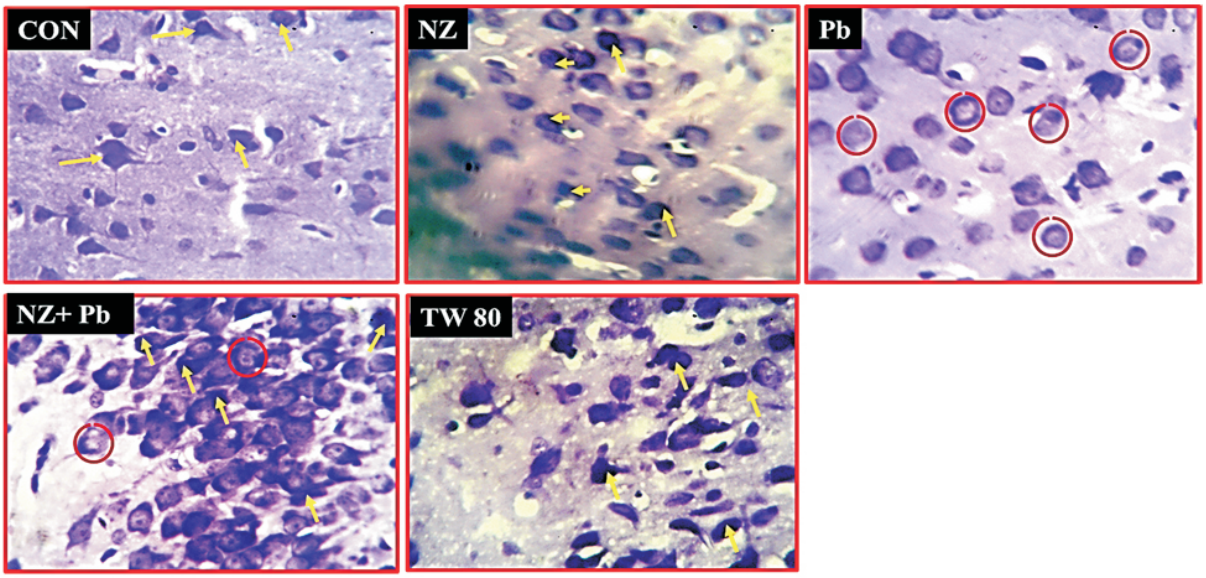

Plate 9: Photomicrograph showing the prefrontal cortex of Pups exposed in 2nd , sacrificed at day 21. CFV; X400. (CON) and (NZ); the prefrontal cortex of control and Niazimicin group showed deeply stained Nissl substance (yellow arrows)throughout the cortical and subcortical layers; (Pb) lead group reveal chromatolytic neurons captured in red circles; (NZ+Pb) evenly distributed deeply stained neurons where present in Niazimicin+lead treated group identified by the yellow arrows as well as chromatolytic neurons (red circle); (TW 80) this group is quite similar to that of the control, but exhibiting less deeply stained Nissl substance (yellow arrows)when compared to control group.

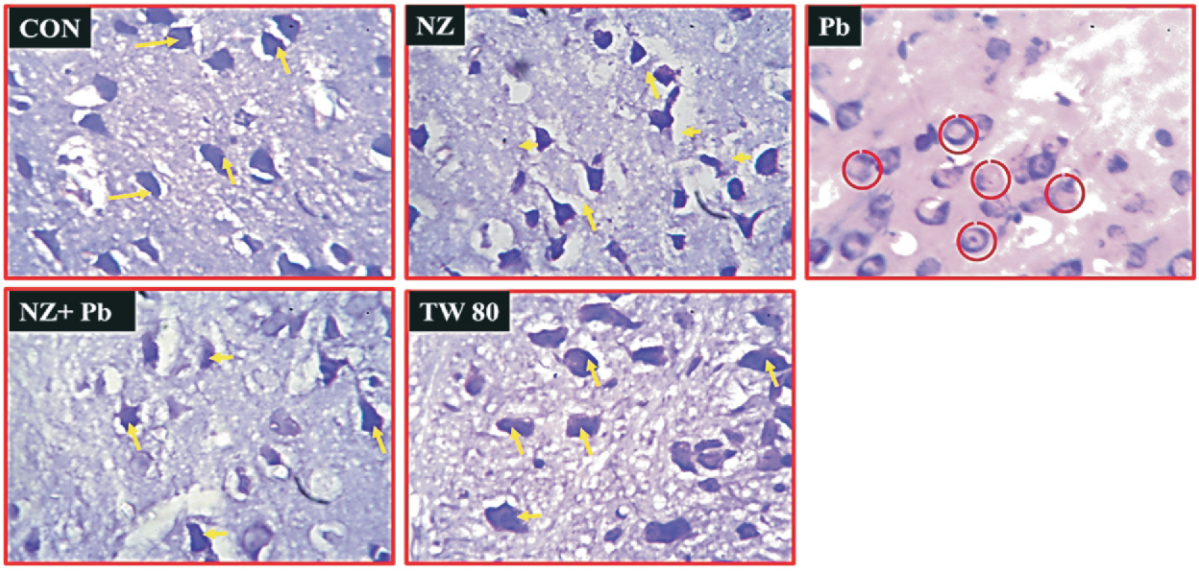

Plate 10: Photomicrograph showing the prefrontal cortex of Pups exposed in 2nd , sacrificed at day 35. CFV; X400. (CON) and (NZ); the prefrontal cortex of control and Niazimicin group showed deeply stained Nissl substance (yellow arrows)throughout the cortical and subcortical layers; (Pb) lead group reveal chromatolytic neurons captured in red circles; (NZ+Pb) few distributed deeply stained neurons where present in Niazimicin+lead treated group identified by the yellow arrows; (TW 80) this group is quite similar to that of the control, but exhibiting less deeply stained Nissl substance (yellow arrows)when compared to control group.

Immunohistochemical Results For Astrocytes In The Pfc:
Glial Fibrillary Acidic Protein (GFAP) for Prefrontal Cortex

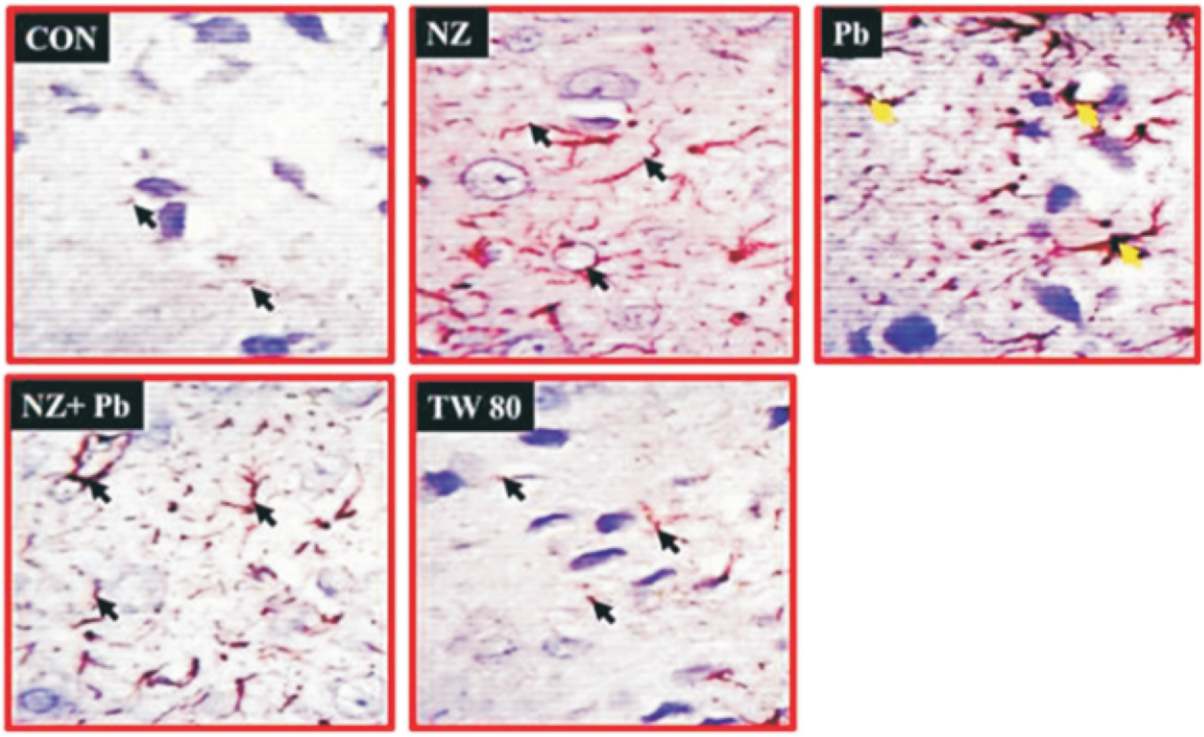

Plate 11:Photomicrograph showing the prefrontal cortex of Pups in 2nd , sacrificed at day 1. GFAP; X400. (CON) and (NZ); the PFC of control and Niazimicin group showed sparse GFAPimmunopositive astrocytes (black arrows) around neurons and the astrocytes exhibit regular processes; however Pb group showed increase in the density of GFAP positive reactive astrocytes; (yellow arrows), an indication of astrogliosis; (NZ+Pb) showed features similar to the niazimicin and control groups, with less reactive astrocytes.

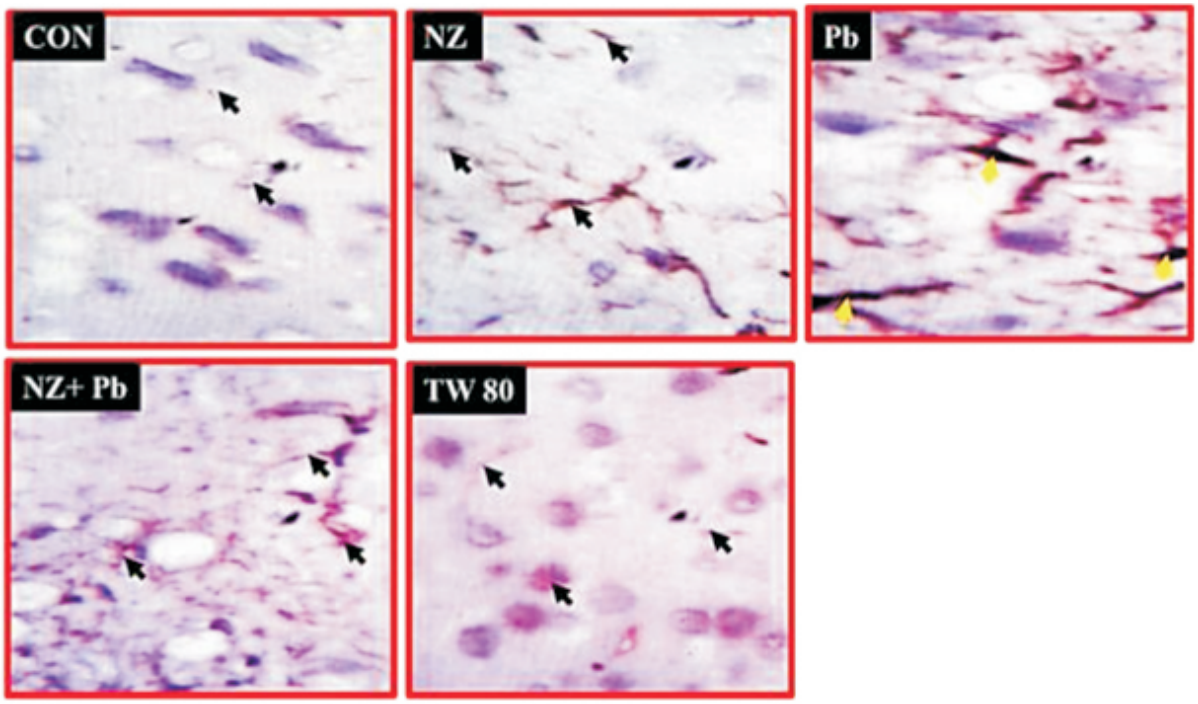

Plate 12: Photomicrograph showing the PFC of Pups in 2nd , sacrificed at day 7. GFAP; X400.(CON) and (NZ); the PFC of control and Niazimicin group showed sparse GFAPimmunopositive astrocytes (black arrows) around neurons and the astrocytes exhibit regular processes; however Pb group showed increase in the density of GFAP positive reactive astrocytes; (yellow arrows), an indication of astrogliosis; (NZ+Pb) showed features similar to the niazimicin and control groups, with less reactive astrocytes.

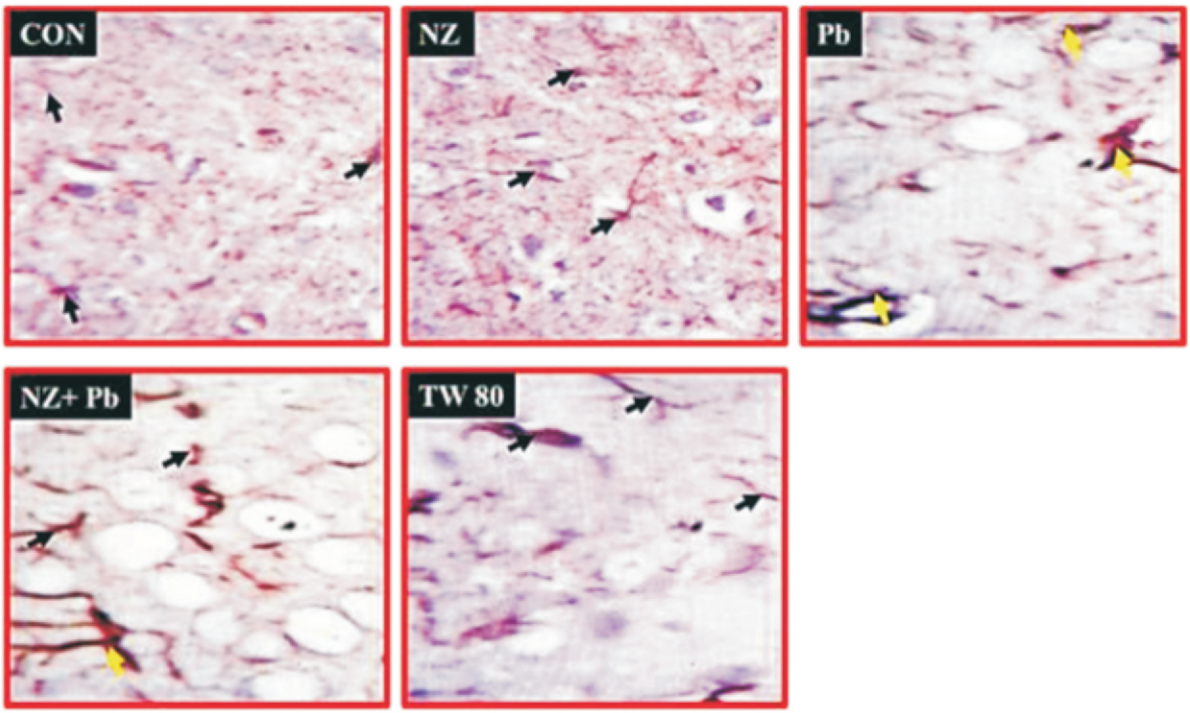

Plate 13: Photomicrograph showing the PFC of Pups in 2nd , sacrificed at day 14. GFAP; X400.(CON) and (NZ); the PFC of control and Niazimicin group showed sparse GFAPimmunopositive astrocytes (black arrows) around neurons and the astrocytes exhibit regular processes; however Pb group showed increase in the density of GFAP positive reactive astrocytes; (yellow arrows), an indication of astrogliosis; (NZ+Pb) showed features similar to the niazimicin and control groups, with less reactive astrocytes.

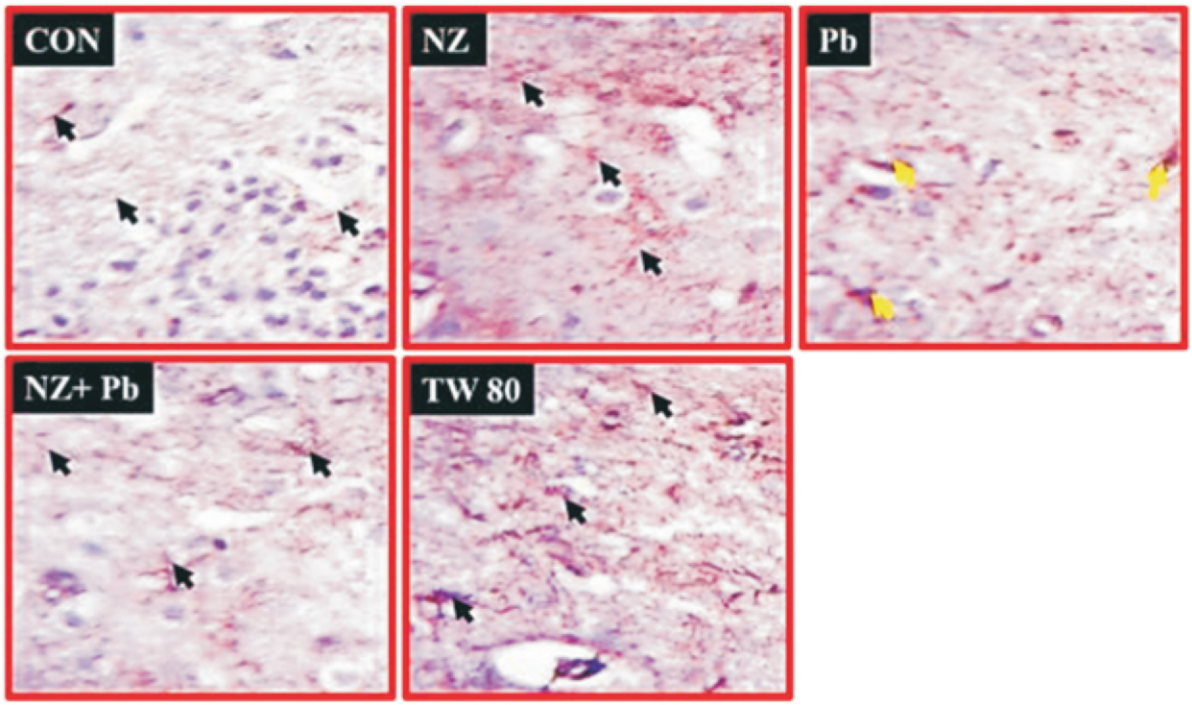

Plate 14: Photomicrograph showing the PFC of Pups in 2nd , sacrificed at day 21. GFAP; X400.(CON) and (NZ); the PFC of control and Niazimicin group showed sparse GFAPimmunopositive astrocytes (black arrows) around neurons and the astrocytes exhibit regular processes; however Pb group showed increase in the density of GFAP positive reactive astrocytes ;(yellow arrows), an indication of astrogliosis; (NZ+Pb) showed features similar to the niazimicin and control groups, with less reactive astrocytes.

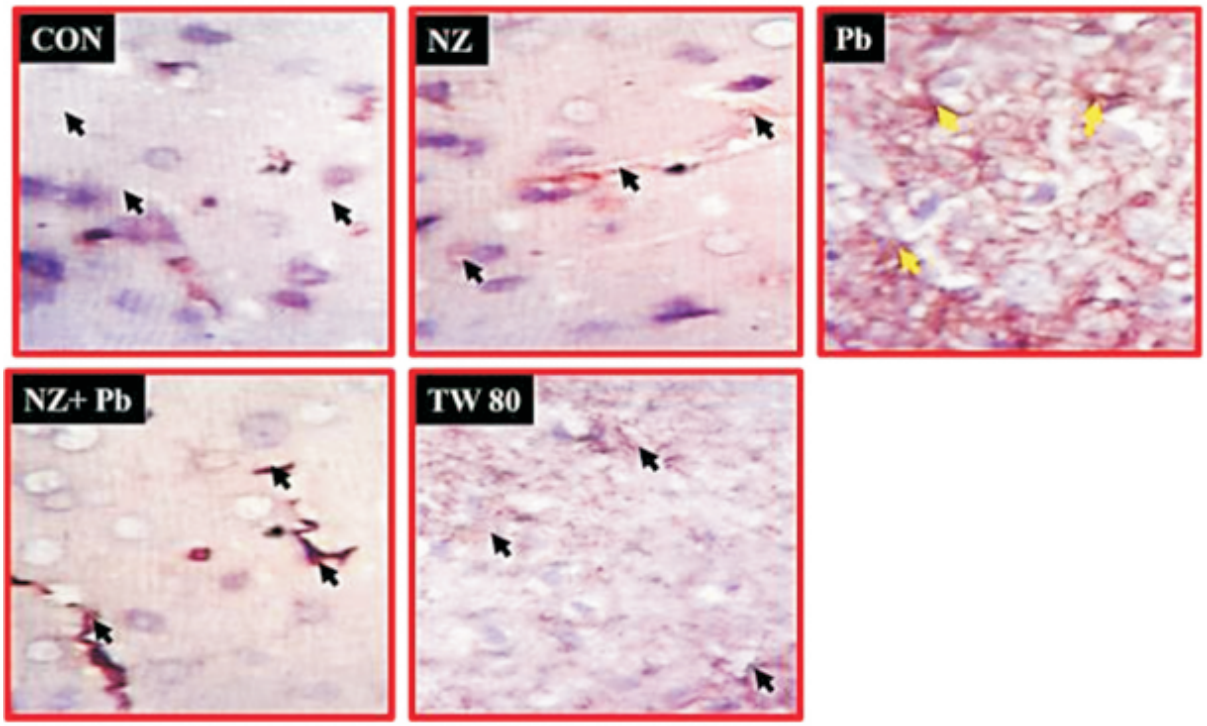

Plate 15: Photomicrograph showing the PFC of Pups in 2nd , sacrificed at day 35. GFAP; X400.(CON) and (NZ); the PFC of control and Niazimicin group showed sparse GFAPimmunopositive astrocytes (black arrows) around neurons and the astrocytes exhibit regular processes; however, Pb group showed increase in the density of GFAP positive reactive astrocytes ;(yellow arrows), an indication of astrogliosis; (NZ+Pb) showed features similar to the niazimicin and control groups, with less reactive astrocytes.

DISCUSSION

Lead (Pb) is considered as one of the earliest well-known and quite extensively studied environmental and occupational heavy metal. Several desired activities of Pbas a means of income, creating boundless possibility for it neurotoxic effect which is more pronounce on children neuropsychological development[22]. Despite all this possible Pb neurotoxic effect, *Nz* in this study, revealed its antioxidant, anti-inflammatory and neuroprotective antagonizing efficacy, which is beneficial in the management/treatment of Pb-induced neurotoxicity as well as prefrontal cortex (PFC) neurodegenerationacross various days in 2nd .

Neuro protective potentials of *Nz* on rat's body and brain weight: Following Pb administration, our study results demonstrated difference in body weight changes across various days in second (2nd) . These differences depleted significantly in day 7, 21 and 35, likewise obviously decreased in day 1 and 14 when compared with control rats. Lead affect treated animal negatively, thus, the negative body weight effect of Pb toxicity were highly worsen in day 35, and this insignificant obvious reduction (day 1 and 14) in body weight maybe as a result of Pb still undergoing the processes of metabolism. However, *Nz* wasn't very protective in sustaining or increasing rats body weight in day 1, 7, 14, 21, rather showed a significant improvement in day 35 as seen in Pb+*Nz* treated rats (table 1). Body weight changes can be an influential factor in the difference seen in experimental animal organ weight. Similarly, Pb accounted for a significant reduction in rat's brain weight in day 1, 14, 21 and 35. Day 7 reveals an obvious reduction which wasn't significant when compared to control (table 2). This body weight as well as brain weight loss, induced by Pb toxicity may indicate that Pb intake by pregnant Wistar rats could deplete the rate of food and water consumption during pregnancy. Contrarily to body weight changes result, Pb+*Nz* treated rats organ weight revealed a slight increased which wasn't significant in day 14, day 21 and 35, with a significant increase in day 1 when compared to Pb treated rats, day 7 slightly decreased, having no statistical significant difference. This finding suggests that, *Nz* could possess the potential ability that works as an antagonist to the symptom and morphological deterioration of Pb-induced neurotoxicity in 2nd . In agreement with Rodrigues, et al.,[23], the cohort who reported a series of reduction in experimental animal body weight in postnatal day 1 to 20, stating that, the regimen of Pb exposure employed in their study, did not affect body weight gain during gestation. Also Ercal, et al.,[24], Kang, et al.,[25] and Singh, et al.,[26] equally documented an obvious reduction in body weight, which is consistent with the present study findings.

Antioxidant potentials of *Nz*on biochemical parameters inPb-toxicated rats: Furthermore, uncontrolled oxidative stress accounts for cells, tissues and organs injury resulting from oxidative damage, and high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipids[27]. Thus, free radical oxidation of polyunsaturated fatty acids in biological systems is referred as lipid peroxidation[27]. The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology and disease[28]. However, Malondialdehyde (MDA) results from lipid peroxidation of polyunsaturated fatty acids and has been an important biomarker in the measurement of oxidative stress in an organism[29,30]. In this study, the result showed a significant increase in MDA level of Pb treated rats across various days in 2nd when compared to control rats, which suggested enhanced lipid peroxidation and oxidative stress. Rats treated with Pb+*Nz*showed a significant reduction of lipid peroxidation as indicated by lower levels of MDA (figure 1). However, the ability of *Nz* to ameliorate the level of MDA following Pb-induced neurotoxicity is due to its antioxidant and free radical properties, making *Nz* a considerable option in antagonizing Pb and related heavy metal neurotoxic damage. The result of this study is in agreement with that of Soltaninejad, et al.,[31], Kalender, et al.,[32] and Saleh[33], who reported a significant increase in MDA level followingPb-induced neurotoxicity. Similarly, oxidative stress is also thought to be increased by a *decrease* in the level of superoxide dismutase (an important antioxidant in virtually all living cells)[34], and has been implicated in the mechanism through which cuprizone induces demyelination[35]. Although, the results of this study showed a statistical significant decrease of SOD activity in the group treated with Pb only, however, there was a significant increase in both the control, *Nz* and Pb+*Nz* treated rat across various days in 2nd when compared with Pb treated rats. The statistical significant increase in Pb+*Nz* treated rats is as a result of the antioxidant and free radical scavenging properties of *Nz* against Pb-induced neurotoxicity. This result is in agreement with[33,26], who reported a decrease in SOD level, following treatment of Pb. Correspondingly, glutathione peroxidase (GPx) possesses high antioxidant defence properties with evidence suggesting that the depletion of GPx with a cell causes cell death[36,37]. The biochemical function of GPx is to reduce lipid hydroperoxidases to their corresponding alcohols and to reduce free H_2O_2 to water[38]. Thus, high expression of this enzyme protects cells against oxidative damage and depletes apoptosis[39], while depletion in GPx levels will increase oxidative damage and cell apoptosis[40]. Nevertheless, we found out that, there was a statistical significant depletion of GPx expression in Pb treated rat across various days in 2nd when compared to control rats, suggesting a higher vulnerability to oxidative stress, but Pb+*Nz* treated rat showed a relatively high level of GPx expression, which was significantly different from Pb treated rat, suggesting an increase in the level of resistance to oxidative stress and the protective adverse effect of *Nz* on the functionality of the brain. This finding is congruent with a study done by Kalender,et al.,[32] and Singh, et al.,[26], who reported a decrease in GPx level in the cerebral cortex, following Pb treatment.

Expression of G_6PDH, LDH and Calcium in Pb-toxicated rats

Glucose-6-phosphate dehydrogenase (**G_6PDH**) is an enzyme found in the cytoplasm of various cells that catalysis the first and rate-limiting step in the hexose monophosphate pathway in which the final product (ribose-5-phosphate) is required for the production of nucleic acid that is essential for cell growth [41]. G_6PDH aids in the stability of catalase and in the regeneration of reduced glutathione. If any condition initiates the release of reactive oxygen species (ROS) and oxidative stress, the expression of G6PDH automatically increases and this has been demonstrated *in vitro* [42,43]and *in vivo*[44]. Also, G_6PDH helps red blood cells in functioning normally, protecting them from potential harmful by-products that can accumulate as a result of the body fighting infections. Therefore the lack of G_6PHD may make red blood cell more vulnerable to breaking down in a process called haemolysis [45]. The result from this study showed a reduced level of G_6PDH across various days following Pb treatment, and it was obvious that Pb+*Nz* treated rats significantly increased in the levels of G_6PDH when compared to Pb treated rats. These statistical significant increases denote the protective effect of *Nz* against Pb-induced neurotoxicity, protecting the red blood cells from accumulated heavy metals such as Pb in the body. Our result is consistent with that of Kang, *et al*.,[46]. Lactate dehydrogenase (LDH) also called lactic acid dehydrogenase, catalyzes the reversible conversion of lactate in the presence of Nicotinamide adenine dinucleotide (NAD^+) to pyruvate and NADH $^+$ $H^+$and plays an important role in cellular respiration (the process by which glucose from food is converted into usable energy for our cells) [47].The expression of LDHin this study showed a significant reduction in Pb treated rats across various days, while Pb+*Nz* treated rat showed a relatively high level of LDH expression, which was significantly different from Pb treated rats across all days. Our result is not in agreement with those of Saleh,[33], who reported a significant increase in LDH level following lead toxicity. Also, calcium is an important nutrient that is basic for many functions in human health such as bone formation and maintenance which is a lifelong development[48]. Research has shown that adequate calcium intake can reduce the risk of fractures, osteoporosis, and diabetes while hypercalcemia (high calcium level in the blood) can cause bone weakening[49]. The results of this study revealed a significantly high level of calcium expression in Pb rats across various days, which was ameliorated in Pb+*Nz* treated rats.Our findings agree with different studies that reported a significant increase in calcium

level following Pb treatment[50,51].

Histological and Immunohistochemical Features in Pb-toxicated rats: The general cyto-achitectural anatomy of the prefrontal cortex in first and second across various days was examined using haematoxylin and eosin (H&E) method likewise crysl fast violet method in this research. Under light microscope, the presentation of pyramidal layer was particularly focused on, because the PFC constitutes large pyramidal neurons with efferent and afferent connection with other brain part. It was shown from this study results that, the PFC of control, *Nz* and Tween treated rats reveals the normal basic histological features of the PFC characterized by large pyramidal neurons, while Pb treated rats prominently demonstrated necrotic and/or pyknotic pyramidal neurons likewise chromatolytic neurons with degenerating pyramidal cells, across various days. All this structural alterations were greatly ameliorated by *Nz*, which exhibited more of large pyramidal neurons similar to that of the control rats, with no or highly depleted necrotic pyramidal neurons unlike Pb treated rats. In addition Glial fibrillary acidic protein (GFAP) is a major protein found in astrocytes. Histological finding are consistent with different studies that reveal structural alteration following Pb toxicity[52,15]. GFAP expression serves as a confirmation of origin and differentiation of astrocytes. It is used to determine glial differentiation which helps with determining disease conditions[53]. In this study, it was observed that GFAP immunohistochemistry showed no major changes in the distribution and structure of astrocytes in the control, *Nz* and tween groups, as they are seen characterized by well-expressed astrocytes with normal sized and numerous processes properly situated within the neutrophil. On the contrary, in the Pb group, the integrity of astrocytes in the PFC were highly compromised, as astrocytic density and thickening was highly increased with appearance of reactive astroglia and hypertrophic cells causing astrogliosis associated with demyelination of neurons. However, rats treated with Pb+*Nz* had very similar appearance with the control and *Nz* groups, with many neurons between the astrocytes having normal morphology and the astrocytes also well expressed with normal sizes and numerous processes. These findings thereby confirm the neuroprotective properties of *Nz* in preventing neonatal Pb-induced neurotoxicity, and results is in agreement with different studies that showed that Pb increase astrocyte expression[54,52] and bioactive component of *Moringa oleifera* has neuroprotective properties[17].

CONCLUSION

Nz was able to ameliorate neonatal Pb-induced neurotoxicity, which account for morphological damages, lipid peroxidation, oxidative stress, metabolic stress and astrogliosis. However, this finding has been able to elucidate the potentials of *Nz* as a neuro-protective agent against Pb-induced neurotoxicity.

CONFLICT OF INTEREST: No conflict of interest

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