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Research Article

Edible Bird's Nest Mitigates Histological Alterations in the Cortexes of Rats' Brains Subjected to Lead Toxicity

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Abstract | Lead (Pb) is a heavy metal which possesses a long half-life and a distinct negative effect on the bodies of humans and animals. Lead precipitates in various tissues of the body, especially the central nervous system, which causes histological structural changes that may persist even after its concentration in the blood reduces. It produces neurotoxicity related to the deterioration of brain functions. Edible bird's nest (EBN) is important natural product that has biological characteristics, such as regenerative effect. The objective of this research was to evaluate EBN's neuroprotective role on the cerebral and cerebellar cortexes of lead acetate-exposed adult female rats. Thirty Sprague Dawley rats were allocated randomly into five groups, with six rats in each group. The control group (C) received solely distilled water. Meanwhile, the treatment groups (T0, T1, T2, and T3) received lead acetate (LA) at a dose of 10 mg/kg BW along with increasing doses of EBN at 0, 30, 60, and 120 mg/kg BW each day, respectively, for five weeks. Behavioural changes were monitored in the various groups. Blood sample for measurement of redox status markers (thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC)) and brain tissue samples for histopathology, were collected after euthanization. Aggression, loss of appetite and uncoordinated body movement were increased in T0 group and absent in the T3 group. Rats pre-treated with EBN showed reduced LA -induced alteration in brain histology and apoptosis attributed to increased TAC and decreased lipid peroxidation (TBARS). These results suggest that EBN's anti-apoptotic, proliferative, and antioxidant properties lessen the neurotoxicity caused by lead acetate.

Keywords | LA, EBN, TAC, TBARS, Cerebellum, Cerebrum

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INTRODUCTION

Lead has an essential role play in the occurrence of insidious hazards and adverse health effects that lead to disorders and diseases especially in oil producing and developing regions (Flora *et al*., 2012). Lead is a multi-organ toxicant affecting almost every organ/system of the human and animal body, especially the central nervous system, reproductive, hepatic, renal, and immune systems (Kali and Flora., 2005; Otong *et al*., 2022). After exposure, lead is mostly deposited in the bones after being primarily retained in the kidneys and liver at first (Satarug *et al*., 2002). Age and hormone production are two examples of conditions that might trigger the re-release of lead into the general circulation (Horiguchi *et al*., 2013). Consequently, lead has lengthy half-lives, as reported by Järup (2003), Rzymski *et al*. (2014), and Liu *et al*. (2014). Trace quantities of lead are eliminated through the urine. Lead accumulates in numerous bodily tissues, primarily in the central nervous system (CNS), from which it is possible that structural changes will endure even after the blood concentration is reduced (Sidhu and Nehru, 2004; Ibrahim *et al*., 2012).

Epidemiological studies have revealed that chronic lead poisoning in young children can affect their growth and cause CNS injury, decreased intelligence, shortterm memory, hearing loss, irreversible brain damage, and mortality (Cleveland *et al*., 2008). The high absorption of lead through the gastrointestinal tract and the permeable blood-brain barrier makes children more susceptible to lead exposure (Jarup, 2003; Liu *et al*., 2015; Barkur and Bairy., 2015).

Over the last decades, the prevalence of neurodevelopmental and neurodegenerative disorders has dramatically increased worldwide. A neurodegenerative disease is caused by the progressive loss of structure or function of neurons. Additionally, research has indicated that behavioural dysfunctions, cognitive impairment, and neurodegeneration can result from even low-level lead exposure (Flora *et al*., 2012; Chen et al., 2022). Increased oxidative stress, alterations of the cholinergic system and glutamate receptor have all been implicated in experimental investigations demonstrating the neurotoxic effects of lead (Shukla et al, 2003; Hossain *et al*., 2016). Previous studies have documented a correlation between elevated levels of lead exposure among occupational workers and heightened production of free radical species, which subsequently results in oxidative harm and a reduction in the antioxidant defence mechanism of affected individuals (Kasperczyk *et al*., 2005; Emam *et al*.,2023). Owing to the generation of free radicals in the tissues, oxidative stress results in lipid peroxidation (LPO), which is the mechanism by which all toxic metals, including lead, induce toxicity (Al-Quraishy *et al*., 2016). Reactive oxygen species (ROS) impair the antioxidant defence system and cause damage to a variety of enzymes, membrane-based lipids and proteins at the same time (Flora *et al*., 2012; Albishtue *et al*., 2020a). Antioxidants are recognised for their ability to absorb and restore damage caused by free radicals. According to the discovery that lead-induced pathogenesis processes produced free radicals, it was hypothesised that antioxidant supplementation would inhibit or reduce the deleterious effects of lead while enhancing the efficacy of chelating agents (Flora *et al*., 2002). In previous studies, EBN provided protection for vital organs exposed to LA such as pituitary, ovary, uterus, liver and kidney (Albishtue *et al*., 2018b; Albishtue *et al*., 2019c; Albishtue *et al*., 2020a). EBN powder contains potential peptides with antioxidant property. Until today, scientific evidence on EBN's role in maintaining integrity of histological structures of cerebral and cerebellar cortexes exposed to lead acetate toxicity has been lacking. The present study was therefore undertaken to evaluate changes in oxidation and micro anatomy of rats' cerebrum and cerebellum subjected to LA and EBN supplement.

MATERIALS AND METHODS

EBN Preparation

EBN was acquired from Nest Excel Resources Sdn Bhd. stored between 25°C -30°C. As the local suppliers specified, EBN extract was prepared in adherence to Chinese tradition. A mixer (BUCHI-400, Switzerland) was utilised to reduce the samples to powder form after they had been cleansed and dried at room temperature. At 4°C, the ground EBN extract was housed. 1 g of EBN powder was dissolved in 100 mL of distilled water to produce EBN solution, which was then heated in a water bath at 60 °C for 45 minutes. Finally, the rats were given doses of the EBN solution based on their weights after it had cooled to room temperature (Albishtue *et al*., 2018e; Albishtue *et al*.,2019d).

Preparation of Lead Acetate Solution

Oxford Lab. Co., India (CAS: 6080-56-4) provided lead acetate with the chemical formula Pb (C2H3O2)2. Individual rats from the treated groups were administered lead acetate using a gavage tube (China, Straight, 18 Gauge) at a dose of 10 mg/kg of body weight. The lead acetate solution was initially produced as a 1% (w/v) solution in distilled water (Albishtue *et al*., 2018b; Albishtue *et al*., 2019c; Albishtue *et al*., 2020a).

Animals and Experimental Design

The Animal Resource Center at the Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), provided 30 female Sprague-Dawley rats (aged 12 weeks) for the study. For a seven-day acclimation phase, the rats were kept together. All rats had unlimited access to water and a regular rat meal (Gold Coin Brand Animal Feed) throughout the study and were housed in plastic cages that were kept at a constant temperature of 25°C ± 2°C. According to the institutional

animal care recommendations and use committee (IACUC) guidelines, the animal management and handling procedures were carried out using the reference number UPM/IACUC/AUP- R009/2016) approved on 11 th April 2016.

Experimental Design and Clinical Observation

Following a 7-day period of acclimation, the rats were divided into 5 random groups of 6 animals each, and 10 mg/kg of LA was given to each group according on Albishtue *et al*. (2018e). Rats were divided into five groups: Control (C) was given distilled water orally. Positive control (T0) was given LA (10 mg/kg). Treatment 1 (T1), which received EBN (30 mg/kg) and LA (10 mg/kg) orally, daily; Treatment 2 (T2), which received EBN (60 mg/kg) and LA (10 mg/kg); and treatment 3 (T3), which received EBN (120 mg/kg) and LA (10 mg/kg). Over the course of 5 weeks, LA and EBN were each given orally once, daily. Following a general anesthesia technique that included the administration of 30 mg ketamine/kg BW and 10 mg xylazine/kg BW and blood collection by heart puncture according to the method of Albishtue et al. (2019d). EBN has been previously studied and confirmed for its potential cognitive enhancing effect, including learning and memory abilities (Xie *et al*., 2018; Mahaq et al., 2020; Loh *et al*., 2022), therefore a group receiving only EBN without lead acetate was not considered in the study design. Clinical monitoring of the rats was done every day. However, important observations, such as aggression, reduced appetite and balance inability, were summarized at each group and presented in scores based on the number of rats showing such signs within a group (Assi *et al*, 2018). CO_2 asphyxiation was used to euthanize rats.

Oxidative Stress Biomarker (OSB) and Antioxidant (AO) Assay

Plasma samples were also collected in order to perform analyses for AOs such as total AO capacity (TAC) and OSBs such as thiobarbituric acid reactive substance (TBARS) which determine marker of lipid peroxidation In accordance with Schmidt *et al*. (2014) and Yew *et al*. (2014) The QuantiChrome™ TBARS Assay Kit, DTBA-100, was utilised to measure the amount of TBARS in order to determine the level of lipid peroxidation in the plasma.A commercial kit called the QuantiChrome™ AO Assay Kit (DTAC-100) was used to assess the TAC. Metmyoglobin oxidises 2,29-azino-di-3-ethybenzthiazoline sulfonate, and this process is blocked by non-enzymatic AOs present in the sample. All the above assay kits were obtained from Bioassay System, San Francisco Bay Area, USA.

Histopathological Examination and Scoring

Following the anesthesia-induced rat sacrifice, tissue samples from the cerebellum and cerebrum were removed and preserved for 48 hours in 10% neutral buffered formalin. Using an automated tissue processor (Leica TP1020, Germany), fixed tissue samples were processed via a series of dehydrations in ethanol. Sample tissues were cut into 4 µm sections using a microtome (Leica 2045, Germany), immersed in paraffin blocks (Leica EG 1150C, Germany), and ribbon-like sections were created. To get them nicely stretched, sections were submerged in a floating water bath (Triangle Biomedical Science) for 15 minutes. Following this, glass slides were used to remove the tissues from the water bath. These slides were then allowed to dry on a hot plate (Leica HI 1220) in preparation for histological analysis using conventional methods of hematoxylin and eosin (H&E). To enable for section preservation, the slides were mounted with cover slip using DPX mounting medium. Stained tissues were inspected using an image analyzer microscope and light microscopy. For every tissue section, three microscopic regions of identical size were examined at different magnifications (20x and 40x). For analysis, sections were chosen at random using Medical Image Analysis (Motic Image plus 2.0, China). The samples were examined under a microscope to check for changes in histomorphology. At magnifications of 20x and 40x, the degeneration score of the cerebral and cerebral cortexes in a given location was recorded. The severity of the lesion was rated on a scale of 0 to +3 (0, none; 1, mild; <30%); 2, moderate; <60%); and 3, severe; <60%) (Albishtue *et al*, 2020a)., the density of granular cells was graded from 0 to +3 at magnification of 20x.

Statistical Analysis

Graph Pad Prism 6.0 (Graph Pad Software, San Diego, California) was used to examine all the data, which were expressed as means (M) ± standard error of mean (SE). The acquired data were subjected to the Shapiro-Wilk test to ensure normal distribution. The concentrations of AOs and OSBs in the sera were compared using a one-way analysis of variance (ANOVA) and the Tukey multiple comparison post-hoc test. Using a Kruskal-Wallis (non-parametric) test, histological lesions in the cerebellum and cerebrum were compared. A value of $p<0.05$ was deemed significant.

RESULTS AND DISCUSSIONS

Clinical Observations

At 35 days from treatment, it was shown that the T0 and T1 groups had greater rates of the major clinical observations that include aggression, appetite loss and inability to coordinate movement, but the T2 group had a smaller proportion while the T3 group had none (Table 1).

Histomorphological Findings in the Cerebrum and Cerebellum of Rats Given EBN Supplementation and Exposed to Lead Acetate

The five experimental groups' typical histological slices of the cerebrum are displayed in Figure 1. Although there were no obvious macroscopic pathological lesions in the rats, the present study showed that lead had penetrated the blood-

brain barrier and altered the normal tissue architecture of the brain. However, histomorphological examination of the T0 group's cerebrum revealed lead-induced histopathological changes, including haemorrhage, edematous and vacuolated tissue with significant cell loss, and the appearance of neural cell pyknotic nuclei (Figure 1).

Table 1: Description of clinical findings that appear after 5 weeks of LA and EBN administration.

Parameters	C	T0	T1	T2	T3
Aggression	0	2.50±0.50^{a}	1.50±.25^{b}	1.00±.25^{ab}	0
Reduced appetite	0	2.50±0. 25^{a}	1.50±0.25^{b}	1.00±.25^{ab}	0
Unable to coordinate movement	0	2.00±0.50^{a}	1.00±0.25^{b}	0	0

The standard error (SE) and mean ± expressed as the data. A significant difference at $p < 0.05$ is indicated by different letters (a and b) within rows.

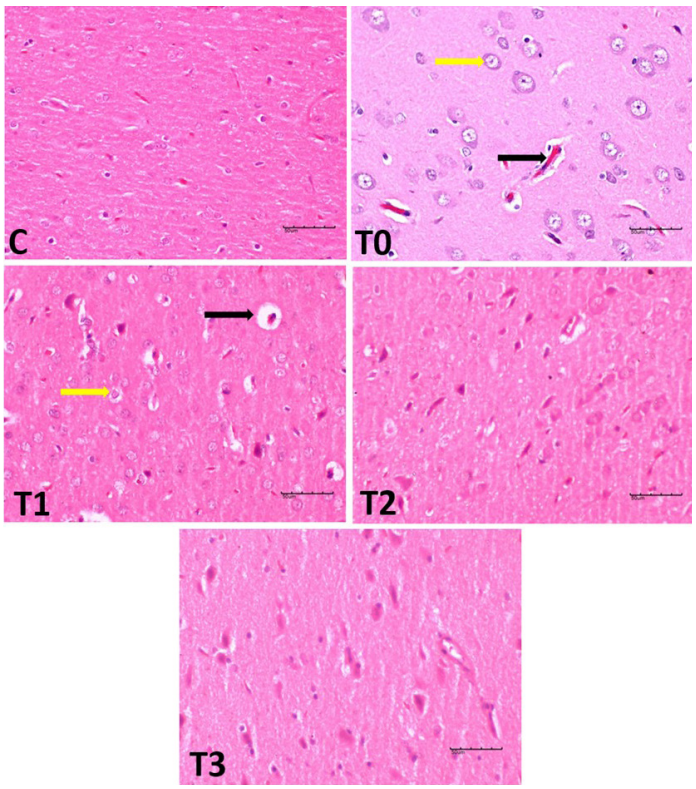

Figure 1: Histological changes in the cerebral cortex of rats exposed to LA and treated with EBN. Note: The control group displayed a typical. The T0 group displayed haemorrhage and pyknotic nuclei, that considered signs of degenerative changes in neural cells. T1 and T2 groups demonstrated neuronal cell protection. T3 showed the regeneration, resemble in the histo-architecture for control.

Table 2 displays the findings of the cerebrum's histological lesion scores. The cerebral lesions with vacuolations and pyknotic neurons varied statistically significantly ($p<0.05$) across the experimental groups, according to the data. Pyknotic neurons and neural vacuolations were lowest in the T2 group and higher in the T0 group (Figure 1). In contrast, T3 supplemented with the greatest dose of EBN exhibited normal cerebral and cerebral cortex structures, similar to those of the control group (Figure 1).

Table 2: Description of histopathological alterations of cerebrum after LA and EBN administration at 5 weeks of experimentation.

Parameters	C	T0	T1	T2	T3
histopathological alterations	0	3.00±0.00^{a}	1.50±.25b	1.00±.25^{b}	0

The standard error (SE) and mean ± expressed as the data. A significant difference at $p < 0.05$ is indicated by different letters (a and b) within rows.

According to the current study, groups C and T3 have normal morphological appearance of the cerebellar cortex when stained with H&E (Figure 2). These groups' cellular morphology is typified by Purkinje cells with prominent cell bodies and dendrites that protrude deeply into the sparsely nucleated molecular layer (M), taking on the form of a fan. Additionally, a large number of compactly arranged tiny granule neurons make up the granule layer (G) in T3 group (Figure 3).

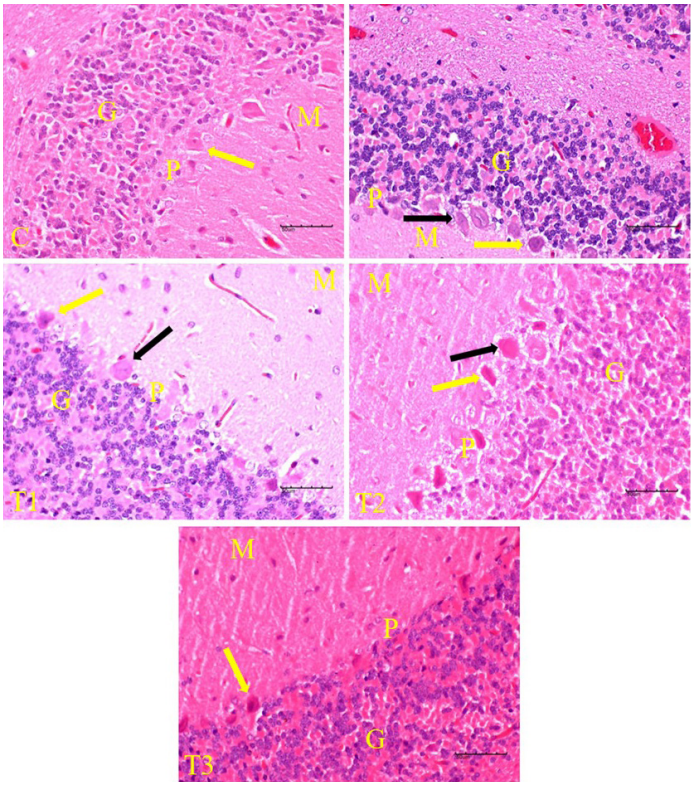

Figure 2: Histological changes in the cerebellar cortex of rats exposed to LA and treated with EBN. Note that darkly stained degenerating pyramidal cells (black arrow) among the healthy pyramidal neurons (yellow arrow) in T0, T1 and T2. Cerebellar layers include molecular(M),purkinje(P) and granular (G).

The cerebellar cortex of rats in the T0, T1, and T2 groups showed histological changes in Purkinje and molecular lay-

er cells including shrinkage and degeneration, as well as dispersed glial cells. Purkinje's cell layer completely separates from the degenerating granular cell layer. Around the hazily defined cerebellar layers, Purkinje cells with their typical pyknotic cell bodies and short dendritic processes may be observed. Unlike the T3 group and the control group the Purkinje cells exhibit empty spaces between the cells, which indicates that the cells are degenerating. In addition, the neutrophils in these groups have irregularly shaped and sized neural cells, giving the impression of fragmentation. Granular cells exhibit morphological deformation as well; in comparison to the control. Moreover, nonparametric one-way ANOVA was used to assess the impact of LA on the cerebellum's purkinje cells. Table 3 displays the findings of the Purkinje cell histopathological lesion scores. The results revealed a significant difference ($p<0.05$) in the neural lesions including vacuolations, pyknotic neurons, and necrosis among the experimental groups. The neural lesions were lower in the T2 group and higher in the T0 group (Figure 3).

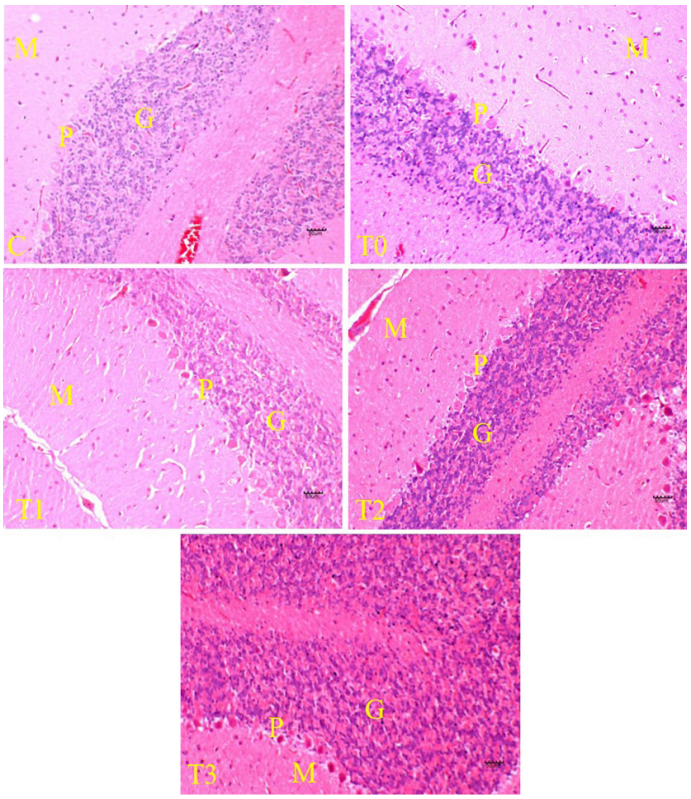

Figure 3: Histological changes in the cerebellar cortex of rats exposed to LA and treated with EBN. Note that the granule layer in T3 group consists of huge numbers of small granule neurons. Cerebellar layers include molecular(M),purkinje(P) and granular (G).

Oxidative Stress and Antioxidant Biomarker Concentrations

The current evaluation, the thiobarbituric acid reactive substance (TBARS) and TAC in the plasma from experimental groups are summarised in Figure 4. The highest concentration of TBARS was found in T0 ($p<0.05$), and the lowest values were found in T3. In T1 and T2, there were no notable alterations. At week 5, TAC levels increased in accordance with the dose of EBN in the treatment groups. TACs concentrations were higher in the T3 and decreased ($p<0.05$) in the T0. These effects improved the enzymatic AO defense and raised the TAC, which altered the redox status. These findings suggest that EBN alters and weakens the redox system.

Table 3: Description of histopathological alterations of cerebellum after LA and EBN administration at 5 weeks of experimentation.

Parameters	C	T0	T1	T2	T3
histopathological alterations	0	3.00±0.00[a]	2.00±.25[b]	1.50±.25[c]	0

The standard error (SE) and mean ± expressed as the data. A significant difference at $p < 0.05$ is indicated by different letters (a , b and c) within rows.

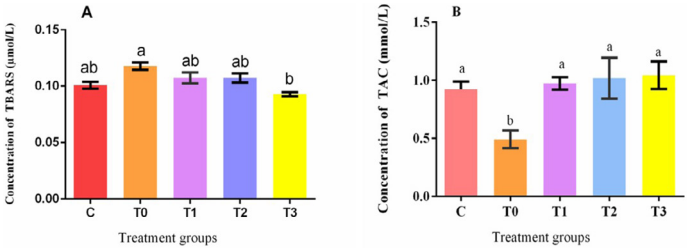

Figure 4: Impact of EBN on the activities of total antioxidant capacity (TAC) and oxidative stress in the plasma of rats exposed to LA. Reactive substance thiobarbituric acid (TBARS). Informations are expressed as means ± S.E.M.

The primary factor contributing to the development of toxicity, which indicates significant pathological damages of important organs, is oxidative stress brought on by exposure to LA (Oyagbemi *et al.*, 2015). Exposure to lead causes encephalopathy, which is characterised by a progressive deterioration of specific brain regions. The major symptoms of encephalopathy include weariness, tremor in the muscles, dullness, irritability, reduced attention span, memory loss, and hallucinations. Elevated exposures result in more severe symptoms such as ataxia, delirium, spasms, unable to coordinate movement and coma (Verina *et al.*, 2007). Similarly, in the present study, rats exposed to LA has shown clinical neurological symptoms including uncoordinated movement, aggression and reduced appetite while the symptoms were observed significantly reduced in the EBN supplemented group. Purkinje cells secrete gamma-aminobutyric acid that inhibits the transmission of nerve impulses by acting on specific neurons. Purkinje cells are capable of coordinating and regulating motor movements by virtue of their inhibitory functions (Britannica, 2015). The clinical observations showed that rat exposure to lead acetate alone was characterized by abnormal body movements and inability to maintain balance due to neurotoxic effects of lead on these cells causes damage and inflammations.

The neurotoxic effects of lead can be attributed to various factors, such as interactions with other micronutrients, the integrity of the blood-brain barrier, lead-binding proteins, and cellular scavengers (e.g. glutathione(GSH)) (Sidhu and Nehru, 2004). One of the ways in which lead damages the central nervous system is by substituting numerous bivalent cations (e.g., Ca2+, Mg2+, Fe2+) with Pb+ ions (Pb2+), which allows lead to cross the blood-brain barrier (BBB) and become trapped in the brain (Sanders *et al*., 2009).

Foetuses and children are particularly vulnerable to the neurological implications of lead because their small intestine's high absorption rate and the blood barrier is more susceptible to heavy metals (Needleman, 2004). Even in cases when lead exposure is minimal, children may exhibit signs of hyperactivity, inattention, and irritability. Elevated lead levels in children can cause hearing loss, short-term memory loss, reduced understanding, and rapid development. At higher concentrations, lead can result in lifelong brain damage and possibly death (Cleveland *et al*., 2008). The progressive deterioration of memory and cognition is the consequence of severe morphological and functional deficits caused by the demise of neurons via apoptosis. Natural antioxidants have been utilised to examine their potential as metal lead chelating agents in the context of lead-induced neurotoxicity (Singh *et al*., 2015; Singh *et al*., 2016). EBN is one of the natural products reported as a potential cognitive enhancer, including learning and memory abilities (Xie *et al*., 2018; Mahaq et al., 2020; Loh *et al*., 2022). Since the Tang and Sung dynasties, EBN has been widely used by humans as a tonic and medicinal diet in traditional Chinese medicine (Zhao *et al*., 2016).

Histopathological findings of the present study showed alterations such as shrinkage, degeneration as well as dispersed glial cells in the cerebellar and cerebral cortexes of rats exposed to only LA while those supplemented with highest dose of EBN demonstrated a preserved histomorphology alike the control group implying a protective role played by the EBN supplement. Previous study revealed that LA causes hypertension and the permeable blood–brain barrier makes susceptible to lead exposure and subsequent brain damage (Järup, 2003; Vaziri and Sica, 2004). Present study confirms these phenomena through congestion of blood vessels and change histological structures. In addition, Pb causes neural degeneration, cerebral oedema, and cerebellar atrophy involving Purkinje, and granular cells have been identified as complications that cause encephalopathy in rats.

Prior in vitro research studies demonstrated that EBN extracts promoted neuronal differentiation, migration, and proliferation by a substantial margin. A study conducted by Yew *et al*. (2019) identified 29 bioactive proteins in EBN extract that may play important roles in various common cellular processes, including neurodevelopment, cell proliferation, migration, formation of extracellular matrix and antioxidation. EBN may also enable to regulate blood hypertension via Angiotensin converting enzyme inhibitory properties of EBN leading to make blood–brain barrier more resistant for LA (Daud et al., 2019). In comparison to rats treated solely with lead, the histopathological alterations induced by lead acetate in the brain regions were significantly diminished and completely restored in the EBN supplemented group.

Recent researches confirm the beneficial effect of an increased intake of EBN in the treatment of neurodegenerative and neurological conditions (Careena *et al*., 2018). EBN contains a diverse group of monosacharides such as sialic acid that plays a distinctive role in repairing and building the human body attributed to its therapeutic and medicinal effects. It is associated with brain development, neurological improvement and intellectual benefits in children, as it serves as a functional portion of brain gangliosides (Wang and Brand-Miller, 2003).

Glycosyl galactosamine (GalNAc), an essential compound for the effective functioning of synapses, is frequently observed to be glycosidically linked with sialic acid. Goh *et al*. (2001) have demonstrated that GalNAc is also capable of improving memory. Further, brain gangliosides, of which sialic acid is a vital constituent, modified neural adhesion cells and enhanced the neurotrophic factor involved in the development of neurons and brain function. Schneider *et al*. (2010) and Wang (2009) both asserted that sialic acid is an essential component in cell-to-cell interactions. Angata and Fukuda (2010) discovered that sialic acids have the ability to regulate synaptic connectivity during memory formation and stimulate neuron outgrowth, axon elongation, migration, and differentiation of NSC at the cellular level. A prior study found that rats exposed to lead showed a decrease in body weight compared to the control group (Albishtue *et al*., 2019d). Lead exposure may be reduced through interaction of lead with appetite-depressant receptors in the gastrointestinal tract (Hammond *et al*., 1989), which is consistent with this research.

Neural stem cells are multipotent precursor cells that have the capacity to generate new neurons, astrocytes, and oligodendrocytes, as well as to respond to trophic factors and self-renew in order to recover functionally from neurodegenerative diseases. Research has indicated that the use of endogenous resident stem cells in combination with exogenous neurotrophic support may be promising for the restoration of damaged or degenerated neurons (Li *et al*., 2013; Xu *et al*., 2014). Worth mentioning is the functional recovery exhibited by a number of natural neurotrophic compounds. Xu *et al*. (2014) concluded that endogenous neural stem cells are stimulate to an exogenous factor and

may be induced to assume specific cell phenotypes in response to the application of suitable signals.

According to the earlier research, the administration of LA raised oxidative stress levels that may lead to flaws in the mitochondrial membrane's permeability, allowing free radicals and cytochrome c to exit the mitochondria and bind to another protein known as apoptotic protease activating factor-1 (Apaf-1), which in turn activates caspase 3 and causes cell death (Liu *et al*., 2012). Experimental studies have demonstrated increased lipid peroxidation and diminished antioxidant defense enzymes in the brain following exposure to lead, indicating increased oxidative stress (Velaga *et al*., 2014, Emam *et al*., 2023). Lead exposure is also related to the depletion of brain GSH content as shown by the ratio GSSG / GSSG + GSH (Adegbesan and Adenuga, 2007). It has been noted that lead forms strong bonds with free-SH affinity groups in enzymes and proteins, which can significantly impair the functionality and efficacy of the enzymes in consideration. Lead-induced neurotransmission was hypothesised to be mediated by oxidative stress, which is generated when the antioxidant equilibrium within the cells is disturbed; cell signalling dysregulation and neurotransmission modification; cell signalling deregulation and alteration of neurotransmission were considered possible mechanisms involved in lead-induced neurotransmission (Bokara *et al*., 2008). In the present study, EBN supplementation showed a dose dependant improvement of redox status of the rats exposed to LA toxicity, the highest EBN dose supplement showing a significant increase in TAC and reduction in lipid peroxidation/MDA level.

EBN contains highly nutritious ingredients such as proteins, mineral salts, vitamins, hormones, and fatty acids (Saengkrajang *et al*., 2013). EBN supplement may have protective effects because of its biological characteristics, which include cell regeneration and proliferation (Muhammad-Azam *et al*., 2022). EBN is very important for avoiding any toxic effects after it is absorbed by cells. According to in-vitro research studies, EBN treatment increased viability and proliferation of cell Caco-2 and neuroblastoma cell (Aswir and Wan Nazaimoon, 2011). This observation could indicate that EBN is absorbed by cells with functional mitochondria, leading to an increase in cell viability absorbance and a potent signal (Mosmann, 1983). EBN contains a high amount of sialic acid which has certain molecular mechanisms that regulate the proliferation of cells by the hormone E2. A diet high in sialic acid reported to increase the number of brain cells in mammals and promotes the expression of genes linked to cognition (Careena *et al*., 2018).

Sialic acid has anti-inflammatory properties, capable of modulating an extensive type of physiological and pathological processes. For example, EBN extract may also reduce the release of factor-alpha (TNF-α) tumour necrosis in a macrophage cell line of a mouse leukaemic monocyte (Aswir and Wan Nazaimoon, 2011). Furthermore, it exhibits a stimulating impact on cell growth and regeneration due to its Epidermal Growth Factor (EGF)-like activity (Zhiping *et al*., 2015). Present study has found that treated group possess highest numbers of glomerular and Purkinje cells even than control although it exposed to LA.

In numerous conditions, including anti-aging, anti-cancer, and immunity enhancement, the EBN has been utilised as a prophylactic hormonal replacement agent. EBN consist of progesterone, testosterone, estradiol, LH, FSH, and prolactin (Ma and Liu., 2012; Albishtue *et al*., 2019c).

According to Jin *et al*. (2001), EBN is also recognised for its composition of VEGF and IL-6, which inhibit cell apoptosis by preventing the activation of caspase three and thereby retinal neuronal apoptosis. Irusta *et al*. (2010) found that VEGF, FSH, and estradiol inhibit caspase 3 activation, promote proliferation, and prevent apoptosis in a synergistic manner. Evidently regulating inflammatory and antioxidant genes, EBN has numerous therapeutic applications (Yida *et al*., 2015).

Pb is capable of traversing the blood-brain barrier and causing damage to glial cells specifically targeting the cerebral cortex, cerebellum, and hippocampus, thereby disrupting the structural components of the brain (Gandhi and Abramov., 2012, Al-Khafaf *et al*., 2021). Due to their diminished capacity to detoxify reactive oxygen species (ROS), neurons are susceptible to increases in ROS levels, which can be generated by lead intoxication (Dringen *et al*., 2005). Thus, altering the histology and neurological functions of the cerebrum and cerebellum. Components of EBN reduced H2O2-induced cytotoxicity and ROS via enhanced scavenging activity, according to a previous study. In addition, EBN induced transcriptional modifications in genes associated with antioxidants that exhibited a propensity for neuroprotection. Furthermore, EBN and its constituents, lactoferrin and ovotransferrin, have the potential to generate synergistic antioxidative effects due to their respective antioxidative capacities (Hou *et al*., 2015). The current study demonstrated the antioxidant properties of EBN by reversing the impact of lead toxicity on MDA and TAC levels. This reversal may be a result of the high concentration of antioxidant factors in the EBN sample. Lead toxicity may be mitigated through additional supplementation of EBN, thereby preserving the phospholipid ratio in the cell membrane. As a result, it is hypothesised that EBN provides protection against lead neurotoxicity via the aforementioned mechanisms and by preventing lead accumulation in the brain via urine elimination.

CONCLUSIONS AND RECOMMENDATIONS

This study revealed that histological alterations of the cerebrum and cerebellum were associated with exposure to lead acetate. EBN could be considered a health-enhancing medicinal food against neurodegenerative diseases. Overall, antioxidant and anti-inflammatory properties of EBN were significantly higher in EBN 60 and 120 mg/kg bwt. Finally, it can be deduced that EBN significantly inhibits neuroinflammatory and oxidative stress processes that can potentially lead to enhanced memory and potent neuroprotection. However, further research studies and clinical trials in humans would be needed to ascertain the benefits of EBN supplement observed in the present study using animal models and to determine the appropriate doses.

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NOVELTY STATEMENT

The present study revealed potential neuroprotective function of EBN against heavy metal (lead) toxicity using rats as a model.

List of Abbreviations

ANOVA= Analysis of variance
ANOVA= a one-way analysis of variance
AO= Antioxidant
Apaf-1= apoptotic protease activating factor-1
Bwt= Body weight
CNS= central nervous system
CNS= Central nervous system
EBN= edible bird's nest
FSH= Follicular stimulating hormone
GalNAc= Glycosyl galactosamine
GalNAc= Glycosyl galactosamine
GSH= Glutathione
H and E= Using hematoxylin and eosin
H2O2= Hydrogen peroxide
IACUC= the institutional animal care recommendations and use committee
LA= Lead acetate
LH= Luteinizing hormone
LPO= lipid peroxidation
OSB= Oxidative Stress biomarker
Pb= Lead
TBARS= Thiobarbituric acid reactive substance
TCA= total antioxidant capacity
TNF-α= factor-alpha

AUTHOR'S CONTRIBUTIONS

This study was conceptualized by AAA, NY, MZAZ, AWH and ASB. The investigations were performed by AAA and NY, under the supervision of NY, MZAZ, AWH, and ASB. Statistical analysis was carried out by AAA and NY. Original manuscript was written by AAA, reviews and editing were finalized by AAA, NY, MZAZ, AWH and ASB. The final manuscript was read and approved by all authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

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