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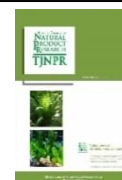

Ameliorative potentials of *Linum usitatissimum (Linn)* (Flax seed) Crude Extract Against Lead Acetate-induced Neuronal Damage on the Hippocampal Histoarchitecture Using Wistar rat Model

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ABSTRACT

Lead-induced neurotoxicity is on the increase and the search for herbal remedy over orthodox intervention is also gaining popularity. The study investigated the ameliorative properties of flaxseed crude extract against lead acetate-induced neuronal damage in the hippocampus of Wistar rat. Thirty adult male Wistar rats (100 – 120 g) were used and assigned randomly into five groups A-E (n=6). Group A is the control, Group B was the negative control, while Groups C, D, E were test groups. Lead acetate was administered at a dose of 5 mg/kg for 14 days, Dimercaptosuccinic acid (DMSA) and flaxseed were later administered for 14 days. Neurobehavioral test (Radial arm maze) was carried out after administration. At the end of the experiment, the animals were sacrificed using cervical dislocation. Hippocampal tissues were collected after animal sacrifice and analyzed for glucose-6-phosphate dehydrogenase activity, Malondialdehyde (MDA), gamma-aminobutyric acid (GABA) levels, and the immunohistochemical expression of Tumor Necrosis Factor α (TNF-α) using enzyme tests. Data collected were analysed using one-way ANOVA, followed by Tukey *post hoc* for multiple comparisons. Flax seed extract reversed the neuronal damage and spatial memory deficit caused by lead acetate exposure to the hippocampus. The study concluded that 200 mg/kg of *Linum usitatissimum* crude extract had an ameliorative effect against memory impairment caused by lead acetate in rat model.

Keywords: Flax seed, Hippocampus, Lead-acetate, Neurotoxicity

Introduction

Lead acetate is a heavy metal that is highly poisonous and injurious to human health. [1] Lead neurotoxicity constitutes a global public health issue, with notable epidemiological incidence recorded in Nigeria, Africa, and globally. [1] The mining of lead-rich ores in Nigeria has caused significant contamination, leading to widespread lead poisoning, especially among youngsters. In 2010, a tragic occurrence transpired in Zamfara State, resulting in the deaths of hundreds of children owing to acute lead exposure from artisanal gold mining operations. [2] As a result of mining and farming activities in developing countries, it is fast becoming a major public health concern. [2] In Africa, lead poisoning is frequently linked to environmental pollution resulting from industrial and mining operations. [3] In 2019, the World Health Organization (WHO) predicted that lead exposure was responsible for roughly one million deaths worldwide. [4]

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Lead accumulation in the body systems can produce deleterious effects in the circulatory, renal, gastrointestinal and central nervous system. [5] Short-term memory loss, nausea, depression, loss of co-ordination, numbness and anaemia are also associated with lead toxicity. [6]
Lead can cross the blood-brain barrier to cause histomorphological changes, memory impairment and neurodegeneration in different brain regions. [7] Low, moderate and high doses of lead have been reported to induce the production of free radicals resulting in oxidative stress damage in brain tissues, [8] ultimately causing the depletion of the brain antioxidant system. [9] At the cellular, molecular and intracellular levels, prefrontal cortex, cerebellum and hippocampus are susceptible to lead-induced damage. [10] The hippocampus plays a major role in learning and memory. [11] Lead preferentially accumulates in the hippocampus resulting in memory impairment and neurobehavioral deficit following its chronic exposure.[12]
Flaxseed (*Linum usitatissimum*) is a plant-derived source of omega-3 fatty acids, lignans, and dietary fibre, recognised for its potential health benefits. [13] Linn is regarded as an essential functional food due to its high content of alpha-linolenic acid (n-3 fatty acids) and dietary fibre, as well as its significant secondary metabolites, lignans, which are found in greater concentrations than in other food sources. [14] Flax serves as a significant source of the lignan known as secoisolariciresinol diglycoside (SDG). SDG is a plant lignan that is metabolised by colonic bacteria in humans (and animals) into mammalian lignans, specifically enterodiol (ED) and enterolactone (EL). [15] Recent research has demonstrated that SDG can scavenge hydroxyl radicals and exhibits significant antioxidant activity. These are biologically active phytochemicals exhibiting notable anticancer and antioxidant properties. [16] Flaxseed has the potential to preserve and enhance brain functions. [17, 18] It has anti-inflammatory, antioxidant and anti-apoptotic properties which have been shown to play a protective role against oxidative stress. [19, 20] Lead exposure may result in astrogliosis and

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microgliosis by activation of a number of signaling cascade and the production of pro-inflammatory cytokines, one of which is tumor necrotic factor-α (TNF-α). [21] One of the mechanisms for reversing heavy metal toxicity is by the actions of metal chelators, Dimercaptosuccinic acid (DMSA) is a common metal chelator used to treat heavy metal toxicity. [22] DMSA a dithiol compound competes with the thiol groups for binding the Pb ions which is then excreted in urine; [23] though commonly used, some side effects have been identified such as renal failure, gastrointestinal discomfort, elevated liver enzymes, among many others. [24] Previous literatures have reported the neuroprotective role of flax seed oil on lead acetate induced neuronal damage in the hippocampus; this study evaluated the anti-inflammatory and anti-oxidant activity of the crude extract of *Linum usitatissimum Linn* (Flax seed) on the hippocampus of Wistar rats following lead acetate-induced neurotoxicity.

Materials and Methods

Chemicals and drug

Anhydrous lead acetate and analytical grade Dimercaptosuccinic acid (DMSA) were acquired from Sigma Aldrich, USA. Materials and reagents acquired for this study were of pure and standard analytical grade.

Animal care and ethics

Wistar rats were acquired and maintained in the animal holding facility at Babcock University Ilishan-Remo, Nigeria, in accordance with the guidelines for the use and care of animals in research and teaching as approved by the Institute of Laboratory Animal Resources and Teaching, National Research Council, DHHS, Pub no. NIH86-23, 1885. Ethical clearance was secured from the Babcock University Health Research and Ethical Committee, reference number BUHREC983/21.

Preparation of the flax seed crude extract

Dried seed of flaxseed was procured from a supermarket in Lagos State Nigeria. A sample was taken to the herbarium at Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria for authentication (FHL: 11331). The seed was thoroughly washed and air-dried. The extraction procedure followed the report by Ahmad *et al.* [25] The seed was ground using a grinder, after which the resulting powder was utilised to prepare the crude extract in a 70 % (v/v) ethanol solution in distilled water. One hundred grams of ground flaxseed were immersed in two litres of 70 % aqueous ethanol solution for a duration of five days, with shaking performed twice daily. After five days, the supernatant was separated by filtration using Whatman filter paper No. 1. The filtrate was pooled and concentrated using a rotary evaporator at 40 °C to obtain the crude extract.

Animal procurement, grouping and drug/extract administration

Thirty (30) male Wistar rats, each weighing between 100 and 120 g, were acquired from the animal holding facility at Babcock University, Ogun State, Nigeria. The animals were housed in adequately ventilated plastic cages maintained at standard temperature, exposed to natural light and dark cycles, and provided with daily access to pelletised feed and water. The rodents were randomly assigned to five groups (n=6): Group A received only distilled water, Group B received 5 mg/kg of lead acetate, Group C received 5 mg/kg of lead acetate and 100 mg/kg of flax seed extract, Group D received 5 mg/kg of lead acetate and 200 mg/kg of flax seed extract, and Group E received 5 mg/kg of lead acetate and 0.2 mg/kg of sodium dimercaptosuccinic acid (See Table 1). The mode of administration for both drug and extract was via the use of oral cannula for 14 days. According to Gad and Pham [26] the oral lethal dose (LD_{50}) for lead acetate is 44 g/kg and the oral lethal dose for DMSA is 3.0/4.0 g/kg. [27]

Neurobehavioural Evaluation

After administration, a neurobehavioral evaluation was conducted on day 14 utilizing the radial arm maze paradigm. The radial arm maze serves as a neurobehavioral assessment tool for evaluating spatial working and reference memory in rodents. [28, 29] The wooden radial arm maze comprises eight equidistant arms, each approximately 4 feet in length, radiating from a central circular platform measuring about 26.67 cm. At the terminus of each arm is a food site capable of holding pelletized feed. During the trials, the rats were trained to identify the feed in any of the four baited arm, identification of the feed by the rats in any of the baited arms served as commencement of the behavioural trial. A video camera was mounted to observe the animal's behaviour and record trials for scoring. On the test day, the apparatus was swabbed with cotton wool dampened with 70 % ethanol. The animals were placed in the testing room one hour prior to the commencement of the test for acclimatization. Following acclimatization, rats were then placed on the central platform from which they would have to locate and collect hidden baits placed at the terminal ends of baited arms. Four arms of the maze were baited alternately with pelletized feed. Each rat was observed for consumption of the pelletized feed; error was considered after failure to consume from any of the four baited arms within 2 minutes. The maze was cleaned with cotton wool soaked in 70 % ethanol after each rat to avoid visual and smell cues.

Animal sacrifice and histological analysis

Following neuro-behavioral assessment, the rats were euthanised using 5 mg/kg ketamine hydrochloride, succeeded by perfusion and cervical dislocation for sacrifice. The hippocampal tissue was carefully extracted from the skull to avoid damage, blotted dry, and weighed using a precision balance. The tissues were fixed in 10 % neutral buffered formalin. Coronal slices of the hippocampus were collected and subjected to standard paraffin embedding and immunohistochemical analysis. Hippocampal tissues for biochemical assays were homogenised in four volumes of ice-cold 0.1 M phosphate-buffered saline at pH 7.2 and subsequently analysed to assess the enzyme activity of G6PDH and the concentration of GABA respectively.

Biochemical Assay

Glucose-6-phosphate dehydrogenase (G6PDH)

Glucose-6-phosphate dehydrogenase (G6PDH) is a cytoplasmic enzyme that serves as the initial catalyst in the pentose phosphate pathway, generating reducing power for cells in the form of NADPH (the reduced variant of nicotinamide adenine dinucleotide phosphate) [28]. The protocol utilised was aligned with that of Owolabi *et al.,* [29] G6PDH demonstrates dual coenzyme specificity; under optimal conditions for each coenzyme, the catalytic activity ratio observed is NAD/NADP = 1.8. The reaction velocity is evaluated by measuring the increase in absorbance at 340 nm resulting from the reduction of NAD or NADP. One unit reduces 1 μmol of pyridine nucleotide per minute at 30 °C and pH 7.8 under the specified conditions.

Assay

The spectrophotometer was calibrated at 340 nm and 30 °C. Reagents were introduced to each cuvette as follows: Prepare a solution comprising 2.7 mL of 0.055 M Tris· HCl buffer at pH 7.8, with the addition of 0.0033 M $MgCl_2$. Introduce 0.1 mL of 0.006 M NADP (or 0.06 M NAD) and 0.1 mL of 0.1 M glucose-6-phosphate. Incubation was conducted in a spectrophotometer at 30°C for 7-8 minutes to ensure temperature equilibration and assess the blank rate. Following this, 0.1 mL of diluted enzyme was introduced, and the change in A340/min was measured over a period of 4-5 minutes. The A340/min was obtained from the initial linear portion of the curve. [30]

$$\text{Units/mg} = \frac{\Delta A340/\text{min}}{6.22 \times \text{mg enzyme/mL reaction mixture}}$$

Malondialdehyde (MDA)

Malondialdehyde (MDA) is a byproduct resulting from lipid peroxidation [33]. The adopted protocol was based on. [34] This method is based on the reaction between 2-thiobarbituric acid (TBA) and

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malondialdehyde (MDA), a byproduct of lipid peroxidation. Heating in an acidic pH results in the formation of a pink complex, which demonstrates maximal absorption at 532 nm and is extractable into organic solvents such as butanol. Malondialdehyde (MDA) functions as a calibration standard for this test, with results expressed as the amount of free MDA produced.

Assay Methodology
A 100 µl aliquot of the test sample was mixed with 400 µl of Tris-KCl buffer, and then 125 µl of 30 % Tricarboxylic Acid (TCA) was added. Following this, 125 µl of 0.75 % TBA was introduced and incubated in a water bath for 45 minutes at 80 °C. The mixture was cooled in ice to room temperature and then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected, and absorbance was measured at 532 nm using a SpectraMax plate reader, with distilled water serving as the reference blank.

Neurotransmitter Assay
Gamma Amino Butyric Acid (GABA)
GABA serves as the primary inhibitory neurotransmitter within the brain. This assay utilizes the competitive inhibition enzyme immunoassay method. Hippocampal homogenates were prepared in phosphate-buffered saline (PBS). The homogenates underwent centrifugation for 5 minutes at 10,000×g, after which the supernatants were promptly collected for assay. Fifty microlitres of samples or standards were added to each well, followed by the addition of 100 microlitres of Horseradish Peroxidase (HRP)-Conjugate reagent to each well, excluding the blank well for control. The wells were then covered with a plate sealer and incubated for one hour at 37 °C. Aspiration and washing were performed three times, followed by the addition of 100 µL of prepared detection reagent B and incubation for 30 minutes at 37 °C. The sample was aspirated and washed five times, followed by the addition of 90 µL substrate solution and incubation for 15-25 minutes at 37 °C. Fifty microlitres (50 µl) of stop solution were added, and the reading at 450 nm was conducted immediately. [29]

Statistical analysis
The collected data were presented as mean ± standard error mean (S.E.M). Statistical significance was assessed through one-way analysis of variance (ANOVA) utilizing GraphPad Prism (version 5). The Tukey post hoc test was employed to discern differences among individual means. The confidence interval was set at 95 %, indicating that a p-value of ≤ 0.05 was deemed significant in all instances.

Results and Discussion

Lead acetate-induced neurotoxicity is underscored by an adverse effect on hippocampal-dependent memory, learning and cognition. Lead can traverse the blood-brain barrier, leading to the generation of reactive oxygen species (ROS), which subsequently induces oxidative stress in various brain regions. Radial arm maze tests for spatial and reference memory in rodents, [34] was evaluated using the spatial explorative behaviour as seen in the number of arm entries and reference memory (see Fig. 1). Lead-acetate impaired reference memory as seen in the group B (Lead acetate only) when compared to the control, whereas flax seed extract attenuated the impairment as seen in group C (Lead acetate and 100mg of flax seed extract), D (Lead acetate and 200 mg/kg flax seed extract) and E (Lead + DMSA) most especially in the high dose group D (Lead acetate and 200 mg/kg flax seed extract), this supports the result obtained from Mason *et al.,* [35] stating that lead acetate has the ability to induce deleterious effects on the memory of rodents. For reference memory, there was no significant difference in number of arm entries across all groups. Group A (3.167 ± 0.3073), Group B (2.500 ± 0.6455), Group C (1.667 ± 0.3333), Group D (4.000 ± 1.000), Group E (3.000± 1.000) (P=0.2326; F=1.586). However, there was an observable increase in number of arm entries in Group D (4.000 ± 1.000) and a decrease in number of arm entries in Group C (1.667 ± 0.3333) When compared to Group A (3.167 ± 0.3073). Values are expressed as mean ± SEM of data obtained. Significant difference (p ≤ 0.05). In the total number of arm entries, there was no significant difference across all groups. Group A (5.833 ± 1.721), Group B (10.75 ± 2.869), Group C (9.000 ± 3.464), Group D (17.33 ± 5.840), Group E (9.000 ± 0.5774). (P=0.1436; F=2.041). There was an observable increase in Group D (17.33 ± 5.840) when compared to Group A (5.833 ± 1.721). Values are expressed as mean ± SEM of data obtained. Significant difference (p ≤ 0.05).

Glucose-6-phosphate-dehydrogenase is an essential rate limiting enzyme that catalyses the dehydrogenation of 6-phospahte glucose to NADPH. [37] G6PDH is important in redox homeostasis especially through the maintenance of catalase and reduced glutathione. [38] The activity of G6PDH in this study shows an increase in all groups when compared to the control group (see Fig. 2). Group E (lead + DMSA) has the highest level of G6PD followed by the higher dose of flaxseed crude extract which is Group D (lead + 200 mg/kg flaxseed). The increased level of G6PDH could be a response to the oxidative stress caused by lead neurotoxicity hence there is a relatively high expression of the enzyme. However, Flora and Pachauri [24] reported otherwise stating a decreased G6PDH activity in the brains of lead exposed rats. Abnormal fluctuations in G6PDH levels, whether reduced or increased, ultimately lead to a compensatory effect that triggers ROS production, which over time can result in symptoms resembling neurodegenerative diseases. [23] The G6PDH level for Group A was 0.01667 ± 0.003756 u/g, Group B was 0.01933 ± 0.004055 u/g, Group C was 0.02333 ± 0.005925 u/g, Group D was 0.02733 ± 0.007055 u/g, Group E was 0.03133 ± 0.008969 u/g. There was an observable increase in all groups when compared to the control group. There was no significant difference across all groups. (P=0.5025; F=0.8939). Values are expressed as mean ± SEM of data obtained. Significant difference (p ≤ 0.05).

Malondialdehyde is an end-product following lipid peroxidation, it is produced when there is depletion of the cellular membrane phospholipids, [41] it is an important biomarker for lipid peroxidation. [40] In this study, there was a reduced level of MDA in the lead + 100mg/kg of flax seed extract when compared to the control, However, the other treated group showed a significant increase compared to the control, indicating a dose-dependent effect of the aqueous flax seed extract in treating lead-induced neurotoxicity. This study however is in contrast with the study conducted by Chera *et al.* [43] stating that flax seed ethanolic extract lowered reactive oxygen species and malondialdehyde. The extremely high level of malondialdehyde seen in the Lead + DMSA group could be accrued to the inability of DMSA to cross the cell membrane and the blood brain barrier. [24] Over the years, the effects of flax seed oil have been reported to be conflicting, which might also be the same issues for the crude extract. Malondialdehyde level for Group A was 0.0150 ± 0.0010 mm/L, Group B was 0.0190 ± 0.0010 mm/L, Group C was 0.0150 ± 0.0100 mm/L, Group D was 0.0390 ± 0.0020 mm/L, Group E was 0.0465 ± 0.0035 mm/L. There was statistical significance in the Lead + DMSA group when compared to the control and the Lead + 100 mg/kg flax seed (P= 0.0156; F=9.262). Values are expressed as mean ± SEM of data obtained. Significant difference (p ≤ 0.05).

The GABAergic system is the major inhibitory system in the central nervous system (CNS). [38] The analysis carried out on the level of GABA shows a slight increase in all the groups when compared to the control group. The use of chelating agent such as odium Dimercaptosuccinic acid as well as other pharmacological intervention increased the level of GABA. This study supports that of Soares *et al.,* [40] stating that chelating therapy is the most effective means of treating metal intoxication and it does not interfere with neurotransmitter systems including the GABAergic system. The mechanism of action pertains to the ways in which aqueous flax seed extract and DMSA mitigate lead neurotoxicity by diminishing lead's detrimental effects on the nervous system. DMSA is a recognized chelator that binds to heavy metals such as lead, facilitating their excretion from the body via urine. DMSA binds to lead ions in the bloodstream and tissues, mitigating lead neurotoxicity by diminishing its capacity to disrupt normal cellular functions. DMSA mitigates lead-induced oxidative stress, hence safeguarding neurons and preventing or diminishing damage to the nervous system. While flax seed extract is not a chelator such as DMSA, it seems to mitigate the harmful effects of lead in a comparable manner via regulating oxidative stress. Flax seeds are abundant in antioxidants such as lignans and omega-3 fatty acids, which assist in neutralizing

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ROS and mitigating oxidative damage to neurons. Lead exposure elevates the generation of reactive oxygen species, hence exacerbating neurotoxicity. The antioxidants in flax seed extract likely diminish ROS formation or neutralize existing ROS, so safeguarding neurons from injury. Both substances serve to safeguard the nervous system against lead poisoning by mitigating oxidative stress; however, DMSA directly eliminates lead, whilst flax seed extract counteracts the detrimental consequences of lead exposure. GABA level for Group A was 02270 ± 0.004041, Group B was 0.2293 ± 0.004631, Group C was 0.2327 ± 0.006009, Group D was 0.2303 ± 0.006888, Group E was 0.2400 ± 0.009165. There was no significant difference across all groups. (P=0.6290; F=0.6675). Values are expressed as mean ± SEM of data obtained. Significant difference ($p \leq 0.05$).

Neuronal observation of the CA1, 2, 3 and DG regions using haematoxylin and eosin and cresyl violet (Plates 1 and 2 respectively) showed excessive neuronal degeneration with cellular manifestations such as pyknosis and vacuolation in the Lead only and Lead+ 100 mg/kg flax seed crude extract. Lead + 200 mg/kg and Lead + DMSA group showed the mild appearance of pyknotic cells and vacuolation suggesting the ameliorative properties of *Linum usitatissimum.* This study supports the report of Abdel-Moneim *et al.,* [44] showing the protective role of flax seed oil on lead-acetate induced neurotoxicity Mahmoud and Sayed [45] also reported extensive neuronal layer disarrangement and vacuolation following lead-acetate assault.

Tumor necrotic factor alpha (TNF-*α*) is a pro-inflammatory cytokine produced in response to injury in the central nervous system. As a key mediator for neuroinflammation cascades, it is generated by astrocytes, microglia and neurons. [46-48] Upregulation of TNF-*α* in the brain especially the hippocampus has been linked to the onset of neurodegenerative diseases like Alzheimer's as well as impairment in spatial memory and other hippocampal-dependent memories. [49] In this study, moderate immunoreactivity was seen in the group B (Lead), C (Lead + 100 mg/kg), D (Lead + 200 mg/kg) and E (Lead + DMSA) which suggests that lead produces inflammation in the brain and the TNF-*α* reactivity is seen as a response to this effect (Plate 3). TNF-α has been discovered to produce a dual effect depending on the receptors expressed; R1 receptors activates the apoptotic cascade hence resulting in neuronal damage while R2 receptors play a neuroprotective function as well as synaptic plasticity. [50] This study did not elucidate the exact mechanism of action in TNF-*α* in lead induced neurotoxicity; whether or not there was a synergistic effect between Lead and Flax seed crude extract, the question remains unanswered and a potential area of research in neurotoxicity.

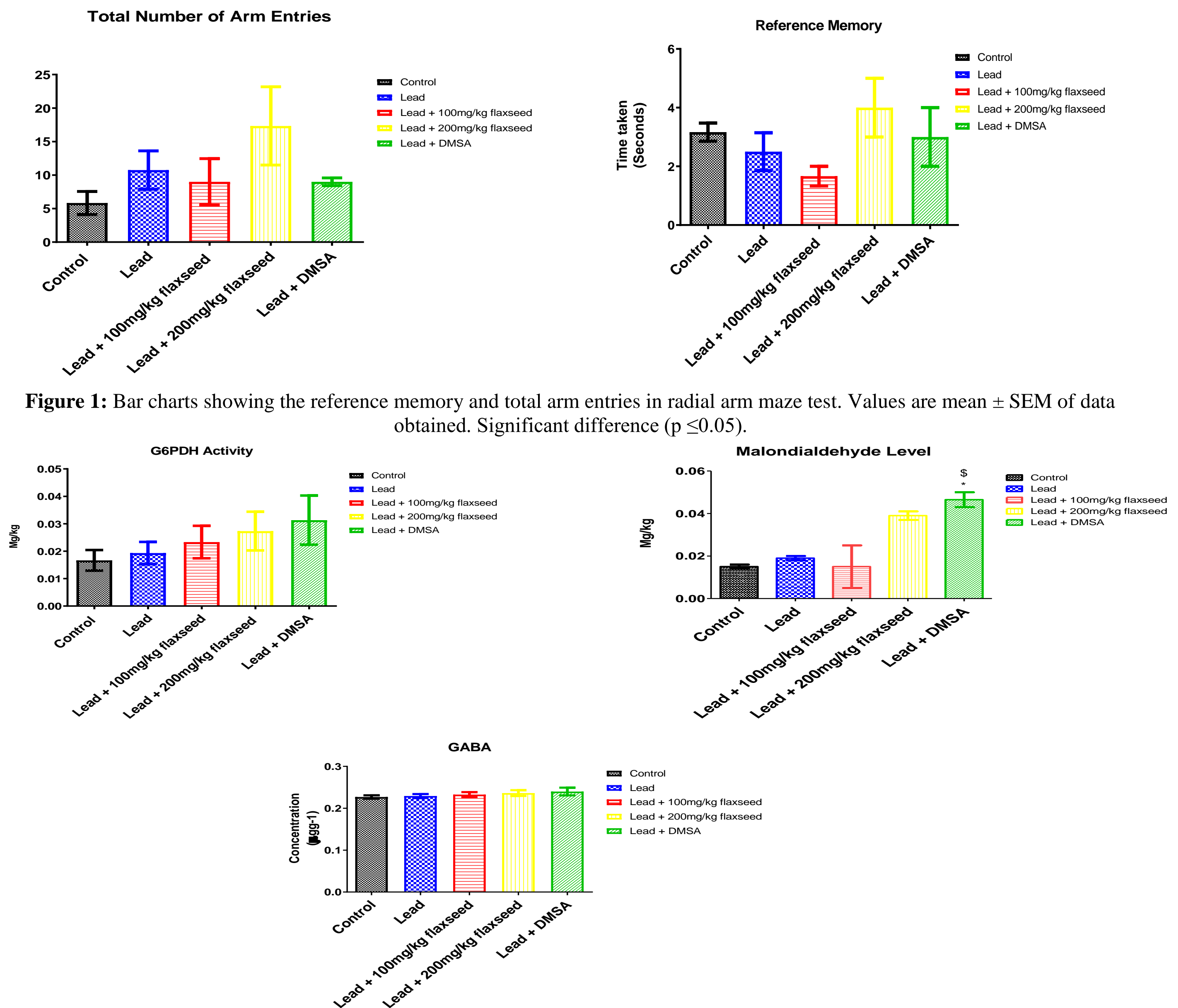

Figure 1: Bar charts showing the reference memory and total arm entries in radial arm maze test. Values are mean ± SEM of data obtained. Significant difference ($p \leq 0.05$).

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Figure 2: Bar charts showing the activity of Glucose-6-phosphate dehydrogenase, the concentration of Gamma Amino Butyric Acid and Malondialdehyde. Values are expressed as mean ± SEM of data obtained. In MDA, there was statistical significance when compared to control and Lead + 100mg/kg difference ($p \leq 0.05$).

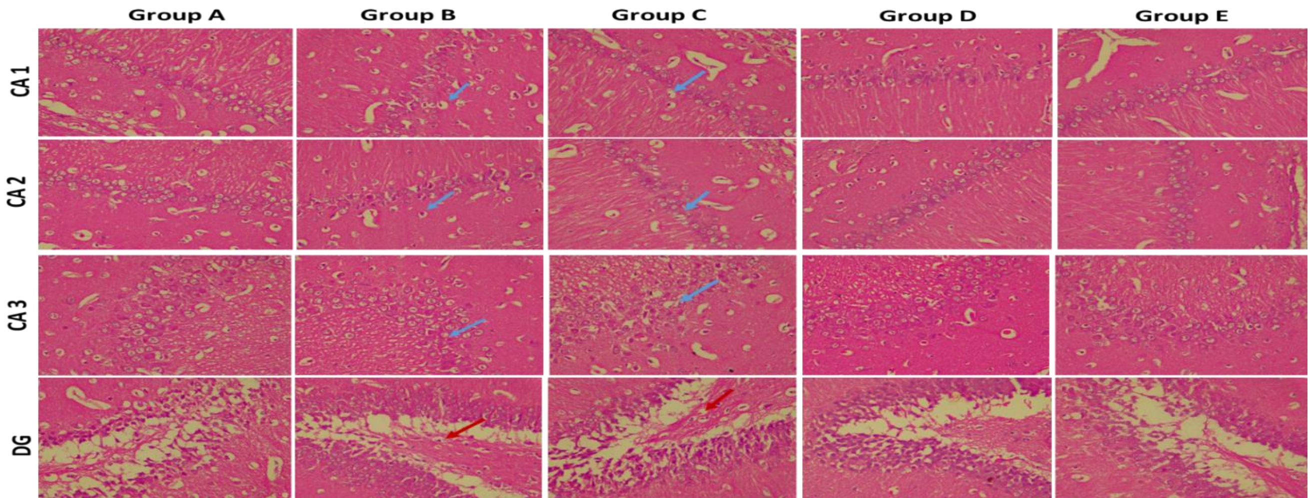

Plate 1: Representative Photomicrographs showing the Cornus Ammonis 1-3 (CA 1-3) and dentate gyrus (DG) region of the hippocampal formation, stained with Haematoxylin and Eosin X400. The control group A shows the normal histoarchitecture of the regions of interest. Group B and C shows evidence of excessive neuronal vacuolation in CA1-3 (Blue arrows) and pyknosis (Red arrows) in DG. Group D and E shows evidence of mild neuronal vacuolation across the regions.

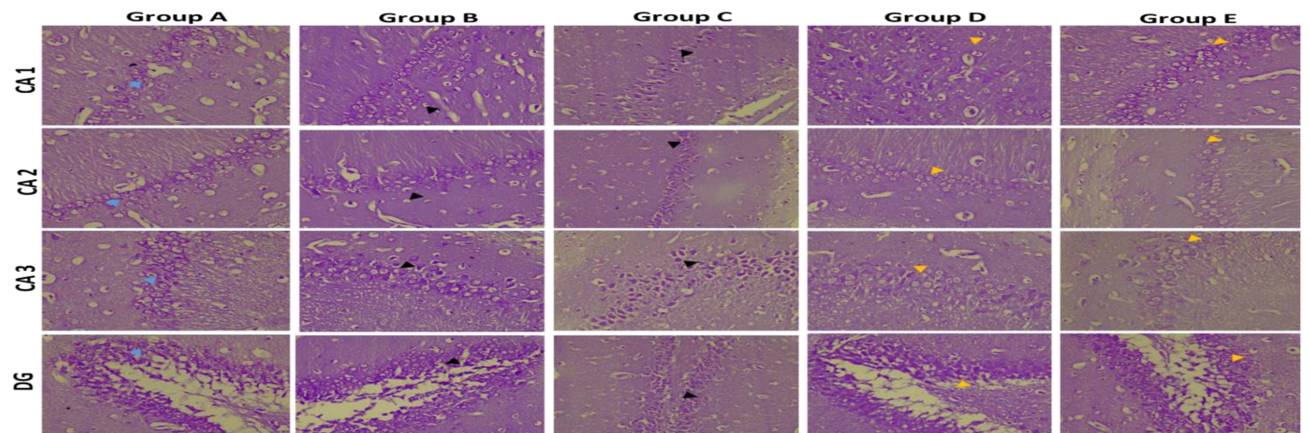

Plate 2: Representative photomicrographs showing Cornus Ammonis 1-3 (CA 1-3) and dentate gyrus (DG) region of the hippocampal formation, stained with Cresyl Fast violet X400. The control group A shows Nissl granules well distributed in the neurons (Blue arrow head). Group Band C shows extensive loss of Nissl granules (Black arrows), Group D and E shows evidence of mild loss of Nissl granules in the regions of interest (Yellow arrows).

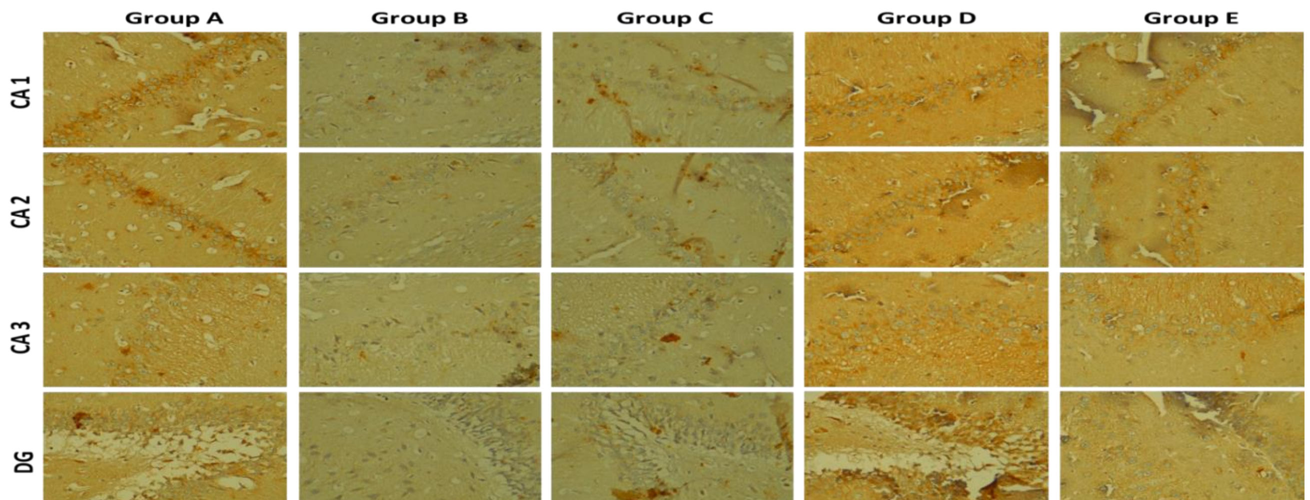

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Plate 3: Representative photomicrographs showing the immunoexpression of Tumor Necrotic Factor-Alpha (X400) in the Cornus Ammonis (CA) 1-3 and dentate gyrus (DG). Across all the regions of interest, there was very high immunoreactivity in Groups A, D and E while low immunoreactivity was observed in Groups B and C.

Table 1: experimental design

GROUPS	NO OF ANIMALS	TREATMENT SCHEDULE	RATIONALE
GROUP A	6	Distilled water	Control group
GROUP B	6	Oral administration of Lead acetate 5 mg/kg for 14 days	Negative control group to induce damage
GROUP C	6	Lead acetate 5 mg/kg + flaxseed extract 100 mg/kg for14 days	Therapeutic effect
GROUP D	6	Lead acetate 5 mg/kg + flaxseed extract 200 mg/kg for 14 days	Therapeutic effect
GROUP E	6	5 mg/kg lead + 0.2mg/kg Dimercaptosuccinic acid (standard drug) for 14days	To compare the action of flaxseed extract with Dimercaptosuccinic acid

Conclusion

The administered neurotoxic agent Lead Acetate caused extensive neuronal damage to the hippocampus resulting in impaired spatial working memory, memory loss and impaired learning in Wistar rats. Higher doses of flax seed crude extract exert an ameliorative effect on the histoarchitecture and functions of hippocampus by modulating the level of oxidative stress markers.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors declare that the work presented in this article is original and that they will assume liability for any claims related to its content.

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