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Neuroprotective Effects of Vitexin and *Cajanus cajan* Extract Against Pb-induced Neurotoxicity in Wistar Rats

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ABSTRACT

Background: The effects of Pb-induced neurotoxicity have been largely established; however, the need for proper neuroprotective agents to mitigate the effects of Pb-induced neurotoxicity remains vague.

Objectives: This study aimed at investigating the neuroprotective effects of pre-treatment with vitexin and *C. cajan* extract against Pb-induced neurotoxicity in Wistar rats.

Methods: Twenty-four male Wistar rats were randomly assigned into four groups (n=6). The control group was administered 0.5 mL of distilled water, Pb group received Pb acetate (200 mg/kg), vitexin+Pb group received Vitexin (50 mg/kg) an hour before Pb acetate (200 mg/kg), and *C. cajan*+Pb group received *C. cajan* (50 mg/kg) an hour before Pb acetate (200 mg/kg). All treatments were done within 28 days. Barnes maze test and novel object recognition (NOR) test were done to ascertain working memory, while the levels of oxidative stress markers (MDA, SOD, and GPx) were also tested. In addition, prefrontal cortical sections were stained with H&E stain, and the immunoreactivity of Iba1 and Nrf2 was examined.

Results: There was a significant decline in working memory in the Pb group, but good working memory was maintained in vitexin and *C. cajan* pretreated groups. In vitexin and *C. cajan* pretreated groups, oxidative stress, neuronal damage, and expression of Iba1 were significantly low compared to the Pb group. Also, Nrf2 expression in the Pb group was significantly low compared to other groups.

Conclusion: Pretreatment with vitexin and *C. cajan* offers neuroprotection against Pb toxicity via antioxidant and anti-inflammation actions. Although both vitexin and *C. cajan* extract showed neuroprotective abilities, vitexin exhibited better results.

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Introduction

Lead (Pb) can transverse the blood-brain barrier and cause neurotoxic effects in both humans and animals. Lead-induced neurotoxicity is influenced by various factors, such as the dose of exposure and duration of exposure [1-4]. Within the brain, Pb promotes protein modifications and causes alteration in signaling pathways and enzyme levels, morphological changes, neuroinflammation, neurodegeneration, and cognitive deficits [1, 2, 5]. Exposure to Pb produces oxidative imbalance via excess production of pro-oxidant with concomitant depletion of anti-oxidant reserves. This, in turn, results in oxidative damage, disruptions in neuronal signaling and neurotransmitter release as well as deficits in behavior [2]. Evidence from previous studies has shown that Pb exposure influences inflammatory processes in the brain [1] and disrupts nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE).

The prefrontal cortex (PFC), a part of the brain, is responsible for working memory, higher reasoning, decision-making, and many other executive functions [6, 7]. The PFC is susceptible to Pb-induced damage [8]. Early-life exposure to Pb may not manifest effects immediately but may manifest at later stages of development or in adulthood [9].

A review of the literature has shown that the consumption of certain mineral elements, vitamins, and flavonoids can offer protection against Pb-induced neurotoxic effects [3]. These nutrients, including flavonoids, have been reported to play a significant role in restoring the imbalance between pro-oxidant and antioxidant molecules. Flavonoids are a group of natural secondary metabolites that are found in plants and possess different phenolic structures [10]. Most flavonoids possess antioxidant properties.

Vitexin is a flavonoid that is extracted mainly from hawthorn plants and other plants, such as *Cajanus cajan* [11]. Vitexin possesses various properties, such as anti-oxidative, anti-tumour, anti-viral, anti-inflammatory, anti-bacterial, anti-hypertensive, anti-nociceptive, anti-diabetic, anti-depressant, and neuroprotection [11, 12]. It is believed that vitexin has an antioxidant effect via suppression of reactive oxygen species (ROS) formation, scavenging ROS generated, and up-regulation of cell antioxidant defenses [12, 13].

C. cajan also known as pigeon pea is a well-known plant source of vitexin [14, 15]. It is a leguminous plant cultivated largely in rain-fed semi-arid tropical regions [15]. *C. cajan* is rich in flavonoids (including vitexin and orientin), saponin, tannins, resins, and terpenoids. It also possesses cajanin, pinostrobin, genistein, genistin, longistylin C, and longistylin A [15]. Vitexin is one of the most abundant flavonoids in the leaves of *C. cajan* with a concentration of 21.03 mg/g [16]. Due to the presence of several compounds, *C. cajan* has been reported to possess antioxidant, anti-cancer, anti-microbial, anti-inflammatory, anti-diabetic, hepatoprotective, and neuroactive properties [14, 15].

Although a few studies have reported the neuroprotective and anti-oxidant properties of vitexin, more data on its neuroprotective and anti-oxidant properties against Pb-induced neurotoxicity are needed. Furthermore, data to support vitexin as a direct substitute for *C. cajan* extract against Pb-induced neurotoxicity is limited or lacking. Hence, this study aimed at investigating the neuroprotective effects of vitexin and *C. cajan* pre-treatment (aqueous leave extract) against Pb-induced neurotoxicity in Wistar rats via the anti-oxidant pathway.

Materials and methods

Preparation of aqueous leave extract of *C. cajan*

The leaves of *C. cajan* were harvested from Mission hill farm in Ugwolawo, Kogi State, Nigeria and authenticated at the Faculty of Agriculture, Kogi State University, Anyigba. The leaves of *C. cajan* were air-dried and ground into powdered form (500 g) using a manual blender. Thereafter, the powder was soaked in distilled water (4 L) for 24 hours at room temperature. After 24 hours, the mixture was agitated vigorously and filtered. The filtrate was evaporated to yield 19.3 w/w. The extracted yield was stored in the airtight bottle at 4°C until use.

Preparation of treatment solutions

Vitexin was purchased from Sigma-Aldrich (St Louis, MO, USA) while Pb acetate and other materials were purchased locally (Ilorin, Nigeria). Distilled water was used to dissolve Pb acetate (0.5 mL), vitexin (0.5 mL), and *C. cajan* (0.5 mL) each morning before treatment.

Experimental design

Animals and grouping

Twenty-four male Wistar rats (eight weeks old, weighing 200-240 g) were procured from Temi animal husbandry center, Ogbomosho. The rats were randomly assigned to four groups (n=6) and acclimatized for seven days under approximately 12 hours light/dark cycle at the animal house of Central Research Laboratory, the University of Ilorin before treatment. All experimental procedures in the study were in accordance with the University of Ilorin Ethical and Review Committee for the use of animals (approval number: UERC/ASN/2018). The experiment also followed the guideline of the National Institute of Health guide for the care and use of laboratory animals (NRC Publication, 8th edition, 2011).

Administration of treatment

The control group received 0.5 mL/kg of distilled water via oral gavage. The Pb group received an oral dose of Pb acetate (200 mg/kg). Vitexin+Pb group received Vitexin solution (50 mg/kg) an hour before Pb acetate (200 mg/kg). *C. cajan*+Pb group received *C. cajan* solution (50 mg/kg) an hour before the oral dose of Pb acetate (200 mg/kg). Treatment was done within 28 days via oral gavage. The doses used in the study were based on a pilot study carried out earlier by the authors to determine appropriate and effective doses.

Behavioral tests

Barnes maze test

This test was carried out to determine the learning memory of rats in the experiment using the modified description of Rosenfeld and Ferguson [17]. The main apparatus of this test consists of a circular table with 20 holes around the perimeter. Nineteen holes open directly to the ground, while the remaining holes end up in a dark box. During the trials, the rats were assisted in their quest to locate the drop box from their initial position at the center of the table. Subsequently, the rats were allowed to find the drop box on their own. Their activities during the test were recorded by a video camera and the following parameters were later analyzed: Errors made by the rats (number of incorrect holes visited before the correct hole) and latency to find the correct hole/dropbox.

Novel object recognition (NOR) test

This test was carried out using the method described by Huang and Hsueh [18]. The apparatus for this test comprises a wooden box (100 c x100 cm x50 cm), colored ping pong balls (one pink and two green), and a video camera for recording. The first two days of this test were used for habituation. The third day was the trial. During the trial, two identical green balls were placed in the test box and each rat was allowed to explore for 10 minutes. On the fourth day (main test), one pink ball (novel object) and one green ball (one of the old objects previously explored) were placed in the box for 10 minutes of exploration by each rat from all the study groups. At the end of this test, the percentage of preference for the new object was calculated using the below Equation 1:

$$1.\quad \%\ \text{Preference for new object} = \frac{\text{Time use for new object X100}}{\text{Total time for objects 1}}$$

Total exploration time for objects

Sample collection and analyses

Biochemical analyses: At the end of the behavioral tests, the rats were euthanized with 90 mg/kg of ketamine and their brains were excised. Afterward, the prefrontal cortices (PFC) were excised from the brain between coordinates 1.8 to 3.1 mm anterior to the bregma. The excised tissues were washed with phosphate buffer saline (PBS). Then, 10%(w/v) of the tissue was homogenized with ice-cold 0.25M sucrose in a Teflon Potter–Elvehjem homogenizer (Hamburg, Germany). The tissue homogenate from each group was centrifuged at 12,000×g for 10 minutes at 4°C. Supernatants obtained from the groups were decanted and subsequently used for biochemical analysis of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), which was done based on the manufacturer's instructions in test kits all purchased from Sigma-Aldrich (St Louis, MO, USA). The absorbance was measured at 532 nm for MDA, 450 nm for SOD, and 340 nm for GPx.

Histopathology analyses: Rats for histological tests were anesthetized with 75 mg/kg of ketamine before transcardiac perfusion with a 4% paraformaldehyde (PFA) solution. The PFC tissues were post-fixed in 4% PFA for 24 hours before dehydration and clearing processes. Tissues were processed as paraffin wax embedded in the blocks and sectioned at 5 µm. Tissues were stained with hematoxylin and eosin (H&E) dye for routine examination of the histoarchitecture using procedures described by Bancroft and Gamble [19].

For the immunohistochemical assay, protein cross-linkages were removed from the sections by applying 0.1% trypsin for 20 minutes at room temperature. Endogenous peroxidase blocking was done using hydrogen peroxide. Anti-Iba1 monoclonal antibody (Abcam, EPR16588; dilution, TBS with 1% BSA, 1:2000) and anti-Nrf2 antibody (ab31163; dilution, TBS with 1% BSA, 1:100) were applied on tissue sections for incubation (60 minutes) at 37°C. The secondary antibody (goat anti-rabbit, 1:200) was diluted in TBS with 1% BSA and incubated with the slides at room temperature (60 minutes). An immunogenic reaction was developed using 3′3′ diaminobenzidine tetrachloride (DAB) for 10 minutes at room temperature. The slides were counter-stained with Haematoxylin and afterward treated with 1% acid alcohol to reduce the counterstain intensity.

Photomicrography

Stained sections were viewed under a light binocular microscope (Olympus, New Jersey, U.S.A.) attached to an Amscope (MD500, CA, USA). The various parts of the PFC were identified using the description of Van De Werd and Uylings [20].

Quantitative Analysis: The H&E-stained, Iba1, and Nrf-2 immuno-stained sections were analyzed with ImageJ software (v. 1.52r). From each tissue block, five sections of six different visual fields were examined at varying magnifications of 25-180µm. For H&E-stained sections, the number of normal pyramidal neurons was counted in layer 2/3 of PFC for each of the groups. Criteria for a normal neuron counted were based on the absence of shrunken or fragmented cytoplasm as well as condensed nuclei. Additionally, the number of immunoreactive Iba 1 cells was counted based on the criteria, which include clearly observed cell bodies with extended processes. The expression of Nrf2 in the tissue was scored from a total of 50 neurons.

Statistical analyses

Data analyses were done with the aid of Graph Pad Prism version 5.0 for Windows. The mean difference was determined using one-way ANOVA followed by Tukey's post hoc test. The significant difference was defined at $P<0.05$. All data were expressed as Mean±SE, with n representing the number of animals used in each experiment.

Results

Influence of vitexin and *C. cajan* on learning memory deficits caused by Pb toxicity

The result of the Barnes maze test (Figure 1A & 1B) showed that rats in all the study groups had the same latency to find the dropbox during the first and second days of acquisition. However, on the third day of acquisition (Figure 1C), the rats in the Pb-treated group showed longer ($P<0.05$) latency vs. the vitexin+Pb group. On the fourth day of acquisition (Figure 1D), rats in the Pb-treated group showed longer latency to find dropbox ($P<0.05$) compared to the control group. Conversely, both vitexin+Pb and *C. cajan*+Pb groups showed shorter latency than the Pb- treated group. The same trend of learning ability observed on the fourth day of the acquisition was also observed on the fifth day (Figure 1E).

On the retention day (Figure 1F), rats in the vitexin+Pb group (29.5±3.1 s) showed similar ($P>0.05$) latency to find the dropbox compared to the control group. On the other hand, rats in the Pb-treated group and *C. cajan*+Pb group showed longer latency to find the dropbox when compared with the control group. Furthermore, the *C. cajan*+Pb treated group showed longer latency to find the dropbox compared to the vitexin+Pb group (Figure 1F).

The result of the NOR test (Figure 2) showed that rats in the Pb-treated group had a lower ($P<0.05$) preference for a new object than the control group. Conversely, rats in the vitexin+Pb group displayed a high preference ($P<0.05$) for the new object compared to the Pb-treated group. Furthermore, there was no significant difference ($P>0.05$) in rats' preference for a new object between the vitexin+Pb and *C. cajan*+Pb groups compared to the control group.

Changes in oxidative stress markers

MDA levels in the Pb-treated group were significantly high ($P<0.05$) compared to the control group (Table 1). Similarly, the levels of MDA in the Pb-treated group were significantly higher ($P<0.05$) compared to the Vitexin+Pb and *C. cajan*+Pb groups. Furthermore, the MDA levels in the vitexin+Pb and *C. cajan*+Pb groups were significantly higher ($P<0.05$) compared to the control group. Conversely, MDA levels in vitexin+Pb and *C. cajan*+Pb groups were not different ($P>0.05$) from the control group.

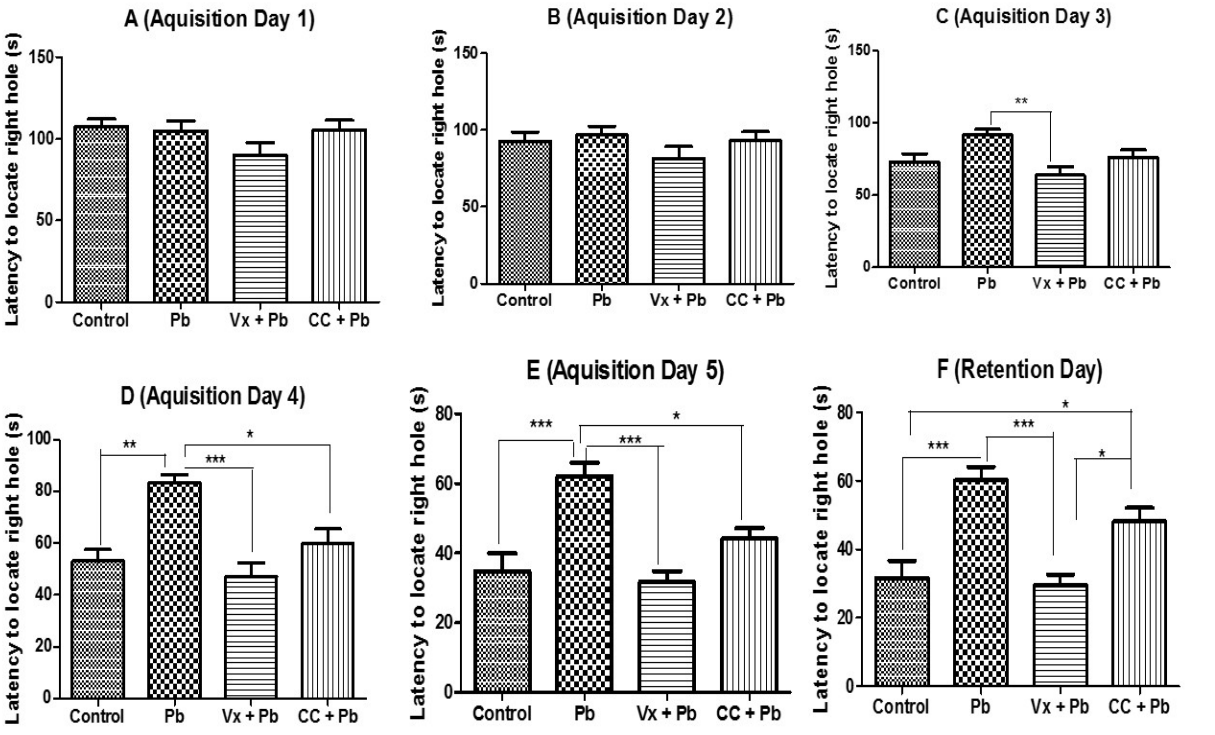

Figure 1. Barnes maze test results

Data are expressed as Mean±SE (n=6); Control: control group; Pb: lead-treated group; Vx+Pb: vitexin+lead group; CC+Pb: *C. cajan*+vitexin group. *P<0.05; **P<0.01; ***P<0.001.

SOD levels in the Pb-treated group were significantly lower (P<0.05) compared to the control group (Table 1). Similarly, the levels of SOD in the Pb-treated group were significantly lower compared to the vitexin+Pb group. Furthermore, the SOD levels in the *C. cajan*+Pb group were significantly lower (P<0.05) compared to the control group. However, no significant difference (P>0.05) was found between the vitexin+Pb group and the control group. Similarly, SOD levels in vitexin+Pb and *C. cajan*+Pb groups were not significantly different (P>0.05). Finally, SOD levels in the Pb-treated group were not significantly different from the *C. cajan*+Pb group.

The levels of GPx in the Pb-treated group were significantly lower (P<0.05) compared to the control group (Table 1). Similarly, the levels of GPx in the Pb-treated group were significantly lower compared to the vitexin+Pb group. Furthermore, GPx levels in the *C. cajan*+Pb group were significantly lower compared to the control group. However, GPx levels in the vitexin+Pb group were not significantly different (P>0.05) than the control group. Similarly, GPx levels in the vitexin+Pb group were not significantly different (P>0.05) from the *C. cajan*+Pb group (Table 1). Also, the GPx levels in the

Table 1. Levels of oxidative stress markers in the study groups

Parameters	Mean±SE			
	Control	Lead (Pb)	Vitexin+Pb	*C.cajan*+Pb
MDA (U/mg)	0.96±0.1	1.75±0.04*	1.29±0.03*#	1.36±0.01*#
SOD (U/mg)	3.07±0.3	1.70±0.4*	2.77±0.2#	2.11±0.3*#
GPx (U/mg)	3.40±0.2	1.62±0.05*	2.97±0.16 #	2.31±0.4*#

Data are represented as Mean±SE (n=5). MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase. *Significantly different(P<0.05) compared to the control group. # Significantly different (P<0.05) compared to the Pb group.

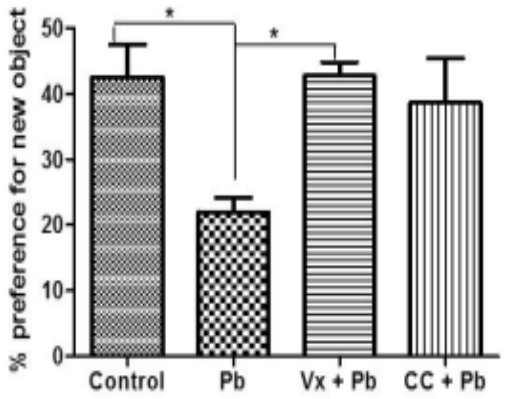

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Figure 2. Novel object recognition test results

Data are expressed as Mean±SE (n=6); Control: control group; Pb: lead-treated group; Vx+Pb: vitexin+lead group; CC+Pb: *C. cajan*+vitexin group. *P<0.05.

Pb-treated group were not significantly different from the *C. cajan*+Pb group.

Effect of vitexin and *C. cajan* on the histoarchitecture of the prefrontal cortex in Pb-exposed rats

The PFC photomicrograph of the control group (Figure 3A) was characterized by numerous large pyramidal neurons (yellow arrow) with very few degenerating neurons (red arrow). The representative photomicrograph of the Pb-treated group (Figure 3A) presented degenerative changes characterized by numerous shrunken neurons with fragmented cytoplasm and condensed nuclei (red arrows). The photomicrograph of the vitexin+Pb group (Figure 3A) presented fewer shrunken neurons and few degenerative changes (red arrow) compared to the Pb-treated group. Furthermore, the photomicrograph of the *C. cajan*+Pb group showed fewer neurons with fragmented cytoplasm compared to the Pb-treated group (Figure 3A). The PFC histoarchitecture in the vitexin+Pb and *C. cajan*+Pb groups appeared similar.

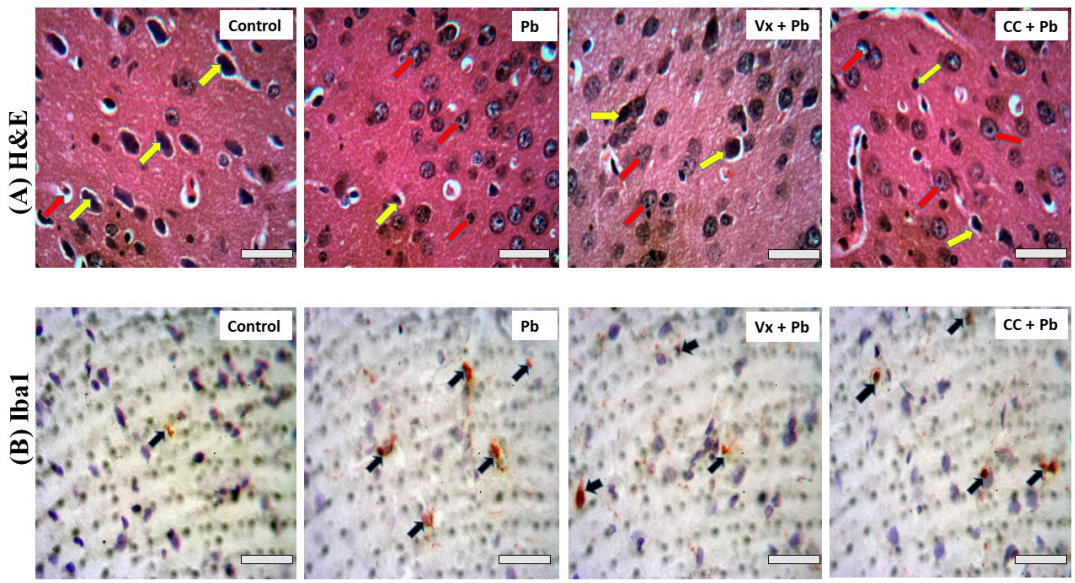

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Figure 3. Representative photomicrograph of hematoxylin and eosin (H&E) stain as well as Iba1 stain in rats

Control: control group; Pb: lead-treated group; Vx+Pb: vitexin+lead group; and CC+Pb: *C. cajan*+lead acetate group; Yellow arrows: pyramidal neurons; Red arrows: degenerative changes; Black arrows: microglial cells: Scale bar=45 µm.

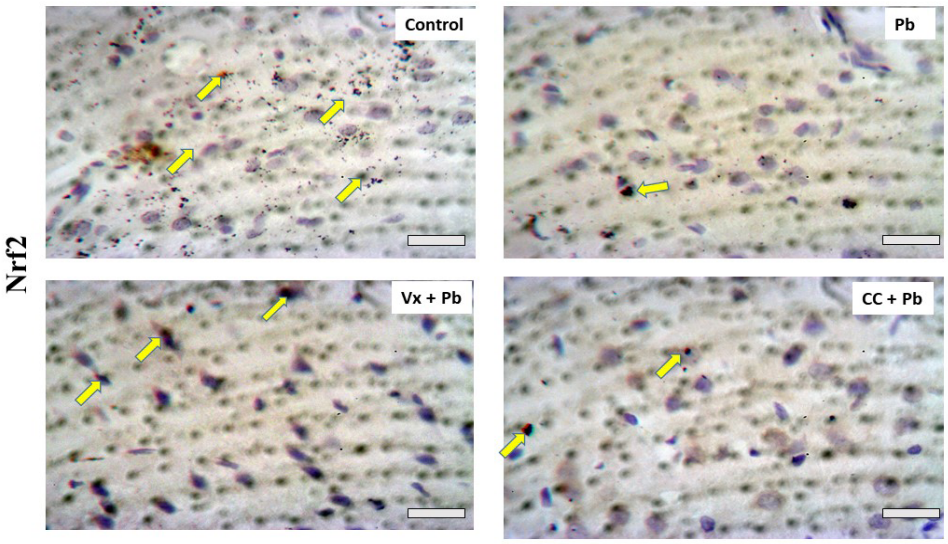

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Figure 4. Representative photomicrograph of nuclear factor erythroid 2-related factor 2 (Nrf2) stain of the prefrontal cortex in rats
Control: control group; Pb: lead-treated group; Vx+Pb: vitexin+lead group; CC+Pb: *C. cajan*+lead acetate group. Yellow arrows: Nrf2 expression; Scale bar=45 μm.

Effect of vitexin and *C. cajan* on microglial cells and Nrf2 expression in rats' prefrontal cortex following Pb exposure

In Figure 3B, the PFC photomicrograph of the Pb-treated group was characterized by numerous activated microglial cells compared to the photomicrograph of the control group. Although vitexin+Pb and *C. cajan*+Pb groups presented activated microglial cells, the number of activated microglial cells appeared fewer than in the Pb-treated group (Figure 3B). Comparatively, activated microglial cells were more in the *C. cajan*+Pb group than in the vitexin+Pb group (Figure 3B).

The expression of Nrf2 in the PFC of all the experimental groups was not up to the level of expression seen in the control group (Figure 4). Furthermore, Nrf2 expression in the vitexin+Pb group was more than in the Pb-treated and *C. cajan*+Pb groups (Figure 4). The expression of Nrf2 in both Pb-treated and *C. cajan*+Pb groups appeared similar.

Discussion

One of the well-known oxidative stress markers used in diagnosing cellular damage is MDA. MDA is one of the bye products of lipid peroxidation [22, 23]. In this study, the MDA levels in the Pb-treated group were significantly higher than the control group (Table 1). However, in groups where vitexin and C. cajan were treated with Pb, the MDA level was significantly low. This result infers that Pb exposure promotes lipid peroxidation as also reported by previous studies [24, 25]. Vitexin treatment reduces lipid peroxidation and oxidative damage [26]. This could be the reason behind the low levels of MDA observed in the vitexin+Pb and *C. cajan*+Pb groups.

Antioxidants, like SOD and GPx, reduce peroxides into innocuous molecules [27]. This study showed low SOD and GPx levels in the Pb-treated group compared to the control group. However, SOD and GPx levels in both vitexin+Pb and *C. cajan*+Pb groups remained significantly high compared to the Pb-treated group [21]. This result infers that vitexin as well as *C. cajan* have the ability to up-regulate and maintain the activity levels of SOD and GPx even after Pb exposure. This result also signifies that superoxide radicals and peroxides will be quickly reduced into innocuous molecules in groups where vitexin and *C. cajan* were administered.

Cellular degeneration occurs when there is an abnormal biochemical function, recognizable structural change, or a combination of both factors. Degeneration is reversible but may progress to necrosis and clinical disease if the injury persists [28]. The Nrf2 is responsible for activating transcription factors, like antioxidant response element (ARE) in response to oxidative stress [29]. Microglia cells (immune cells of the CNS) are activated in response to toxic or pathogenic insults, which leads to neuronal damage and also participates in the removal of damaged cells [30].

The histologic observation (Figures 3 and 4) in the Pb-treated group of this study showed high degenerative changes, numerous activated microglia cells, and low expression of Nrf2 compared to the control group as well as groups where vitexin and *C. cajan* were administered (vitexin+Pb and *C. cajan*+Pb groups). These results indicate that Pb administration (Pb-treated group) will cause neuronal damage. In response to the damage, numerous microglial cells were activated to remove the damaged cells. Vitexin and *C. cajan* pre-treatments may have reduced Pb-induced insult and in turn, reduced the number of activated microglial cells in the vitexin+Pb and *C. cajan*+Pb groups. Furthermore, the low expression of Nrf2 in the Pb-treated group suggests that prefrontal cortical neurons in this group will be susceptible to oxidative stress. An earlier study stated that neurons lacking Nrf2 are more susceptible to oxidative stress [31]. The rationale behind the low expression of Nrf2 in the Pb-treated group in this study is not fully understood but it is possible that Pb may have interfered with the oxidation of sulfhydryl groups on specific cysteines in Keap1 (kelch-like ECH-associated protein) [32], which in turn reduced the release of Nrf2 by Keap1.

Neurons of the PFC have been linked to cognitive functions as they communicate with other parts of the brain, particularly the hippocampus [33, 34]. Loss of these neurons suggests that cognitive functions will be impaired. The tissue damage and loss of neurons in the Pb-treated group of this study (Figure 3A) could be the reason behind deficits observed in learning memory (Figures 1 and 2). Pretreatment with vitexin and *C. cajan* before Pb exposure reduced tissue damage and loss of neurons. This outcome could be the reason behind the good learning memory maintained by rats pretreated with either vitexin or *C. cajan* in this study.

Conclusion

The results of this study showed that pretreatment with vitexin and *C. cajan* significantly reduced oxidative stress, neuronal damage, and microglial activation that would have resulted from Pb toxicity. Furthermore, vitexin and *C. cajan*, helped to maintain good working memory in rats. Although both vitexin and *C. cajan* showed neuroprotective abilities, vitexin showed better results. Further studies are recommended to identify how vitexin and *C. cajan* influence the expression of Nrf2 after Pb exposure.

Ethical Considerations

Compliance with ethical guidelines

Animals were handled according to the requirements and approval of the Ethical Committee of the University of Ilorin and also in line with the recommendations of the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Publication 1985).

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Authors' contributions

Conceptualization, literature review, final manuscript approval: Nathaniel Ohiemi Amedu; Data analysis, the initial manuscript writing: Michael Olim Obu; laboratory tests: the both authors.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Wani AL, Ara A, Usmani JA. Lead toxicity: A review. Interdiscip Toxicol. 2015; 8(2):55-64. [DOI:10.1515/intox-2015-0009] [PMID] [PMCID]

[2] von Stackelberg K, Guzy E, Chu T, Claus Henn B. Exposure to mixtures of metals and neurodevelopmental outcomes: A multidisciplinary review using an adverse outcome pathway framework. Risk Anal. 2015; 35(6):971-1016. [DOI:10.1111/risa.12425] [PMID] [PMCID]

[3] Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Interdiscip Toxicol. 2012; 5:47-58. [DOI:10.2478/v10102-012-0009-2] [PMID] [PMCID]

[4] Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: A review. Rev Environ Health. 2009; 24(1):15-45. [DOI:10.1515/REVEH.2009.24.1.15] [PMID] [PMCID]

[5] Mitra P, Sharma S, Purohit P, Sharma P. Clinical and molecular aspects of lead toxicity: An update. Crit Rev Clin Lab Sci. 2017; 54:506-28. [DOI:10.1080/10408363.2017.1408562] [PMID]

[6] Funahashi S. Working memory in the prefrontal cortex. Brain Sci. 2017; 7(5):49. [DOI:10.3390/brainsci7050049] [PMID] [PMCID]

[7] Afifi A, Bergman R. Functional neuroanatomy: Text and atlas. New York: McGraw Hill Professional; 2005. [Link]

[8] Mahmoud YI, Sayed SS. Effects of L-cysteine on lead acetate induced neurotoxicity in albino mice. Biotech Histochem. 2016; 91(5):327-32. [DOI:10.3109/10520295.2016.1164897] [PMID]

[9] Hou S, Yuan L, Jin P, Ding B, Qin N, Li L, et al. A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children. Theor Biol Med Model. 2013; 10:13. [DOI:10.1186/1742-4682-10-13] [PMID] [PMCID]

[10] Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016; 5:e47. [DOI:10.1017/jns.2016.41] [PMID] [PMCID]

[11] He M, Min JW, Kong WL, He XH, Li JX, Peng BW. A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia. 2016; 115:74-85. [DOI:10.1016/j.fitote.2016.09.011] [PMID]

[12] Babaei F, Moafizad A, Darvishvand Z, Mirzababaei M, Hosseinzadeh H, Nassiri-Asl M. Review of the effects of vitexin in oxidative stress-related diseases. Food Sci Nutr. 2020; 8:2569-80. [DOI:10.1002/fsn3.1567] [PMID] [PMCID]

[13] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci World J. 2013; 2013:162750. [DOI:10.1155/2013/162750] [PMID] [PMCID]

[14] Gu CB, Ma H, Ning W, Niu L, Han H, Yuan X, et al. Characterization, culture medium optimization and antioxidant activity of an endophytic vitexin-producing fungus dichotomopilus funicola y3 from pigeon pea [cajanus cajan (l.) millsp.]. J Appl Microbiol. 2018; 125(4):1054-65. [DOI:10.1111/jam.13928] [PMID]

[15] Pal D, Mishra P, Sachan N, Ghosh AK. Biological activities and medicinal properties of cajanus cajan (l) millsp. J Adv Pharm Technol Res. 2011; 2(4):207-14. [DOI:10.4103/2231-4040.90874] [PMID] [PMCID]

[16] Wu N, Fu K, Fu YJ, Zu YG, Chang FR, Chen YH, et al. Antioxidant activities of extracts and main components of pigeonpea [cajanus cajan (l.) millsp.] leaves. Molecules. 2009; 14(3):1032-43. [DOI:10.3390/molecules14031032] [PMID] [PMCID]

[17] Rosenfeld CS, Ferguson AS. Barnes maze testing strategies with small and large rodent models. J Vis Exp. 2014; 84:e51194. [DOI:10.3791/51194] [PMID] [PMCID]

[18] Huang TN, Hsueh YP. Novel object recognition for studying memory in mice. Bioprotocol. 2014; 4(19):1249. [DOI:10.21769/BioProtoc.1249]

[19] Bancroft JD. Theory and practice of histological techniques. Amsterdam: Elsevier Health Sciences; 2008. [Link]

[20] Van De Werd HJ, Uylings HB. Comparison of (stereotactic) parcellations in mouse prefrontal cortex. Brain Struct Funct. 2014; 219(2):433-59. [DOI:10.1007/s00429-013-0630-7] [PMID] [PMCID]

[21] Amedu NO, Omotoso GO. Lead acetate- induced neurodegenerative changes in the dorsolateral prefrontal cortex of mice: The role of vitexin. Environ Anal Health Toxicol. 2020; 35(1):e2020001. [DOI:10.5620/eaht.e2020001] [PMID] [PMCID]

[22] Ayala A, Muñoz MS, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev. 2014; 2014:e360438. [DOI:10.1155/2014/360438] [PMID] [PMCID]

[23] Tang Q, Su Y-W, Xian CJ. Determining oxidative damage by lipid peroxidation assay in rat serum. Bio Protoc. 2019; 9(12):e3263. [DOI:10.21769/BioProtoc.3263] [PMID] [PMCID]

[24] Barkur RR, BairyLK. Assessment of oxidative stress in hippocampus, cerebellum and frontal cortex in rat pups exposed to lead (Pb) during specific periods of initial brain development. Biol Trace Elem Res. 2015; 164(2):212-8. [DOI:10.1007/s12011-014-0221-3] [PMID]

[25] Amedu NO, Omotoso GO. Influence of vitexin on ataxia-like condition initiated by lead exposure in mice. Toxicol. Environ. Health Sci. 2020; 12:305-13. [DOI:10.1007/s13530-020-00041-x]

[26] Nurdiana S, Goh YM, Hafandi A, Dom SM, Nur Syimal'ain A, Noor Syaffinaz NM, et al. Improvement of spatial learning and memory, cortical gyrification patterns and brain oxidative stress markers in diabetic rats treated with ficus deltoidea leaf extract and vitexin. J Tradit Complement Med. 2018; 8(1):190-202. [DOI:10.1016/j.jtcme.2017.05.006] [PMID] [PMCID]

[27] Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med. 2018; 54:287-93. [DOI:10.1016/j.ajme.2017.09.001]

[28] Chandrasoma P, Taylor CR. Cell degeneration & necrosis. In: Chandrasoma P, Taylor CR, editors. Concise pathology. New York: McGraw Hill Professional; 1998. [Link]

[29] Ramsey CP, Glass CA, Montgomery MB, Lindl KA, Ritson GP, Chia LA, et al. Expression of Nrf2 in neurodegenerative diseases. J Neuropathol Exp Neurol. 2007; 66(1):75-85. [DOI:10.1097/nen.0b013e31802d6da9] [PMID] [PMCID]

[30] Carniglia L, Ramirez D, Durand D, Saba J, Turati J, Caruso C, et al. Neuropeptides and microglial activation in inflammation, pain, and neurodegenerative diseases. Mediators Inflamm. 2017; 5048616. [DOI:10.1155/2017/5048616] [PMID] [PMCID]

[31] Kraft AD, Johnson DA, Johnson JA. Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. J Neurosci. 2004; 24(5):1101-12. [DOI:10.1523/JNEUROSCI.3817-03.2004] [PMID] [PMCID]

[32] Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A. 2002; 99(18):11908-13. [DOI:10.1073/pnas.172398899] [PMID] [PMCID]

[33] Petanjek Z, Sedmak D, Džaja D, Hladnik A, Rašin MR, Jovanov-Milosevic N. The protracted maturation of associative layer IIIC pyramidal neurons in the human prefrontal cortex during childhood: A major role in cognitive development and selective alteration in autism. Front Psychiatry. 2019; 10:122. [DOI:10.3389/fpsyt.2019.00122] [PMID] [PMCID]

[34] Kritzer MF, Goldman-Rakic PS. Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefrontal cortex in the rhesus monkey. J Comp Neurol. 1995; 359(1):131-43. [DOI:10.1002/cne.903590109] [PMID]

[35] Aglan HS, Gebremedhn S, Salilew-Wondim D, Neuhof C, Tholen E, Holker M, et al. Regulation of Nrf2 and NF-kB during lead toxicity in bovine granulosa cells. Cell Tissue Res. 2020; 380(3):643-55. [DOI:10.1007/s00441-020-03177-x] [PMID]