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Neurobehavioral and Immune-Histochemical Effects of *Daucus Carota* Ethanolic Leaf Extract in Cadmium-Induced Toxicity of the Hippocampus and Prefrontal Cortex of Adult Wistar Rats

Okechukwu Anyigor-Ogah[1], *Chijioke Stanley Anyigor-Ogah[2], Clinton Ogbonna Njoku[3], Idika Mba Idika[4], Toochukwu Nnamdi Nnama[1], Miracle Erinma Chukwuonye[2], Agatha Nkechinyere Ekechi[2], Albert N. Eteudo[3], Ndudim Ogwuegbu Okezie[2],

[1]Department of Human Anatomy, Alex Ekwueme Federal University, Abakaliki, Ebonyi State, Nigeria

[2]Department of Family Medicine, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

[3]Department of Human Anatomy, Ebonyi State University, Abakaliki, Nigeria

[4]Department of Family Medicine, David Umahi Federal University, Teaching Hospital, Uburu, Ebonyi State, Nigeria

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ABSTRACT

Introduction: Chronic exposure to cadmium has been linked to neuro-degenerative disorders due to its ability to induce neuro-inflammation and apoptosis in critical brain regions such as the hippocampus and prefrontal cortex.

Objectives: This study evaluated the neurobehavioral and immune-histochemical effects of *Daucus carota* ethanolic leaf extract (CLE) in cadmium-induced toxicity of the hippocampus and prefrontal cortex of adult wistar rats.

Materials and methods: Thirty adult male Wistar rats (weighing 150–180g) were assigned into five groups (6 per group). Group 1 (normal control) received water, Group 2 (Cadmium-only) received cadmium chloride (5 mg/kg). Group 3 received only CLE (400 mg/kg). Groups 4 and 5 received cadmium chloride and CLE at doses of 200 mg/kg and 400 mg/kg, respectively. Neuro-behavioral tests were conducted to assess cognitive and emotional responses. Brain tissues were harvested for biochemical analysis as well as immune-histochemical evaluation of neuronal integrity. Data were analyzed using GraphPad Prism version 8 and presented as Mean ± SEM. Statistical comparisons were made using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

Results: Cadmium exposure significantly impaired cognitive function and triggered neuro-inflammation. Group C showed weight loss compared to the control group, indicating systemic toxicity. However, CLE treatment ameliorated these changes in a dose-dependent manner. The Cadmium + CLE (200 mg/kg) and Cadmium + CLE (400 mg/kg) groups exhibited significant improvements compared to the Cadmium-only group. The highest dose (400 mg/kg) demonstrated the most pronounced neuroprotective effects, with weight parameters approaching those of the control group. Histamine level were lowered significantly in group B when compared to the control group A at $p<0.05$, acetylcholine level was significantly lowered in all the treated groups when compared to group B at $p<0.05$.

Conclusion: The CLE exhibits potent neuroprotective properties against cadmium-induced neurotoxicity, hence could serve as a promising natural intervention for mitigating heavy metal-induced cognitive and neuronal impairments.

Key words: Neurobehavioral, Immune-histochemical, Cadmium, Hippocampus, Prefrontal

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INTRODUCTION

Heavy metal toxicity is one of the oldest environmental problems and remains a serious health concern today.[1] The toxicity and perceived permanency of heavy metal contaminations in the environment have sparked growing concern.[2] High concentrations of these indestructible and non-biodegradable metals are hazardous to all forms of life.[3] One of these heavy metals known to be part of this is cadmium (Cd).[4] Therefore, it is recognized as an important environmental contaminant.[5]

The greatest exposure to Cd occurs in the metallurgical industry (in zinc smelters or in units where pig iron is purified).[6] Countries with a significant prevalence of this metal toxicity in humans include Nigeria, India, the Philippines, Indonesia, Pakistan, Peru, the United States of America, China, Brazil, France, and Mexico.[7] The continued use of Cd in industry drastically affects the environment, resulting in high exposure of humans to the element.[8]

Traditional medicine is becoming the mainstay of basic healthcare in developing and poor nations.[9] Comparatively speaking, plant-derived medications are more affordable and readily available than their synthetic equivalents. An example of this medicinal plant is the Carrot (*Daucus carota*).[10] The greatest nutritional interest in carrots stems from their bioactive content, but research has also focused on carrots as a source of fiber. Studies have shown that carrot extract only or in combination with other natural contents possess neuro-curative roles on different parts of the brain when exposed to different toxicants due to its rich source of bioactive compounds (mainly polyphenols, nitric pigments, and saponins).[11] While the health benefits of carrot root are well-documented, the potential neuroprotective effects of its leaves remain largely unexplored. However, preliminary research suggests that plant-derived antioxidants can effectively scavenge free radicals, restore cellular homeostasis, and prevent neuronal damage,[12] making them promising candidates for mitigating heavy metal-induced neurotoxicity.[13] This study aims to investigate the potential neuroprotective role of carrot leaf extract (CLE) in cadmium-induced toxicity in the hippocampus and prefrontal cortex of adult Wistar rats.

MATERIALS AND METHODS

A. Plant Collection and Identification Extract Preparation

Fresh carrot leaves were obtained from Ndufu Echara Community in Ikwo Local Government Area of Ebonyi State, Nigeria. It was identified and authenticated by a Botanist in the Department of Science and Biotechnology, University of Nigeria, Nsukka, with herbarium number 1017b.

Carrot leaves (2g) were collected and allowed to dry under shade for two weeks to prevent the direct effects of sunlight on the active constituents of the leaves, after which they were grounded into powdery form in a milling machine. The powder was sieved to obtain uniform particle size that was used in the extraction process by the maceration method. The leaves were dissolved in ethanol at a ratio of 1:7, using 450 ml of ethanol. The mixture was stirred every 6 hours over 48 hours. After this duration, it was sieved to extract the liquid content and subsequently strained again using litmus paper. The obtained solution was dried with a water bath at 40°C, and 2.5g, 5g and 7.5g of the extract were mixed with 30ml, 50ml and 70ml of water for the low dose, medium dose and high dose treatment, respectively.[14]

B. Animal procurement

Thirty-five (35) Adult Wistar rats weighing 100-120g were purchased from the animal house of the study institution. The animals were housed in well-ventilated wired cages and allowed to acclimatize for two weeks in the animal house. They were maintained under standard photoperiodic conditions of 12 hours of light/dark cycle at a temperature of 27^0C -30^0C and relative humidity of 50 ± 0.05C. The animals were fed with rat pellets (Top Feed Ltd, Nigeria) and allowed unrestricted drinking water access.

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C. Experimental Design

Cadmium chloride was purchased from Zayo-Sigma Chemicals Ltd, Jos, Northern part of Nigeria, with molecular weight 201.32g/mol, batch number 117 09, product number 80683 and pack size 250g, which was used as the toxin for this experiment, the LD50 of cadmium is 88mg/kg. It was prepared using 1g of cadmium which was dissolved in 50ml of distilled water. The constituted solution was shaken for proper dissolution and then preserved in a refrigerator for use.

The study involved thirty-five (35) adult Wistar rats randomly divided into five (5) groups (Groups A-E), each comprising seven (7) randomized rats, which were tagged and housed in separate cages. In preparing the stock solution, 20g of CLE was dissolved in 100 ml of distilled water, from which subsequent concentrations for administration were derived. According to Ijomone *et al*. (2020), 20 mg of Cadmium chloride ($CdCl_2$) does not cause morbidity.[15] Furthermore, according to Ahmad *et al*. (2023), 800 mg/kg of CLE does not cause morbidity.[16]

a. Group A (Control): Received normal rat feed and water only, orally for 28 days.

b. Group B received 8-mg/kg body weight (bwt) of $CdCl_2$ orally for 28 days.

c. Group C was administered 400-mg/kg bwt of CLE orally for 28 days.

d. Group D received 8-mg/kg (bwt) of $CdCl_2$ in saline and 200-mg/kg bwt of CLE for 28 days

e. Group E was administered 8-mg/kg bwt of $CdCl_2$ in saline and 400-mg/kg bwt of CLE for 28 days.

D. Animal Sacrifice and Sample Collection

The animals were sacrificed at the end of 28 days using cervical dislocation after 24 hours of fasting, and blood samples were collected from the apex of the heart for biochemical analysis while the skull was excised, the prefrontal cortex and hippocampus were harvested and fixed in 10% formalin for histological studies.

E. Neurobehavioral Studies

Neuro-behavioural studies of the experimental animals were carried out using T-Maze, Sociability chamber and Novel object recognition (NOR) test methods. These methods were used to ascertain the learning, memory, and cognitive functions of the adult Wistar rats respectively.

F. Learning and Memory Test using T-Maze

The rats were subjected to a learning and memory test in a T-maze. The experiment was conducted according to Maodaa *et al*. (2016).[17] The rats were starved for 24h, given only water, before the experiment. The maze was a wooden device consisting of three arms that formed a T shape: the right arm, the left arm, and the main long arm. On the second day, animals were placed in the device for 3 minutes and allowed to explore each of the arms. Afterwards, animals were returned to their cages for 3 hours and again placed in the T-maze for 5min per animal.

Several parameters of behaviour, time spent in the left arm, number of entrances into the left arm, number of entrances into the right arm, time spent in the right arm, number of entrances into the main arm, and time spent in the main arm, were recorded for 5 min for each animal.

G. Novel Object Recognition Test

The NOR is specifically used to evaluate recognition memory and object recognition memory and is very useful to study short-term memory, intermediate-term memory, and long-term memory via manipulation of the retention interval.[18] The task procedure consists of three phases; habituation, familiarization and test phase. During the habituation phase, each Wistar rat was allowed to explore the open-field arena freely in the absence

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of objects and then removed from the arena and placed in its holding cage. During the familiarization phase, a single rat was placed in the open-field arena containing two identical sample objects, of the same colour, shape, texture and size, for a few minutes (retention interval) for the rats to familiarize themselves with the objects. To prevent coercion to explore the objects, the rats were released against the centre of the opposite wall with their backs to the objects. During the test phase, the rats were returned to the open-field arena with two sample objects, one of which was familiar with, and the other the novel object (A + B).[19] During the familiarization and the test phase, the objects were placed in opposite and symmetrical corners of the open-field arena and the location of novel versus familiar objects was counterbalanced.[20] After the test phase, the following parameters were collected namely; mean time spent sniffing familiar object, mean time spent exploring familiar object, mean time spent exploring novel object, percentage of object discrimination (%), and discrimination index

A. Calculation of discrimination index:

$$\frac{\text{time with novel object} - \text{time with familiar object}}{\text{time with novel object} + \text{time with familiar object}}$$

B. Calculation of Percentage of object discrimination (%):

$$\frac{(\text{time with novel object})}{\text{time with novel object} + \text{time with familiar object}} \text{ X } 100$$

H. Immuno-histochemical Studies

Immuno-histochemical studies, was done using paraffin sections of the brain which were deparaffinised with xylene, followed by antigen retrieval by heating in citrate buffer (10 mM, for 20 min). This was followed by endogenous peroxidase blocking in 3% H_2O_2 for 10 min and incubated with rabbit anti-mouse rabbit anti-GFAP (1:500 Santa Cruz Biotechnology).

After washing the slides with phosphate-buffered saline, the sections were incubated with suitable fluorescent secondary antibodies, goat anti-rabbit AlexaFluor 488 (1:200) at room temperature for 1 hour, followed by detection with 3-amino-9-ethylcarbazole, a chromogen. The slides were appropriately counterstained and mounted in Paramount aqueous mounting medium.

Coronal sections were examined from the rostral anteroposterior (- 2.1 mm) to the anteroposterior (- 4.5 mm) direction, as defined by the bregma of the brain atlas. Images were obtained at ×40 magnification using the IMAGE PRO PLUS System (version 4.0; Media Cybernetics, Silver Spring, MD, USA) on a computer attached to a light microscope (Zeiss Axioskop, Oberkochen, Germany), which interfaced with a charge-coupled device video camera (Kodak Mega Plus model 1.4 I). Each image was stitched to avoid overlap of adjacent tiles and exported in TIFF format

I. Immuno-labelling

Immuno-reactivity was quantified using the Image J software (National Institute of Health, USA). The percentage of positively stained area in the hippocampus and cortex was measured for each section and 9 fields were selected (randomly). The total field and immune-histochemical (IHC) stained areas were calculated and the percentage of IHC stained area was calculated as follows:

$$\text{Percentage of IHC stained area} = \frac{\text{IHC stained area}}{\text{Total area}} \text{ X } 100$$

J. Image Analysis of Immuno-Stained Slides

A digital bright-field microscope was used to examine immune-stained slides as photomicrographs were taken. At x10 magnification, non-overlapping pictures of the hippocampus and prefrontal cortex were acquired and

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used for image analysis. Photomicrographs were analyzed and quantified using image analysis and processing for Java (image J). The number of GFAP-positive cells were counted using the Image J cell counter.

K. Data Analysis

The experimental data obtained was analyzed using Graph Pad prism version 9.0.1.5 and results were presented in a tabular form as Mean ± SEM. The significant differences between means were established using a Two-Way Analysis of Variance (ANOVA). Multiple comparisons of Turkey's Post Hoc Test were adopted to check the significance level at $p \leq 0.05$.

RESULTS

A. Body Weight Analysis

As shown in Table 1, group A, B, C, D and E had a weight changes of 10.30±3.94g (6.34±2.42%), -22.90±4.11g (-12.84±2.31%), 9.40±1.98g (5.88±1.24%), -9.20±2.44g (-5.94±1.58%), and -0.80±2.61g (-0.49±1.61%) respectively.

Table 1: Effects of *Daucus carota* ethanolic leaf extract on cadmium toxicity on animal weight change.

Groups	Initial weight (g)	Final weight (g)	Weight Change (g)	Percentage Weight Change (%)
A	162.50±5.96	172.80±9.90	10.30 ± 3.94	6.34 ± 2.42
B	178.40±5.98	155.50±10.09	-22.90 ± 4.11	-12.84 ± 2.31
C	159.90±3.66	169.30±4.51	9.40 ± 1.98	5.88 ± 1.24
D	154.90±4.25	145.70±6.69	-9.20 ± 2.44	-5.94 ± 1.58
E	162.00±3.93	161.20±6.54	-0.80 ± 2.61	-0.49 ± 1.61

No Significant difference.

B. Neuro-behavioural Study Analysis

The study showed the results of neurobehavioral studies of the animals assessed for Spatial memory using the T-maze and recognition memory using the Novel object recognition tests, respectively as given below:

1). *T-Maze Result*

As shown in Table 2, in the T-maze test, TSIA A was 68.00±22.52 secs, 58.60±27.70 secs, 92.14±47.23 secs, 101.40±30.25 secs, and 99.36±25.29 secs in groups A to E respectively. TSIA B was 118.40±43.56 secs, 44.00±12.91 secs, 63.67±24.10 secs, 76.92±15.68 secs, and 76.92±15.68 secs in groups A to E respectively, while TSNA was respectively 258.00±14.84 secs, 271.70±28.33 secs, 300.60±0.60 secs, 303.30±3.25 secs, and 303.30±3.25 secs in groups A to E. There was no significant difference among the groups.

Table 2: Effects of *Daucus carota* ethanolic leaf extract on cadmium neurotoxicity in rodent's spatial memory.

Groups	TSIA A (secs)	TSIA B (secs)	TSNA (sec)
A	68.00±22.52	118.40±43.56	258.00±14.84
B	58.60±27.70	44.00±12.91	271.70±28.33

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C	92.14±47.23	63.67±24.10	300.60±0.60
D	101.40±30.25	76.92±15.68	303.30±3.25
E	99.36±25.29	76.92±15.68	303.30±3.25

No Significant difference.

KEYS: TSIA A= Time spent in arm A; **TSIA B=** Time spent in arm B; **TSNA=** Time spent in no arm

2). ***Novel Object Recognition***

In the novel object test, group B (cadmium only) showed a decrease in Tn (7.27±0.89s) and Tf (4.00±1.68s) compared to group A (Control: Tn = 10.93±2.99s, Tf = 12.31±5.65s). Group C (200 mg/kg CLE) showed a significant improvement (Tn = 21.65±3.23s, Tf = 23.96±3.04s, $p<0.05$) compared to group B. Groups D (Tn = 14.09±5.49s, Tf = 16.20±4.78s) and E (Tn = 7.37±2.52s, Tf = 7.62±3.13s) showed an increase in Tn and Tf compared to group B, but the changes were not statistically significant as shown in table 3.

Table 3: Effects of *Daucus carota* ethanolic leaf extract in rodent's impaired recognition memory due to cadmium toxicity.

Groups	Tn(secs)	Tf(secs)
A	10.93±2.99	12.31±5.65
B	7.27±0.89	4.00±1.68
C	21.65±3.23	23.96±3.04[b]
D	14.09±5.49	16.20±4.78
E	7.37±2.52	7.62±3.13

a = Significant difference when compared to A; b = Significant difference when compared to B; c = Significant difference when compared to C; d = Significant difference when compared to D; e = Significant difference when compared to E.

KEYS: Tn - Time with Novel object; Tf = Time with Familiar object.

C. Effects of cadmium and carrot leaves extract on Neurodegenerative Proteins and apoptotic marker

As shown in Figures 1a and b, the Caspase-3 level was significantly increased in group B when compared to the control group A. The carrot leave extract treated groups showed significant decrease in the level Caspase-3 when compared to group B at $p<0.05$ (Figure 1a). The results showed a significant decrease in the level of the Tau proteins in group B compared to the control group A at $p<0.05$ (Figures 2b). The result also showed that the extract treatment significantly increased the concentration of the Tau protein compared to the untreated group B at $p<0.05$.

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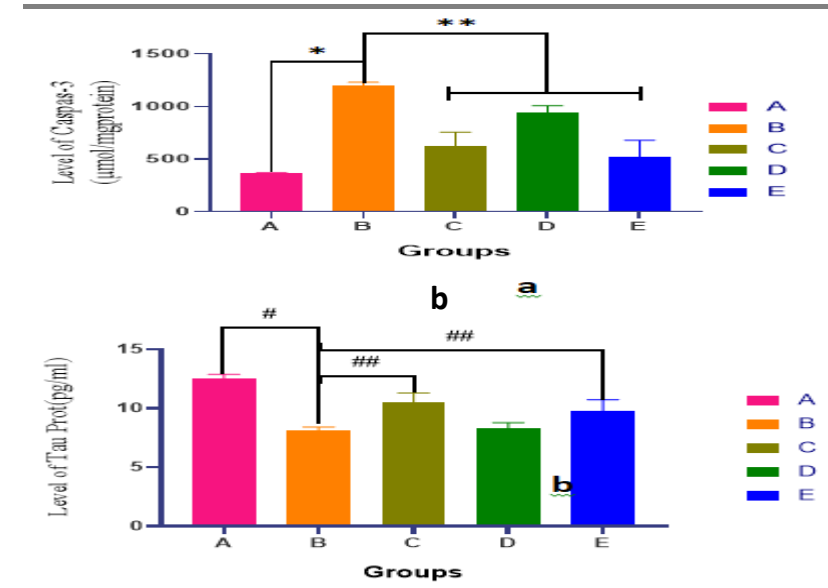

Fig. 1: Effects of cadmium and carrot leaves extract on the levels of neurodegenerative protein and apoptotic markers during the experiment. (a) Caspase-3 and (b) Tau protein. *Significant increase at $p<0.05$; **Significant decrease at $p<0.05$; #Significant decrease at $p<0.05$; and ##Significant increase at $p<0.05$

D. Effects of cadmium and carrot leaves extract on the levels of Neurotransmitters

Regarding the histamine (Fig 2a), the levels were lowered significantly in group B when compared to the control group A at $p<0.05$. The carrot leaves extract significantly increased the levels of histamine in the brain when compared to group B at $p<0.05$. The level of Acetylcholine in the brain homogenate (Figure 2b), showed that cadmium administration significantly increased the brain-acetylcholine concentration in group B when compared to the control group A at $p<0.05$, while the level of acetylcholine was significantly lowered in all the treated groups when compared to group B at $p<0.05$.

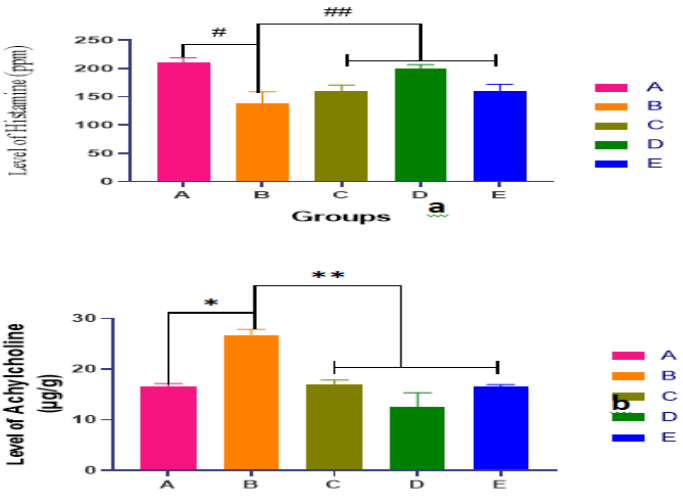

Fig 2: The effect of cadmium and carrot leaves extract on the levels of neurotransmitters (a) Histamine, and (b) Acetylcholine. #Significant decrease at $p<0.05$ compared to A; ##Significant increase at $p<0.05$ compared to B; *Significant increase at $p<0.05$ compared to A; **Significant decrease at $p<0.05$ compared to B

DISCUSSION

This study provided significant insights into the potential neuro-curative effects of *Daucus carota* ethanolic leaf extract against cadmium-induced toxicity in the hippocampus and prefrontal cortex of adult Wistar rats. The hippocampus and prefrontal cortex, which are essential for learning, memory, and executive functions are particularly vulnerable to cadmium-induced damage.[21,22]

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A. Effects of CLE and Cd on the body weights

The body weight analysis highlights the detrimental effects of cadmium exposure and the potential curative role of *Daucus carota* ethanolic leaf extract (CLE). As shown in table 1, the control group (group A) exhibited normal weight gain (6.34%), consistent with expected physiological growth in Wistar rats. However, cadmium exposure in group B resulted in significant weight loss (-12.84%), which aligns with a previous study that reported reduced body weight in cadmium-exposed animals due to its toxic effects on metabolism and organ function.[23] Cadmium toxicity has been shown to disrupt lipid and protein metabolism, increase oxidative stress, and impair nutrient absorption, all of which contribute to weight loss.[24] In line with these findings, Adegoke *et al*. (2017) also reported a significant decrease in final body weight following cadmium exposure.[25] Contrary to this, another study has shown that low-dose cadmium exposure can lead to weight gain over time, possibly due to metabolic compensatory mechanisms that increase adiposity following initial toxicity.[26] These variations may be influenced by the duration of exposure, dosage, and species differences.

Group C, which received CLE alone (400 mg/kg bwt), exhibited a weight gain of 5.88%, suggesting that CLE does not induce weight loss under normal conditions but may instead support metabolic homeostasis. This aligns with a previous study suggesting that carrot consumption has beneficial metabolic effects, with carotenoids such as β-carotene helping to regulate adipose tissue function.[27] Similarly, Mahesh *et al*. (2021) reported that carrot juice is low in calories and rich in fiber, which may contribute to improved metabolic function.[28] Contradictory findings exist, as a study by Khan (2019) found no significant impact of carrot extract on body weight.[29] These discrepancies may be due to differences in the composition of the extract, duration of administration, or the presence of other dietary factors influencing metabolism. Group D, which was exposed to cadmium and treated with a lower dose of CLE (200 mg/kg bwt), showed weight loss (-5.94%). Although this loss was less severe than in group B, it indicates that CLE at this dose only partially mitigated cadmium-induced metabolic disruption. This aligns with previous study suggesting that while carrot extracts possess antioxidant and anti-inflammatory properties, their ability to counteract cadmium toxicity may be dose-dependent.[30] However, it is important to note that some researches have implicated *Daucus carota* in weight loss due to its fiber content, which promotes satiety and reduces caloric absorption.[31] A study by Ramirez *et al*. (2023) demonstrated that a high-fiber diet from carrot sources led to a reduction in body weight by 10-12% over 12 weeks.[30] This suggests that while CLE helps mitigate cadmium-induced weight loss, its fiber content may also contribute to weight regulation by limiting energy intake.

Group E, which was exposed to cadmium and treated with a higher dose of CLE (400 mg/kg bwt), exhibited minimal weight change (-0.49%), suggesting a stronger curative effect at this higher dose. This finding aligns with a study indicating that plant extracts at higher concentrations may exert stronger anti-oxidative and metabolic regulatory effects.[32] The improved weight maintenance in this group may be attributed to the digestion-stimulating properties of *Daucus carota* extract, which has been reported to enhance nutrient absorption and counteract metabolic disturbances.[32] Furthermore, a clinical study reported an inverse relationship between fiber intake and BMI, fat oxidation, and energy storage, reinforcing the idea that *Daucus carota* may support weight maintenance rather than promoting excessive weight gain.[33]

Comparing these findings with previous research highlights the variability in both cadmium's and *Daucus carota*'s effects on body weight. While cadmium exposure in this study led to weight loss, some studies have reported compensatory weight gain following prolonged exposure.[26] Similarly, while CLE helped prevent excessive weight loss in cadmium-exposed groups, its potential role in weight regulation remains complex. Studies have reported both weight-maintaining and weight-reducing effects, likely due to variations in fiber content, dosage, and metabolic response.[30,32] The findings of this study suggest that while CLE provides some ameliorating against cadmium-induced weight loss, its fiber content may also contribute to weight regulation, depending on dosage and individual metabolic responses.

C. Neurobehavioral effects of CLE in Cadmium-induced toxicity

The neurobehavioral assessments in this study were conducted using the T-maze and novel object recognition (NOR) tests to evaluate spatial and recognition memory, respectively. The results indicate that cadmium exposure impaired recognition memory, while treatment with CLE demonstrated varying degrees of neuro-

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amelioration. In the T-maze test (table 2), TSIA A was slightly reduced in group B (cadmium-only) compared to the control (group A), while groups C, D, and E exhibited prolonged times. The TSIA B was notably lower in group B than in the control, indicating possible cadmium-induced deficits in spatial learning and decision-making. Despite these differences, no statistically significant variations were found among the groups.

Previous research has established that cadmium exposure impairs spatial memory and learning ability in rodents. Enogieru & Inegbedion (2022) reported that cadmium-treated rats displayed lower spontaneous alternation behavior in maze tests, suggesting deficits in spatial working memory.[34] Another study also demonstrated that cadmium exposure leads to reduced exploratory behavior and impaired decision-making in maze-based tests.[35] In contrast, the lack of significant changes in the present study suggests that the cadmium dose and duration of exposure may not have been sufficient to induce severe deficits in spatial memory. Additionally, factors such as compensatory neuroplasticity or individual variability among animals may have influenced the outcomes.

The NOR test (table 3) revealed a marked decrease in time spent with the novel object (Tn) and the familiar object (Tf) in group B, indicating significant impairments in recognition memory due to cadmium exposure. These findings are consistent with previous studies demonstrating that cadmium reduces exploratory behavior and lowers discrimination index scores in rodents.[35] The ability of cadmium to impair memory function has been attributed to its neurotoxic effects, including oxidative stress, neuro-inflammation, and synaptic dysfunction.[36] Interestingly, group C (400 mg/kg CLE) showed a significant improvement in Tn and Tf, suggesting that CLE at this dose provided notable neuro-amelioration against cadmium-induced memory deficits. This aligns with findings that plant-derived antioxidants can mitigate neurotoxic damage and support cognitive function. The improvement in group C may be attributed to the antioxidant properties of *Daucus carota*, which has been shown to reduce oxidative stress and enhance synaptic plasticity.

Groups D (Cd + 200 mg/kg CLE) and E (Cd + 400 mg/kg CLE) showed moderate increases in Tn and Tf compared to group B, but the changes were not statistically significant. While previous research on *Daucus carota* and cognitive function is scarce, studies on other plant-based antioxidants have suggested that their neuro-curative effects may be dose-dependent. It is possible that the higher CLE dose in groups D and E did not provide additional cognitive benefits beyond those observed in group C. Alternatively, high concentrations of certain bioactive compounds could have modulated neurotransmitter levels in a way that did not significantly enhance recognition memory.

Cadmium has been shown to cross the blood-brain barrier, accumulate in the hippocampus, and cause neuronal damage leading to cognitive impairment.[37] Conversely, Akinyemi *et al*. (2017) demonstrated that cadmium exposure caused severe recognition memory dysfunction, whereas Pulido *et al*. (2019) observed no statistical differences in memory performance between cadmium-treated and control animals at certain time points.[38,39] These discrepancies may be explained by differences in cadmium dosage, exposure duration, and species-specific responses to heavy metal neurotoxicity.

Furthermore, behavioral alterations associated with cadmium exposure extend beyond memory deficits. A study has shown that cadmium exposure can induce anxiety-like behaviors, social deficits, and altered locomotor activity.[40] Clinical evidence also suggests that cadmium exposure in humans is linked to learning disabilities, hyperactivity, and lower IQ.[41] The present findings contribute to this growing body of evidence by demonstrating that cadmium impairs recognition memory, reinforcing its role as a neuro-toxicant with cognitive consequences.

D. Effects of cadmium and carrot leaves extract on Neurodegenerative Proteins and apoptotic marker

This study (Fig 1a) showed that the level of Caspase-3 was significantly increased (at $p<0.05$) in group B animals when compared to the control (group A). Meanwhile, the groups (C, D and E) treated with carrot leave extract showed a significant decrease in the level of Caspase-3 when compared to group B at $p<0.05$. This indicates the potentiality of carrot leave extract in ameliorating apoptosis. Therefore, carrot leave extract exerted an inhibitory effect on the mitochondrial and death receptor pathways involved in Cd-induced apoptosis. It has been suggested that Cd can cause apoptosis in a variety of cells, and can occur in a dose-and

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time-dependent manner.[42] Pathak and Khandelwal (2006) reported that 6 h exposure to 25 μmol/l Cd induced apoptosis in rat thymus cells.[43] Caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins.[44] Caspase-3 is a key factor in apoptosis, and is directly involved in the chromosome condensation and DNA fragmentation processes.

Also, this study (Fig 1b) showed a significant decrease at $p<0.05$) in the level of the Tau proteins in group B animals compared to the control (group A) while the treatment with carrot leave extract significantly increased (at $p<0.05$), the concentration of the Tau protein compared to the untreated group B. This could mean that the abnormal tau protein that accumulated as a result of cadmium exposure were not affected by administration of carrot leaves extract. Meanwhile, the finding aligns with research suggesting that cadmium disrupts cytoskeletal integrity by impairing tau protein phosphorylation.[45] While CLE treatment in groups D and E increased tau protein levels, these changes were not statistically significant, suggesting that further investigation is needed to understand the precise role of *Daucus carota* in tau protein regulation. Hyperphosphorylation of tau protein is proposed to be an early event for the evolution of tau pathology, and may play an important role in Cd-induced neurodegeneration.[46]

E. Effects of cadmium and carrot leaves extract on the levels of Neurotransmitters

This study (Fig 2a) showed that the levels of histamine was lowered significantly (at $p<0.05$) in the cadmium untreated animals (group B) when compared to the control (group A). However, the carrot leaves extract significantly increased the levels of histamine in the brain when compared to group B at $p<0.05$. Recent evidence suggests that aberrant histamine signaling in the brain may also be a key factor in addictive behaviors and degenerative disease such as Parkinson's diseases and multiple sclerosis.[47] Cadmium exposure has been implicated to affect brain histamine levels and related signaling pathways, potentially contributing to neuro-inflammation and neurotoxicity.[48] Cadmium stimulates mast cell degranulation, leading to increased histamine release and inflammatory mediator release, as well as disrupting redox signaling and calcium influx.[49] Furthermore, cadmium can alter the binding activity of histamine receptors in the brain, particularly subtype 2, in certain regions.

Acetylcholine is a crucial neurotransmitter in the brain, playing a vital role in various cognitive functions like memory, learning, and attention, as well as influencing mood and motivation.[50] Cadmium exposure (Fig 2b) significantly increased acetylcholine (ACH) levels in group B, indicating disrupted cholinergic neurotransmission. Studies suggest that cadmium interferes with acetylcholine metabolism by inhibiting acetylcholinesterase activity, leading to excessive ACH accumulation and neuronal hyperactivity.[51] This dysregulation has been implicated in cognitive impairments and neurodegenerative conditions.[52] Meanwhile, carrot leaf extract (CLE) treatment significantly reduced ACH levels in Groups C, D, and E, suggesting that it may help restore cholinergic balance and improve cognitive function.

CONCLUSION

This study comprehensively evaluated the neurobehavioral and immune-histochemical effects of *Daucus carota* ethanolic leaf extract in cadmium-induced toxicity of the hippocampus and prefrontal cortex of adult wistar rats, assessing its impact on body weight, neurobehavior, oxidative stress markers, and neurotransmitters in the hippocampus and prefrontal cortex. The findings confirmed that cadmium exposure led to significant weight loss, cognitive impairments, increased oxidative stress, and severe neuronal degeneration in a dose-dependent manner.

The observed neuronal regeneration in CLE-treated groups further support its neuro-ameliorating potential, likely attributed to its rich antioxidant and anti-inflammatory bioactive compounds. While this study highlights the potential of CLE as a natural therapeutic agent against cadmium-induced neurotoxicity, further research is needed to explore its precise mechanisms of action and long-term effects in both experimental and clinical settings.

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Ethical Approval

Ethical approval was sought and got from the Research Ethics Committee of the Faculty of Basic Medical Sciences Ebonyi State University, Abakaliki, Ebonyi State, with number EBSU/REC/2024/7299. This research also complied with the Helsinki declaration of 2013 as it concerns animal studies.

Conflict of Interests: The authors have no potential conflict of interests to declare

Data Availability Statement: All data about this research is available on reasonable request to the corresponding author on ogashstanly90@yahoo.com

Author Contributions: The authors contributed equally to all aspects of this research

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