ORIGINAL ARTICLE

Zingerone improves memory impairment in Wistar rats exposed to cadmium via modulation of redox imbalance

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Abstract

Background: Our environment exposes us to various neurotoxins that can invariably affect cognitive ability. *Aim and Objectives*: To evaluate the modulatory role of zingerone on cadmium induced memory impairment, oxidative stress and apoptosis on the hippocampus of male Wistar rats. *Material and Methods:* Thirty (30) adult male Wistar rats used were grouped into six (6) groups, (n = 5) as follows; Control group (A), Group B was given 5 mg/kg of cadmium for twenty one days, Group C received 200 mg/kg b.w.t. of zingerone (C) for 21 days, the treatment study Groups D-F were given 5 mg/kg of cadmium for seven days and treated using varying dose of zingerone for 14 days (50 mg/kg, 100 mg/kg and 200 mg/kg b.w.t.). Zingerone and cadmium chloride were dissolved in normal saline, the route of administration was oral and the administration period was twenty-one days. Y –maze neurobehavioral test was used to evaluate spatial activity and spatial memory using number of arm entries, time spent in novel arm, number of correct spontaneous alternation and percentage alternation. On day 22, the animals were sacrificed, brain was removed and samples collected for biochemical analysis, histological and immunohistochemical investigations. *Results*: Administration of zingerone resulted in significant increased ($p< 0.05$) time in novel arm, number of arm entries, spontaneous alternations and percentage alternations when compared to rats that received cadmium only. This is indicating that zingerone improve the memory ability of Wistar rats exposed to cadmium. Cadmium intoxication increased the levels of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxide (GPx) and decreased level of Malondialdehyde (MDA) as shown in Group B, however this was attenuated in Groups C-F that were treated with zingerone. Result also showed neuronal cell death, degenerative changes fragmented pyramidal and granular layer, loss of cytoplasmic content and pyknotic nuclei, evident in the histology and immunohistochemical study of the hippocampus of rats in Group B; however, these effects were reversed by zingerone in all Groups D-F. *Conclusion:* These findings suggest that zingerone may protect against cadmium mediated memory impairment, apoptosis and oxidative stress in the hippocampus.

Keywords: Cadmium, Zingerone, Hippocampus, Cognitive Impairment, Y-Maze

Introduction

Exposure to heavy metals like Cadmium (Cd), Aluminum Chloride ($AlCl_3$) and Lead (Pb) has been implicated in neurotoxicity and some neurodegenerative disorders. [1-2]. Cadmium is a fluffy, silvery-white metal that resembles zinc, placed in group XI on the periodic table of chemical elements. Cadmium (Cd) is a ubiquitous industrial and environmental toxin that accumulates in both animals and human beings. About 24,000 metric tons of it are produced yearly

worldwide [3] and have a variety of industrial uses. Cadmium is found in our surroundings due to the use of fossil fuels, burning of metal ores, burning of garbage, inhalation of cigarette smoke, and plant uptake into the food chain. [4] Studies have linked neuronal cell damage, oxidative stress, mitochondrial dysfunction, inflammation and activation of glial cells, memory impairment to exposure to cadmium toxicity [5-6]. Cadmium may contribute to the onset of neurodegenerative diseases like Alzheimer's Disease (AD) and Parkinson's Disease (PD) [5-6].

Studies have shown that exposure to high cadmium levels typically results in the excessive production of Reactive Oxygen Species (ROS) by up-regulating the expression of NADPH oxidase II and the depletion of the antioxidant molecule, Glutathione (GSH). However, increased levels of cadmium have also been shown to impair the functionality of the Blood Brain Barrier (BBB) and induce an increase in the BBB permeability. As a result, there is increased oxidation of lipids, proteins, and nucleic acids in a variety of tissues, including the lung, brain, kidney, and liver, according to Areba *et al*. [7].

Cadmium can accumulate in the brain over time and impair memory, which has a significant impact on both general health and disease risk in an aging population [3]. Due to the ability of antioxidant agents to treat many neurodegenerative diseases, the therapeutic use of antioxidants from our everyday diet is receiving more attention in scientific studies. Therefore, cadmium-induced memory impairment, oxidative damage, and neurodegeneration may be treated with natural chelating-antioxidants like zingerone. In many cultures around the world, ginger is used as a spice and condiment to enhance the flavor and sweetness of food. In addition to its use as a dietary spice, ginger is increasingly being used in traditional medicines all over the world. Ginger has hepatoprotective, nephroprotective, neuroprotective, anti-inflammatory, anti-obesity, anti-oxidative, anti-cancer, anti-microbial, and anti-diabetic effects [8-9].

Zingerone (4-(4-hydroxy3-methoxyphenyl)-2-butanone) is a major constituent of ginger and accounts for about 9.5% of ginger component. [9]. It is a non-volatile, pungent phenolic compound and shares a close structural relationship with other non-volatile substances which include gingerol and shogaol [8-9]. Due to its potent anti-inflammatory and antioxidant qualities, zingerone may be beneficial in the prevention and treatment of a number of conditions, including cancer, diabetes, cardiovascular disease, arthritis, renal damage, AD, PD, and cognitive impairment [9-10]. During a normal metabolic process, an increase in free radical generation causes oxidative stress and lipid peroxidation, which can cause DNA damage and tissue toxicity [11]. Zingerone has been reported to easily metabolize in rats and humans and cross BBB quickly, achieving a respectable blood concentration. It has been found that it prevents the degeneration of endogenous antioxidants that scavenge ROS and safeguards brain mitochondria [12]. Hence, the purpose of this study was to evaluate the modulatory role of zingerone on cadmium induced memory impairment, oxidative stress and apoptosis on the hippocampus of male Wistar rats.

Material and Methods

Drugs and treatment

Zingerone (4-(4-hydroxy3-methoxyphenyl)-2-butanone), Cadmium chloride ($CdCl_3$) and all other reagents used for this study were purchased from Sigma-Aldrich USA. All chemicals were of analytic standard grade.

Experimental animals

A total of thirty adult male Wistar rats were obtained from the animal house of Veterinary Department, University of Nigeria Nsukka. Rats were housed in clean, well-ventilated cages with a controlled temperature and 12-hour light/dark cycles in the animal house at the Department of Anatomy, University of Nigeria's Enugu campus. The animals were allowed unrestricted access to food and water. The animals were acclimatized for two weeks prior to the start of administration.

Experimental design

Rats were weighed and randomly divided into six groups of five rats each. Group A (Normal control Group/received normal saline only), Group B (Cadmium 5 mg/kg only b.w.t.), Group C (Zingerone 200 mg/kg only b.w.t.), Group D (Cadmium 5 mg/kg + Zingerone 50 mg/kg only b.w.t.), Group E (Cadmium 5 mg/kg + Zingerone 100 mg/kg only b.w.t.), Group F (Cadmium 5 mg/kg + Zingerone 200 mg/kg only b.w.t.). Zingerone and cadmium chloride were dissolved in normal saline, the route of administration was oral and the administration period was twenty-one days. The dose for Zingerone was according to previous studies [13-15]. The dose and route of administration for cadmium were from previous works by other authors [16-17].

Ethical approval

The study was approved by the College of Medicine Research Ethics Committee of University of Nigeria, Enugu Campus and it complies with the Institutional Animal Ethics Committee's (IAEC) rules for the care and use of laboratory animals, which are established by the U.S. National Institutes of Health (NIH).

Neurobehavioral testing (Y-Maze test)

The Y-maze test was carried out according to the method previously described by Onaolapo *et al*., [18]. Y-maze apparatus was made of wooden arms at 120 degrees between each arm and with a dimension of 35×5×10cm and the arms were labelled A, B and C. The Y-maze test is used to assess and evaluate cognitive ability, locomotive activity, spatial memory and memory ability of experimental animals. The test was conducted in a quiet environment and 5% alcohol was used to clean the apparatus after every test phase. The procedure for Y-maze behavioral test were in two phases (training and testing phase). In the training phase (T1), the animals were introduced into the apparatus and allowed to explore the y-maze with two arms open and one arm closed for five minutes followed by only two open arms for five minutes. The next phase was the testing phase where the rats were reintroduced into the apparatus and allowed to explore all arms freely without restriction. The whole process was recorded with a digital camera, placed in an elevated position above the apparatus to capture all arms. The time spent in novel arm, number of arms entries, spontaneous alternation and percentage alternation were recorded and used as parameters to evaluate memory ability of the rats.

Animal sacrifice and sample collection

Twenty four hours after the last neurobehavioral test, the animals were weighed, sacrificed by cervical dislocation and transcardiac perfusion was performed with 0.9% saline followed by paraformaldehyde in 0.1M phosphate buffer. Brain samples were carefully dissected out of the skull, weighed and fixed in formaldehyde. Blood sample and brain tissues were taken to the laboratory for biochemical assay analysis, immunohisto-chemistry and histological investigations.

Biochemical assay: antioxidant enzyme assay

Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) activities were measured using commercial assay kits according to the manufacturer's instructions.

Measurement of SOD

The activity of SOD was obtained by Marklund and Marklund's method [19]. The reaction mixture (3 ml) contained 2.95 ml of 0.05 M sodium carbonate buffer, pH 10.2, 0.02 ml of specimen. 0.3 ml of epinephrine in 0.01N HCl were used to set off the reaction process. Enzyme activity was then calculated and estimated by measuring the alteration in absorbance level at 480 nm for 5 min and the enzyme activity was expressed as U/mg protein.

Measurement of GPx

The method described by Mohandas *et al*., [20] for the activity of GPx and the result was calculated in nmol NADPH oxidized/min/mg protein. GPx activity was determined by the method of Mohandas *et al*., (1984). The reaction assay consisted of phosphate buffer (0.05 M, pH 7.0), EDTA (1mM), sodium azide (1 mM), glutathione reductase (1 EU/ml), glutathione (1 mM), NADPH (0.2 mM), hydrogen peroxide (0.25 mM) and 0.1 ml of mitochondria in the final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein by using molar extinction coefficient of $6.22 \times 103\ M^{-1}cm^{-1}$.

Measurement of catalase activity

CAT activity was calculated according to Claiborne [21]. Specifically, 0.05 ml of post mitochondrial supernatant, 0.1 ml of phosphate buffer (0.1 M, pH 7.4), and 180 mM hydrogen peroxide (PMS) were used. For three minutes, the changes were seen at 240 nm per minute. Using nmol H_2O_2 consumed/ min/mg protein and a molar extinction coefficient of 43.6 103 M-1 cm, the catalase value was computed. CAT activity was calculated in terms of nmol H_2O_2 consumed/min/mg protein.

Measurement of MDA

Lipid peroxidation (LPO) such as MDA was analyzed by colorimetric method, after the reaction with thiobarbituric acid in acidic medium at 95°C for 30 min forming thiobarbituric acid reactive product. The resulting substance absorbance was estimated at 534 nm according to method described by Ohkawa *et al.* [22] by usage of commercially accessible diagnostic kits.

Histological examination

The fixed brain tissues were subjected to the routine method for paraffin wax embedment to obtain paraffin wax-embedded tissue blocks. PFA-fixed tissues were put through routine tissue processing procedures to obtain tissue blocks for staining procedures. The following procedures were followed;

- **Dehydration**

The hippocampal tissues in tissue cassettes were passed through ascending grades of alcohol (50%, 70%, 90%, absolute 1-100%, and absolute 2-100%).

- **Clearing**

Dehydrated tissues in cassettes were put through two changes of Xylene for 1hr each to facilitate the removal of alcohol which is immiscible with paraffin wax.

- **Infiltration**

The cleared tissues were put in two changes of melted paraffin wax at 60°C for one hour each.

- **Embedding**

The infiltrated tissues were removed from the tissue cassettes and embedded in paraffin wax using a stainless-steel embedding mould. The orientations of the tissues were carefully managed to enable capturing complete sections of the hippocampus during sectioning.

- **Trimming**

Tissue blocks were trimmed to expose the tissues for sectioning.

- **Sectioning**

Tissue blocks were sectioned at a thickness of 5 microns to obtain tissue ribbons. Tissue ribbons were cut and numbered in threes to maintain seriality across all the experimental groups. Tissue ribbons were floated in a hot water bath at 40°C to facilitate stretching and easy picking on a slide. Slides were placed on a hot plate at 40°C to facilitate the removal of water and coronal sections of the hippocampus were stained using Hematoxylin and Eosin (H&E) for histological evaluation of the hippocampus. The prepared, stained, and mounted slides of hippocampal tissues were examined using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA).

Immunohistochemical examination

Immunostaining of the thin section (5 μm) of the brain tissues for BCL2 protein expression in the hippocampus was carried out using standard immunohistochemical protocol. The method, whose principle is based on the antigen-antibody reaction, was used to demonstrate the expression of specific proteins (antigen) of interest in the hippocampus of rats. The protocol involved antigen retrieval and protein and peroxidase block. Primary antibody (anti-BCL-2) was diluted (1:100) in blocking buffer (10% goat serum with 5% bovine serum albumin (BSA) and 0.1% Triton X-100 in 10 mM PBS). Sections were then incubated in 500μl of rabbit respective antibodies solution overnight at (4°C).

Secondary antibody

Slides were then incubated in Horse Radish Peroxidase (HRP) conjugated secondary antibodies (diluted in PBS + 5% BSA) for one hour at room temperature. Quantification of the apoptotic neuron expressivity across the *cornu ammonis* layer of the hippocampus was determined from captured brain images (n=5 per group per analysis) using Image-J (NIH, USA).

Data analysis

The data obtained from the neurobehavioral and biochemical tests were subjected to statistical analysis using IBM SPSS Version 22.0. Analysis of Variance (One way ANOVA) followed by Tukey's Post Hoc test were used to check for significance. Results were expressed as Mean ± Standard Error of Mean (SEM) and $P \leqslant 0.05$ was considered as statistically significant.

Results

Role of zingerone on cognitive ability of Wistar rats using Y-maze test

The Y–maze test was used to evaluate spatial activity and spatial memory by calculating the number of arm entries, time spent in novel arm, number of correct spontaneous alternation and percentage alternation. From the result, in the group that received cadmium alone, we recorded a significant decrease in number of arm entries, time spent in novel arm, alternation and percentage alternation when compared to the normal control group ($p \leqslant 0.05$). In all zingerone treated groups (50 mg/kg b.w.t., 100 mg/kg b.w.t. and 200 mg/kg b.w.t.), there was a significant increase in spatial activity, evident by an increase in time spent in the novel arm, number of arm entries, alternation and percentage alternation value when compared to the group that received cadmium alone. The result also showed that the group which received high dose of zingerone (200 mg/kg b.w.t.), recorded high values for all the evaluated parameters, which shows that 200 mg/kg b.w.t. of zingerone increased spatial activity and cognitive ability better.

Anti-oxidative role of zingerone on cadmium induced oxidative stress

From the result, we observe a significant difference in the level of SOD, GPx, MDA and CAT. The level of SOD, CAT and GPx significantly decreased in Group B (cadmium 5 mg/kg only b.w.t.) when compared to the normal control group at p value $\leqslant$ 0.05. In all treatment group, administration of zingerone at different doses significantly increased SOD, MDA, CAT level when compared to the cadmium only group. There was significant increase in lipid peroxidation (MDA) level in Group B (cadmium 5 mg/kg only b.w.t.), when compared to the normal group. The treatment group (Zingerone 50 mg/kg b.w.t., 100 kg/mg b.w.t. and 200 mg/kg b.w.t.) showed a significant decrease in MDA level for all zingerone treated groups ($p \leqslant 0.05$) when compared to the cadmium Group B.

Table 1: Role of zingerone on cognitive ability of Wistar rats using y-maze test

Groups	Time in novel arm	Number of arm entries	Number of correct spontaneous alternation	Percentage alternation (%)
Group A	187.25 ± 17.92	6.25 ± 2.01	2.75 ± 2.136	47.55 ± 27.5
Group B	105.00 ± 07.63*	3.33 ± 0.88*	0.00 ± 0.000*	00.00 ± 0.00*
Group C	127.50 ± 14.93#	8.25 ± 0.62#	1.25 ± 0.63#	36.75 ± 12.27#
Group D	160.50 ± 14.61#	5.00 ± 1.29#	1.75 ± 0.75#	43.75 ± 15.72#
Group E	181.25 ± 39.81#	6.25 ± 2.01#	1.00 ± 0.70#	46.00 ± 22.93#
Group F	201.75 ± 51.94#	7.25 ± 1.97#	2.25 ± 1.32#	54.50 ± 23.59#

*Values were expressed as Mean ± SEM. *-showed significant difference compared with the Control Group A (P<0.05). #-showed significant difference when compared to Group B (cadmium only) group (P<0.05).*

Table 2: Anti-oxidative role of zingerone on cadmium induced oxidative stress

Groups	SOD	Catalase	GPx	MDA
A (Normal control)	13.15 ± 0.55	35.75 ± 0.45	30.90 ± 0.60	4.45 ± 0.25
B (Cadmium 5 mg/kg only)	9.65 ± 0.65[a]	31.20 ± 0.60[a]	23.80 ± 0.20[a]	7.00 ± 0.10[a]
C (Zingerone 200 mg/kg only)	11.30 ± 0.25	33.75 ± 1.75	25.20 ± 1.55	5.10 ± 010
D (Zingerone 50 mg/kg + cadmium)	10.85 ± 0.35[b]	35.25 ± 0.75[b]	28.40 ± 0.30[b]	5.80 ± 0.60[b]
E (Zingerone 100 mg/kg + cadmium)	11.20 ± 0.30[b]	35.95 ± 0.65[b]	26.85 ± 0.15[b]	5.25 ± 0.25[b]
F (Zingerone 200 mg/kg + cadmium)	11.25 ± 0.55[b]	34.50 ± 0.30[b]	27.65 ± 0.25[b]	6.17 ± 0.08[b]

Values were expressed as Mean ± SEM. [a]-showed significant difference compared with the Control Group A (P<0.05). [b]-showed significant difference when compared to Group B (cadmium only) group (P<0.05). MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: Catalase, Gpx: Glutathione Peroxidase

Effect of zingerone on the histology of the hippocampus (× 40)

Photomicrographs showing panoramic views of hippocampal general histomorphological presentations in rats in Groups A-F. (H&E) staining × 40. **Group A**: Normal control group showed a normal morphology of the hippocampal region, fine array of cells can be seen distantly arranged in the *cornus ammonis* (CA). **Group B:** Cadmium only group showed degenerative changes in the hippocampus characterized by fragmented pyramidal and granular layer, with loss of cellular process, loss of nuclear and cytoplasmic content surrounded by pyknotic nuclei. **Group C:** (Zingerone 200 mg/kg only) showed a normal morphological representation of the hippocampus. **Group D:** mildly similar in morphology with normal control group, characterized by succinctly expressed neurons with appreciable axons and dendrites observable within the neuropil, gradual restoration of cytoplasmic content indicated by yellow arrows. **Group E**: showed a slight alteration in the morphology of the hippocampus indicated by red arrow. **Group F:** Histomorphological presentation of the hippocampus appears normal, with slight alteration when compared to the normal control, with same features as seen in Group D. Profiles with a marked alteration is indicated by red arrows while groups with a mild alteration is indicated by yellow arrows.

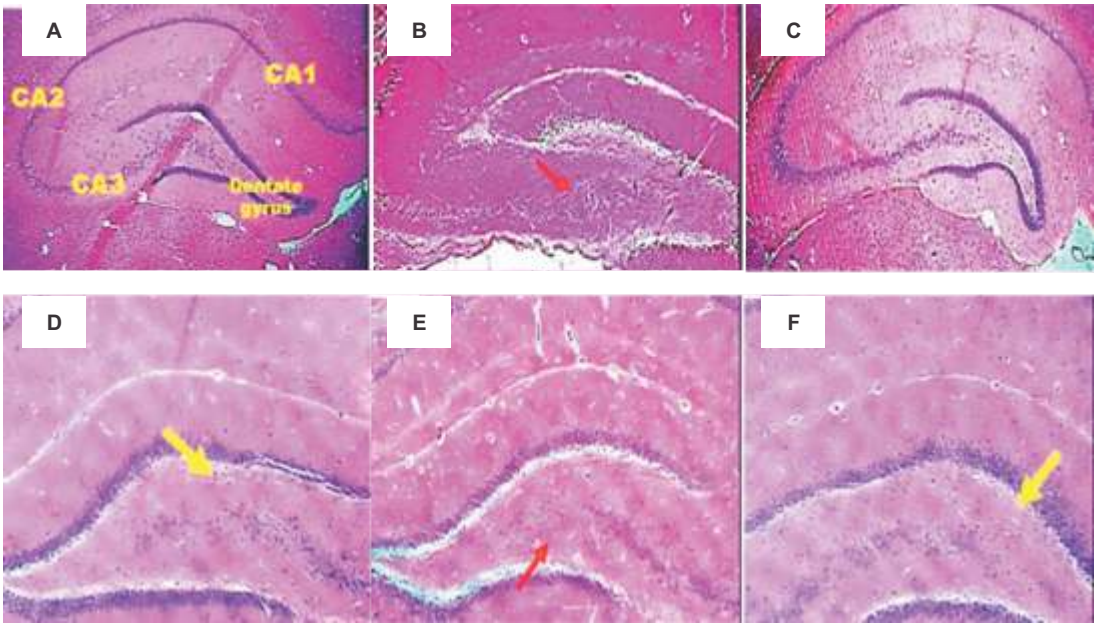

Figure 1: Showing the magnified views of the hippocampus general micromorphological presentations in Wistar rats across the study groups A-F (H&E) staining × 40. Dentate Gyrus (DG) composed of both pyramidal and granule cells, *cornu amonus* (CA1-3) containing pyramidal cells, are well demonstrated. Red arrows indicate profiles with significant cellular distortion, with evidence of degenerative changes in the hippocampus characterized by loss of cytoplasmic content and pyknotic nuclei while yellow arrows indicate profiles with normal histology of the hippocampus with mild alterations.

Effect of zingerone on the histology of the hippocampus (×100)

Showing magnified views of micro-morphological presentations hippocampus CA3 region in Wistar rats across the study groups A-F (H&E) staining ×100. The *cornu amonus* 3 (CA-3) containing granule and pyramidal cells, are well demonstrated. Red arrows indicate profiles with a significant cellular distortion, with evidence of degenerative changes in the hippocampus characterized while yellow arrows indicate profiles with normal histology of the hippocampus with mild alterations.

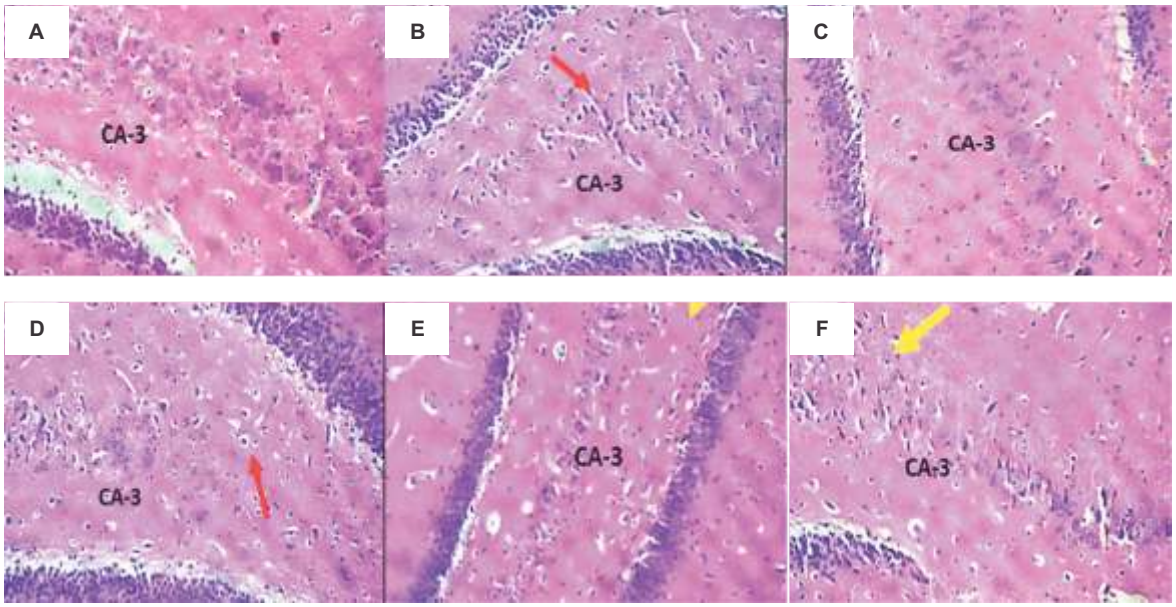

Figure 2: Showing magnified views of micromorphological presentations of hippocampus CA3 region in Wistar rats across the study groups A-F (H&E) staining ×100. *cornu ammonis* 3 (CA-3) containing granule and pyramidal cells, are well demonstrated. Red arrows indicate profiles with a significant cellular distortion, with evidence of degenerative changes in the hippocampus characterized by loss of cytoplasmic content and pyknotic nuclei while yellow arrows indicate profiles with normal histology of the hippocampus with mild alterations.

Immunohistological results: BCL2 (apoptotic neural cell expression)

Photomicrographs of hippocampal general micromorphological presentations in adult rats across Groups A-F. BCL-2 protein immunohistochemical expression by rat anti-BCL-2 antibody (scale bar 50um). Apoptotic neuron expressivity across the *cornu ammonis* layers of the hippocampus is demonstrated across the groups. Apoptotic immunopositive cells are indicated by red arrows across micrographs. **Group A**: showed increased observable apoptotic activity relative to other treatment. **Groups D-F** showed a relatively mild apoptotic activity.

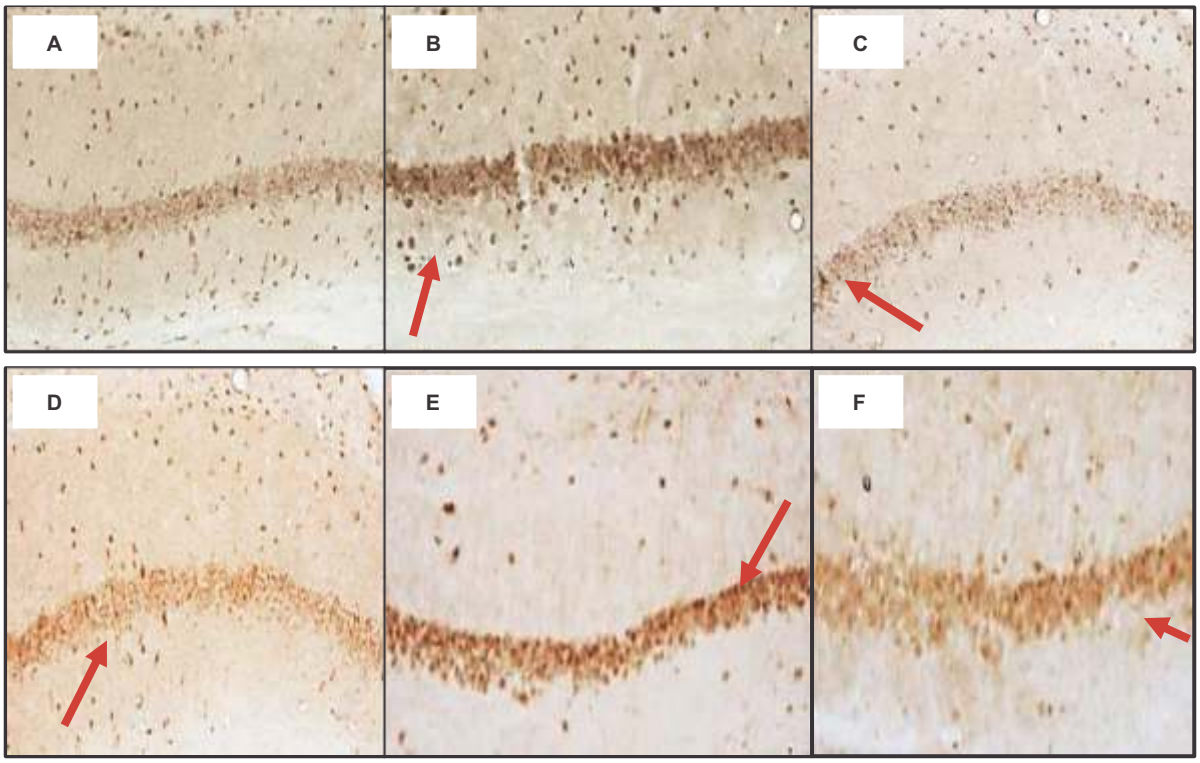

Figure 3: Micromorphological presentations of CA 3 region of the hippocampus across Groups A-F using BCL-2 protein immunohistochemical expression by rat anti-BCL-2 antibody (scale bar 50um). Apoptotic neuron expressivity across the layers of the hippocampus is demonstrated across study groups. Apoptotic immunopositive cells are indicated by red arrows.

BCL-2 expressivity in the hippocampal cornu ammonus region of the hippocampus

Figure showing number of BCL-2 positive cells across groups within the *cornu ammonis* region of the hippocampus by Image-J software. Values were expressed as Mean ± SD; # showed a significant difference compared with the normal control Group A ($p<0.05$) * showed significant difference when compared to the Group B ($p<0.05$).

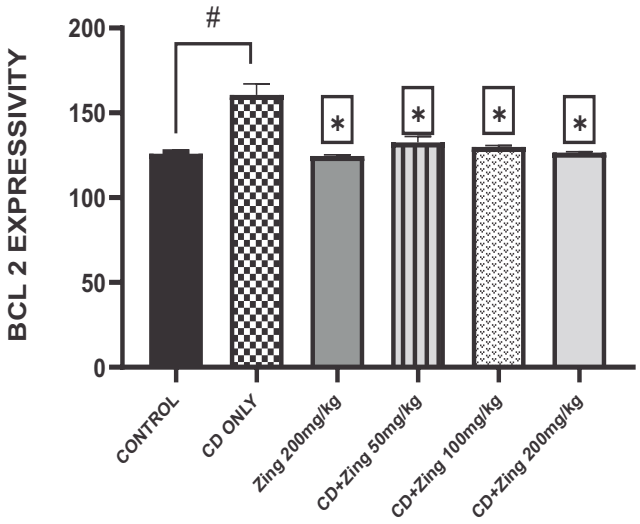

Figure 4: BCL-2 expressivity in the hippocampal cornu ammonus region of the hippocampus

Discussion

Exposure to cadmium over time may have a negative impact on cognitive function associated with neuronal cell death and oxidative stress in the hippocampus and cortical areas of the brain [23]. Reactive oxygen species, apoptosis, inflammation, and DNA repair disruption may result from cadmium poisoning, and this plays a major role in the development of neurodegenerative diseases including AD and PD [24]. Memory impairment has been implicated in cadmium induced toxicity [23]. Y- maze test was used to assess learning, short term memory and locomotor activity. Data from our study showed that cadmium administration caused a significant decrease in the value of total arm entries, time spent exploring novel arm and alternations. Previous studies showed that cadmium adversely impaired learning and memory abilities and this was evident in our study from the observable reduction in spatial memory parameters [23]. Similarly reported research showed that cadmium toxicity induced memory deficits, with reduced spontaneous alternations in mice exposed to cadmium [26]. However, in the group treated with only zingerone at 200 mg/kg b.w.t. and groups treated with zingerone after cadmium exposure, we recorded an increased in number of arm entries, increased in time spent exploring the novel arm and increased spontaneous alternation as compared to the group that received only cadmium. This indicates that zingerone may have the potential to improve cognitive ability, as previously reported about zingiber offinale and its component ameliorating cognitive impairment [27].

This study showed that cadmium evidently reduced the levels of SOD, CAT, GPx and increased lipid peroxidation level (MDA) similar to various findings which reported that cadmium may cause oxidative stress due to it's ability to cross the BBB [7]. Reported facts showed that cellular enzymatic antioxidants SOD, CAT and GPx significantly decreased as a result of the cadmium-induced lipid peroxidation. This is linked to the fact that oxidative stress can trigger DNA damage, inflammation and apoptosis in the brain, which can result in cognitive decline [27]. One of the earliest biochemical signs of neurodegeneration is considered to be caused by oxidative stress and neuroinflammation triggered by neurotoxins [25].

Administration of zingerone only and in the groups co-treated with zingerone after cadmium exposure, results showed that there was significant increase in the level of SOD, CAT, GPx activities and significant decrease in lipid peroxidation activity (MDA level). This shows that zingerone possess high anti-oxidant properties for scavenging free radicals and attenuating oxidative stress produced by toxins like cadmium which agrees with previous reports [9, 14]. The anti-oxidative potential of zingerone in scavenging free radical is attributed to the fact that zingerone can easily cross the BBB.

Progressive loss of neuronal content and structure can lead to cognitive impairment. Neurodegeneration, inhibition of neurogenesis in the hippocampus, debilitated mature neurons and cognitive impairment are all factors implicated in cadmium toxicity [24]. Histopathological findings from this study showed that varying doses of zingerone were beneficial in restoring neurogenesis and alteration caused by cadmium toxicity in the hippocampus. Histological examination of the hippocampus confirmed that cadmium only treated rats showed degenerative changes in the hippocampus, with loss of neuronal content, loss of cytoplasmic cellular content, reduced neuronal differentiation and axonogenesis, vacuolation and pyknotic nuclei. These observable histomorphological features in the hippocampus of rats exposed to cadmium was previously reported [16]. The histomorphological distortion seen in the hippocampus of rats exposed to cadmium accounted for cognitive deficit and reduced spatial activity observed in the Y-maze test. Zingerone showed great potential in reversing the alteration seen in the cadmium alone group, this was evident in the histological examination of rats in group treated with zingerone after cadmium exposure. The hippocampus showed a normal microanatomy with axon and dendrites clearly seen, clear cytoplasmic content and improved neurogenesis. This observation was well improved in the group which received higher dose of zingerone. This proves that high dose of zingerone can better protect the hippocampus against neurodegeneration and neural cell death. This finding is in agreement with previous studies which reported that zingerone has neuroprotective potential against brain damage and brain mitochondrial toxicity [14].

Apoptosis is a physiological process that eliminates unneeded cells from the body during development and keeps tissues in a state of equilibrium. Apoptotic cells can exhibit some morphological and biochemical alterations, such as cell shrinkage and chromatin condensation [28]. Research has linked oxidative stress to trigger apoptosis as increased production of ROS can result in neural cell death [29]. Growing research suggest that cadmium can negatively impact nervous system functions by causing neuronal death [30]. But however, the particular process by which cadmium induces apoptosis is unclear. Apoptotic neuron expressivity across the *cornu ammonis* layers of the hippocampus was demonstrated using BCL-2 protein immunohistochemical expression by rat anti-BCL-2 antibody. Apoptotic neuron expressivity across *cornu ammonis* layer of the hippocampus showed an observable increased in apoptotic activity in cadmium alone treated rats as compared to other groups. However, varying doses of zingerone administration showed a reduction in apoptotic activity.

Conclusion

Our findings showed that oxidative stress, apoptosis, degenerative changes with cellular alterations in histoarchitecture of the hippocampus as a result of cadmium exposure are all implicated in cognitive impairment. However, treatment with various doses of zingerone showed improved cognitive behavior, spatial memory, improved anti-oxidant activities and normal histology in the hippocampus of Wistar rats. This study also showed that high dose of zingerone exhibit more potential in improving memory. More research is needed to determine the exact mechanism by which zingerone enhances memory.

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References

1. Liu J, Lewis G. Environmental toxicity and poor cognitive outcomes in children and adults. *J Environ Health* 2014; 76(6):130-138.
2. Obasi KK, Anyanwu GE. Fisetin potentiates anxiolysis in rat models of aluminium chloride-induced Alzheimer-like disease. *J Krishna Inst Med Sci Univ* 2022; 11(3): 39-49.
3. Tolcin, AC. Cadmium. In: Mineral Commodity Summaries, U.S. Government Publishing Office, Washington DC 2016; 42-43.
4. Munisamy R, Ismail SNS, Praveena SM. Cadmium exposure via food crops: a case study of intensive farming area. *Am J Appl Sci* 2013;10:1252-1262.
5. Das, SC, Al-Naemi, HA. Cadmium toxicity: oxidative stress, inflammation and tissue injury. *Occup Dis Environ Med* 2019;7: 144-163.
6. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The Effects of Cadmium Toxicity. *Int J Environ Res Public Health* 2020; 17(11):3782.
7. Areba GO, Khalid R, Ngure RM, Maloba F, Nyaga N, Moseti KO, *et al*. Neuroprotective effects of tea against cadmium toxicity. *Bioactive Compounds Health Dis* 2019; 2(12): 230-246.
8. Shukla Y, Singh M. Cancer preventive properties of ginger: A brief review. *Food Chem Toxicol* 2007;45(5):683-690.
9. Rehman AB, Amin MU, Arif I, Rasool A, Bhat S, Afzal SA, *et al*. A review on pharmacological properties of Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). *Sci World J 2015*, 816364.
10. Kumar L, Harjai K, Chhibber S. Recent update on multiple pharmacological benefits of zingerone: a quick review. *Am J Phytomed Clin Ther* 2014;2:693-704.
11. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 2001; 49: 5165-70.
12. Safhi MM. Zingerone protects the Tellurium toxicity in the brain mitochondria of rats. *Metabolomics* 2015; 5: 156.
13. Mohammad FA, Saeed A, Essam A, Mohammad AA, Tarique A, Khan G. Nephroprotective effects of 4-4(hydroxyl-3 methoxyphenyl)-2-butane against sodium tellurite induced acute kidney dysfunction by attenuating oxidative stress and inflammatory cytokines in rats. *Arabian J Chem* 2022;15(6):1878-5352.
14. Alam MF. Neuroprotective effects of Zingerone against carbon tetrachloride (CCl4) induced brain mitochondrial toxicity in Swiss albino mice. *J Appl Nat Sci* 2018;10(2):548-552.
15. Libakhshi T, Khodayar MJ, Khorsandi L, Rashno M, Zeidooni L. Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity. *Biomed Pharmacother* 2018;10(5): 225-232.
16. Vijaya P, Kaur, Garg, N, Garg N, Sharma, S. Protective and therapeutic effects of garlic and tomato on cadmium-induced neuropathology in mice. *J Basic Appl Zoo* 2020; 81: 23.

17. Shati AA. Effects of Origanum majorana L. on cadmium induced hepatotoxicity and nephrotoxicity in albino rats. *Saudi Med J* 2011; 32(8):797-805.
18. Onaolapo OJ, Onaolapo AY, Akanji OO, Abiodun OR. Elevated plus maze and Y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. *IOSR-J Pharm Biol Sci* 2012; 3(4):21.
19. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974;47:469-474.
20. Mohandas J, Marshal JJ, Duggin GG, Horvath JS, Tiller DG. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. *Cancer Res* 1984;44(11): 5086–5091.
21. Clairborne A. Catalase activity. In: CRS Handbook of Methods in Oxygen Radical Research. Greenwald RA ed. CRS Press, Boca Raton 1985: 283-284.
22. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95(2): 351-358.
23. Wang H, Zhang L, Abel GM, Storm DR, Xia Z. Cadmium exposure impairs cognition and olfactory memory in male C57BL/6 mice. *Toxicol Sci* 2018; 161(1):87-102.
24. Wang B, Du Y. Cadmium and its neurotoxic effects. *Oxid Med Cell Longev* 2013:898034.
25. Atuadu VO, Oyem JC, Finbarrs-Bello E, Ezeah C. Mucuna puriens root extract: A novel therapeutic agent for lead induced polyphagia and hippocampal neurotoxicity in adult Wistar rats. *J Krishna Inst Med Sci Univ* 2022; 11(1):14-29.
26. Lukawski K, Nieradko B, Sieklucka-Dziuba M. Effects of cadmium on memory processes in mice exposed to transient cerebral oligemia. *Neurotoxicol Teratol* 2005; 27(4):575-584.
27. Franzoni F, Scarfò G, Guidotti S, Fusi J, Asomov M, Pruneti C. Oxidative stress and cognitive decline: the neuroprotective role of natural antioxidants. *Front Neurosci* 2021;15:729-757.
28. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta* 2016;1863(12):2977-2992.
29. Robertson JD, Orrenius S. Molecular mechanisms of apoptosis induced by cytotoxic chemicals. *Crit Rev Toxicol* 2000; 30(5): 609-627.
30. Yuan Y, Jiang CY, Xu XH, Sun H, Hu FF, Bian JC, *et al*. Cadmium-induced apoptosis in primary rat cerebral cortical neurons culture is mediated by a calcium signaling pathway. *PLoS One* 2013;8(5)e64330.

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