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Modulatory Effects of Ferulic Acid on Cadmium-Induced Brain Damage

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Stephen A. Adefegha, PhD[1], Olasunkanmi S. Omojokun, Mtech[1], Ganiyu Oboh, PhD[1], Olasunkanmi Fasakin, BTech[1], and Opeyemi Ogunsuyi, BTech[1]

Abstract

Studies have shown the pharmacological relevance of phenolics like ferulic acid (FA) in promoting health. This study sought to investigate the modulatory effects of FA on cadmium-induced brain damage in rats. Brain damage was induced in Wistar strain rats by oral administration of cadmium (5 mg/kg body weight) for 21 days. Assays for malondialdehyde (MDA) content, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidase (MAO), and Na^+/K^+-ATPase activities were carried out. The study revealed significant ($P < .05$) increases in the MDA content and all enzymes' (AChE, BChE, MAO, and Na^+/K^+-ATPase) activity investigated following cadmium administration. However, rats administered FA (10 and 20 mg/kg body weight) alongside cadmium significantly ($P < .05$) protected the brain by reversing the level of lipid peroxidation as measured by the MDA content as well as the enzymes' activity. This study, therefore, substantiates the neuroprotective potentials of FA especially in the management of cadmium-induced toxicity.

Keywords

cadmium, ferulic acid, cholinesterases, monoamine oxidase, Na^+/K^+-ATPase, malondialdehyde

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Cadmium is one of the heavy metals that contributes to serious industrial and environmental pollution. Cadmium is nonbiodegradable; thus, the main source of exposure is its bioaccumulation in plants grown on cadmium-polluted soil, some of which later serve as food for humans.[1] Cadmium is used in the production of materials such as batteries, plastics, metal coatings, and pigments, which may contaminate the soil on disposal. In addition, occupational exposure in the course of manufacturing these products is another source of exposure to cadmium. Furthermore, cadmium can accumulate in several organs and tissues and has been reported to induce damage to organs such as testis, kidney, liver, and brain.[1,2] One of the proposed mechanisms for organ damage induced by cadmium is the induction of free radical generation and impairment of cellular antioxidant defense mechanisms, thus inducing oxidative stress.[3] Oxidative stress, on the other hand, can induce oxidative and peroxidative damages to cellular components such as DNA, protein, and lipid, leading to overall organ damage.

Ferulic acid (FA; Figure 1) is a natural phenolic, a derivative of caffeic acid with wide distribution in fruits and vegetables.[4] It is rapidly absorbed in the guts and does not undergo degradation by stomach acidic contents.[5,6] Several findings have reported therapeutic potentials of FA, which has been largely linked to its antioxidant properties. Srinivasan et al[7] reported the ability of FA to prevent carbon tetrachloride–induced liver damage in female rats by reducing plasma liver damage indices as well as lipid and protein oxidations. Similarly, Roy et al[8] reported that FA was able to restore the levels of endogenous antioxidant enzymes (superoxide dismutase and catalase) in pancreatic tissue of streptozotocin-induced diabetic rats.

One of the most susceptible organs to cadmium toxicity is the brain. This is because cadmium can cross the blood-brain barrier and promote oxidative damages[1] as a result of the high oxygen demand, high content of polyunsaturated fatty acids, and weak endogenous antioxidant defense system of the brain.[9] These ultimately lead to oxidative stress in the brain and thus induce several neurotoxic effects that have been reported to

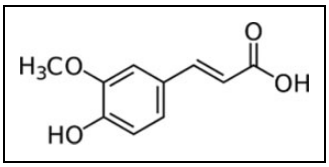

Figure 1. The chemical structure of ferulic acid.

[1] Federal University of Technology, Akure, Ondo State, Nigeria

Corresponding Author:
Ganiyu Oboh, Funct Foods, Nutraceuticals and Phytomedicine Unit, Department of Biochemistry, School of Sciences, Federal University of Technology, Akure, PMB 704, Akure 340001, Ondo State, Nigeria.
Email: goboh2001@yahoo.com

produce symptoms such as headache, impairment in learning and memory, and impaired peripheral nerve functions.[1]

Monoamine oxidase (MAO), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and Na^+/K^+-ATPase are unique enzymes identified as therapeutic targets in neurodegenerative conditions. MAO is involved in the oxidation of amine neurotransmitters and excessive elevation in its activity may favor progression of depression and other neurodegenerative conditions such as Alzheimer's disease.[10] Decreased brain cholinergic neurotransmission can lead to impairment in memory and cognitive functions.[10] AChE and BChE are enzymes of the cholinergic system that hydrolyze acetylcholine and their modulation has been identified as possible therapeutic strategies in the management of neurodegeneration.[10] Na^+/K^+-ATPase is an enzyme predominant at neuronal cell membranes and functions to maintain membrane potential essential for neuronal excitability by moving (Na and K) ions across neuronal plasma membrane.[11] Consequently, impairment in the activity of Na^+/K^+-ATPase has been linked to neuronal damage and neurodegenerative processes in the brain.[11,12]

In addition, cadmium has been reported to produce diverse modulatory effects on the cholinergic and monoaminergic neuronal systems.[13] On the other hand, FA has been linked to potential neuroprotective properties partly due to their antioxidant effects; in an in vitro experiment, Trombino et al[14] reported the ability of FA to reverse lipid peroxidation induced in reconstituted rat brain microsome by peroxy radicals.

Therefore, in this study, we aimed to investigate the neuromodulatory properties of FA in cadmium-induced brain damaged rats. FA (10 and 20 mg/kg body weight [bw]) were administered over the course of 21 days, and its protective ability was investigated by assessing the level of lipid peroxidation and its effect on Na^+/K^+-ATPase cholinergic and monoaminergic enzyme activities in the rat brain tissue homogenate.

Materials and Methods

Chemical and Reagents

Chemicals such as ferulic acid, acetylthiocholine iodide, butyrylthiocholine iodide, semicarbazide, benzylamine, adenosine triphosphate (ATP), ouabain, ammonium molybdate, thiobarbituric acid (TBA), dinitrophenylhydrazine (DNPH), sodium acetate, sodium dodecyl sulfate, phosphorus red, ascorbic acid, magnesium chloride, phosphate buffer of different molarity, adenosine monophosphate (AMP), sodium hydroxide, benzene, and 5,5′-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma-Aldrich, ChemieGmH (Steinheim, Germany). Acetic acid was procured from BDH Chemical Ltd (Poole, England). Cadmium was sourced as cadmium chloride. Except otherwise stated, all other chemicals and reagents were of analytical grade while water was glass distilled. A JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo WORLD SCIENTIFIC, Dunmow, UK) was used to measure absorbance throughout the experiment.

Experimental Design

Animals. Male Wistar strain rats, weighing between 150 and 160 g, were purchased from the Central Animal House, University of Ibadan, Ibadan, Oyo State, Nigeria. They were acclimatized for 1 week in a stainless steel cage under controlled conditions of a 12-hour light/dark cycle, 50% humidity, and 28°C temperature. The rats were allowed access to food and water ad libitum. All animals were used in accordance with the guidelines of the Ethical Committee on Care and Use of Experimental Animals of The Federal University of Technology, Akure, Ondo State, Nigeria.

Treatment Groups. The animals were divided into 7 groups of 5 animals each, and all samples (cadmium, FA, and prostigmine) were administered orally via intubation for 21 days:

Group 1: Normal rats, administered normal saline (Basal group).
Group 2: Rats administered 5 mg/kg bw of cadmium (negative control group)
Group 3: Rats administered cadmium (5 mg/kg bw) and 2.5 mg/kg bw of prostigmine (positive control group)
Group 4: Rats administered cadmium (5 mg/kg bw) and FA (10 mg/kg bw)
Group 5: Rats administered cadmium (5 mg/kg bw) and FA (20 mg/kg bw)
Group 6: Rats administered FA (10 mg/kg bw) only
Group 7: Rats administered FA (20 mg/kg bw) only

Preparation of Tissue Homogenates. The rats were dislocated at the cervical region and the cerebral tissue (whole brain) was rapidly dissected, placed in phosphate buffer (pH 7.4) on ice, and weighed. This tissue was subsequently rinsed with the phosphate buffer (pH 7.4) and later homogenized with the phosphate buffer, pH 7.4 (1:5 w/v), with about 10 up and down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 minutes at 3000 *g* to yield a pellet that was discarded and the supernatant was used for all assays.

Methods

Lipid Peroxidation and Thiobarbituric Acid Reactions

The lipid peroxidation assay was carried out according to the modified method of Ohkawa et al.[15] Briefly, 300 µL of the brain homogenate was added to 300 µL of 8.1% sodium dodecyl sulfate (SDS), 500 µL of acetic acid/HCl buffer (pH 3.4), and 500 µL of 0.6% thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 hour, and thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm using a spectrophotometer. Malondialdehyde (MDA) was used as standard and TBARS produced was reported as MDA equivalent.

Enzyme Assays

Monoamine Oxidase Activity Assay. The assay for MAO activity was performed according to the modified method described by Turski et al.[16] In brief, the reaction mixture contained 0.025 M phosphate buffer of pH 7, 0.0125 M semicarbazide, 10 mM benzylamine (pH adjusted to 7), and 0.67 mg of tissue. After 30 minutes, 250 µL of acetic acid was added and boiled for 3 minutes in a boiling water bath followed by centrifugation. The resultant supernatant (250 µL) was mixed with equal volume of 0.05% of 2,-DNPH and 1.25 mL of benzene was added after 10 minutes of incubation at room temperature. After separating the benzene layer, it was mixed with equal volume of 0.1 N NaOH. The alkaline layer was decanted and heated at

80°C for 10 minutes. The orange-yellow color that developed was measured at 450 nm in a spectrophotometer. The enzyme's activity was expressed in mmol/mg protein.

Cholinesterase (AChE and BChE) Activity Assay. The AChE activity was investigated according to the modified method of Ellman et al.[17] The AChE activity was determined in a reaction mixture containing 50 µL of brain homogenate, 50 µL of 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB), 1175 µL of 0.1 M phosphate-buffered solution (pH 8.0). After incubation for 20 minutes at 25°C, 25 µL acetylthiocholine iodide solution was added as the substrate. A total of 25 µL of butyrylthiocholine iodide was used as a substrate to assay for BChE activity, while all the other reagents and conditions remained the same. The AChE and BChE activities were determined as changes in absorbance reading at 412 nm for 3 minutes at 25°C using a spectrophotometer, and the enzyme's activities expressed as µmol/mg protein.

Na^+,K^+-ATPase Activity Assay. The Na^+/K^+-ATPase activity was performed according to the modified method described by Wyse et al.[18] The reaction mixture for Na^+,K^+-ATPase assay contained 120 mMTris–HCl, 0.4 mM EDTA, 200 mM NaCl, 20 mM KCl, and 24 mM $MgCl_2$ (pH 7.4), in a final volume of 200 µL. After 10 minutes of preincubation at 37°C, the reaction was initiated by addition of 50 µL adenosine triphosphate (ATP) to a final concentration of 3.0 mM, and was incubated for 20 minutes. Controls were carried out under the same conditions with the addition of 50 µL of 1.0 mM ouabain. The reaction was terminated by the addition of 50 µL of 10% (w/v) trichloroacetic acid. The amount of inorganic phosphate (Pi) released was quantified using a reaction mixture that contained 250 µL of ammonium molybdate (100 mM), 200 µL of reaction mixture from first grid, 500 µL of distilled water, and 50 µL of ascorbic acid (8%). Released inorganic phosphate (Pi) was measured at 620 nm in a spectrophotometer. Specific activity of the enzyme was expressed as nmol of Pi released per minute per mg of protein.

Statistical Analysis

The results of replicate experiments were represented as mean ± standard deviation (SD). Statistical analyses were done using Statistical Program for Social Science (SPSS) 16.0 (SPSS Inc, Chicago, IL). One-way analysis of variance was carried out followed by Duncan's test for the post hoc treatment. A value of $P < .05$ was considered to be statistically significant.

Results

Figure 2 shows that there was an increase in the brain MDA content of rats administered 5 mg/kg bw of cadmium (negative control group) when compared to rats administered normal saline (basal group) and those administered 5 mg/kg bw of cadmium and 2.5 mg/kg bw of prostigmine (positive control group). However, the brain MDA level was significantly reduced ($P < .05$) in rats administered cadmium (5 mg/kg bw) with FA (10 and 20 mg/kg bw) dose dependently. Nevertheless, no significant difference ($P > .05$) was observed between the brain MDA content of the basal rat group and normal rats treated with FA (10 and 20 mg/kg bw) alone.

The results of the effect of cadmium-induced brain damage on MAO activity is shown in Figure 3. The results showed that

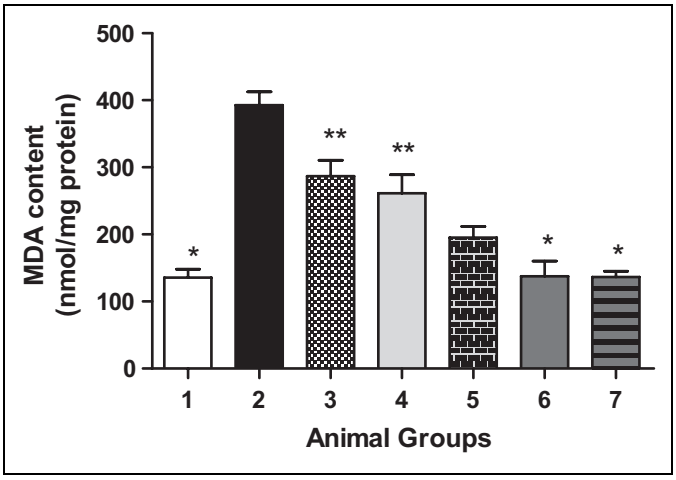

Figure 2. Effect of ferulic acid on lipid peroxidation (MDA content) in cadmium-induced brain damage in rats.
Key—*Group 1:* Rats administered normal saline (basal group); *Group 2:* negative control rats administered 5 mg/kg body weight (bw) of cadmium (Cd); *Group 3:* positive control rats administered Cd and prostigmine (2.5 mg/kg bw); *Group 4:* rats administered Cd and 10 mg/kg bw of ferulic acid; *Group 5:* rats administered Cd and 20 mg/kg bw of ferulic acid; *Group 6:* rats administered 10 mg/kg bw of ferulic acid; *Group 7:* rats administered 20 mg/kg bw of ferulic acid.
Values represent mean ± standard deviation (n = 5).
*No significant difference at $P < .05$. **No significant difference at $P < .05$.

cadmium induced an increase in the MAO activity in rats administered cadmium (negative control group), when compared with the basal group (administered normal saline) and positive control group (administered cadmium and prostigmine). However, this was significant reversed ($P < .05$) in rats administered cadmium and FA (10 and 20 mg/kg bw) dose dependently, while there was no significant ($P > .05$) difference between the enzyme's activity in the basal group and rat groups administered FA (10 and 20 mg/kg bw) alone.

The effect of cadmium-induced brain damage on cholinesterase activities in rats showed that there was an increase in the AChE activity (Figure 4) of rats administered cadmium, when compared to basal rat group and rats administered cadmium and prostigmine. However, there was a significant reversal ($P < .05$) in the AChE activity of rats administered cadmium and FA (10 and 20 mg/kg bw). There was no significant ($P > .05$) difference between the enzyme's activity in the basal group and rat groups treated with FA (10 mg/kg bw) alone. Similarly, Figure 5 shows that there was an increase in the BChE activity of rats administered cadmium when compared to the basal group and rats administered cadmium and prostigmine. However this was reversed significantly ($P < .05$) in rat groups administered cadmium and FA (10 and 20 mg/kg bw). There was, in addition, no significant ($P > .05$) difference between the enzyme's activity in the basal rat group, rats administered cadmium and 20mg/kg bw of FA, and normal rat groups treated with FA (10 and 20 mg/kg bw) alone.

We report the effect of cadmium-induced brain damage on Na^+/K^+-ATPase activity in rats in Figure 6. This revealed that

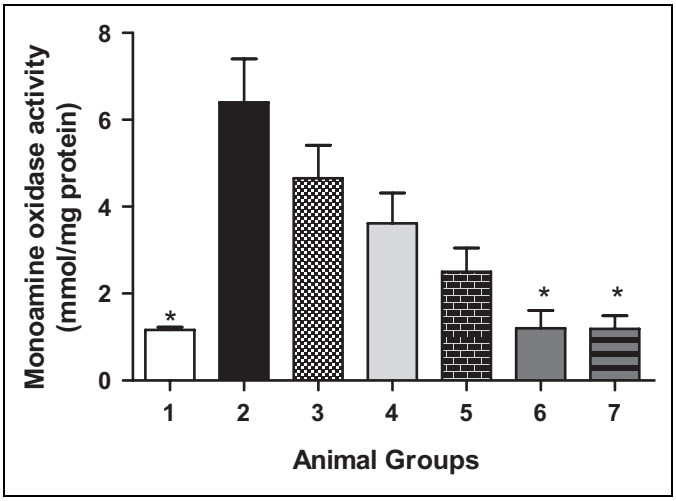

Figure 3. Effect of ferulic acid on monoamine oxidase (MAO) activity in cadmium-induced brain damage in rats.
Key—*Group 1:* Rats administered normal saline (basal group); *Group 2:* negative control rats administered 5 mg/kg body weight (bw) of cadmium (Cd); *Group 3:* positive control rats administered Cd and prostigmine (2.5 mg/kg bw); *Group 4:* rats administered Cd and 10 mg/kg bw of ferulic acid; *Group 5:* rats administered Cd and 20 mg/kg bw of ferulic acid; *Group 6:* rats administered 10 mg/kg bw of ferulic acid; *Group 7:* rats administered 20 mg/kg bw of ferulic acid.
Values represent mean ± standard deviation (n = 5).
*No significant difference at $P < .05$.

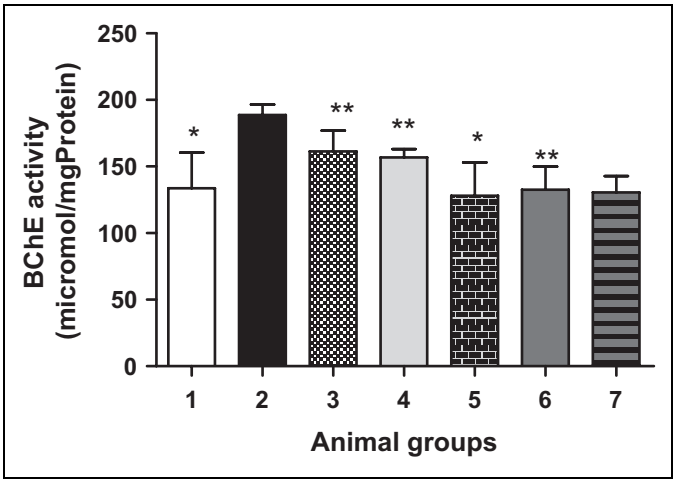

Figure 5. Effect of ferulic acid on butyrylcholinesterase (BChE) activity in cadmium-induced brain damage in rats.
Key—*Group 1:* Rats administered normal saline (basal group); *Group 2:* negative control rats administered 5 mg/kg body weight (bw) of cadmium (Cd); *Group 3:* positive control rats administered Cd and prostigmine (2.5 mg/kg bw); *Group 4:* rats administered Cd and 10 mg/kg bw of ferulic acid; *Group 5:* rats administered Cd and 20 mg/kg bw of ferulic acid; *Group 6:* rats administered 10 mg/kg bw of ferulic acid; *Group 7:* rats administered 20 mg/kg bw of ferulic acid.
Values represent mean ± standard deviation (n = 5).
*No significant difference at $P < .05$. **No significant difference at $P < .05$.

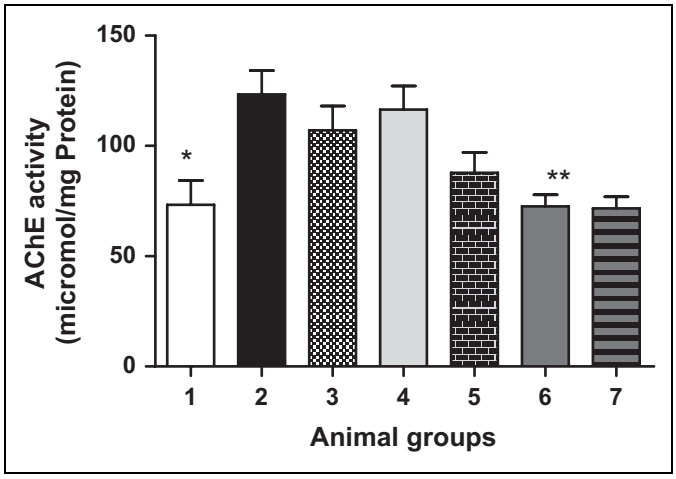

Figure 4. Effect of ferulic acid on acetylcholinesterase (AChE) activity in cadmium-induced brain damage in rats.
Key—*Group 1:* Rats administered normal saline (basal group); *Group 2:* negative control rats administered 5 mg/kg body weight (bw) of cadmium (Cd); *Group 3:* positive control rats administered Cd and prostigmine (2.5 mg/kg bw); *Group 4:* rats administered Cd and 10 mg/kg bw of ferulic acid; *Group 5:* rats administered Cd and 20 mg/kg bw of ferulic acid; *Group 6:* rats administered 10 mg/kg bw of ferulic acid; *Group 7:* rats administered 20 mg/kg bw of ferulic acid.
Values represent mean ± standard deviation (n = 5).
*No significant difference at $P < .05$.

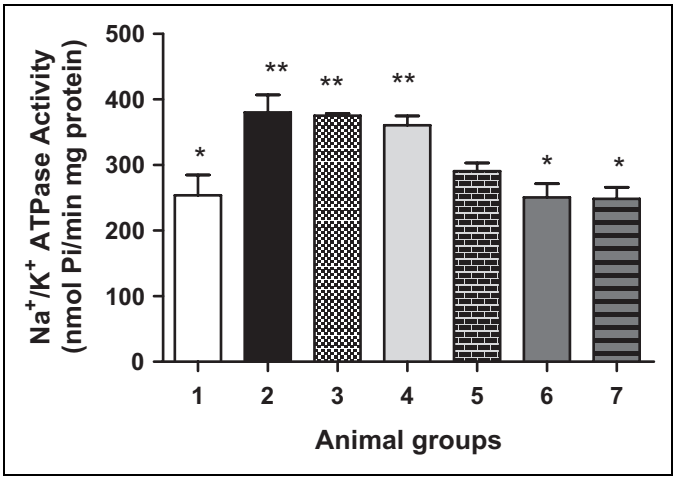

Figure 6. Effect of ferulic acid on Na^+/K^+-ATPase activity in cadmium-induced brain damage in rats.
Key—*Group 1:* Rats administered normal saline (basal group); *Group 2:* negative control rats administered 5 mg/kg body weight (bw) of cadmium (Cd); *Group 3:* positive control rats administered Cd and prostigmine (2.5 mg/kg bw); *Group 4:* rats administered Cd and 10 mg/kg bw of ferulic acid; *Group 5:* rats administered Cd and 20 mg/kg bw of ferulic acid; *Group 6:* rats administered 10 mg/kg bw of ferulic acid; *Group 7:* rats administered 20 mg/kg bw of ferulic acid.
Values represent mean ± standard deviation (n = 5).
*No significant difference at $P < .05$. **No significant difference at $P < .05$.

cadmium induced a significant ($P < .05$) rise in the activity of this enzyme in rats administered cadmium (negative control group) when compared to the basal group rats. However, there was significant decrease ($P < .05$) in the enzyme's activity only in rats administered cadmium and 20mg/kg bw of FA. Moreover, there was also no significant ($P > .05$) difference between the enzyme's activity in the basal group rats and normal rat groups administered FA (10 and 20 mg/kg bw) alone.

Discussion

There is growing public and research interest in the use of phytochemicals derived from dietary sources to combat human diseases.[19] Ferulic acid; a phytochemical commonly found in fruits and vegetables, has been reported to exhibit a wide range of therapeutic potentials against various diseases such as cancer, diabetes, and cardiovascular and neurodegenerative diseases.[4,19] FA is found in abundance in food sources such as vegetables and fruits and consumption of these food sources have been estimated to a daily intake of about 150 to 250 mg of FA.[20] It has been established that FA is not degraded by the acidic nature of the stomach and is well absorbed into the blood plasma in its active form.[5,6]

The increased MDA content in cadmium-administered rats suggests enhanced lipid peroxidation induced by cadmium administration. Oxidative attack on polyunsaturated fatty acids of biological membrane may compromise their integrity and functions.[21] The damage caused by lipid peroxidation is highly detrimental to the functioning of cells and its survival.[22] However, this agrees with previous reports that cadmium induced its cellular toxicity by indirectly stimulating reactive oxygen production and thus stimulating oxidative stress.[3] However, there was significant reduction in the MDA content of rats treated with cadmium and FA in a dose-dependent manner (10 and 20 mg/kg bw). This could be as a result of the antioxidant property of FA as previously reported; Srinivasan et al[19] reported the antioxidant property of FA and stated that it can effectively scavenge superoxide anion radicals and inhibit lipid peroxidation, while Trombino et al[14] also reported that FA inhibited peroxyl radical–induced lipid peroxidation in reconstituted rat brain microsomes.

Excessive MAO activity has been linked to increased generation of free radicals in the brain and, consequently, neuronal damage.[23] Therefore, possible inhibition of MAO activity may be a therapeutic target toward the management of Alzheimer's disease and other neurodegenerative conditions.[24] The increased MAO activity in cadmium-administered rats (negative control group) may therefore be another indicator of impaired neuronal functions leading to brain damage. However, the significant and dose-dependent reduction in the MAO activity in rats administered cadmium and FA (10 and 20 mg/kg bw) may indicate that FA was able to inhibit the formation of hydrogen peroxide and ammonia, which are potentially harmful products of amine degradation, thereby acting as MAO inhibitors and exerting its neuroprotective potentials.[25]

Cholinesterases (AChE and BChE) are enzymes relevant to cognitive functions and memory, and they have been shown to be therapeutic targets in the management of several neurodegenerative diseases, especially Alzheimer's disease.[26] Therefore, the use AChE and BChE inhibitors to reduce the hydrolysis of acetylcholine may be a good approach toward the treatment and management of neurodegeneration.[24] Normally, in the healthy brain, AChE is predominant. However, on neuronal damage BChE activity rises while AChE activity remains unchanged or diminishes.[27] The observed increases in both AChE and BChE activities in cadmium-administered rats are potential indicators of neuronal damage induced by cadmium. Interestingly, the administration of cadmium with FA (10 and 20 mg/kg bw) led to a significant reversal in the enzymes' activities and could further corroborate the neuroprotective potentials of FA. It is noteworthy that administration of cadmium with 10kg/mg bw reduced the BChE activity to a level not significantly different from that produced when cadmium was administered with the standard cholinesterase inhibitor—prostigmine. Similarly, rats administered cadmium with 20 mg/kg bw of FA reduced the rise in BChE activity induced by cadmium to the levels not significantly different from those observed in basal rats or rats administered FA alone.

Furthermore, we observed that brain damage induced by cadmium in this study led to the increase in the activity of Na^+/K^+-ATPase. This enzyme is essential for proper neuronal function; it helps in maintaining action potential across membranes and acts as a secondary active transporter.[12] Therefore, impairment in its function by overstimulation can lead to neuronal damage. However, administration of FA with 20 mg/kg bw gave a significant reversal in the increase of Na^+/K^+-ATPase activity induced by cadmium.

Conclusion

This study has been able to show that cadmium at a dose of 5 mg/kg body weight was able to induce brain damage in rats as shown by elevated MDA contents as well as increased activities of Na^+/K^+-ATPase, cholinergic, and monoaminergic enzymes essential for proper brain functioning. However, rats administered cadmium with FA (10 and 20 mg/kg bw) showed a significant reduction in both MDA contents and enzymes' (AChE, BChE, MAO, and Na^+/K^+-ATPase) activities. This could therefore further substantiate the possible neuroprotective potentials of FA, especially in the management of heavy metal toxicity; nevertheless, further experimental studies and clinical trials are recommended.

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Author Contributions

SAA and GO participated actively in the design of the experiment and provided mentorship support. OF and OO participated in the design of the experiment, conducted the experiment, and carried out data analysis.

OSO participated in the design of the research, supervised the experiment, and drafted the article. All authors read and approved the article for publication.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The ethics regulation standards of the Helsinki Declaration of 1975 as revised in 2000 was followed strictly in accordance with Federal University of Technology, Akure, institutional guidelines for laboratory experiments and the protection of animals during experiments (Reference Number FUTA/SOS/1502).

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