Neuroprotective role of zingerone: investigating the effective doses of zingerone in lead acetate-induced brain dysfunctions in rats

Abstract

This experiment was designed to determine the effective dose of zingerone against the sublethal dose of lead acetate that induced brain dysfunctions in rats through using different successive doses of zingerone on some parameters related to oxidative stress for 28 days. Thirty-six adult male rats were randomly selected and divided equally into six experimental groups and treated for 28 days as follows: Control group: administered (orally) sterile distilled water. G1 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 25mg/kg for 4 weeks, the rats were then dissected. G2 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 50mg/kg for 4 weeks, the rats were then dissected. G3 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 100mg/kg for 4 weeks, the rats were then dissected. G4 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 150mg/kg for 4 weeks, the rats were then dissected. G5 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 200mg/kg for 4 weeks, the rats were then dissected. Blood samples were collected, by heart punched under anesthesia, at the end of the experiment for measuring the serum malondialdehyde, neuroglobulin, and dopamine concentrations. The result showed a significant ($P<0.05$) positive correlation between successive doses of zingerone and dopamine and neuroglobulin concentrations, while a significant ($P<0.05$) negative correlation between successive doses of zingerone and the concentration of malondialdehyde in all animals that are treated with lead acetate compared to the control group. Concluded from this research show that the zingerone has potent antioxidants and neuroprotective effects at the dose 125 mg/kg BW may result in a significant improvement of the neurotransmitter levels and decrease in the production of oxidative stress to the brain tissue.

Keywords: Brain damage, Effective dose, Neurodegenration diseases, Oxidative stress, Zingerone.

Introduction

“Lead (Pb) is a heavy metal that is utilized extensively in many different forms despite concerns of its harmful effects being well established (1). Pb exposure causes a number of negative effects, especially in the brain, even at low levels (2). As a neurotoxicant, lead reaches the brain through the blood-brain barrier and causes oxidative stress (3), morphologic damage, neurodegeneration, and cognitive impairment, especially in developing brains” (4–5). “Zingerone is a nontoxic and inexpensive compound with varied pharmacological activities. It is the least pungent component of Zingiber officinale. Zingerone is absent in fresh ginger, but cooking or heating transforms gingerol to zingerone. Zingerone is closely

related to vanillin from vanilla and eugenol from clove. Zingerone has potent antiinflammatory, antidiabetic, antilipolytic, antidiarrhoeic, antispasmodic, and so forth properties. Besides, it displays the property of enhancing growth and immune stimulation. It behaves as an appetite stimulant, anxiolytic, antithrombotic, radiation protective, and antimicrobial. Also, it inhibits the reactive nitrogen species, which are important in causing Alzheimer's disease and many other disorders" (6,7). "Ginger is a source of a large number of antioxidants and also plays an important role in the reduction of lipid oxidation and inhibits the pathogenesis of diseases. Previous studies reported that ginger extract possesses antioxidant characteristics and shows a role in scavenging superoxide anion and hydroxyl radicals" (8), and gingerol inhibited ascorbate/ferrous complex-induced lipid peroxidation in rat liver microsomes (9). Additionally, "a fraction of the dried ginger powder abundant in polyphenols showed high antioxidant activity based on data from FRAP, oxygen radical absorbance capacity, and cellular antioxidant activity assays" (10). "Several studies have indicated that ginger was effective for protection against oxidative stress. The underlying mechanisms of antioxidant action were investigated in cell models" (11). "Ginger extract showed antioxidant effects in human chondrocyte cells, with oxidative stress mediated by interleukin-1β (IL-1β). It stimulated the expression of several antioxidant enzymes and reduced the generation of ROS and lipid peroxidation" (12). Additionally, ginger extract could reduce the production of ROS in human fibrosarcoma cells with H2O2-induced oxidative stress (13). Recently, "many investigations have revealed that ginger positively affects memory function and exhibits anti-neuroinflammatory activity, which might contribute to the management and prevention of neurodegenerative diseases" (14). "Further experiments in mouse hippocampi and rat C6 glioma cells revealed that ginger extract promoted the formation of synapses in the brain through the activation of extracellular signal-regulated kinase (ERK) induced by nerve growth factor (NGF) and cyclic AMP response element-binding protein (CREB) (15). Another study found that 6-shogaol exhibited neuroprotective activity by activating Nrf2, scavenging free radicals, and elevating the levels of several phase II antioxidant molecules, such as NQO1 and HO-1, in neuron-like rat pheochromocytoma PC12 cells" (16). The aim of the present study is to determine the effective dose of zengeron supplement as a natural antioxidant on the modulation of toxic effects and oxidative stress induced by sublethal doses of lead exposure in rats.

Material and methods

Zengerone supplement

The ginger powder that was used in this study comes from ginger rhizomes from controlled organic cultivation in India. Naturally, no pesticides were used during the cultivation process. Each capsule is free from any sort of additive, including gelatin, making the product suitable for vegans, as shown in HPLC analysis (Figure 1) with the following specifications: 450 mg organic ginger powder per capsule, 100% certified organic ginger, 180 capsules/bottle, 100% vegan, Made in Germany, Free from additives, pesticides, and non-GMO, Produced according to ISO 9001, HACCP, and GMP standards.

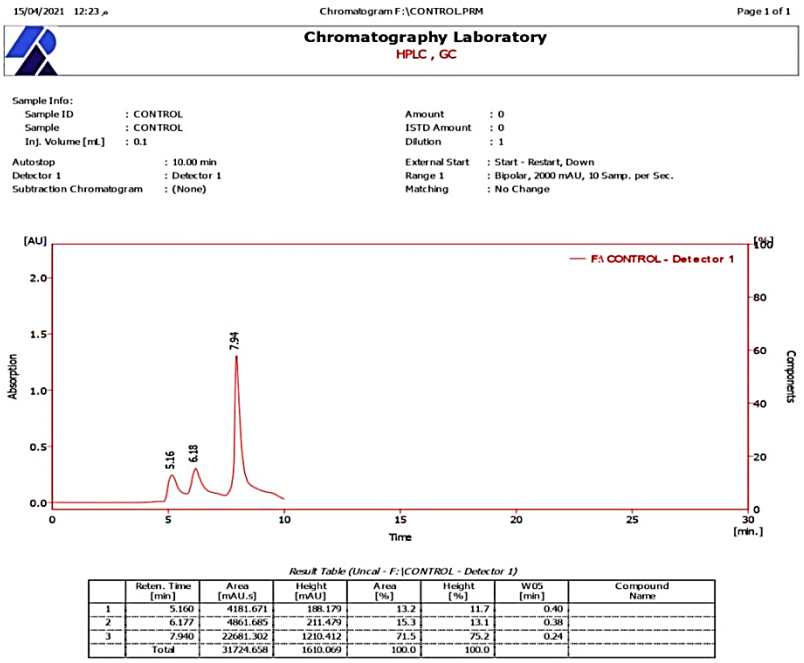


Fig 1: HPLC Analysis

Experimental design

Forty albino adult male rats, weighing 190–220 g, were used and housed in an animal house (College of Veterinary Medicine/Baghdad University). The animals were kept at 22–25˚C with a 12-hour light/dark cycle. Animals were allowed freely access to water and pellets along the experimental period. After acclimatization for 15 days, will be randomly selected and divided equally into six experimental groups and treated for 28 days as follows:

- Control group: administered (orally) sterile distilled water.
- G1 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 25mg/kg for 4 weeks, the rats were then dissected.
- G2 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 50mg/kg for 4 weeks, the rats were then dissected.
- G3 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 100mg/kg for 4 weeks, the rats were then dissected.
- G4 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 150mg/kg for 4 weeks, the rats were then dissected.
- G5 group: administered (orally) (1/280 from LD50) of lead acetate and treated with 200mg/kg for 4 weeks, the rats were then dissected.

Blood samples

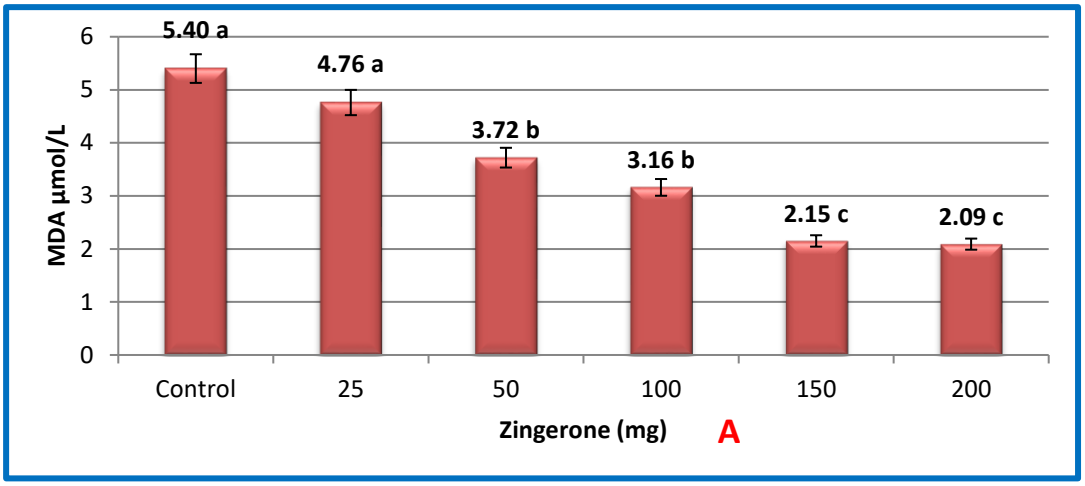
Blood samples were collected, by heart punched under anesthesia by using ketamine and xylene, at the end of the treatment for measuring the following criteria: Serum malondialdehyde (MDA); the level of serum MDA was determined by a modified procedure described by (17); the serum neuroglobulin and dopamine concentration (pg/mL) was measured by using the commercially available ELISA Kit (CEA851Ge, Cloud-Clone Corp., USA) according to the manufacturer's instructions.

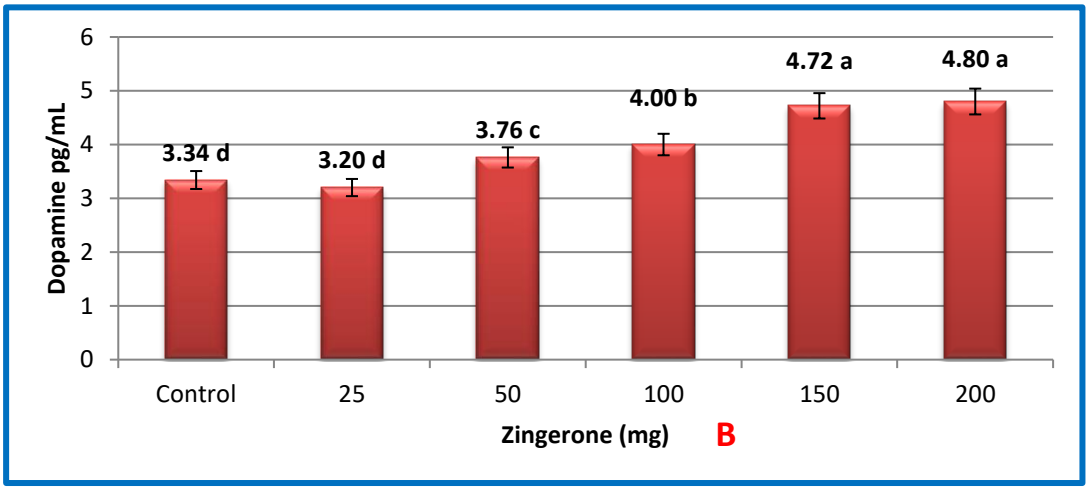
Statistical analysis

Data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant (18).

Results

The results shown in figure 2 (A, B, and C) after treatment of rats with zingerone supplement (25, 50, 100, 150, and 200 mg/kg B.W.) against LD50 of lead acetate (1/280 from rats in the previous experiment) for 28 days. A significant ($P<0.05$) decrease is shown in MDA concentration (figure 2-A) with successive zingerone supplement doses in all treated groups, while there were no differences noticed between G1 (25 mg) and control, between G2 (50 mg) and G3 (100 mg), and between G4 (150 mg) and G5 (200 mg). The data in figure 2-B showed a significant ($P<0.05$) increase in the concentration of dopamine concomitant with a zingerone supplement dose increase. Besides, the results showed no-significant ($P > 0.05$) differences between G1 (25 mg) and control and between G4 (150 mg) and G5 (200 mg), while there were significant differences between G2 (50 mg) and G3 (100 mg). Concerning neuroglobine, figure 2-C showed no significant ($P > 0.05$) increase in neuroglobine concentration in G1, G2, and G3 rats treated with a zingerone supplement against a sublethal dose of lead acetate (1/280 from the LD50 of PbAc) compared to the control. There is a significant increase in neuroglobine concentration related to a zingerone dose increase in G4 (150 mg) and G5 (200 mg) treated groups compared to control and other treated groups.





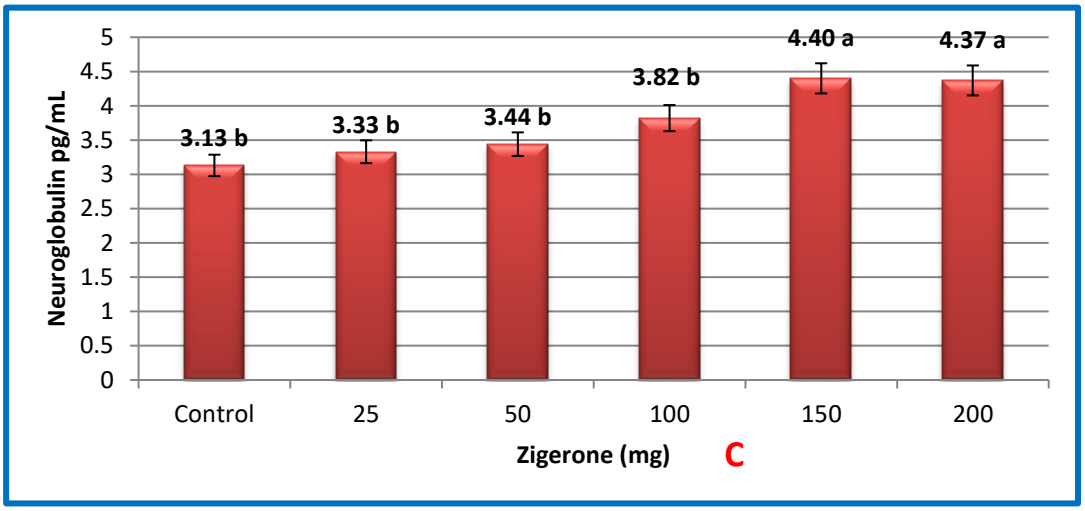
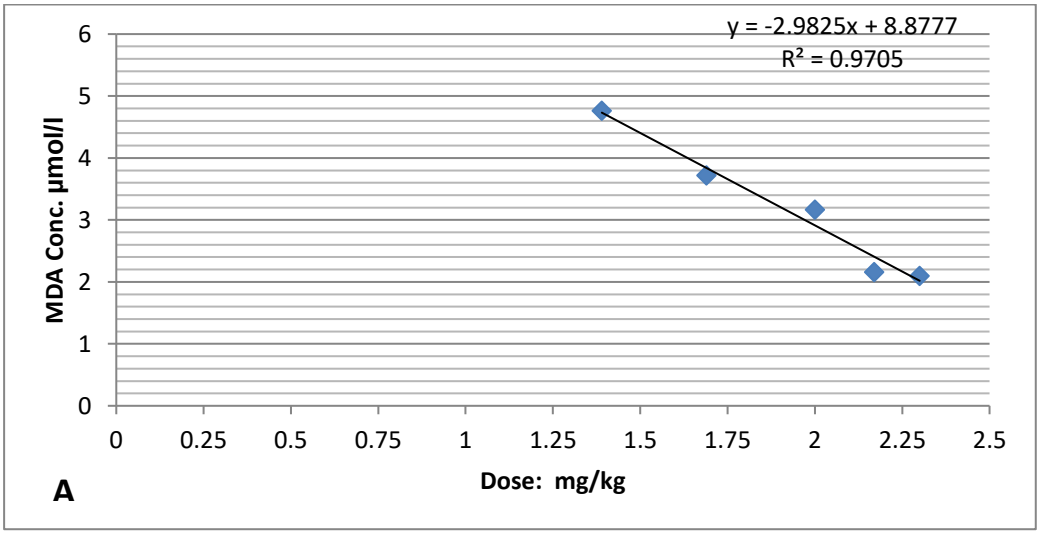


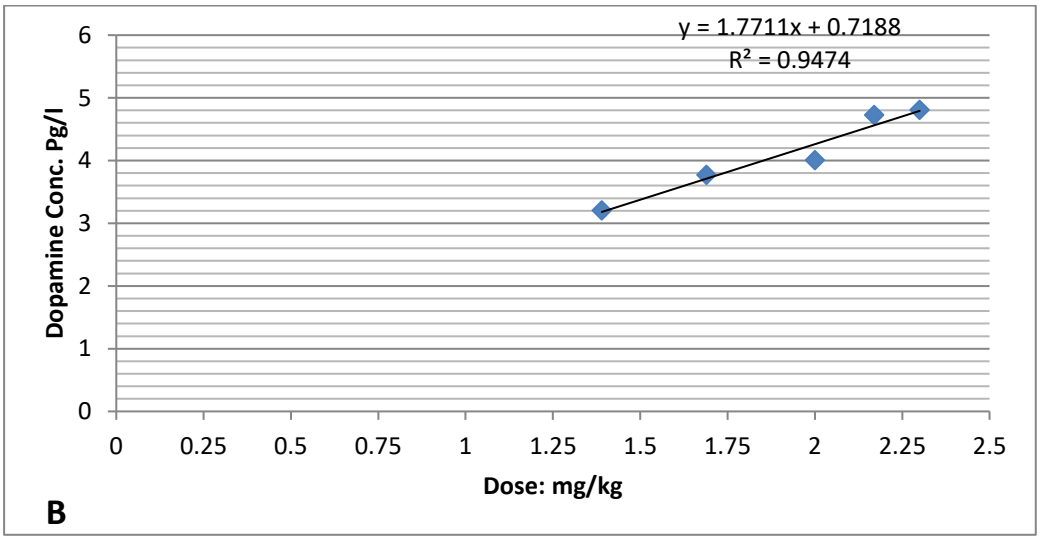
Figure 2: Shows the effect of different successive doses of zingerone supplement on MDA (Figure 2-A), dopamine (Figure 2-B) and neuroglobulin concentrations (Figure 2-C) after 28 days in adult male rats.
Values are expressed as mean ±SE. n= 6. Small letters denote significant differences between groups (P< 0.05).

Determination the effective dose (ED) of zingerone

Depending on the results shown in figures 3 (A, B, and C), maximally significant changes in the above parameters were recorded after 28 days of zingerone supplement with a sublethal

dose (1/280 of LD50) of Pb-treated rats. Accordingly, the results show the estimation of ED of zingerone as follows: Figure 3-A explains highly significant ($P<0.05$) decreases in serum MDA concentration accompanied by successive increases in the dose of zingerone supplement. Were the estimated ED of zingerone equal to 125 mg/kg B.W., a positive relationship was observed between serum dopamine and neuroglobulin concentrations and successive doses of zingerone, as shown in figures 3 (B and C). To determine the ED of zingerone, which was obtained from the equations of the straight line for the previous parameters, the arithmetic mean of ED of zingerone in rats received a sub-lethal dose (1/280 of LD50) of Pb on serum MDA, dopamine, and neuroglobulin concentrations, which equaled 125 mg/kg BW according to probit analysis.





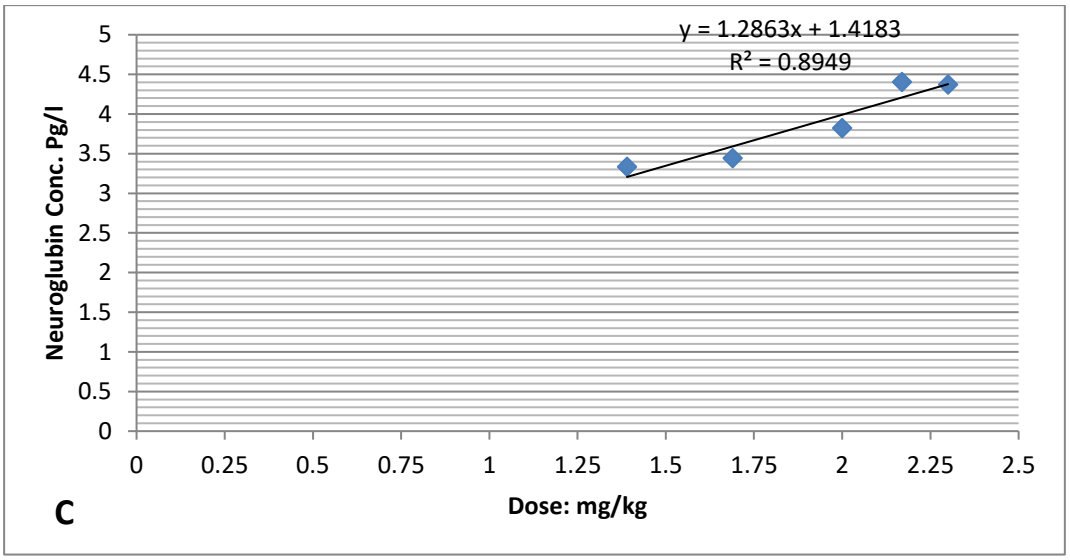


Figure 3: Reveals effect of different successive doses of zingerone supplement on serum MDA (A), dopamine (B) and neuroglobuline (C) concentrations after 28 days in rats . n= 6, ED= 125 mg / kg, BW.

Discussion

Lipid peroxidation is the most important cause of reperfusion injury. Increased free radicals initiate lipid peroxidation in neuronal cells, plasma, organelle membranes, vascular endothelial cell membrane and myelin (19). Administration of zingerone ED with lead acetate sublethal dose 1/280 of LD50 showed a significant decrease in serum MDA concentration at dose 125 mg/kg B.W. according to probit method, as compared with lower and higher doses; the current results were in line with results of (20) and (21), who reported that ginger suppresses lipid peroxidation and recovers antioxidant concentration. Also (22) found that ginger lead to lowered Lipid peroxidation by maintaining the activities of antioxidant enzymes such as SOD, catalase, and glutathione peroxidase (GPx) in rats. So, current results confirm the ability of zingerone to reduce the oxidative stress induced by PbAc exposure (23, 24). The result showed a significant increase in the concentrations of dopamine and neuroglobine in treated groups, which indicates the zingerone supplement works on the neuronal function improvement and inhibits the neurodegenerative disorders. The results are going in line with (25), who reported a neuroprotective effect of ginger through protecting dopaminergic cells via the inhibition of neuroinflammatory responses of microglia. (26) suggested that 6-shogaol may play a role in inhibiting glial cell activation and reducing memory impairment. It has been suggested that ginger crude extract might be a potential neuroprotective agent for the treatment of lipopolysaccharide (27) and monosodium glutamate (MSG)-induced neurodegenerative diseases (28), due to the polyphenolic compounds content of ginger. Ginger has a high antioxidant activity to inhibit the hydroxyl radicals, due to the presence of bioactive phytochemicals like zingerone gingerols, shogaols, paradols, and gingerdiols. Zingerone superoxide anion scavenges peroxyl radicals and also inhibits the production of NO; it is the major bioactive constituent responsible for the antiinflammatory and antioxidant activities of ginger (29, 30). It seems that ginger, given its antioxidant, immunomodulatory, and anti-inflammatory capacity, has the ability to intercept all the main elements involved in the development of multiple sclerosis as well as to attenuate the symptoms of neurological diseases (31, 32). The prophylactic role of ZS against the oxidative

stress caused by a sublethal dose of PbAc counteracts the progression of neurodegenerative diseases. These results are in agreement with (33) who showed that ginger can be a candidate to treat neurodegenerative diseases through bioactive compounds and may improve neurological symptoms and pathological conditions by modulating cell death or cell survival signaling molecules. Collectively, our findings may be helpful in understanding the modulation of brain injury under lead acetate toxicity. Zingerone is considered a promising edible option for reducing the deleterious effects of lead due to its strong antioxidant and modulatory capabilities.

Conclusions

Zingerone has potent antioxidants and neuroprotective effects at the dose of 125 mg/kg BW, which may result in a significant improvement of the neurotransmitter levels and a decrease in the production of oxidative stress in the brain tissue.

Acknowledgments

This study was funded by the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. The animals have adapted to the animal's habitat, which is within the aforementioned college, and tests were conducted within its laboratories

Authors' contribution

The final manuscript draft was reviewed by all authors, who also gave their approval.

Ethical Approval

The local ethics group confirms that these experiments were approved by the College of Medicine Board, University of Fallujah, Ramadi.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.
2.
3.

References

1. Thangarajan S, Vedagirl A, Somasundaram S, Sakthimanogaran R, Murugessan M. Neuroprotective effect of morin on lead acetate- in-duced apoptosis by preventing cytochrome c translocation via regulation of Bax/Bcl-2 ratio. Neurotoxicol Teratol 2018; 66:35-45.

2. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy met-als induced imbalances in redox systems. Biomed Res Int 2014; 640754
3. Barkur RR, Bairy LK. Assessment of oxidative stress in hippocampus, cerebellum and frontal cortex in rat pups exposed to lead (Pb) during specific periods of initial brain development. Biol Trace Elem Res 2015;164(2): 212-218.
4. Mason LH, Harp JP, Han DY. Pb neurotoxicity: neuropsychological ef-fects of lead toxicity. Biomed Res Int, 2014: 840547.
5. Zhang L, Tu R, Wang Y, Hu Y, Li X, Cheng X et al. Early-Life exposure to lead induces cognitive impairment in elder mice targeting SIRT1 phosphorylation and oxidative alterations. Front Physiol 2017; 8: 446
6. Huh, Eugene et al. “6-Shogaol, an Active Ingredient of Ginger, Improves Intestinal and Brain Abnormalities in Proteus Mirabilis-Induced Parkinson's Disease Mouse Model.” Biomolecules & therapeutics vol. 31,4 (2023): 417-424. https://doi:10.4062/biomolther.2023.098
7. Yang, Ah Young et al. “6-Shogaol Ameliorates Liver Inflammation and Fibrosis in Mice on a Methionine- and Choline-Deficient Diet by Inhibiting Oxidative Stress, Cell Death, and Endoplasmic Reticulum Stress.” Molecules (Basel, Switzerland) vol. 29,2 419. 15 Jan. 2024. https://doi:10.3390/molecules29020419
8. Shamsabadi, Shahnam et al. “Promising influences of zingerone against natural and chemical toxins: A comprehensive and mechanistic review.” Toxicon : official journal of the International Society on Toxinology vol. 233 (2023): 107247. https://doi:10.1016/j.toxicon.2023.107247
9. Afzal, Nasima et al. “Prospective randomized double-blind comparative study of topical acetyl zingerone with tetrahexyldecyl ascorbate versus tetrahexyldecyl ascorbate alone on facial photoaging.” Journal of cosmetic dermatology vol. 23,7 (2024): 2467-2477. https://doi:10.1111/jocd.16292
10. Meyer, Thomas A et al. “Acetyl Zingerone: A Photostable Multifunctional Skincare Ingredient That Combats Features of Intrinsic and Extrinsic Skin Aging.” Antioxidants (Basel, Switzerland) vol. 12,6 1168. 29 May. 2023, https://doi:10.3390/antiox12061168
11. Meyer, Thomas A et al. “Acetyl Zingerone: A Photostable Multifunctional Skincare Ingredient That Combats Features of Intrinsic and Extrinsic Skin Aging.” Antioxidants (Basel, Switzerland) vol. 12,6 1168. 29 May. 2023, https://doi:10.3390/antiox12061168
12. Mehrzadi, Saeed et al. “Zingerone Mitigates Carrageenan-Induced Inflammation Through Antioxidant and Anti-inflammatory Activities.” Inflammation vol. 44,1 (2021): 186-193. https://doi:10.1007/s10753-020-01320-y
13. Ayustaningwarno, Fitriyono et al. “A critical review of Ginger's (Zingiber officinale) antioxidant, anti-inflammatory, and immunomodulatory activities.” Frontiers in nutrition vol. 11 1364836. 6 Jun. 2024. https://doi:10.3389/fnut.2024.1364836
14. Im, Hyeri et al. “Trichosanthis Semen and Zingiberis Rhizoma Mixture Ameliorates Lipopolysaccharide-Induced Memory Dysfunction by Inhibiting Neuroinflammation.” International journal of molecular sciences vol. 23,22 14015. 13 Nov. 2022. https://doi:10.3390/ijms232214015

15. Yousefi, Omid et al. “Effect of a Boswellia and Ginger Mixture on the Memory Dysfunction of the Mild Traumatic Brain Injury Patients: A Randomized, Double-Blind Controlled Trial.” Bulletin of emergency and trauma vol. 10,4 (2022): 157-164. https://doi:10.30476/BEAT.2022.97106.1400
16. Pázmándi, Kitti et al. “The "root" causes behind the anti-inflammatory actions of ginger compounds in immune cells.” Frontiers in immunology vol. 15 1400956. 28 Jun. 2024. https://doi:10.3389/fimmu.2024.1400956
17. Guidet, B, and S V Shah. “Enhanced in vivo H2O2 generation by rat kidney in glycerol-induced renal failure.” The American journal of physiology vol. 257,3 Pt 2 (1989): F440-5. https://doi:10.1152/ajprenal.1989.257.3.F440
18. Abdul, M.R., Rahim, S.M., Saleh, A.H. Cardioprotective Activity of Costus Root Ethanol Extract in Experimentally-Induced Hypothyroidism in Female Albino Rats. HAYATI Journal of Biosciences, 2023; 30(6): 1054–1060.
19. Mori T, Tateishi N, Kagamiishi Y, Shimoda T, Satoh S, Ono S, et al. Attenuation of a delayed increase in the extracellular glutamate level in the peri-infarct area following focal cerebral ischemia by a novel agent ONO-2506. Neurochem Int 2004;45:381–7.
20. El-Sharaky, A S et al. “Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats.” Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association vol. 47,7 (2009): 1584-90. https://doi:10.1016/j.fct.2009.04.005
21. Amin, Insha et al. “Zingerone prevents lead-induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats.” Journal of food biochemistry vol. 45,3 (2021): e13241. https://doi:10.1111/jfbc.13241
22. Alibakhshi, Tuba et al. “Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity.” Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie vol. 105 (2018): 225-232. https://doi:10.1016/j.biopha.2018.05.085
23. Wali, A. F., Rehman, M. U., Raish, M., Kazi, M., Rao, P. G. M., Alnemer, O., Ahmad, P., and Ahmad, A. Zingerone [4-(3-Methoxy-4-hydroxyphenyl)-butan-2] Attenuates Lipopolysaccharide-Induced Inflammation and Protects Rats from Sepsis Associated Multi Organ Damage. Molecules 2020, 25(21), 5127; https://doi.org/10.3390/molecules25215127
24. Hassanpour, Shahram et al. “Effect of zinc oxide nanocomposite and ginger extract on lipid profile, glucose, pancreatic tissue and expression of Gpx1 and Tnf-α genes in diabetic rat model.” Molecular biology reports vol. 51,1 11. 12 Dec. 2023, https://doi:10.1007/s11033-023-08963-8
25. Rashid, Summya et al. “Zingerone Targets Status Epilepticus by Blocking Hippocampal Neurodegeneration via Regulation of Redox Imbalance, Inflammation and Apoptosis.” Pharmaceuticals (Basel, Switzerland) vol. 14,2 146. 11 Feb. 2021, https://doi:10.3390/ph14020146
26. Moon, Minho et al. “6-Shogaol, an active constituent of ginger, attenuates neuroinflammation and cognitive deficits in animal models of dementia.” Biochemical

and biophysical research communications vol. 449,1 (2014): 8-13. https://doi:10.1016/j.bbrc.2014.04.121

27. Kongsui, Ratchaniporn et al. “The Neuroprotective Effect of Zingiber cassumunar Roxb. Extract on LPS-Induced Neuronal Cell Loss and Astroglial Activation within the Hippocampus.” BioMed research international vol. 2020 4259316. 25 May. 2020. https://doi:10.1155/2020/4259316
28. Boarescu, Ioana et al. “Zingiber officinale Root Capsule Extract Synergistically Enhance the Anti-Inflammatory Effects of Diclofenac Sodium in Experimental Acute Inflammation.” International journal of molecular sciences vol. 25,3 1781. 1 Feb. 2024. https://doi:10.3390/ijms25031781
29. Rita I Odo, Edmund C Mbegbu, Lawrence N Anyanwu. Effect of aqueous ginger (Zingiber officinale) extract on sperm quality and haematology in lead acetate-treated male albino rats. Tropical Journal of Pharmaceutical Research July 2020; 19 (7): 1481-1485. http://dx.doi.org/10.4314/tjpr.v19i7.21
30. Angelopoulou, Efthalia et al. “Elucidating the Beneficial Effects of Ginger (Zingiber officinale Roscoe) in Parkinson's Disease.” ACS pharmacology & translational science vol. 5,10 838-848. 7 Sep. 2022. https://doi:10.1021/acsptsci.2c00104
31. Arcusa, Raúl et al. “Potential Role of Ginger (Zingiber officinale Roscoe) in the Prevention of Neurodegenerative Diseases.” Frontiers in nutrition vol. 9 809621. 18 Mar. 2022. https://doi:10.3389/fnut.2022.809621
32. Pázmándi, Kitti et al. “The "root" causes behind the anti-inflammatory actions of ginger compounds in immune cells.” Frontiers in immunology vol. 15 1400956. 28 Jun. 2024. https://doi:10.3389/fimmu.2024.1400956
33. ALmohaimeed, Hailah M et al. “Synergistic Anti-inflammatory and Neuroprotective Effects of Cinnamomum cassia and Zingiber officinale Alleviate Diabetes-Induced Hippocampal Changes in Male Albino Rats: Structural and Molecular Evidence.” Frontiers in cell and developmental biology vol. 9 727049. 8 Sep. 2021, https://doi:10.3389/fcell.2021.727049