Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Neurobehavioral toxicity of copper sulfate accompanied by oxidative stress and histopathological alterations in chicks' brain

Sh.I. Alnuaimi and Y.Z. Al-Abdaly

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received March 30, 2022
Accepted July 04, 2022
Available online July 04, 2022

Keywords:
Chicks
Copper sulfate
Nervous system
Oxidative stress

Correspondence:
Y.Z. Al-Abdaly
yalabdaly@yahoo.com

Abstract

The aim is to investigate the sub-acute neurotoxic effects of copper sulfate in chicks on motor and neurobehavioral activity and its relation to oxidative stress and histopathological changes in chick brain tissue. Thirty chicks were employed in this experiment, randomly separated into 5 groups of 6 chicks. They were given the following concentration 2.5, 5, 10, and 15% of LD_{50}. Each of the chicks is put through the following behavioral tests response to tonic immobility test, righting reflex, testing the motor activity of the chicks inside the open field box. Orally LD_{50} was 772 mg/kg, Recording an inhibition in the animal's movement in the open field and an increase in the chicks' dormancy duration. The effects are directly proportional to the increase in the chicks' dose. Copper sulfate in 2.5, 5, 10, and 15% of the LD_{50} showed a significant increase in malondialdehyde concentration, while 15% of LD_{50} recorded a significant decrease in glutathione and cholinesterase activity. All doses substantially decreased total antioxidant capacity in brain and liver tissue. Chick brain of copper sulfate 15% of LD_{50} shows in the cortex of cerebrum severe gliosis, satellitosis, perivascular and periaxonal edema, necrosis (karyorrhexis) of neuron, and apoptosis. The rest of the concentrations had histopathological alterations proportionate to the rise in the given dose. We concluded from this work that high concentrations of copper sulfate in the brain generated oxidative stress and histopathological alterations, which influenced chicks' neurobehavior and motor activity in the open environment.

DOI: 10.33899/ijvs.2022.133416.2224,

Introduction

Heavy metals refer to any metallic chemical element with a relatively high density and are toxic at a low concentration (1). Continuous exposure to many heavy metals that may contain copper, cadmium, zinc, nickel, lead, and arsenic has a wide range of harmful effects on living organisms (2). Although some heavy metals have a role in biochemistry and affect low concentrations, they negatively affect physiological functions at high concentrations due to bioaccumulation in vital organs (3-5). Except in rare hereditary diseases and deficiencies that result in the inability to eliminate copper. Copper is not considered a dangerous substance for living creatures, but it can cause liver, kidney, and brain damage if consumed excessively unless special conditions exist (6-8). Copper-containing enzymes are required for several important activities. Copper is received from food, and the liver ensures that it is transported safely. On the other hand, inorganic copper essentially bypasses the liver and enters the bloodstream directly, which is potentially harmful because copper can cross the blood-brain barrier (9-11). Even though copper attaches to the protein, it can uncouple and release it and accelerate the generation of highly reactive hydroxyl and other reactive oxygen species (12-14). The current study aimed to investigate the sub-acute neurotoxic effects of copper sulfate in chick's neurobehavioral and motor activity in the open field-

moreover, the effect of previous treatments on oxidative stress levels by measuring glutathione and malondialdehyde. The total antioxidant capacity and cholinesterase activity support the result histopathological examination of changes in changes in chick brain tissue will be done.

Materials and methods

Ethical approval

The College of Veterinary Medicine at the University of Mosul provided all ethical clearances for the humane treatment of laboratory animals, and the form number was UM.VET.2021.35.

The animals

Local chickens of both sexes were used in this study, with an estimated number of 40 chicks (10 chicks for LD_{50}) raised in the poultry hall in the animal house of the College of Veterinary Medicine, University of Mosul, for 7 days before the experiment began. Specially adapted for this purpose and given plenty of water and forage and the proper circumstances.

The Chemicals

The following chemical substances were used, hydrated copper sulfate from Karlsruhe, Germany, Zeppelinstrassa Thaiobarbturic Acid (TBA) Merk Company, HCl at a concentration of 0.25 N, Trisodium-citrate buffer at a concentration of 10%, Na2HPO4 buffer (0.3 M, 5.5 Th) DTNB (L-Glutathione, formalin, neutralized 10% utilized diagnostic kit) 2- Nitrobenzoic acid. Elabscience produced a Total Antioxidant Capacity (T-AOC) colorimetric measurement kit.

Preparing the potion

After estimating the specific dose for each item, copper sulfate doses were produced and dissolved in distilled water, then administered to the animals using a gavage oral syringe, with a 5ml volume of administration.

Blood sample collection

The chicks' jugular veins were cut to collect blood in special tubes, and the blood was separated into two groups, one with anticoagulants to extract blood plasma and the other without anticoagulants to extract blood serum. The serum and plasma samples were collected for 15 minutes and centrifuged at 3000 rpm for 15 minutes. Before being stored in special plastic tubes and frozen at -20°C until laboratory biochemical testing was completed.

Organ collection

After the chickens were slaughtered, the brains and livers were taken. Each sample was divided into 2 sections and placed in numbered bags in the freezer at -20ºC (for use in Tissue Homogenisis). The remaining piece of each sample was placed in special containers containing diluted formalin at a concentration of 10% for preservation until it was completed, taking into account the numbering of each group separately. A brain histopathological investigation will be performed.

Experiment design

The first experiment was conducted to determine the median lethal dose (LD_{50}) of copper sulfate in chicks. The up and down approach was used to determine the LD_{50} (15). Second, encounter neurobehavioral tests used to determine the response to different dosages of copper sulfate. Thirty chicks were employed in this experiment, randomly separated into five groups of six chicks each. Several dosages of copper sulfate were administered to the chicks in this experiment, based on the median lethal dose determined in the preceding experiment. They were given the following concentrations: 2.5, 5, 10, and 15 % of LD_{50} equal to 19.3 mg/kg, 38.6 mg/kg, 77.5 mg/kg and, 115.8 mg/kg. The therapy lasted three days before being recorded on the fourth day, which marked the end of the experiment's treatments. Each of the chicks is put through the following behavioral tests:

Response to tonic immobility test

The test is performed by placing each chick alone on its right side and attempting to calm it for 15 seconds. Then quietly withdraw the hand and measure the time of the chick to remain silent until resistance and movement began, with a maximum period of 300 seconds, and repeat the chick that failed to calm for five attempts, with a 30-second gap between each try (16).

Righting reflex

The chicks in the preceding groups are placed on one of their sides and calculated the time it takes to adjust their body position or their failure to do so (17).

Testing the motor activity of the chicks inside the open field box

In an isolated room, a rectangular wooden box with dimensions of 60*60*30 cm and a floor partitioned into 24 equal squares with a side length of around 15 cm was employed. This assessment examines the chicks' general movement and activity within the open field box for each chick (18).

Measuring some biochemical variables

Some biochemical parameters of treated chicks' plasma and blood serum were measured by studying oxidative stress by measuring malondialdehyde (19), serum glutathione (20), total antioxidant status (TAC), and measurement of cholinesterase activity in plasma.

Statistical analysis

For non-parametric data, use the Mann-Whitney test. Parametric data were statistically evaluated using the SPSS one-way analysis of the variance test program, after which the LSD test was used to determine the level of significant difference. The probability was less than 0.05 (21).

Results

The first experiment determined the median lethal dose of copper sulfate (LD_{50}) in chicks. When given orally, LD_{50} was 772 mg/kg, with a 200 mg/kg value for the rise and decrease in dose. The presence of puffy feathers, tiredness in animals, lack of movement and eating, difficulty breathing, bloody diarrhea, and eventually death were all indicators of poisoning in chicks (Table 1).

In the second experiment, a neurobehavioral test was used to measure the reaction to different dosages of copper sulfate. This experiment was carried out with concentrations of 2.5 % (19.3 mg/kg), 5% (38.6 mg/kg), 10% (77.5 mg/kg) and 15% (115.8 mg/kg) of the median lethal dose. The number of squares crossed by chicks was directly related to the increase in the provided dose compared to the control. The behavioral changes reflected by the delay in the time of starting movement were recorded with a significant decrease in the number of squares crossed (Table 2). In the body posture correction (Righting Test), the administered dose resulted in a substantial increase in posture correction time of 5, 10, and 15% compared to the control and the other groups. The groups treated with concentrations of 5, 10, and 15% of the mean lethal dose recorded a significant and apparent increase in the dormancy period of the chicks compared to the control group in the Tonic immobility test. At the same time, the 2.5 percent group also recorded an increase in the chicks' dormancy period, but less than the other concentrations (Table 2).

Table 1: The oral median lethal dose of copper sulfate in chicks

Variable	Results
LD_{50} (orally)	772 mg/kg
Dosage range	1200-600 mg/kg
First dose	1200 mg/kg
Last dose	600 mg/kg
No. chicks used	(xxoxxo) 6
Dose change	200 mg/kg
Time of toxicity	minutes 3-10
Signs of poisoning	Fluffy feathers, breath difficulty, bloody diarrhea, slimy secretions from the mouth, and finally, death.

X means the chick is dead and 0 means survives the chick.

Copper sulfate in 2.5, 5, 10, and 15% of the LD_{50} showed a significant increase in malondialdehyde concentration compared with the control group. While 15% of LD50 recorded a significant decrease in glutathione compared to the other groups, all doses substantially decreased total antioxidant capacity (TAC) in brain and liver tissue compared with the control group (Table 3).

Table 2: Motor and neurobehavioral response to different doses of copper sulfate

Neurobehavior	Control	19.5 mg/kg	38.6 mg/kg	77.5 mg/kg	115.8 mg/kg
Start movement/sec	1±0.1	0.1± 3*	1±5*[a]	2±17*[ab]	33±30 *[abc]
Number of squares	2±20	2±14*	1.1±4*[a]	1± 4 *[ab]	1±2 *[ab]
Body correction/sec	1±0.1	0.2±2	0.1± 3*	0.3±3*	1.4± 3 *
Tonic immobility/sec	56±5	5.6±66*	23±232*[a]	35±300*[ab]	66±300*[ab]

Each group consists of 6 animals. Data as mean ± SE. *This means different in the control group. a- This means a significant difference from 19.5 mg/kg. b- This means a significant difference from 38.6 mg/kg. c- This means a significant difference from 77.5 mg/kg.

Table 3: Oxidative stress status by measuring malondialdehyde, glutathione, in serum and TAC in the tissue of chicks

Groups	In Serum		TAC (U/ml)	
	MDA (nmol/ml)	GSH (nmol/ml)	Brain Tissue	Liver Tissue
Control	2.29±1	0.08±0.01	3.1±0.4	4.1±1
19.5 mg/kg	3.89±1 *d	0.02±0.01 *d	1.1±0.03*	1.0±0.02*
38.6 mg/kg	3±0.5 *d	0.021±0.001 *d	0.7±0.05*	0.9±0.01*
77.5 mg/kg	94.3±1 *d	0.01±0.005 *d	0.5±0.04*	0.7±0.02*
115.8 mg/kg	5.69±1 *	0.005±0.002 *	0.3±0.02*	0.4±0.02*

Each group consists of 6 animals. Data as mean ± SE. *This means different in the control group. d- This means a significant difference from 115.8 mg/kg.

The level of inhibition of cholinesterase activity in concentrations of 2.5, 5, 10, and 15% of the lethal median dose resulted in a significant decrease in cholinesterase activity compared to the control group. While the concentrations of 10 and 15% of the lethal median dose resulted in a significant decrease in cholinesterase activity compared to the control group and two groups of 2.5 and 5% (Table 4).

Table 4: Effect on cholinesterase activity in plasma of chicks treated with different concentrations of LD_{50}

Groups	Cholinesterase activity in plasma
Control	2.95 ± 0.01
19.5 mg/kg	0.96 ± 0.007*
38.6 mg/kg	0.90 ± 0.004*
77.5 mg/kg	0.80 ± 0.002*^{ab}
115.8 mg/kg	0.69 ± 0.004*^{ab}

Each group consists of 6 animals. Data represented as mean ± SE. *This means different in the control group. a- This means a significant difference from 19.5 mg/kg. b- This means a significant difference from 38.6 mg/kg.

Histopathological changes

Figure 1 appeared the chick brain of the control group shows the cortex of the cerebrum with standard architecture represented by neurons, glial cells, and blood vessels. In figure 2 chick brain of copper sulfate, 2.5% of LD_{50} dose treated group shows the cerebrum cortex with intact neurons with mild cytogenic edema and mild congestion of blood vessels with mild perivascular edema. Figure 3 for chick brain of copper sulfate 5% of LD_{50} dose treated group shows the cortex of cerebrum with intact neurons and gliosis, congestion of blood vessels, and perivascular edema.

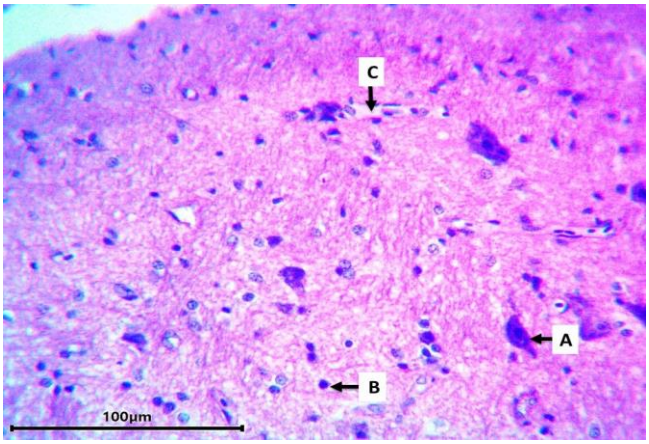

Figure 1: photomicrograph of chick brain of control group shows the cortex of cerebrum with standard architecture represented by neurons (A), glial cells (B) vessels(C). H&E stain, Scale bar = 100µm.

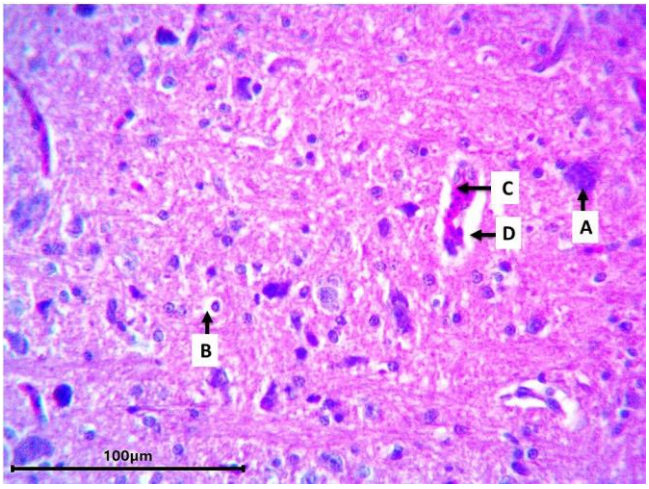

Figure 2: photomicrograph of chick brain of copper sulfate 2.5% of LD50 dose treated group shows the cortex of cerebrum with intact neurons (A) with mild cytogenic edema (B) with mild congestion of blood vessels (C) and mild perivascular edema (D). H&E stain, Scale bar =100 µm.

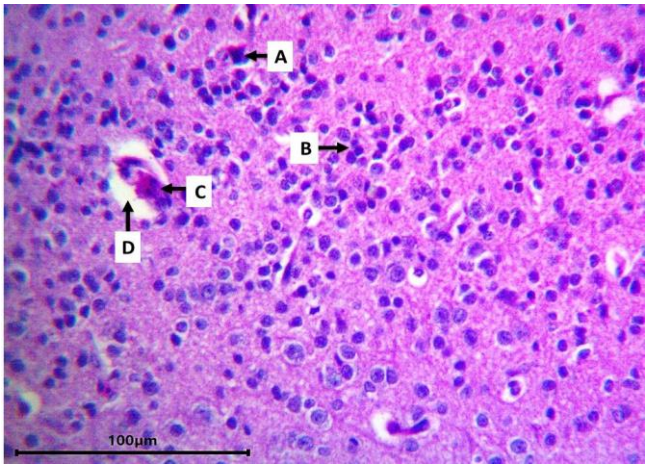

Figure 3: photomicrograph of chick brain of copper sulfate 5% of LD_{50} dose treated group shows the cortex of cerebrum with intact neurons (A) with gliosis (B), congestion of blood vessels (C), and perivascular edema (D). H&E stain, Scale bar = 100µm.

In figure 4, the chick brain of copper sulfate 10% of LD_{50} dose treated group shows the cortex of cerebrum with congestion of blood vessels, diffuse perivascular edema, periaxonal edema, satellitosis by glial cells around neurons, and neuronophagia. Also Figure 5 showed chick brain of copper sulfate 15% of LD_{50} dose treated group the cortex of cerebrum appeared with liquefactive necrosis of neurons and neuropil, gliosis, neuronophagia congestion of blood vessels, perivascular edema and periaxonal edema. Figure

6 for chick brain of copper sulfate 15% of LD_{50} dose treated group shows the cortex of cerebrum with severe gliosis, satellitosis by glial cells around neurons, congestion of blood vessels, perivascular edema, periaxonal edema, necrosis (karyorrhexis) of neuron and apoptosis**.**

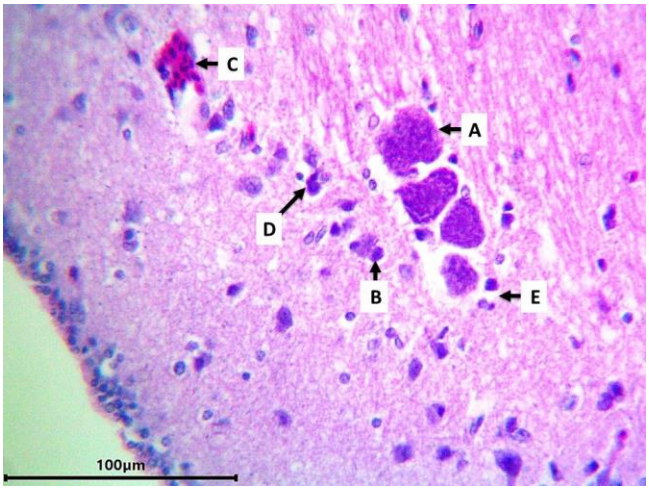

Figure 4: photomicrograph of chick brain of copper sulfate 10% of LD_{50} dose treated group shows the cortex of cerebrum with a cluster of sizeable multiple pyramidal cells with necrosis (A), satellitosis by glial cells around neurons (B), hemorrhage (C), apoptosis (D) and periaxonal edema (E). H&E stain, Scale bar = 100µm.

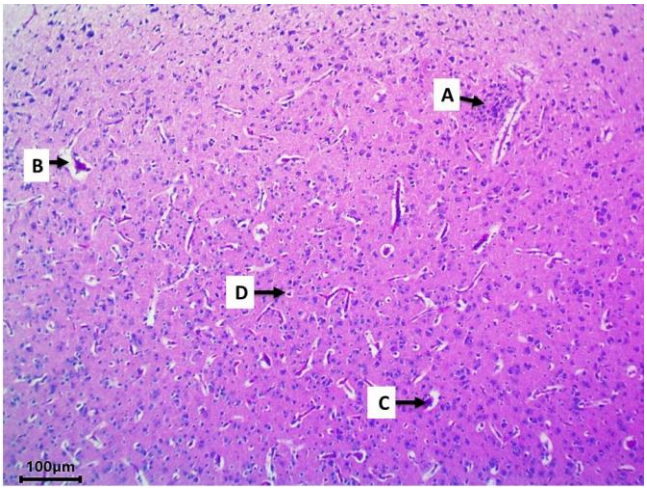

Figure 5: photomicrograph of chick brain of copper sulfate 15% of LD_{50} dose treated group shows the cortex of cerebrum with severe gliosis (A), diffuse perivascular edema (B), congestion of blood vessels (C) and periaxonal edema (D), satellitosis by glial cells around neurons (D) and neuronophagia (E). H&E stain, Scale bar = 100µm.

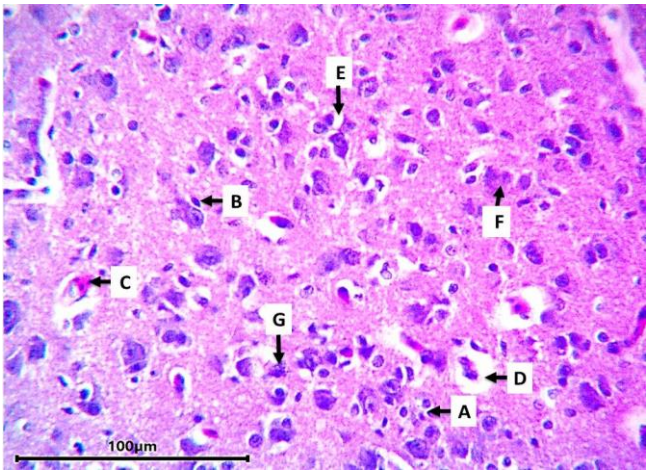

Figure 6: photomicrograph of chick brain of copper sulfate 15% of LD_{50} dose treated group shows the cortex of cerebrum with severe gliosis (A), satellitosis by glial cells around neurons (B), congestion of blood vessels (C), perivascular edema (D) periaxonal edema (E), necrosis (karyorrhexis) of neuron (F) and apoptosis (G). H&E stain, Scale bar = 100µm.

Discussion

Behavior is an appropriate end aim for environmental toxicology research because it integrates information from various levels of biological information and has direct and intuitive relevance to environmentally significant outcomes such as growth, reproduction, and survival. It is also an excellent early warning indication, with a sensitivity of 10-1000 times that of more severe endpoints like mortality (22,23). In our current investigation, the median lethal dose of copper sulfate in chicks was 772 mg/kg, with acute clinical signs. The goal of utilizing several percentages of the median lethal dosage of copper sulfate in chicks (2.5 percent, 5 percent, 10 percent, and 15 percent) was to discover the lowest hazardous dose that could indicate the latent effects on the nervous system without causing visible toxic effects on the chicks. In the tensile immobility test, there was a decrease in the chicks' activity and movement inside the open field and an extension of the chicks' dormancy phase.

These effects were directly proportional to the dose value, as the dose of 2.5 percent was substantially better than the other doses, although the toxic effects were quite apparent in the other doses. The remaining doses and results are consistent with several studies that have shown that copper sulfate has harmful effects on the central nervous system as a result of its capacity to cross the blood-brain barrier, where copper showed an effect on human astrocytes exposed to copper (24,25).

According to one study, the effect of copper chloride on membrane function and integrity in astrocytes in vitro after

low doses of copper chloride was applied. According to these tests, the copper content of astrocytes exponentially with time and in a concentration-dependent manner (26,27). Chronic copper toxicity generates copper accumulation in the cortex, striatum, and cerebellum areas of male Wistar rats, which is linked to cognitive deficits (28,29). Excessive copper administration via oral administration increased the amount of copper unbound to ceruloplasmin in plasma, causing oxidative damage through excessive production of reactive oxygen species in the cortex and hippocampus (30,31).

The cerebellum is essential in balance, motor control, and different brain functions such as emotional regulation and cognition (32,33). Oxidative stress appears to be the primary source of neurological impairments following copper accumulation. Because the cerebellum is one of the brain regions affected by copper toxicity, it is logical to believe that elevated copper levels in the cerebellum could impact its structure and function (34,35). Also, because our results showed a significant decrease in the cholinesterase enzyme activity in plasma of all treated groups compared to the control group, cholinesterase inhibition may play a role in the decreased activity of chick movement inside the open field to muscle fatigue (weakness). This is consistent with other researchers who found that metal ions in high concentrations (especially copper) can inhibit cholinesterase activity by binding to azole imide. Metal ions also interact with other amino acids in the active protein, such as tryptophan, 9-metho, cysteine, and d phenylalanine (36,37).

Copper is also one of the few heavy metals that inhibits cholinesterase activity in a non-competitive manner (38,39). The prior effects were not isolated from the oxidative stress generated by various concentrations of copper sulfate, where the concentration of malondialdehyde increased significantly and was linked to a drop in serum glutathione levels. The overall antioxidant capacity in liver and brain tissue was much lower than in the control group. These findings are consistent with other research that suggests the cause of this is due to free copper ions acting as potent catalysts for the creation of (ROS), (Cu_2^+) to copper (Cu^+) in the presence of superoxide (O_2-), which can catalyze the production of reactive hydroxyl radicals (OH) from the decomposition of hydrogen peroxide (H_2O_2) (40-42). Hydroxyl radicals are the most potent oxidizing radicals found in living systems and can catalyze lipid peroxidation, which causes tissue injury (43,44). The histological study confirmed the findings, revealing apparent degenerative alterations on the neurons whose intensity correlated with the dose administered, with the effects being more severe at a concentration of 15% of the typical lethal dose. These findings show that oxidative stress and free radicals produced by high copper dosages play a role in cell damage, neuronal degeneration, and other pathological alterations, including programmed death.

Necrosis is a type of cell death that occurs when tissue is damaged accidentally and includes activating a specific cellular program (40). Copper-generated free radicals appear to have caused the early loss of plasma membrane integrity. Moreover, the expansion of the cell body is followed by the cell's explosion, and as the cell explodes, hazardous compounds are released from the mitochondria, causing damage to the surrounding cells. Dying cells shrink, compress, and eventually break apart during apoptosis, releasing small membrane-bound apoptosis bodies filled by immune cells (macrophages).

Conclusion

We concluded that copper sulfate had toxicity at high doses, as evidenced by neurobehavioral and motor activity, several serum indices of oxidative stress and tissue in the brain, and several enzymes and histological alterations.

Acknowledgment

The researchers are appreciative of the University of Mosul's College of Vets. Medicine for providing all of the resources needed to complete this work

Conflict of interest

The researchers promise that they have no links to any of the parties involved in this study.

Reference

1. Arias VJ, Koutsos EA. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult Sci. 2006;85(6):999-1007. DOI: 10.1093/ps/85.6.999
2. Sabullah MK, Sulaiman MR, Shukor MY, Syed MA, Shamaan NA, Khalid A, Ahmad SA. The assessment of cholinesterase from the liver of Puntius javanicus as detection of metal ions. Sci World J. 2014;2014. DOI: 10.1155/2014/571094
3. Sabullah MK, Ahmad SA, Shukor MY, Gansau AJ, Syed MA, Sulaiman MR, Shamaan NA. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction, and proteomics approaches. Inter Food Res J. 2015. [available at]
4. Brewer GJ. The risks of copper toxicity contribute to cognitive decline in the aging population and Alzheimer's disease. J Am Coll Nutrit. 2009;28(3):238-42. DOI: 10.1080/07315724.2009.10719777
5. Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicol. 2003;189(1-2):147-63. DOI: 10.1016/S0300-483X(03)00159-8
6. Scheiber IF, Schmidt MM, Dringen R. Zinc prevents the copper-induced damage of cultured astrocytes. Neurochem Inter. 2010;57(3):314-22. DOI: 10.1016/j.neuint.2010.06.010
7. Pal A, Prasad R. Regional distribution of copper, zinc, and iron in brain of Wistar rat model for non-Wilsonian brain copper toxicosis. Indian J Clin Biochem. 201;31(1):93-8. DOI: 10.1007/s12291-015-0503-3
8. Arnal N, Morel GR, de Alaniz MJ, Castillo O, Marra CA. Role of copper and cholesterol association in the neurodegenerative process. Inter J Alzheimer's Dis. 2013;2013. DOI: 10.1155/2013/414817
9. Baldaçara L, Ismael F, Leite V, Pereira LA, Dos Santos RM, Gomes VD, Calfat EL, Diaz AP, Périco CA, Porto DM, Zacharias CE.

Brazilian guidelines for the management of psychomotor agitation. Part 1. Non-pharmacological approach. Brazil J Psychiat. 2018;41:153-67. DOI: 10.1590/1516-4446-2018-0163
10. Wang C, Liu L, Zhang L, Peng Y, Zhou F. Redox reactions of the μ - synuclein - Cu 2 complex and their effects on neuronal cell viability. Biochem. 2010;49:8134-8142. DOI: 10.1021/bi1010909
11. Ajuwon OR, Idowu OM. Vitamin C attenuates copper-induced oxidative damage in broiler chickens. African J Biotech. 2010;9(44):7525-30. DOI: 10.5897/AJB10.776
12. Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxic. 2003;189(1-2):147-63. DOI: 10.1016/S0300-483X(03)00159-8
13. Gaggelli E, Kozlowski H, Valensin D, Valensin G. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chem Rev. 2006;106(6):1995-2044. DOI: 10.1021/cr040410w
14. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mole Cellu Bioch. 2010;345(1):91-104. DOI: 10.1007/s11010-010-0563-x
15. Dixon WJ. Efficient analysis of experimental observations. Annual Rev Pharma Toxico. 1980;20(1):441-62. DOI: 10.1146/annurev.pa.20.040180.002301
16. Heiblum R, Aizenstein O, Gvaryahu G, Voet H, Robinzon B, Snapir N. Tonic immobility and open field responses in domestic fowl chicks during the first week of life. Appl Anim Beh Sci. 1998;60(4):347-57. DOI: 10.1016/S0168-1591(98)00157-9
17. Jones RB. The tonic immobility reaction of the domestic fowl: a review. World's Poult Sci J. 1986;42(1):82-96. DOI: 10.1079/WPS19860008
18. Jones RB, Blokhuis HJ, Beuving G. Open-field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. British Poul Sci. 1995;36(4):525-30. DOI: 10.1080/00071669508417798
19. Miller DM, Aust SD. Studies of ascorbate-dependent, iron-catalyzed lipid peroxidation. Archives Bioch Biophys. 1989;271(1):113-9. DOI: 10.1016/0003-9861(89)90261-0
20. James RC, Goodman DR, Harbison RD. Hepatic glutathione and hepatotoxicity: changes induced by selected narcotics. J Pharma Experiment Therap. 1982;221(3):708-14. [available at]
21. Elliott AC, Woodward WA. Statistical analysis quick reference guidebook: With SPSS examples. NY: Sage; 2007.
22. Gupta, P. Concepts and applications in veterinary toxicology. Switzerland: Springer Inter Publishing; 2019. 242-244 p.
23. Kalita J, Kumar V, Misra UK, Bora HK. Memory and learning dysfunction ollowing copper toxicity: Biochemical and immunohistochemical basis. Mol Neurobiol. 2018;55:3800-3811. DOI: 10.1007/s12035-017-0619-y
24. Nordberg GF. Neurotoxic effect of metals and their compounds. Oxford: Pergamon Press; 1980. 3-15 p.
25. Ozcelik D, Uzun H. Copper intoxication; antioxidant defenses and oxidative damage in rat brain. Bio Trace Element Res. 2009;127(1):45-52. DOI: 10.1007/s12011-008-8219-3
26. Opazo CM, Greenough MA, Bush AI. Copper: from neurotransmission to neuroproteostasis. Fron Aging Neuro. 2014;6:143. DOI: 10.3389/fnagi.2014.00143
27. Pal A, Prasad R. Regional distribution of copper, zinc and iron in brain of Wistar rat model for non-Wilsonian brain copper toxicosis. Indian J Clin Bioch. 2016;31(1):93-8. DOI: 10.1007/s12291-015-0503-3
28. Pastor-Maldonado CJ, Suárez-Rivero JM, Povea-Cabello S, Álvarez-Córdoba M, Villalón-García I, Munuera-Cabeza M, Suárez-Carrillo A, Talaverón-Rey M, Sánchez-Alcázar JA. Coenzyme q10: Novel formulations and medical trends. Inter J Mole Sci. 2020;21(22):8432. DOI: 10.3390/ijms21228432
29. Opazo CM, Greenough MA, Bush AI. Copper: from neurotransmission to neuroproteostasis. Fron Aging Neuro. 2014;6:143. DOI: 10.3389/fnagi.2014.00143
30. Peng X, Dai C, Zhang M, Das Gupta S. Molecular mechanisms underlying protective role of quercetin on copper sulfate-induced nephrotoxicity in mice. Fron Vet Sci. 2021;7:934. DOI: 10.3389/fvets.2020.586033
31. Przybyłkowski A, Gromadzka G, Wawer A, Bulska E, Jabłonka-Salach K, Grygorowicz T, Schnejder-Pachołek A, Członkowski A. Neurochemical and behavioral characteristics of toxic milk mice: an animal model of Wilson's disease. Neuro Res. 2013;38(10):2037-45. DOI: 10.1007/s11064-013-1111-3
32. Ranjan A, Kalita J, Kumar V, Misra UK. MRI and oxidative stress markers in neurological worsening of Wilson disease following penicillamine. Neurotoxic. 2015;49:45-9. DOI: 10.1016/j.neuro.2015.05.004
33. Rotilio G, Carr MT, Rossi L, Ciriolo MR. Copper-dependent oxidative stress and neurodegeneration. IUBMB life. 2000;50(4-5):309-14. DOI: 10.1080/15216540051081074
34. Sabullah MK, Sulaiman MR, Shukor MY, Shamaan NA, Khalid A, Ahmad SA. In vitro and in vivo effects of *Puntius javanicus* cholinesterase by copper. Frese Enviro Bulletin. 2015;234:78. [available at]
35. Sabullah MK, Sulaiman MR, Shukor MY, Syed MA, Shamaan NA, Khalid A, Ahmad SA. The assessment of cholinesterase from the liver of Puntius javanicus as detection of metal ions. Sci World J. 2014:14-20. DOI: 10.1155/2014/571094
36. Sabullah MK, Ahmad SA, Shukor MY, Gansau AJ, Syed MA, Sulaiman MR, Shamaan NA. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. Inter Food Res J. 2015. [available at]
37. Kalita J, Kumar V, Misra UK, Bora HK. Memory and learning dysfunction following copper toxicity: biochemical and immunohistochemical basis. Mol Neurobiol. 2018;55(5):3800-11. DOI: 10.1007/s12035-017-0619-y
38. Sanoobar M, Eghtesadi S, Azimi A, Khalili M, Jazayeri S, Reza Gohari M. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with relapsing-remitting multiple sclerosis. Int J Neurosci. 2013 ;123(11):776-82. DOI: 10.3109/00207454.2013.801844
39. Saravu K, Jose J, Bhat MN, Jimmy B, Shastry BA. Acute ingestion of copper sulphate: A review on its clinical manifestations and management. Indian J Crit Care. 2007;11:74-80. DOI: 10.4103/0972-5229.33389
40. Scheiber IF, Schmidt MM, Dringen R. Zinc prevents the copper-induced damage of cultured astrocytes. Neuro Inter. 2010;57(3):314-22. DOI: 10.1016/j.neuint.2010.06.010
41. Alabbasi EH, Alabdaly YZ. Effect of boric acid on sodium fluoride toxicity in chicks. Iraqi J Vet Sci. 2022;36(1):123-31. DOI: 10.33899/ijvs.2021.129497.1653
42. Alabsy EH, Alabdaly YZ. Therapeutic effect of taurine on sodium fluoride toxicity in chicks. Iraqi J Vet Sci. 2022;36(1):223-38. DOI: 10.33899/ijvs.2021.129854.1692
43. Al-Abdaly YZ, Saeed MG, Al-Hashemi HM. Effect of methotrexate and aspirin interaction and its relationship to oxidative stress in rats. Iraqi J Vet Sci. 2021;35(1):151-6. DOI: 10.33899/ijvs.2020.126490.1335
44. Alabdaly YZ, Al-Hamdany EK, Abed ER. Toxic effects of butylated hydroxytoluene in rats. Iraqi J Vet Sci. 2021;35(1):121-8. DOI: 10.33899/ijvs.2020.126435.1322

السمية السلوكية العصبية لكبريتات النحاس وعلاقتها بالإجهاد التأكسدي والتغييرات النسيجية المرضية في دماغ أفراخ الدجاج

شهد إسماعيل النعيمي و يمامة زهير صالح العبدلي

فرع الفسلجة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

الهدف هو دراسة التأثيرات السمية العصبية تحت الحادة لكبريتات النحاس في الأفراخ على النشاط الحركي والسلوك العصبي وعلاقته بالإجهاد التأكسدي والتغيرات النسيجية المرضية في نسيج دماغ الأفراخ. استخدام ثلاثين فرخا في هذه التجربة، قسمت عشوائيا إلى ٥ مجاميع من ٦ فراخ. تم إعطاؤهم التراكيز التالية ٢,٥ و ٥ و ١٠ و ١٥٪ من الجرعة المميتة الوسطية، وإخضاع كل الأفراخ للاختبارات السلوكية التالية وهي الاستجابة لاختبار عدم الحركة الشدي، واختبار تصحيح الجسم، واختبار النشاط الحركي للأفراخ داخل صندوق الحقل المفتوح. كانت الجرعة المميتة الوسطية عن طريق الفم ٧٧٢ ملغم / كغم، وأدت إلى تثبيط حركة الحيوان في الحقل المفتوح، وزيادة مدة سكون الأفراخ. التأثيرات تناسبت طرديا مع زيادة الجرعة. أظهرت كبريتات النحاس في ٢,٥، ٥، ١٠، ١٥٪ من الجرعة المميتة الوسطية زيادة معنوية في تركيز المالونديالديهايد بينما سجل ١٥٪ من الجرعة المميتة ٥٠ انخفاضًا معنويًا في نشاط الكلوتاثيون والكولين استريز. أدت جميع الجرع إلى انخفاض كبير في إجمالي قدرة مضادات الأكسدة في أنسجة الدماغ والكبد. يُظهر دماغ الأفراخ المعاملة بكبريتات النحاس بنسبة ١٥٪ من الجرعة المميتة الوسطية، تجمع الخلايا الدباقية والنجمية حول الخلايا العصبية ووذمة حول الأوعية الدموية، تكسر الأنوية ونخر في الخلايا العصبية، وموت الخلايا المبرمج. كان لبقية التراكيز تغيرات نسيجية مرضية تتناسب مع ارتفاع الجرعة المعطاة. خلصنا من هذا العمل إلى أن التراكيز العالية من كبريتات النحاس في الدماغ تولد الإجهاد التأكسدي والتغيرات النسيجية المرضية، والتي أثرت على السلوك العصبي والنشاط الحركي للأفراخ في البيئة المفتوحة.