Journal of Trace Elements in Medicine and Biology 52 (2019) 186–191

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

The neurotoxic impact of subchronic exposure of male rats to copper oxychloride

Heba N. Gad El-Hak[a,*], Yomn M. Mobarak[b]

[a] Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
[b] Zoology Department, Faculty of Science, Suez University, Suez, Egypt

ARTICLE INFO

Keywords:
Cerebellum
Cerebral cortex
LDH
Neurotoxicity
Copper oxychloride

ABSTRACT

Background: The target of this animal study was to clarify the influence of Copper oxychloride (COC) (at concentrations of 50, 100 and 200 mg/kg b.wt.) administration for ninety days on the brain tissues to evaluate its possible neurotoxicity.

Methods: Thirty male albino rats were divided up into control and four experimental groups. Group-II (rats were fed corn oil daily through oral gavage) and Group-III-A, Group-III-B, Group-III-C (rats were fed orally with COC in a dosage of 50 mg/kg, 100 mg/kg and 200 mg/kg b.wt., respectively, daily for ninety days. Various biochemical analyses and histopathological assessment of rat forebrain were investigated.

Results: the brains of the treated rats at the three chosen doses of COC recorded a significant ($p \leq 0.05$) elevation of lipid peroxidation. The measured brain lactate dehydrogenase (LDH) revealed non significant ($p \geq 0.05$) differences among the studied groups. Besides, there was a significant ($p < 0.05$) decrease in the brain manganese concentration (Mn) of COC treated rats. In addition, there were significant ($p < 0.05$) increase in zinc (Zn) brain concentration and non significant change in copper (Cu) brain concentration among groups. The brain, cerebrum showed marked histopathological damage than cerebellum. The cerebral cortex of COC treated animals exhibited severe degenerative changes.

Conclusion: The present results concluded that consumption of food contaminated even with modest amount of COC can enter the brain barrier resulted in neurotoxicity in the brain of albino rats.

1. Introduction

Pesticides are compound substances accustomed to kill, repel, or manage the development of biological organisms [1]. The study of exposure assessment of pesticides is therefore extremely critical to understand the potential unsafe impacts to the human health [2]. Pesticides are broadly sprayed on agricultural crops and fruits to manage insects and fungi that can turn into a toxicological hazard for human health [3]. The COC is also commonly used owing to its antifungal activity depends on soluiblization and release of ionic copper [4]. Despite the production of a wide variety of synthetic organic fungicides, COC still predominate the field of antifungal plant disease management and in crop protection [5] and used to control leaf twist [6], spot [7], spoil [8], dark spot [9] and other fungal diseases such as control downy mildew built up in lettuce [10], cucumber [11] and grapes [12], early blight in tomatoes and potatoes [13], purple blotch in onion [14], leaf spot in strawberry [15], sunflower [16] and beans. It is also used in compensating Cu deficiency of wheat grains [17]. Copper is relatively safe from a handling perspective, but there is some concern regarding its buildup in agricultural soils. After application on plants in the field, residual Cu typically accumulates in the upper 15 cm of soil, bound to the organic matter and fine clay fraction [18]. The ecological risk assessments indicate that copper is relatively non toxic for use as a broad-spectrum fungicide on many food and ornamental crops, and for direct use in water applications as an algicide, aquatic herbicide, bactericide, and molluscicide [19]. Copper-based agrochemicals especially COC can affect human health through inhalation and ingestion causing different types of cancer, degenerative diseases, and many immune, hematological, neurological and reproductive disorders [20]. Gunay, et al. [21] reported four patients with chronic copper poisoning and one patient with acute poisoning consumed copper pesticide-treated vegetables. Food, vegetables are also important sources of entry of COC and population such as substance farmers that consume locally grown products are at particular risk. Toxic response in humans has been observed at concentration 11 mg/kg of copper [22].

Copper has an important role for healthy brain development [23].

* Corresponding author.
E-mail addresses: heba_ahmed@science.suez.edu.eg (H.N. Gad El-Hak), y.mobarak@suezuni.edu.eg (Y.M. Mobarak).

https://doi.org/10.1016/j.jtemb.2018.12.015
Received 20 October 2018; Received in revised form 14 December 2018; Accepted 29 December 2018
0946-672X/

Cu deficiency likewise its abundance can seriously influence brain functions [24] which associated to brain damage disease as Menkes disease, Wilson's disease (WD) and Alzheimer's disease [25]. An animal model of Cu toxicity for longer duration may recreate the pathological changes similar to WD patients [26]. Central nervous system (CNS) is protected against most of the harmful agents and infections owing to of blood brain and blood cerebrospinal barriers. However, free Cu is succeed in crossing blood brain barrier resulting within it oxidative cell harm [27]. Copper imbalance increase or decrease found adverse effect has been strongly implicated in neurodegenerative diseases caused by the induction of oxidative damage [28,29].

The purpose of the present investigation was to explore the neurotoxic impact of copper-based pesticide compound (copper oxychloride) as a well known antifungal pesticide in developing nations to the rat brain at different doses after 90 days treatment. The information on its neurotoxic impact still warrants further work. The neurotoxic impact was evaluated by assessing the rat brain lipid peroxidation, lactate dehydrog enase activity, different trace element concentration and histopathological examinations.

2. Material and methods

Copper oxychloride (concentration of copper, 96.6%, CAS No 1332-40-7, registration number 1118 manufactured by Delta for agriculture chemical, Egypt) was obtained from local agrochemical stores. For the experiments, Copper oxychloride doses were separately dissolved in corn oil.

2.1. Experimental animal treatment

In the laboratory earlier to the experiment beginning, rats were acclimatized for seven days together with free access to water and food. The animals kept within an air-conditioned room with ordinary light and dark cycle. The work was approved by the Animal Local Ethics Committee in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and related guidelines.

The experimental animals consisted of thirty male albino rats (*Rattus rattus*) (body weight = 205 ± 10 g) provided with food and tap water *ad libitum* Six rats served as control (Group-I) received water daily through oral gavage while the remaining twenty four rats were separated into 4 groups of 6 rats per group of three animals per cage for experimental treatment. In the experimental group, Group-II (rats received corn oil daily through oral gavage) and Group-III-A, Group-III-B, Group-III-C (rats received COC in a dosage of 50 mg/kg, 100 mg/kg and 200 mg/kg b.wt. respectively, daily for 90 days through oral gavage. The dosages utilized in the present investigation were adjusted according to the LD_{50} calculations carried out by [30] and the doses selected according to the study of Alkolaly [31] on the Copper oxychloride residue in lettuce. After 90 days, all the animals were fasted for 24 h and blood was collected by venipuncture from all the animals and processed for the determination of Cu concentration in the whole blood according to Kazi, et al. [32] after anesthetized with 4% halothane. Then, Rats were sacrificed by decapitation. Coronal section from the frontal lobe of the brain were removed for histopathological and biochemical analyses and cerebellum for only histopathological examination. All the processing of histopathological and biochemical analyses were ended within one week.

2.2. Biochemical analyses

1 g of brain specimens were homogenized in 50 m M potassium phosphate buffer with 1 m M EDTA (pH 7.4). the. 200 µL of the supernatant from each homogenized sample was utilized for the colorimetric determination of malondialdhyde (MDA) as indicator of Lipid peroxidation following the manufacturer's protocol (Lipid Peroxidation (MDA) Assay Kit, Sigma-Aldich, Egypt) at the absorbance 532 nm (A532) [33] and 100 µL of the supernatant from each homogenized sample was utilized for the lactate dehydrogenase (LDH) following the manufacturer's protocol (LDH-LQ Assay Kit, SPINREACT, SPAIN) at the absorbance 340 nm. [34]. 0.5 g of brain specimen was used for determination of copper, zinc, manganese and iron concentrations according to Clegg, et al. [35]. Brain samples were then placed into Pyrex screw glass vials and then 700 µl of nitric acid (65%, w/w, Sigma-Aldich, Egypt) and 300 µl of hydrogen peroxide (30%, w/w, Bio-Basic, Canada) were added. Samples were then digested on the hot plate at 120–150 °C for 40 min. Afterwards, the digested samples were filtered, transferred to a 100 ml plastic volumetric bottle and fulfilled with deionized water to the end of the measure. The Cu, Zn, Mn and iron (Fe) concentrations in brain tissues were given as µg per g of wet weight. Estimation was carried out using a Perkin Elmer AS 2380 flame atomic absorption spectrophotometer. [36] Determine the concentration of Cu, Zn, Mn and iron (Fe) using the procedures listed in the "Standard Conditions" section of [37]. Standards are prepared by diluting the stock standard solution with deionized water. Deionized water used as a blank solution. Blank determination was carried out for calibration of the instrument. All reagents were from Merck, Darmstadt (Germany).

2.3. Histological preparations

The specimens were prepared, fixed as very thin sliced in buffered 10% neutral formalin for 24 h., the tissues were rinsed and then dehydrated immediately as the brain tissues avoided to left in alcohol longer than 24 h, embedded in paraffin wax, sectioned coronally on a microtome at a thickness of 4 µm, and stained with standard stain haematoxylin and eosin. Also, Cresyl Violet Acetate solution was applied to stain Nissl substance in the cytoplasm of the nerve cells. The resulting slides were then investigated under the light microscope to compare tissue sections of the control group with the treated group. The histological examination focused mainly on degeneration, atrophy or pyknosis of pyramidal cells, congestion of blood vessel, hemorrhage, vacuolation, white and grey matter with pyknotic nuclei.

2.4. Statistical analysis

Statistical evaluation was run out with the use of SPSS version 15 (SPSS Inc., Chicago, USA). One way analysis of variance (ANOVA) used to examine the variation difference in measured indices among experimental groups. Individual differences among the groups were tested successively using the Duncan test. Significance was assumed at $p < 0.05$. All data were reported as mean ± standard error of mean (SEM).

3. Results

No mortality was recorded during the observational study and the treated rats with COC showed decrease in weight in comparison to control group Exposure of rats to COC for ninety days in Group-III-A, Group-III-B and Group-III-C, respectively caused a significant $p < 0.05$ increase in CU concentration in the blood by increasing the COC dose in comparison to control group. Non significant change in CU concentration in the blood was observed in corn oil group compared to control group (Fig. 1).

Exposure of rats to COC in Group-III-A, Group-III-B and Group-III-C, respectively caused a higher significant $p < 0.05$ MDA levels in the brain as compared with controls. Non significant change in brain MDA levels was observed among rats treated with corn oil compared to controls (Fig. 2). Treatment of rats with corn oil and 50, 100 and 200 mg/kg b.wt. COC caused non significant change in the LDH activities in brains of all treated rats compared to the control group (Fig. 3).

The obtained results indicated that there was a significant ($p < 0.05$) decrease in Mn brain concentration and a nonsignificant increase of Cu brain concentration of the treated rats with COC. While the brain concentration of Fe in treated rats exhibited a significant ($p <$

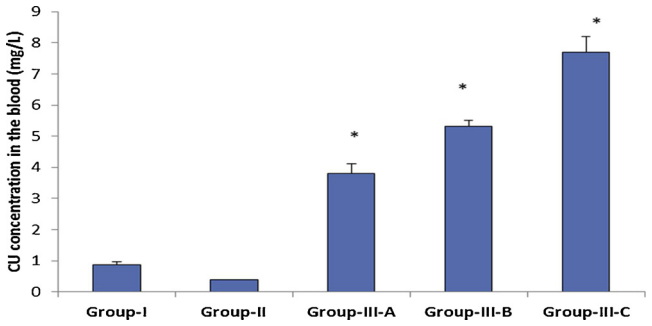

Fig. 1. Cu concentration levels in the whole blood of control and COC treated rats. The results are presented as means ± SEM. (*) showed $p \leq 0.05$ when compared to control group (ANOVA and Duncan test as a post hoc).

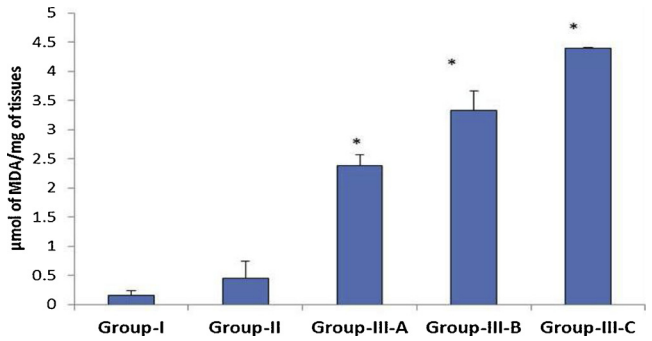

Fig. 2. Lipid peroxidation levels in the brains of control and COC treated rats. The results are presented as means ± SEM. (*) showed $p \leq 0.05$ when compared to control group (ANOVA and Duncan test as a post hoc).

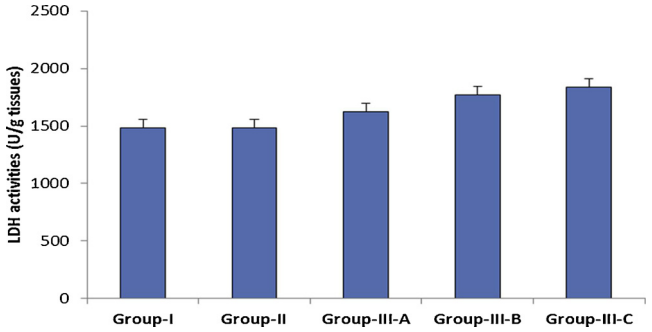

Fig. 3. LDH activity in the brains of control and COC treated rats. The results are presented as means ± SEM. (ANOVA and Duncan test as a post hoc).

Table 1
Trace element concentration in brain of different rat groups.

Experimental group	CU (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)
Group-I	0.239 ± 0.0	0.147 ± 0.0	0.261 ± 0.0	0.375 ± 0.1
Group-II	0.281 ± 0.0	0.147 ± 0.0	0.261 ± 0.0	0.442 ± 0.0
Group-III-A	0.281 ± 0.0	0.182 ± 0.0*	0.398 ± 0.0*	0.250 ± 0.1*
Group-III-B	0.335 ± 0.1	0.271 ± 0.0*	0.391 ± 0.0*	0.230 ± 0.0*
Group-III-C	0.394 ± 0.1	0.327 ± 0.1*	0.631 ± 0.1*	0.250 ± 0.1*

Values are expressed as mean ± SEM (n = 6/group).
** $p < 0.05$; statistical significance with respect to controls.

0.05) increase in the Group-III-A, Group-III-B and Group-III-C (Table 1) comparing to the control group. Likewise, the brain concentration of Zn values showed a significant ($p < 0.05$) increase among the treated group in comparison to the control group (Table 1).

Histopathological examination of the cerebral cortex from control rats (Fig. 4-A, Fig. 5-A & Fig. 6-A) and rats received corn oil for 90 days showed normal histological structure of the meninges, neurons and the six cerebral layers. Examination of the cerebral cortex of treated rats with different doses of COC for 90 days showed the severity of damage associated with the COC doses increase. Examination of the cerebral cortex of treated rats with 50 mg/kg b.wt. COC for 90 days (Fig. 4-B, Fig. 5-B & Fig. 6-B) showed obvious disruption of the normal arrangement of cell layers. Examination of the cerebral cortex of treated rats with 100 mg/kg b.wt. COC for 90 days (Fig. 4-C & Fig. 5-C) showed with severe congestion in the blood vessels of the meninges with larger space, associated with marked pyramidal neurons pyknosis and chromatolysis and fibrous degeneration of cerebral cortex neurons. Examination of the cerebral cortex of treated rats with 200 mg/kg COC for 90 days (Fig. 4-D, Fig. 5-D& Fig. 6-D) showed marked neurons pyknosis, many vacuoles of variable size and spreading of red blood cells (hemorrhage).

The histopathological examination of control, cerebellum (Fig. 7-A) and rat received corn oil for 90 days composed of three successive layers of cell which is molecular layers, normal Purkinje cell layer arrangement with perikaryon and granular layer. The treatment with different doses of COC did not interrupt the normal arrangement of the three layers compared to the control layers (Fig. 7-B)

4. Discussion

Copper toxicity may impact human health through persistent exposure to small doses over a long period of time and its exposure to small doses can produce both acute and chronic tissue injury. The effect of copper on the brain has been described by many authors. So the neurotoxic impact was chosen as a target in this work. Gaggelli, et al. [38] and Kaden, et al. [39] suggested that copper itself may be considered an acute phase reactant and marker for neurological disease which caused by the disturbance of copper homeostasis.

Treatment of rats orally daily with COC in a dose of 50, 100 and 200 mg/kg b.wt. for 90 days showed an increase to the Cu concentration in the blood and the fore brain tissue of treated rats compared to the control groups.

The ROS have a critical part in the pathological process of many diseases, particularly neurological and mental illness [40]. The brain has comparatively greater vulnerability to oxidative damage [41]. Exposure to copper reported to enhance oxidative stress and cause neurotoxicity. The brain is more vulnerable target to injury by lipid peroxidation products than other tissues because its expensive in oxygen consumption, high lipid content, especially polyunsaturated fatty acids, and low mitotic rate and antioxidant levels [42–44]. Membrane lipid derangements, including lipid peroxidation, have been reported to contribute significantly to membrane dysfunction in neurodegenerative diseases [45]. In our experimental situation, the increase blood copper level followed the increased levels of lipid peroxidation are consistent with earlier studies [29].

The COC caused a breakdown of brain metal (Cu, Zn, Fe and Mn) homeostasis mechanisms by significantly diminished the brain Mn levels and significantly increased the brain, iron and zinc levels in all the COC treated rats. It is difficult to explain the mechanism by which such an effect is produced. Manganese is needed for development and performance of the brain and acts as one of necessary component of glutamine syntheses and mitochondrial superoxide dismutase [46,47] and its decrease is toxic to the brain according to Takeda [48]. It may affect the neural activity and causes neurological disorder to the experimental animal and human. Manganese has additionally been stated in previous studies of rats to negatively correlate with iron [49–51]. The increased brain metal levels (copper, zinc and iron) and imbalance of the brain homeostasis has been associated with normal aging and a variety of diseases [52]. The copper, iron and zinc levels, may adversely interact with the amyloid-β peptide causing its aggregation and the production of neurotoxic hydrogen peroxide (H_2O_2), contributing to the

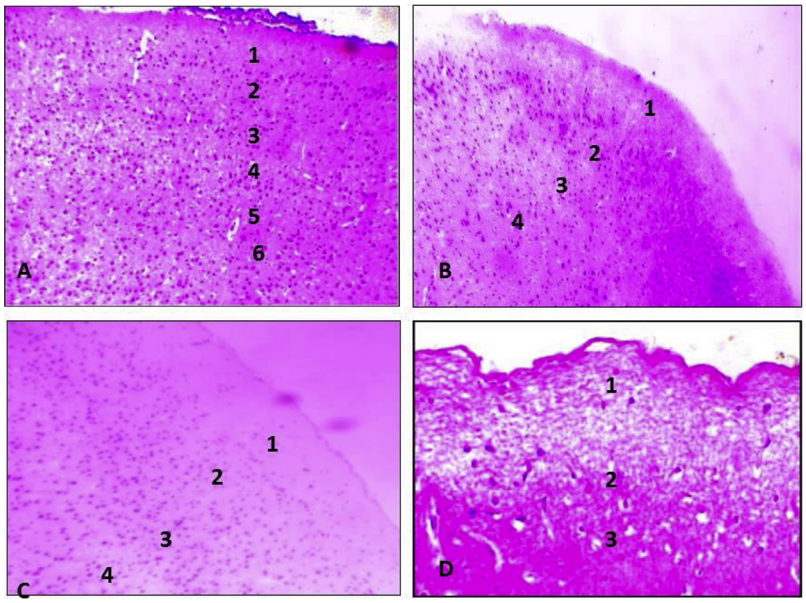

Fig. 4. photomicrographs of sections in the frontal cortex of rats. (A) a control rat showing normal cortex layer H&E. × 10. (B) Rat treated with 50 mg/kg COC for 90 days showing degeneration of outer molecular layer H&E. × 10. (C) Rat treated with 100 mg/kg COC for 90 days showing degeneration of outer molecular layer and external granular layer H&E. × 10. (D) Rat treated with 200 mg/kg COC for 90 days showing degeneration of outer molecular layer and external granular layer H&E. × 20. The six layers of the frontal cortex can be identified: 1- outer molecular layer; 2- external granular layer; 3- external pyramidal cell layer; 4- internal granular layer; 5- ganglionic layer; 6- multiform layer.

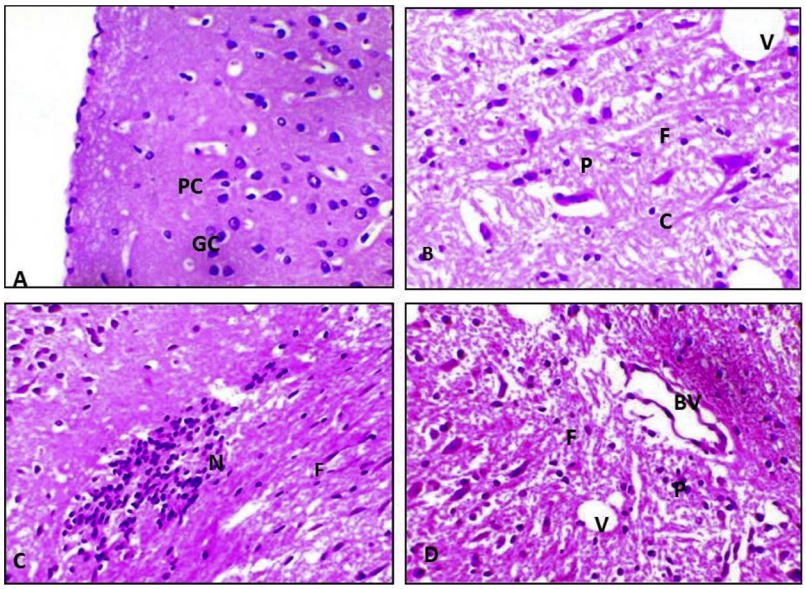

Fig. 5. photomicrographs of sections in the frontal cortex of rats. (A) a control rat showing normal outer molecular layer and external granular layer with normal pyramidal cells (PC) and glial cells (GC). (B) Rat treated with 50 mg/kg COC for 90 days showing vacuolar degeneration (V), pyramidal neurons pyknosis (P), pyramidal neurons chromatolysis (C) and fibrous degeneration (F). (C) Rat treated with 100 mg/kg COC for 90 days showing Focal necrosis (N) with dense pyknotic neurons and fibrous degeneration (F). (D) Rat treated with 200 mg/kg COC for 90 days showing congestion of the cerebral blood vessels with increased perivascular space (BV), vacuolar degeneration (V), pyramidal neurons pyknosis (P) and fibrous degeneration (F) (G). H&E X40.

pathological process of Alzheimer's disease [53–58]. Incontrast to Pal and Prasad [59] found intraperitoneally injected copper lactate (0.15 mg /100 g body weight) daily for 90 days significantly increased Cu content and non-significant changes for the Zn and Fe content compared to the control. Based on the relative increase of copper with disturbance copper homeostasis affect the brain tissue [60].

Brain is especially sensitive to increase copper levels in the blood owing to copper transport activity in the blood brain barrier [24,40,61]. Copper increase in the blood was associated with marked widespread CNS lesions and neurohistological alteration which noted in the cerebrum when compared to control ones. The brain cerebrum is part of brain control the learning and memory [62]. In the present study, the cerebral cortex was noted vascular congestion, neuronal degeneration, necrosis, vacuoles formation accompanying by gliosis and increased density of inflammatory cells. The pyramidal cells showed chromatolysis appeared as cell swelling and Nissl's dissolution. In additional findings of dark neuron cells appeared with the higher dose of COC, which may indicated signs of inflammatory changes and may have resulted from the death of these brain tissues as reported by [63] or may be reflection of certain phase of apoptosis [64] or may be due to abnormalities in the structural capillary wall of the blood brain barrier [65,66]. These outcomes were in the line with past examinations that demonstrated an increase in neural cell vacuolization and an increase degeneration, referring to neuronal damage induced by copper [67,68]. The increased vacuolation in the cerebral cortex of treated rats was result of brain lipid peroxidation confirmed by the bichemical studies

The cerebellum is basically known for its coordination of somatic motor function, muscle tone, balance, equilibrium [69] and nonmotor activities of the brain, such as cognition, emotions, behavior, remember and learning processes through its connections with other areas of the brain [70]. Discoveries from this examination have demonstrated that in all the COC treated rats had no injuries or impacts on the cells of the cerebellum including Purkinje cells. This is in opposition to Deibel, et al. [71] and Butterfield, et al. [72] that reported cerebellum effect with copper increase.

LDH activities is energy metabolite for brain cells [73], its elevation give s an indication of brain aging and encephalopathy syndrome [74] In the present study, treatment within all the COC treated rats did not cause significant alteration in LDH activities in the brain regions with

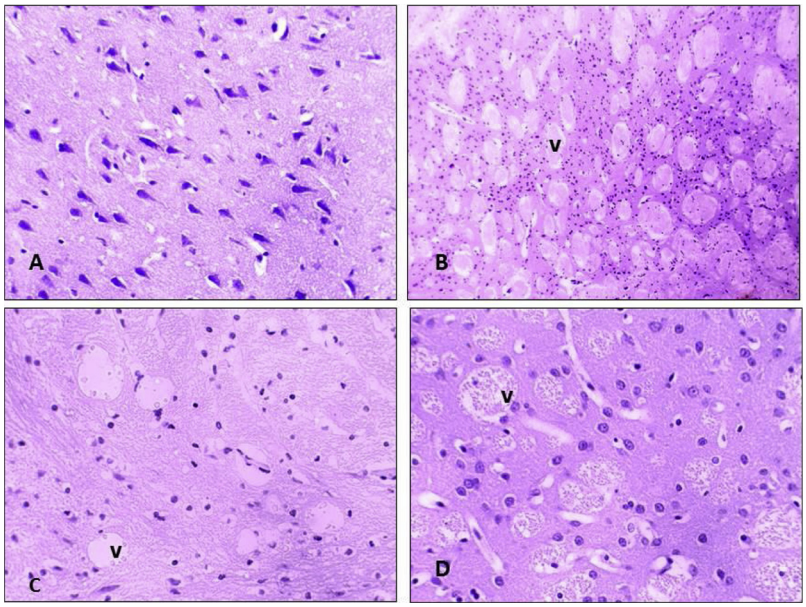

Fig. 6. photomicrographs of sections in the frontal cortex of rats. (A) a control rat showing normal outer molecular layer and external granular layer with normal cells. (B) Rat treated with 50 mg/kg COC for 90 days showing vacuolar degeneration (V) and neuron damage. (C) Rat treated with 100 mg/kg COC for 90 days showing vacuolar degeneration (V) and neuron damage. (D) Rat treated with 200 mg/kg COC for 90 days showing vacuolar degeneration (V) and neuron damage. Cresyl Violet X20.

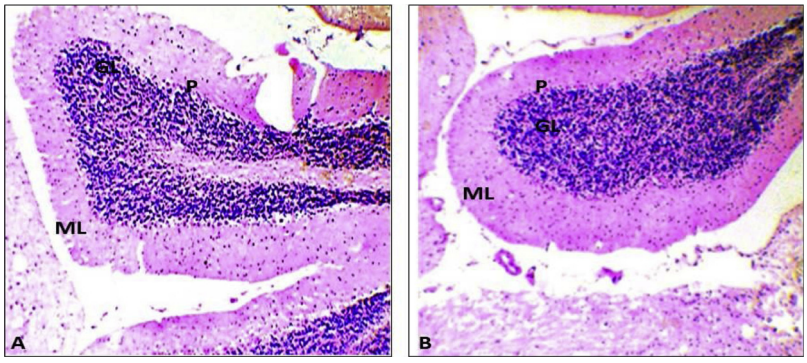

Fig. 7. photomicrographs of sagittal sections of cerebellum of the control (A) and rat treated with 200 mg/kg COC for 90 days (B) showed well developed molecular layer (ML), granular layer (GL), Purkinje cell layer (P). H&E X20.

the increase in the copper levels in the rat brain.

5. Conclusions

In the present study, oral administration of the antifungal Copper oxychloride at various dosages brough t neurotoxic damage caused by Cu increase in the blood. Equally well, it produced oxidative stress in the rat brain. The extent of brain damage is correlated with the quantity of metal content in the brain tissue. These alterations may contribute to the neurotoxicological effects of this antifungal. Therefore, total avoidance should be employed in the use of these agents as pesticides long time even with low doses. There is a critical need to discover neuroprotective agents that will secure the body against unfavorable impact of copper oxychloride and neutralize its toxicity.

Conflict of interest

There is no conflict of interest to declare by all authors

References

[1] S. Igbedioh, Effects of agricultural pesticides on humans, animals, and higher plants in developing countries, Arch. Environ. Health Int. J. 46 (4) (1991) 218–224.
[2] K. Jaga, C. Dharmani, Sources of exposure to and public health implications of organophosphate pesticides, Revista panamericana de salud pública 14 (3) (2003) 171–185.
[3] C.A. Damalas, I.G. Eleftherohorinos, Pesticide exposure, safety issues, and risk assessment indicators, Int. J. Environ. Res. Public Health 8 (5) (2011) 1402–1419.
[4] M.I. Gharieb, M.I. Ali, A.A. El-Shoura, Transformation of copper oxychloride fungicide into copper oxalate by tolerant fungi and the effect of nitrogen source on tolerance, Biodegradation 15 (1) (2004) 49–57.
[5] G.M. Sanders, L. Korsten, F. Wehner, Survey of fungicide sensitivity in Colletotrichum gloeosporioides from different avocado and mango production areas in South Africa, Eur. J. Plant Pathol. 106 (8) (2000) 745–752.
[6] J. Heaton, S. Dullahide, Control of celery leaf curl disease caused by Colletotrichum acutatum, Australas. Plant Pathol. 22 (4) (1993) 152–155.
[7] M. Kamhawy, Host range and control of Phyllosticta sp. The cause of banana leaf spot and blight, Egyptian J. Phytopathol. 34 (2) (2006) 1–15.
[8] G. Johnson, A. Cooke, A. Mead, I. Wells, Stem End Rot of Mango in Australia: Causes and Control, (1989), pp. 288–295.
[9] G. Schutte, K. Beeton, J. Kotze, Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa, Plant Dis. 81 (8) (1997) 851–854.
[10] U. Gisi, Chemical Control of Downy Mildews. Advances in Downy Mildew Research, Springer, 2002, pp. 119–159.
[11] S. Chaudhry, J. Iqbal, A. Mustafa, Efficacy of different fungicides for the control of downy mildew of cucumber, J. Anim. Plant Sci 19 (4) (2009) 202–204.
[12] S.P. Falk, R.C. Pearson, D.M. Gadoury, R.C. Seem, A. Sztejnberg, Fusarium proliferatum as a biocontrol agent against grape downy mildew, Phytopathology 86 (10) (1996) 1010–1017.
[13] N.M. Sallam, Control of tomato early blight disease by certain aqueous plant extracts, Plant Pathol. J. (Faisalabad) 10 (4) (2011) 187–191.
[14] B. Chethana, G. Ganeshan, B. Manjunath, Screening of genotypes and effect of fungicides against purple blotch of onion, J. Agric. Technol. 7 (5) (2011) 1369–1374.
[15] A. Alippi, M. Carranza, B. Ronco, Angular leaf spot of strawberry, a new disease in Argentina. Comparative control with antibiotics and fungicides, Adv. Hortic. Sci. [rivista dell'ortofloroftutticoltura italiana] 3 (N 1) 3 (1) (1989) 1000–1004 1989.
[16] N. Mathivanan, V. Prabavathy, Effect of carbendazim and mancozeb combinationon Alternaria leaf blight and seed yield in sunflower (Helianthus annus L.), Arch. Phytopathol. Plant Prot. 40 (2) (2007) 90–96.

[17] R. Brennan, Effectiveness of some copper compounds applied as foliar sprays in alleviating copper deficiency of wheat grown on copper-deficient soils of Western Australia, Aust. J. Exp. Agric. 30 (5) (1990) 687–691.
[18] L. Epstein, S. Bassein, Pesticide applications of copper on perennial crops in California, 1993 to 1998, J. Environ. Qual. 30 (5) (2001) 1844–1847.
[19] V. Husak, Copper and copper-containing pesticides: metabolism, toxicity and oxidative stress, J. Vasyl Stefanyk Precarpathian Natl. Univ. 2 (no. 1) (2015) 39–51.
[20] A. Ngowi, T. Mbise, A. Ijani, L. London, O. Ajayi, Smallholder vegetable farmers in Northern Tanzania: pesticides use practices, perceptions, cost and health effects, Crop. Prot. 26 (11) (2007) 1617–1624.
[21] N. Gunay, C. Yildirim, O. Karcioglu, N.E. Gunay, M. Yilmaz, C. Usalan, A. Kose, I. Togun, A series of patients in the emergency department diagnosed with copper poisoning: recognition equals treatment, Tohoku J. Exp. Med. 209 (3) (2006) 243–248.
[22] S.B. Goldhaber, Trace element risk assessment: essentiality vs. toxicity, Regul. Toxicol. Pharmacol. 38 (2) (2003) 232–242.
[23] D.J. Waggoner, T.B. Bartnikas, J.D. Gitlin, The role of copper in neurodegenerative disease, Neurobiol. Dis. 6 (4) (1999) 221–230.
[24] I.F. Scheiber, J.F. Mercer, R. Dringen, Metabolism and functions of copper in brain, Prog. Neurobiol. 116 (2014) 33–57.
[25] D. Strausak, J.F. Mercer, H.H. Dieter, W. Stremmel, G. Multhaup, Copper in disorders with neurological symptoms: alzheimer's, Menkes, and Wilson diseases, Brain Res. Bull. 55 (2) (2001) 175–185.
[26] D. Huster, T.D. Purnat, J.L. Burkhead, M. Ralle, O. Fiehn, F. Stuckert, N.E. Olson, D. Teupser, S. Lutsenko, High copper selectively alters lipid metabolism and cell cycle machinery in the mouse model of Wilson disease, J. Biol. Chem. 282 (11) (2007) 8343–8355.
[27] K. Jomova, D. Vondrakova, M. Lawson, M. Valko, Metals, oxidative stress and neurodegenerative disorders, Mol. Cell. Biochem. 345 (1-2) (2010) 91–104.
[28] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, Nat. Rev. Drug Discov. 3 (3) (2004) 205.
[29] L.M. Gaetke, C.K. Chow, Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology 189 (1-2) (2003) 147–163.
[30] E.F.S. Authority, M. Arena, D. Auteri, S. Barmaz, A. Brancato, D. Brocca, L. Bura, L. Carrasco Cabrera, A. Chiusolo, D. Court Marques, Peer review of the pesticide risk assessment of the active substance spinosad, Efsa J. 16 (5) (2018) e05252.
[31] A.M. Alkolaly, S.E. El-Nahas, T. A Abdel Rahman, A residual effect of certain copper fungicides used in controlling downy mildew of Lettuce, Egypt J Agric Res 95 (1) (2017) 65–71 2007;95(1).
[32] T.G. Kazi, H.I. Afridi, N. Kazi, M.K. Jamali, M.B. Arain, N. Jalbani, G.A. Kandhro, Copper, chromium, manganese, Iron, nickel, and zinc levels in biological samples of diabetes mellitus patients, Biol. Trace Elem. Res. 122 (1) (2008) 1–18.
[33] D. Bagchi, A. Garg, R. Krohn, M. Bagchi, D. Bagchi, J. Balmoori, S. Stohs, Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice, Gen. Pharmacol. Vasc. Syst. 30 (5) (1998) 771–776.
[34] P.G. Cabaud, F. Wróblewski, Wt T.A. ok, V. Ruggiero, Colorimetric measurement of lactic dehydrogenase activity of body fluids, Am. J. Clin. Pathol. 30 (3) (1958) 234–236.
[35] M.S. Clegg, C.L. Keen, B. Lönnerdal, L.S. Hurley, Influence of ashing techniques on the analysis of trace elements in animal tissue, Biol. Trace Elem. Res. 3 (2) (1981) 107–115.
[36] H. Agemian, A.-Y. Chau, An atomic absorption method for the determination of 20 elements in lake sediments after acid digestion, Anal. Chim. Acta 80 (1) (1975) 61–66.
[37] P.-E. Corporation, Analytical Methods for Atomic Absorption Spectrophotometry, (1964).
[38] E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis), Chem. Rev. 106 (6) (2006) 1995–2044.
[39] D. Kaden, A.I. Bush, R. Danzeisen, T.A. Bayer, G. Multhaup, Disturbed copper bioavailability in Alzheimer's disease, Int. J. Alzheimers Dis. 2011 (2011).
[40] Y. Gilgun-Sherki, E. Melamed, D. Offen, Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier, Neuropharmacology 40 (8) (2001) 959–975.
[41] A. Reynolds, C. Laurie, R.L. Mosley, H.E. Gendelman, Oxidative stress and the pathogenesis of neurodegenerative disorders, Int. Rev. Neurobiol. 82 (2007) 297–325.
[42] E. Mariani, M. Polidori, A. Cherubini, P. Mecocci, Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview, J. Chromatogr. B 827 (1) (2005) 65–75.
[43] R.P. Bazinet, S. Layé, Polyunsaturated fatty acids and their metabolites in brain function and disease, Nat. Rev. Neurosci. 15 (12) (2014) 771.
[44] S. Hussein, A.-A. El-Saba, M.K. Galal, Biochemical and histological studies on adverse effects of mobile phone radiation on rat's brain, J. Chem. Neuroanat. 78 (2016) 10–19.
[45] M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol. 39 (1) (2007) 44–84.
[46] J.R. Prohaska, Functions of trace elements in brain metabolism, Physiol. Rev. 67 (3) (1987) 858–901.
[47] C.G. Fraga, Relevance, essentiality and toxicity of trace elements in human health, Mol. Aspects Med. 26 (4-5) (2005) 235–244.
[48] A. Takeda, Manganese action in brain function, Brain Res. Rev. 41 (1) (2003) 79–87.
[49] K.M. Erikson, Z.K. Shihabi, J.L. Aschner, M. Aschner, Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations, Biol. Trace Elem. Res. 87 (1-3) (2002) 143–156.
[50] K.M. Erikson, T. Syversen, J.L. Aschner, M. Aschner, Interactions between excessive manganese exposures and dietary iron-deficiency in neurodegeneration, Environ. Toxicol. Pharmacol. 19 (3) (2005) 415–421.
[51] S.J. Garcia, K. Gellein, T. Syversen, M. Aschner, Iron deficient and manganese supplemented diets alter metals and transporters in the developing rat brain, Toxicol. Sci. 95 (1) (2006) 205–214.
[52] C.J. Maynard, R. Cappai, I. Volitakis, R.A. Cherny, A.R. White, K. Beyreuther, C.L. Masters, A.I. Bush, Q.-X. Li, Overexpression of Alzheimer's disease amyloid-β opposes the age-dependent elevations of brain copper and iron, J. Biol. Chem. (2002).
[53] A.I. Bush, R.E. Tanzi, Therapeutics for Alzheimer's disease based on the metal hypothesis, Neurotherapeutics 5 (3) (2008) 421–432.
[54] C. Ha, J. Ryu, C.B. Park, Metal ions differentially influence the aggregation and deposition of Alzheimer's β-amyloid on a solid template, Biochemistry 46 (20) (2007) 6118–6125.
[55] T. Chen, X. Wang, Y. He, C. Zhang, Z. Wu, K. Liao, J. Wang, Z. Guo, Effects of cyclen and cyclam on zinc (II)-and copper (II)-induced amyloid β-peptide aggregation and neurotoxicity, Inorg. Chem. 48 (13) (2009) 5801–5809.
[56] A.I. Bush, The metallobiology of Alzheimer's disease, Trends Neurosci. 26 (4) (2003) 207–214.
[57] R.A. Cherny, C.S. Atwood, M.E. Xilinas, D.N. Gray, W.D. Jones, C.A. McLean, K.J. Barnham, I. Volitakis, F.W. Fraser, Y.-S. Kim, Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice, Neuron 30 (3) (2001) 665–676.
[58] C.J. Maynard, R. Cappai, I. Volitakis, R.A. Cherny, A.R. White, K. Beyreuther, C.L. Masters, A.I. Bush, Q.-X. Li, Overexpression of Alzheimer's disease amyloid-β opposes the age-dependent elevations of brain copper and iron, J. Biol. Chem. 277 (47) (2002) 44670–44676.
[59] A. Pal, R. Prasad, Regional distribution of copper, zinc and Iron in brain of wistar rat model for non-wilsonian brain copper toxicosis, Indian J. Clin. Biochem. 31 (1) (2016) 93–98.
[60] W. Zheng, A.D. Monnot, Regulation of brain iron and copper homeostasis by brain barrier systems: implication in neurodegenerative diseases, Pharmacol. Ther. 133 (2) (2012) 177–188.
[61] J.F. Mercer, The molecular basis of copper-transport diseases, Trends Mol. Med. 7 (2) (2001) 64–69.
[62] N.M. White, R.J. McDonald, Multiple parallel memory systems in the brain of the rat, Neurobiol. Learn. Mem. 77 (2) (2002) 125–184.
[63] Y.S. Kim, T.H. Joh, Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease, Exp. Mol. Med. 38 (4) (2006) 333.
[64] M. Ankarcrona, J.M. Dypbukt, E. Bonfoco, B. Zhivotovsky, S. Orrenius, S.A. Lipton, P. Nicotera, Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function, Neuron 15 (4) (1995) 961–973.
[65] H.C. Grant, P. Lantos, C. Parkinson, Cerebral damage in paraquat poisoning, Histopathology 4 (2) (1980) 185–195.
[66] S. Nag, J.L. Takahashi, D.W. Kilty, Role of vascular endothelial growth factor in blood-brain barrier breakdown and angiogenesis in brain trauma, J. Neuropathol. Exp. Neurol. 56 (8) (1997) 912–921.
[67] C. Armstrong, W. Leong, G.J. Lees, Comparative effects of metal chelating agents on the neuronal cytotoxicity induced by copper (Cu+ 2), iron (Fe+ 3) and zinc in the hippocampus, Brain Res. 892 (1) (2001) 51–62.
[68] A. Pal, R.K. Badyal, R.K. Vasishta, S.V. Attri, B.R. Thapa, R. Prasad, Biochemical, histological, and memory impairment effects of chronic copper toxicity: a model for non-Wilsonian brain copper toxicosis in Wistar rat, Biol. Trace Elem. Res. 153 (1-3) (2013) 257–268.
[69] M.G. Paulin, The role of the cerebellum in motor control and perception, Brain Behav. Evol. 41 (1) (1993) 39–50.
[70] A.E. Cavanna, M.R. Trimble, The precuneus: a review of its functional anatomy and behavioural correlates, Brain 129 (3) (2006) 564–583.
[71] M. Deibel, W. Ehmann, W. Markesbery, Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress, J. Neurol. Sci. 143 (1-2) (1996) 137–142.
[72] D.A. Butterfield, J. Drake, C. Pocernich, A. Castegna, Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β-peptide, Trends Mol. Med. 7 (12) (2001) 548–554.
[73] P.J. Magistretti, L. Pellerin, Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging, Philos. Trans. R. Soc. Lond., B, Biol. Sci. 354 (1387) (1999) 1155–1163.
[74] J.M. Ross, J. Öberg, S. Brené, G. Coppotelli, M. Terzioglu, K. Pernold, M. Goiny, R. Sitnikov, J. Kehr, A. Trifunovic, N.-G. Larsson, B.J. Hoffer, L. Olson, High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio, Proc. Natl. Acad. Sci. 107 (46) (2010) 20087–20092.