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Research Article
Nanocurcumin, Promising Potential Protective Agent Against Histopathological Damage in the Cerebral Cortex of Mice Induced by Aluminum Chloride

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Abstract

Background and Objective: Aluminum (Al) is widely used in many aspects of daily life, such as food packaging, cooking utensil components, food additives, cosmetics and water distillation. This study aimed to evaluate the protective role of nanocurcumin on the cerebral cortex of one and two-month-old mice exposed to 200 mg kg^{-1} b.wt., aluminium. **Materials and Methods:** The Swiss Webster mice were used in this study. The control group only received sterile distilled water, the Al group was administered 200 mg kg^{-1} b.wt., of $AlCl_3$ solution and the Al+Na Cur group was administered 200 mg kg^{-1} b.wt., $AlCl_3$+200 mg kg^{-1} nanocurcumin by intraperitoneal injection. The nanocurcumin was administered one hour after $AlCl_3$ exposure and then on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30. All the mice were anaesthetized and their brains were collected and fixed in a neutral formalin buffer solution for histological analysis. The paraffin method was used in this study. **Results:** The death of granular neuron cells and karyolysis cells and the vacuolation of the pyramid cell layer of the cerebral cortex could be prevented by the intraperitoneal administration of nanocurcumin. The effect of nanocurcumin administration on the Al group at two months of age was more effective than on the Al group at one month of age. **Conclusion:** Nanocurcumin can be a promising candidate protective agent against cerebral cortex changes after aluminium administration.

Key words: Aluminum chloride, cerebral cortex, nanocurcumin, protective agent

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aluminium (Al) is widely used in many aspects of daily life, such as food packaging, cooking utensil components, food additives, cosmetics[1], deodorants, sunblocks, adjuvant vaccines and medicines, such as antacids[2]. Iron, as a cofactor, plays a role in cellular homeostasis. The accumulation of iron caused by the aluminium can disruption of iron homeostasis and lead to induce oxidative stress[3]. Oxidative stress, such as increased lipid peroxidation and free radical production, is related to cytotoxicity, which can lead to cell and tissue damage[4,5].

The absorption of Al^{3}+ions mainly occurs in the gastrointestinal tract and these ions then spread throughout the body and subsequently accumulate in different tissues. These ions can pass through the BBB (The blood-brain barrier) by binding to transferrin. The Al^{3}+ions are highly reactive and tend to form a compound. The highest Al accumulation in the body is the brain and these conditions can induce the formation of ROS. The membrane of the brain contains a lot of polyunsaturated fatty acids, so this condition is at risk of exposure to Al and makes the brain very susceptible[6]. Therefore, accumulation of this ion in the brain can induce oxidativestress[7], which impacts laminated disorganization of the cerebral cortex[8].

In recent years, plants characterized by antioxidants have gained importance in oxidative stress-related diseases[9]. Antioxidants have long been known to neutralize free radicals and ameliorate oxidative stress-related diseases[10]. Curcuma longa (turmeric), a member of the ginger family (Zingiberaceae), includes curcumin as the most active polyphenol compound. Curcumin has antioxidant potential, is neuroprotective[11] and can suppress oxidative stress, inflammation, cognitive deficits and amyloid accumulation[12]. Moreover, this compound seems to have great promise in the treatment of neurodegenerative diseases due to its ability to cross the BBB. The polyphenol structure of curcumin allows it to cross the blood-brain barrier and bind to redox metal ions, which makes curcumin a promising prophylactic agent in the treatment of neurodegenerative diseases in the future[13]. In addition, the ketone group in curcumin has a lone pair of electrons that can form the aluminium-curcumin complex[14]. However, the use of curcumin is limited because of its low bioavailability due to its low absorption in the body, high metabolism, easy hydrolysis and release from the body[15]. Increasing the absorption and bioavailability and preventing the hydrolysis of curcumin in the body can be achieved by producing it in the form of nanocurcumin[16]. Therefore, the present study investigated the neuroprotective effect of nanocurcumin on the cerebral cortex of aluminium-treated mice.

MATERIALS AND METHODS

Study area: This study was conducted from April to December, 2019, this study was conducted April to December, 2019, at the Biomedical Center and Basic Health Technology and the Laboratory of Animal Structure and Development, State University of Jakarta.

The equipment for this study included mouse cages and drinking bottles, syringes and syringes, sample bottles, surgical instruments, digital scales, microtome, slide and cover glass, water baths, staining jar Helen dahl and microscope.

The study material consisted of male mice, nanocurcumin, aluminium chloride, pure alcohol solution, neutral formalin buffer, sodium chloride solution, Hematoxylin-Eosin solution, xylol, entellan, glycerin.

Preparation of aluminum chloride and nanocurcumin: Aluminium chloride (Merck, CAT-No: 7784-13-6) was diluted with sterilized distilled water and administered at a dose of 200 mg kg^{-1} b.wt. Curcumin standard (high-quality turmeric root extract powder 95%, bulk pure curcumin) was purchased from Palmed Green Science Limited, Xi'an, China. Nanocurcumin preparation was based on Kakkar[17]. Eight grams of curcumin powder were dissolved in the emulsifier mixture. The components of the emulsifier mixture included Tween 80 (45.45%) solution, soy lecithin (0.58%) and water. The mixture of the curcumin-emulsifier was added to a lipid solution (7.27%), which was melted at a temperature of 80°C until a nanoemulsion was obtained. The nanoemulsion was mixed with 400 mL PBS (2°C), stirred for 1.5 hrs and stored in a freezer. Furthermore, the curcumin levels were calculated using HPLC and PSA.

Animal sample: In total, 15 male mice (4 weeks of age) and 15 male mice (8 weeks of age) of the Swiss webster strain were obtained from Biomedical Center and Basic Health Technology, Jakarta, Indonesia University, Indonesia. The animals were housed under standard conditions with a 12 hrs light/dark cycle. Every morning, food and drink are given ad libitum. The cages were given husks and changed every day. The treatment was carried out after the acclimatization process ended (14 days). The study protocols were approved by the Medical Ethics and Care of Experimental Animals Committee (No. KET-496/UN2.F1/ ETIK/PPM.00.02/2019).

The mice were randomly divided into three groups (n = 5). The animals in the control group received sterile distilled water solution, the Al group was administered 200 mg kg^{-1} b.wt., $AlCl_3$ solution and the Al+NaCur group was administered 200 mg kg^{-1} b.wt., $AlCl_3$+200 mg kg^{-1} nanocurcumin. The administration of aluminium and nanocurcumin was performed by intraperitoneal injection. Nanocurcumin was administered one hour after $AlCl_3$ exposure[18] and then on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30[19]. The mice were weighed on the same days to monitor their body weight. All the mice were anaesthetized and their brains were collected and washed with NaCl solution. All the brains were fixed in a neutral buffer solution for further analysis.

Histological preparation: The histological preparations of the cerebral cortex were generated at the Veterinary Research Center, Bogor, Indonesia. The brains were fixed in NBF solution and dehydrated in alcohol solution (60, 70, 80, 90 and 100%) for 10 min at every step. The clearing was performed in xylol solutions and paraffin infiltration was carried out in an oven at a temperature between 57 and 60°C. The brain samples were embedded in a paraffin block and sliced to 5 µm using a rotary microtome. Finally, the specimens were stained with hematoxylin and eosin[20].

RESULTS

The cerebral cortex plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. The histological structures of the cerebral cortex of the control group at one in Fig. 1a and two months of age in Fig. 1b, the Al group at one in Fig. 1c and two months of age in Fig. 1d and the Al+NaCur group at one month of age in Fig. 1e and two months of age in Fig. 1f were composed of the molecular zone (MZ), cortical plate zone (CP), subplate zone (SP), intermediate zone (IZ), subventricular zone (SVZ), and ventricular zone (VZ). The thickness of each cerebral cortex zone was not different between the treatment groups and the control group at one month of age.

The molecular zone (MZ) layer of the control group was composed of some relatively small neuron cells, round morphology and fusiform in Fig. 2a and b. At the cellular level, the cerebral cortex of the Al group in Fig. 2c and d and the Al+Na Cur group in Fig. 2e and f at both one and two months of age exhibited congestion and haemorrhage. The MZ zone of the Al group and the Al+Na Cur group showed a very similar ultrastructure to that of the control group (Fig. 2a, b). The CP and SP zones of the Al group at one month of age and two months of age showed marked karyolitic cell death, cell size shrinkage and cell vacuolation. The cell death of the Al

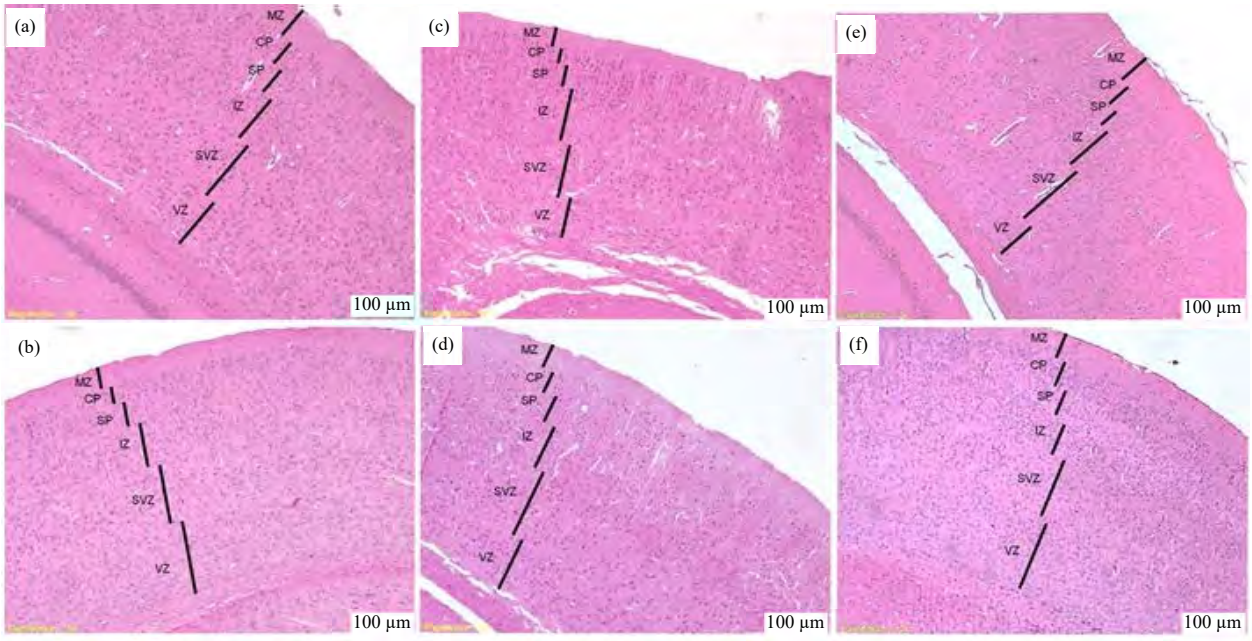

Fig. 1(a-f): Cross-section of cerebral cortex, (a) Control groups in one month age, (b) Control groups in two months' age, (c) Al group in one month age, (d) Al group in two months age, (e) Al+Na Cur group in one month age and (f) Al+Na Cur group in two months age

MZ: Molecular zone, CP: Cortical plate zone, SP: Subplate zone, IZ: Intermediate zone, SVZ: Subventricular zone, VZ: Ventricular zone and H&E (100x)

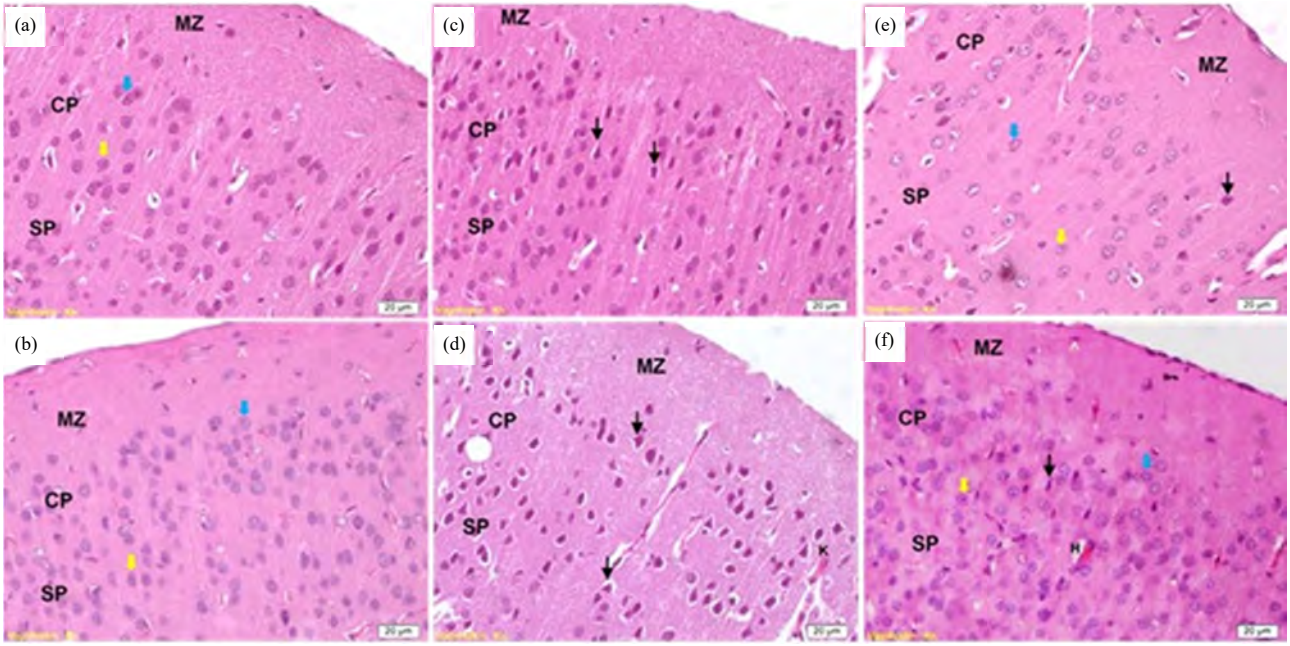

Fig. 2(a-f): Cross-section of cerebral cortex, (a) Control groups in one month age, (b) Control groups in two months' age, (c) Al group in one month age, (d) Al group in two months age, (e) Al+Na Cur group in one month age and (f) Al+Na Cur group in two months age

Blue arrows: MZ, CP and SP zones, granular neuron cells, Yellow arrows: Pyramid cells, Black arrows: Cell death, MZ: Molecular zone, CP: Cortical plate zone, SP: Subplate zone, H: Haemorrhage, K: Congestion and H&E (400x)

group at two months of age (Fig. 2d) was more prominent than the cell death of the Al group at one month of age (Fig. 2c). After administration of nanocurcumin, the morphology of the granule neurons was round and pale in colour and some pyramidal cells had a characteristic pyramidal shape with a well-defined round nucleus (Fig. 2e, f). The effect of nanocurcumin in the Al group at two months of age was more effective than the Al group at one month of age. However, the number of cell death in this zone is still relatively small.

The intermediate (IZ) zone of the cerebral cortex in the control group at one month and two months of age were characterized by many granular neuron cells and pyramidal cells. The composition of the cells was less dense, as characteristic of the IZ zone in Fig. 3a and b. However, the IZ zone neurons of the cerebral cortex in the Al group at one month of age in Fig. 3c and two months of age in Fig. 3d exhibited extensive neuronal death. The cells morphology of the Al group at one and two months of age in this layer was similar, but the cell density of the granular neuron cells in the Al group at two months of age in Fig. 3d was relatively denser than the granular neuron cells in the Al group at one month of age in Fig. 3c. The cell repair process induced by nano curcumin in the Al group at two months of age in Fig. 3f was better than the repair process in the Al group at the age of one month in Fig. 3e. Therefore, the administration of nanocurcumin could decrease neuronal and pyramidal cell death.

The SVZ zone contains many large pyramidal cells, which are cone-shaped and some granular neuron cells in Fig. 4a and b. The SVZ zone of the cerebral cortex in the Al groups at one month in Fig. 4c and two months of age in Fig. 4d showed karyolitic, which led to cell death and some pyramidal cells undergoing cell death. The pyramidal cell size in the Al group in both age groups was relatively smaller than the control group. The administration of nanocurcumin stimulated the proliferation of neuronal cells in both the Al groups at one month in Fig. 4e and two months of age in Fig. 4f. Although, some dead cells were still found the numbers were relatively small.

In the VZ zone of the cerebral cortex in mice, many cells are observed, including granular neuron cells, pyramidal cells, fusiform neuron cells and progenitor cells that actively proliferate in Fig. 5a and b. The morphology of granular

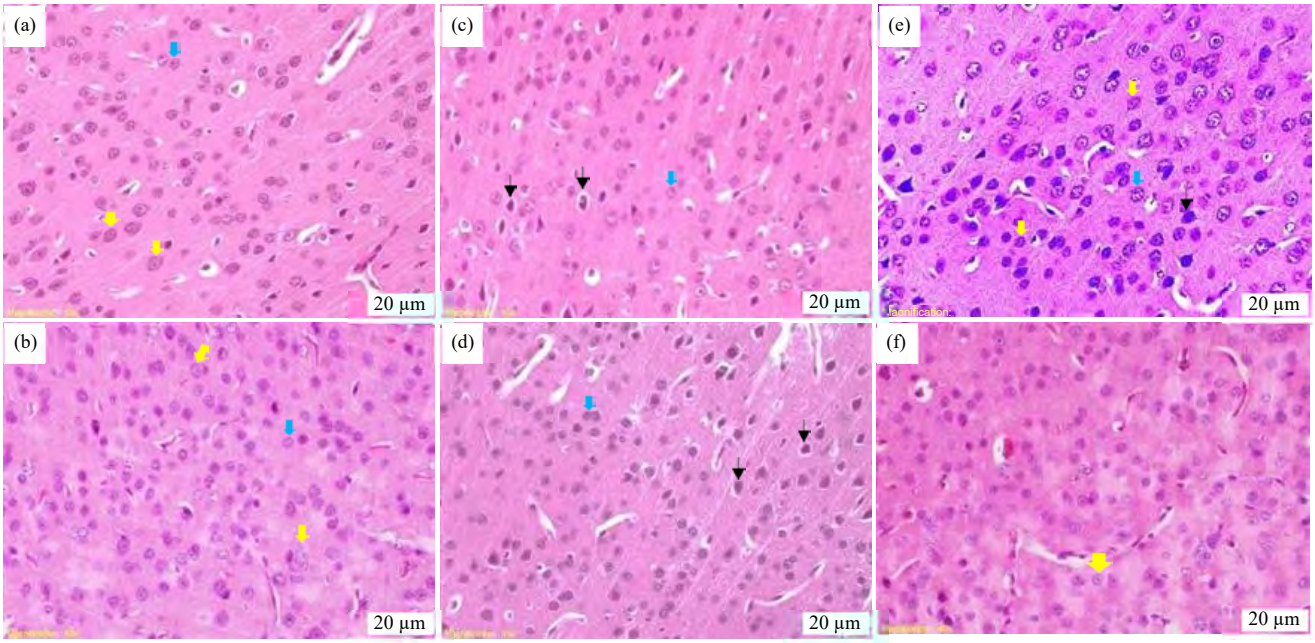

Fig. 3(a-f): Cross-section of cerebral cortex, (a) Control groups in one month age, (b) Control groups in two months' age, (c) Al group in one month age, (d) Al group in two months age, (e) Al+Na Cur group in one month age and (f) Al+Na Cur group in two months age

Blue arrows: IZ zone, granular neuron cells, Yellow arrows: Pyramid cells, Black arrows: Cell death and H&E (400x)

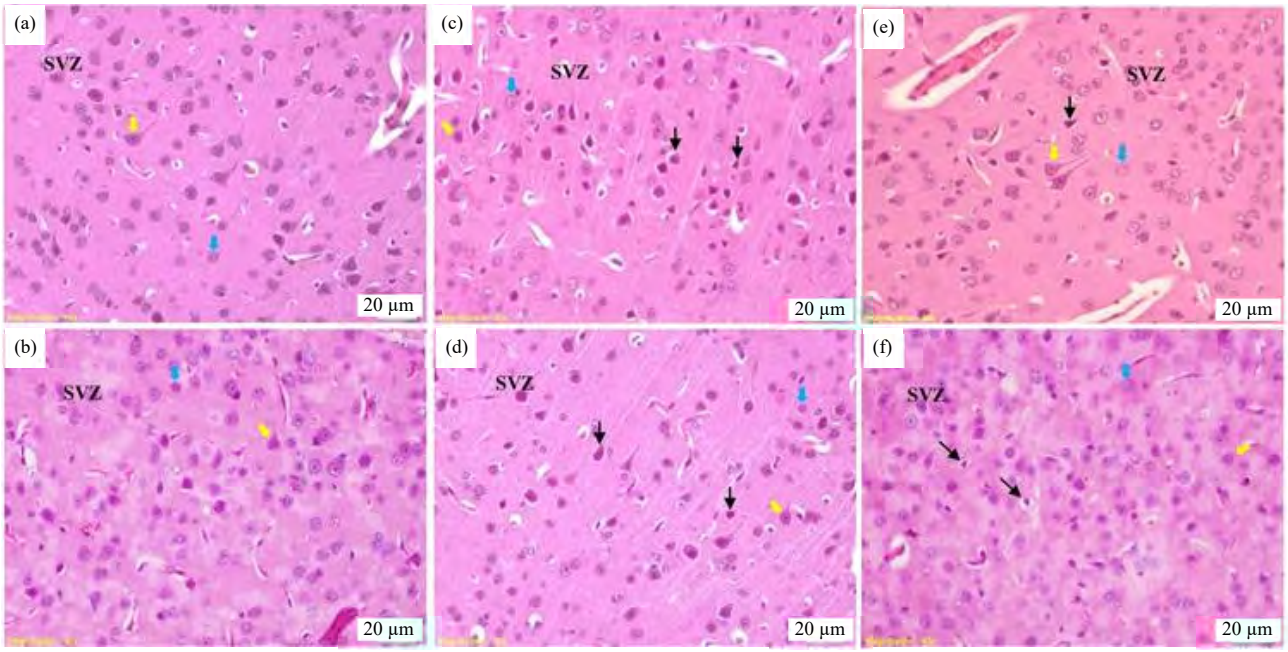

Fig. 4(a-f): Cross-section of cerebral cortex, (a) Control groups in one month age, (b) Control groups in two months age, (c) Al group in one month age, (d) Al group in two months age, (e) Al+Na Cur group in one month age and (f) Al+Na Cur group in two months age

Blue arrows: SVZ zone, granular neuron cells, Yellow arrows: Pyramid cells and Black arrows: Cell death and H&E (400x)

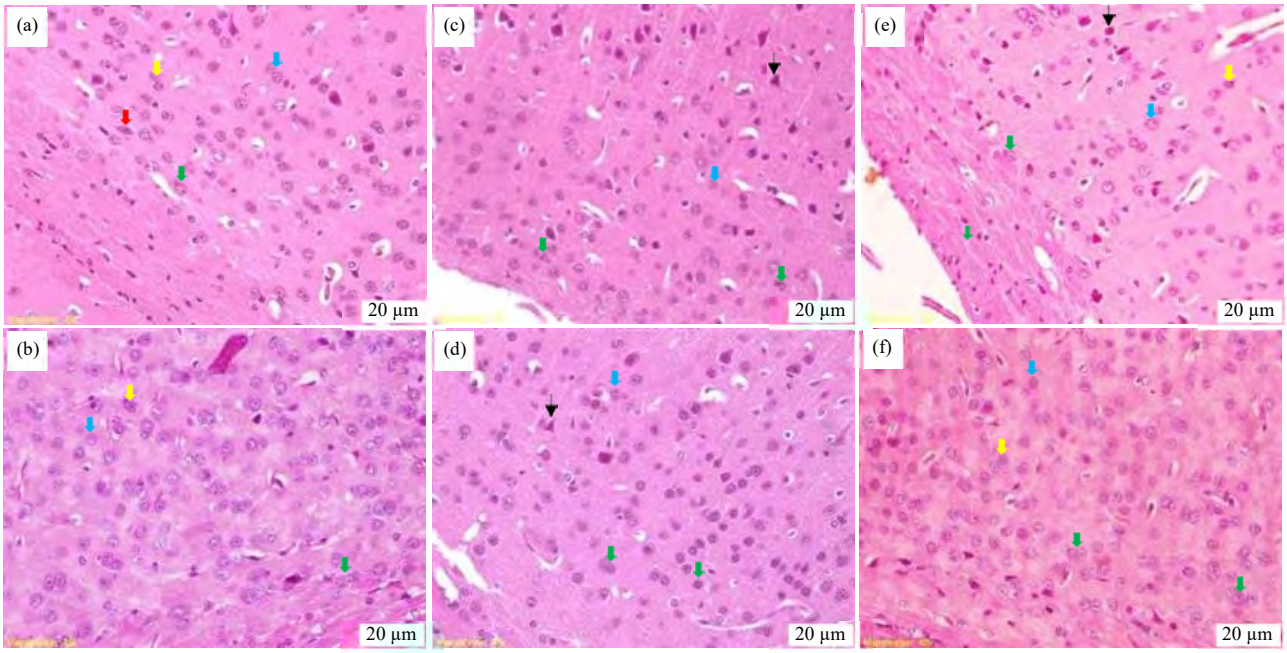

Fig. 5(a-f): Cross-section of cerebral cortex, (a) Control groups in one month age, (b) Control groups in two months age, (c) Al group in one month age, (d) Al group in two months age, (e) Al+Na Cur group in one month age and (f) Al+Na Cur group in two months age

VZ: Ventricular zone, Blue arrows: Granular neuron cell, Yellow arrows: Pyramid cells, Red arrows: Fusiform neuron cell, Black arrows: Cell death, Green arrows: Progenitor cells and H&E (400x)

neuron cells in the VZ zone is very small and the cell body is round. Epithelial cells in the walls of the VZ zone can proliferate actively. Epithelial cells will differentiate into neuroblasts and glial cells if they migrate to the layer above, the IZ zone. The characterization of the neuron cells of the IZ zone in the Al groups at one month in Fig. 5c and two months of age in Fig. 5d showed cell death. The administration of nanocurcumin decreased cell death, which was shown by the density of cells of the IZ zone at one month in Fig. 5e and two months of age in Fig. 5f. The density of granular neuron cells in the IZ zone on the Al groups at two months of age was denser than granular neuron cells in the IZ zone on the Al groups at one month of age. It can be concluded that the effect of administration nanocurcumin on the Al group at two months of age was more effective than the Al group at one month of age.

DISCUSSION

This study showed that the administration of nano curcumin caused a decrease in cell death of granular neurons that make up the cerebral cortex. Cell death is characterized by karyolitic cells and cell size shrinkage. Al-Griw *et al.*[21] stated that the administration of heavy metals to brain tissue can cause changes in the structure of neurons, decrease the number of neurons, damage the nucleus of neurons or cause karyolitic. Abd El-Rahman[22] also reported similar results, karyolitic cells, cell size shrinkage and cell death were observed in the cerebral cortex of mice treated with aluminium chloride.

At the cellular level, the CP zone is composed of granular neuron cells and the SP zone is composed of pyramidal cells and a small number of granular neuron cells. The characteristics of granular neuron cells are round and pale in colour and these cells are arranged in a regular pattern. Pyramid cells have a characteristic pyramidal shape with a well-defined spherical nucleus, a characteristic general shape and a large centrally located nucleus. Research by Yuan *et al.*[23] showed that administration of aluminium increased the concentration of ROS and lipid peroxidation in the rat brains. High ROS content causes mitochondria to release caspase signals and activate the apoptotic pathway[24]. In this study, it was suspected that Al-induced ROS production causes lipid peroxidation in cell membranes, thus leading to granular neuronal cell death.

The decrease in cell density in the CP and SP zones was caused by Al, which interferes with granular neuronal cell differentiation, impairs cell migration and increases the rate of cell death. Cellular response to Al occurs through increased production of ROS[25] which can attack proteins and lipids in cell membranes, thereby affecting the histoarchitecture of CP and SP zones[26]. It is suspected that disruption of cell membranes causes loss of integrity and stability of cell membranes and can even cause karyolysis[27]. These results are following the research of Irnidayanti and Aprilyanti[8], Al causes cell death in the cerebral cortex. This study showed cell death in the CP and SP zones at one month and two months of age caused by Al. Precursor granular cell neurons express Reelin in superficial areas to stimulate neuronal migration and CP zone formation[28]. It is suspected that the administration of Al inhibits neuronal migration in the formation of CP zones and causes granular neuronal cell death.

Aluminium is stored in the vacuoles of the cells so as not to disturb the nucleus of granular neuron cells. The accumulation of aluminium in this organelle causes vacuolization if it exceeds the carrying capacity of the organelle[29]. In addition, the induction of ROS and lipid peroxidation by Al causes a charge imbalance in the cell membrane and ultimately leads to membrane lysis and cell death. This condition caused a decrease in the density of pyramidal cells or granular neuron cells in the cerebral cortex in the Al group. This study agrees with Buraimoh *et al.*[30], who reported vacuolization, necrosis and a decrease in the number of pyramidal cells in the cerebral cortex due to the administration of aluminium chloride.

Nanocurcumin as a natural chelating agent can bind single metal ions[31]. The chemical structure of curcumin has a ketone group, which contains a lone pair of electrons. Therefore, nano curcumin can form an aluminium-curcumin complex[14]. This complex can pass through the BBB and reduce Al^{3}+ions to stabilize ROS. In addition, the decrease in ROS due to aluminium-curcumin binding triggers the production of the enzyme catalase, superoxide dismutase and glutathione to break down ROS in tissues. At the cellular level, curcumin directly increases the activation of nuclear factor 2 (Nrf2), which plays a role in regulating the expression of antioxidant proteins to protect against oxidative stress caused by toxic compounds[11,32]. Increased nuclear factor 2 (Nrf2) in cells can increase the production of peroxidase enzymes. Therefore, the number of dead cells in the nanocurcumin group was relatively less than that in the Al group. Thus, nanocurcumin has the potential as a neuroprotective agent against ROS-mediated cell death, because of Al administration. These results are in line with Kumar *et al.*[10], who showed that curcumin can reduce ROS levels and significantly reduce the amount of lipid peroxidation in heavy metal-treated rat brain[33] and reduce cell damage[13]. Nanocurcumin can attenuate the reactivity of reactive oxygen species and increase the activity of antioxidant enzymes, such as superoxide dismutase, glutathione S-transferase and glutathione. These data are supported by the report of Ibrahim *et al.*[34], who showed that curcumin can interfere with cell signalling, apoptosis and proliferation pathways can upregulate protein binding and can induce p50 degradation in the NF-B complex, thereby downregulating NF-B production. It was concluded that nanocurcumin has the potential to reduce Al toxicity.

Endothelial cells in the blood-brain barrier function as a specialized brain microvascular system that protects the brain from toxic substances. Therefore, there is a correlation between functional and molecular changes in brain microvascular endothelial cells and loss of tight junction protein integrity[35]. The Al^{3}+ions are reported to directly disrupt the blood-brain barrier by reducing phosphate levels in cell membranes. Microvascular endothelial cells membranes that should be semipermeable become permeable. It was causing an imbalance of ionic charge in blood vessels and can lead to the damaged endothelial structure at the blood-brain barrier and ruptured blood vessels[36]. It can be assumed that the haemorrhage in the Al group is caused by the rupture of blood vessels and blood cells accumulate in the cerebral cortex. This result is supported by Said and Rabo[37], who reported that aluminium can cause congestion and haemorrhage in the blood vessels of the cerebral cortex. Haemorrhage and congestion can interfere with nutrient supply and cause pyramidal cell death and a decrease in granular neuron cell density which is relatively low compared to controls. In this study, the administration of nanocurcumin can reduce the haemorrhage caused by aluminium. Yuan's research results show that curcumin can reduce blood-brain barrier disruption due to subarachnoid haemorrhage in brain rats. Curcumin maintains the integrity of the blood-brain barrier through downregulation of matrix metallopeptidase-9 and can increase membrane permeability[38].

CONCLUSION

These findings suggest that nanocurcumin is a protective agent against neuronal cell death, karyolitic and pyramidal cell vacuolation in the layers of the cerebral cortex induced by aluminium administration. The administration of nano curcumin in the Al of the group at two months of age was more effective than in the Al of the group at one month of age.

SIGNIFICANCE STATEMENT

The current study shows that nanocurcumin can be a therapeutic agent in cerebral cortex damage due to aluminium chloride. For this reason, this study recommends avoiding water provided by municipal service that mainly uses Al for purification, products related to aluminium. It is recommended to use curcumin as a protective agent, to avoid damage due to Al poisoning.

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