Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v45i1.64651

Curcumin exerts its antioxidant and neuroprotective effects against aluminum-induced oxidative stress and neurotoxicity in male albino rats

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ABSTRACT. Aluminum is a neurotoxicant and one of the most harmful metals in the environment; it is producing tissue inflammation and oxidative stress. Curcumin is an effective antioxidant and neuroprotective compound with medicinal potential. Curcumin's effect on AL toxicity was investigated in this study. Two groups of 70 mature adult albino rats were used, each of which was subdivided into five groups: Control, Vehicle, Curcumin, Aluminum, and Curcumin + Aluminum group. For two periods of 20 and 40 days, animal models were administered orally $AlCl_3$ (20 mg kg^{-1} bw) and/or Curcumin (100 mg kg^{-1} bw). In the cerebral cortex, aluminum caused a significant rise ($p < 0.05$) in lipid peroxidation and DNA fragmentation, as well as a significant decrease ($p < 0.05$) in antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. In the brain hippocampus, aluminum caused a major reduction ($p < 0.05$) in neurotransmitters (dopamine and serotonin), while Acetylcholine esterase activity increased sharply ($p < 0.05$). Aluminum also triggered histological analyses in the hippocampus of the brain. Curcumin co-administration considerably reduced the increase in lipid peroxidation and DNA fragmentation, but also enhanced the depletion of antioxidant enzymes. Curcumin also reversed the decline in neurotransmitters, the increase in Acetylcholine esterase, and the distortion in the brain hippocampus.

Keywords: heavy metals; cortex; lipid peroxidation; superoxide dismutase; hippocampus; neurotransmitters.

Received on September 21, 2022.
Accepted on January 26, 2023.

Introduction

Aluminum (Al) is a well-known harmful metal in the environment and industry, as well as the third most common element in the earth's crust, contributing to 8.13 % of the planet's surface (Exley, 2004a; Hirata-Koizumi et al., 2011; Lin, Chen, Lu, Liu, & Yang, 2015). Aluminum is found in antacids, antiperspirants, the water purification process, and as a pollutant in drinking water and cooking utensils (Exley, 2012; Kijak, Rosato, Knapczyk, & Pyza, 2014). The brain is the organ most vulnerable to aluminum's damaging effects (Chiroma et al., 2019). Aluminum can pass the blood-brain barrier (BBB) and accumulate in the brain due to its high affinity for transferrin receptors (Liaguat et al., 2019). According to subsequent research, aluminum accumulates in the brain, primarily in the frontal cortex and hippocampus (Cheng et al., 2019).

Aluminum exposure can stimulate the generation of reactive oxygen species (ROS) and impair the functioning of antioxidant defense systems, both enzymatic and non-enzymatic (Esparza, Gomez, & Domingo, 2019; Sahebkar, Panahi, Yaribeygi, & Javadi, 2018). Reduced axonal mitochondria turnover leads to the release of oxidative products such as malondialdehyde (MDA), peroxynitrites, carbonyls, and enzymes such as superoxide dismutase (SOD) inside neurons, contributing to an increase in the creation of oxygen-derived free radicals (Prakash & Kumar 2009; Sahebkar et al., 2018). Treatment with aluminum chloride causes a significant increase in oxidative stress in the brain, as seen by a decrease in catalase and GPx activity (Auti & Kulkarni, 2019; El-Gendy, 2011). Treatment with aluminum also tends to affect cytochrome oxidase activity, which enhances mitochondrial membrane permeability, DNA damage, and apoptosis, all of which promote neuronal death (Kumar, Dogra, & Prakash, 2009; Garcia et al., 2010). Aluminum neurotoxicity was caused by a mix of processes, including oxidative stress and brain injury, apoptosis induction and neuronal death, neuroglia inflammation, and impaired neurotransmitter production (Kawahara & Kato-Negishi, 2011;

Zatta, Lucchini, van Rensburg, & Taylor, 2003). According to many researchers aluminum causes neurochemical, neurophysiological, neurobehavioral, and neuropathological alterations (Kumar, Prakash, & Dogra, 2011; Walton, 2013).

Several chemical components extracted from medicinal plants have pharmacological and therapeutic effects. Polyphenolic chemicals derived from natural sources have popularity as a safe treatment for a number of oxidative stress-related disorders (Cheraghi & Roshanaei, 2019; Molino et al., 2016; Reddy et al., 2016). Curcumin is the major curcuminoid in turmeric, which is a natural polyphenolic chemical. Curcumin's phenolic groups allow it to react with reactive species, which is likely one of the processes by which it protects cells from oxidative damage.

According to several studies (Begum et al., 2008; Mythri & Bharath, 2012; Esatbeyoglu et al., 2012), curcumin is a powerful antioxidant, antiseptic, antibacterial, antimicrobial, anti-inflammatory, antidepressant, antimutagenic, and antitumor suppressor. Curcumin decreased neuro-apoptosis and inhibited ROS production by enhancing antioxidant capacity, according to a previous study (Adams et al., 2005). Curcumin has been observed to effectively stimulate the expression of antioxidant proteins such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) (Trujillo et al., 2013; Samarghandian, Azimi-Nezhad, Farkhondeh, & Samini, 2017). Due to its capacity to pass the blood-brain barrier, curcumin has a great potentiality in neurodegenerative disorders (Mythri & Bharath, 2012) and has neuroprotective properties in the hippocampus and other brain locations (Jayasena et al., 2013). Curcumin has been reported to suppress acetylcholinesterase activity, an enzyme related to neurodegenerative conditions (Akinyemi, Oboh, Ogunsuyi, Abolaji, & Udofia, 2018; Chen et al., 2018; Akinyemi et al., 2016). The proposed study was designed to evaluate the protective effect of curcumin in the management of biochemical and neurochemical alterations caused by aluminum.

Material and methods

Chemicals

$AlCl_3$ was purchased from the Acmatic Company, Egypt. Curcumin was purchased from Sigma Aldrich Co. (USA), DA and 5-HT Elisa kits were purchased from My BioSource Company, Southern California, San Diego. Glutathione peroxidase was purchased from the bio-diagnostic Company, Egypt. All other chemicals were of the highest purity commercially available.

Experimental design

Seventy adult male albino rats (weighing 140 ± 20 g) were obtained from the Serum and Vaccine Laboratory - Helwan Farm. Animals were housed in a well-ventilated clean cage maintained under a 12h:12h schedule of light: dark cycle at 25 ± 2ºC with a relative humidity of 50 ± 5% at the Department of Zoology, Aswan University, Aswan, Egypt. The animals were fed a standard diet and given water *adlibitum* and were they allowed to acclimate for two weeks before they were used. No excitement or chance of a fight was allowed for the groups under investigations. The animals were treated between 10:00 am and 12:00 pm, throughout the period of study to avoid time variations. The rats were randomly divided into five groups 14 rats each as follows: Group I (Control): received saline. Group II(Vehicle): received 30% DMSO. Group III (Curcumin): received (100 mg kg^{-1} bw) of Cur. Group IV (Aluminum): received (20 mg kg^{-1} bw) of $AlCl_3$. Group V (Aluminum + Curcumin) received 100 mg Cur per kg body weight, followed by 20 mg $AlCl_3$ kg^{-1} body weight after one hour. Curcumin was freshly dissolved in 30% DMSO before administration. Similarly, aluminum was freshly prepared and dissolved in distilled water every day before administration. The dose of aluminum and curcumin changed according to animal weight every week of the experimental period.

Collection of brain hippocampal and frontal cortex tissue

Animals were sacrificed by decapitation and specimens were taken after 20 and 40 days of treatment. Twenty-four hours after the last dose of treatment (20 and 40 days), the brain was immediately dissected on an ice-cold plate and then the cortex, hippocampus were isolated and homogenized separately with phosphate buffer saline (pH 7.4), then centrifuged at 7,000 rpm for 10 min. The supernatant was immediately separated and placed in cleaned Eppendorf tubes and kept at -80°C for further analysis, and brain tissues were kept in neutral formalin for further histological examinations.

Biochemical analysis

MDA is an indicator of lipid peroxidation and was quantitatively measured by Ohkawa, Ohishi, and Yagi (1979), in the frontal cortex regions. According to the method of Beers and sizer (1952), catalase was assayed in tissues homogenate. According to the method of Misra and Fridovich (1972), Superoxide dismutase was measured in tissues homogenate based on the inhibitory effect of SOD on epinephrine oxidation. Glutathione peroxidase was assayed in homogenate tissues according to Paglia and valentine (1967). The procedure of Kurita-Ochiai, Fukushima, and Ochiai (1999) was used to determine DNA fragmentation using a spectrophotometer set to 600 nm and a reagent blank. The following formula was used to calculate the proportion of fragmented DNA: % of fragmented DNA = (fragmented DNA/fragmented + intact DNA) X 100.

Determination of brain monoamine neurotransmitters levels

Dopamine and serotonin were determined using Elisa kits purchased from My BioSource Company. The method produced by the kit was used to measure DA and 5-HT.

Acetylcholinesterase (AChE) activity estimation

In the brain tissue homogenate, according to Ellman, Courtney, Andres, and Featherstone (1961). AChE content was measured. The change in absorbance was measured at 412 nm for 2 min. following the increase in yellow color produced from thiocholine reacting with dithiobisnitro benzoate ions (DTNB reagent).

Histological preparations

The conventional technique of paraffin embedding was used to process of the fixed brain. Sections of 5 µm thick were obtained from the prepared paraffin blocks using a microtome. Then, these sections were stained with hematoxylin and eosin (H&E) (Gabe, 1976). Microscopic fields of examined sections were randomly selected and magnified using high-power light microscope (Olympus BX43F Tokyo163-0914 Japan). Image analysis was done using a personal computer, camera software (Olympus DP74 Tokyo 163-0914 Japan), and an optical microscope.

Statistical analysis

Results of all quantitative data from the biochemical assays were expressed as means ± SEM. Differences between means were tested by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls T-test using Minitab 19 software so that the data obtained can be compared and statistically evaluated.

Results

Lipid peroxidation level in the brain cortex

The cortical tissue homogenates of the AL- treated group exhibited a higher level of MDA than those of the control group, as indicated by mean ± SE values of (33.8 ± 0.3 nmol g^{-1} tissue and 38.267 ± 0.93 nmol g^{-1} tissue) after 20 and 40 days of administration respectively. Additionally, the AL+Cur group exhibited a significantly ($p < 0.05$) lower mean ± SE value (22.25 ± 2.09 nmol g^{-1} tissue and 17.467 ± 0.422 nmol g^{-1} tissue) for MDA after 20 and 40 days, respectively than those of the treated groups (Figure 1).

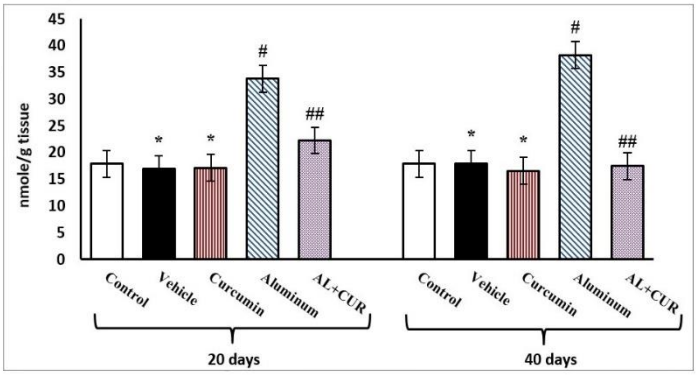

Figure 1. levels of MDA in the brain cortex of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

The antioxidant enzymes activity index in the brain cortex

The tissue homogenates of the brain cortex of the AL-group showed a significant decrease ($p < 0.05$) in antioxidant enzymes activity (CAT, SOD and GPx) than those of the control group as measured by mean± SE values of (2.47 ± 0.172, 7.85 ± 0.654, and 15.73 ± 0.663 U min.$^{-1}$ g^{-1} tissue, respectively) and (1.70 ± 0.066, 6.5 ± 0.564, and 12.3 ± 1.03 U min.$^{-1}$ g^{-1} tissue, respectively) after 20 and 40 days of administration respectively (Figures 2, 3, and 4). Additionally, the AL+ Cur group exhibited a significantly ($p < 0.05$) increased mean ±SE value for the antioxidant enzymes (CAT, SOD and GPx) with values of (5.012 ± 0.0816, 12.767 ± 0.263, and 25.10 ± 0.712 U min.$^{-1}$ g^{-1} tissue, respectively) and (6.09 ± 0.124, 13.75 ± 0.311, and 27.94 ± 0.64 U min.$^{-1}$ g^{-1} tissue, respectively) after 20 and 40 days respectively compared to those of the treated group (Figures 2, 3 and 4).

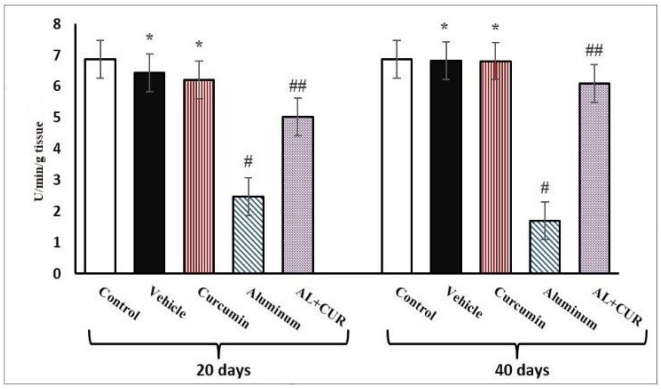

Figure 2. Activity of CAT in the brain cortex of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

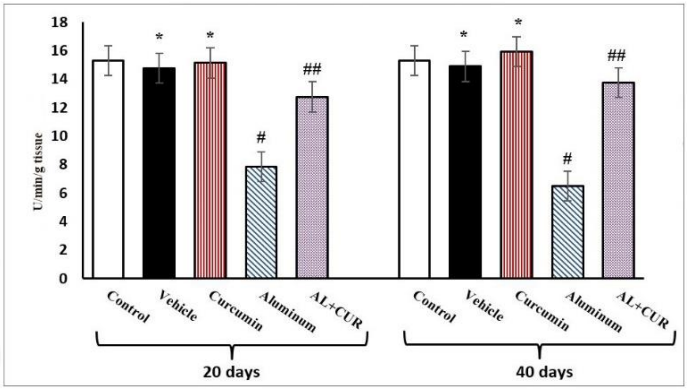

Figure 3. SOD activity in the brain cortex of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

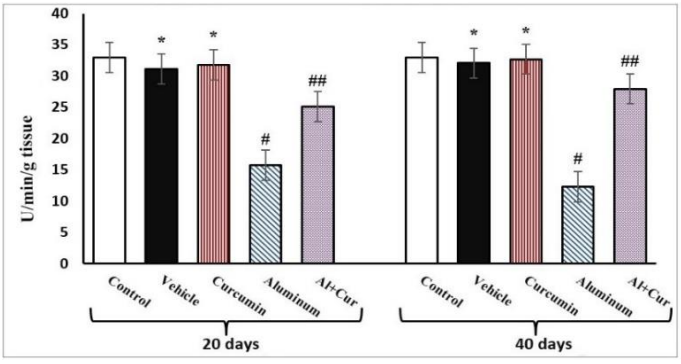

Figure 4. Activity of GPx in the brain cortex of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

DNA fragmentation percentage in the brain cortex

The tissue homogenates from cortex of the AL- treated group revealed high significant ($p < 0.05$) increase in the fragmented DNA percentage compared to control group by mean± SE values of (47.24 ± 2.15 and 64.44 ± 0.807%) after 20 and 40 days of administration, respectively. On the other hand, the AL+Cur treated group exhibited a significantly ($p < 0.05$) depletion of mean ±SE value for the fragmented DNA as indicated by (23.099 ± 3.27 and 19.33 ± 3.1%) after 20 and 40 days of administration, respectively compared to those of the treated group (Figure 5).

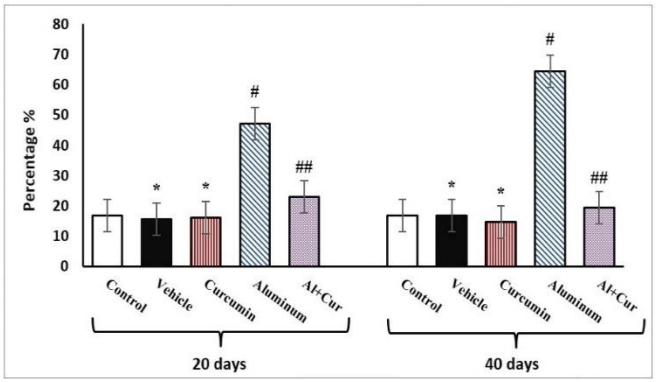

Figure 5. Percentage of DNA fragmentation in the brain cortex of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

Determination of brain monoamines (DA and 5-HT) in the brain hippocampus

The tissue homogenates of brain hippocampus of the AL-treated group showed a high significant ($p < 0.05$) decrease in the concentration of brain neurotransmitters (DA and 5-HT) compared to the control group by mean± SE values of (2.079 ± 0.132 and 0.2626 ± 0.0158 ng mg^{-1} tissue, respectively) and (1.832 ± 0.104 and 0.1922 ± 0.0171 ng mg^{-1} tissue, respectively) after 20 and 40 days of administration, respectively (Figures 6 and 7). Co-treatment of curcumin exhibited a significant ($p < 0.05$) increase of mean ±SE value by (2.932 ± 0.258 and 0.542 ± 0.0664 ng mg^{-1} tissue, respectively) and (3.189 ± 0.289 and 0.637 ± 0.104 ng^{-1} mg tissue, respectively) after 20 and 40 days of administration, respectively (Figures 6 and 7).

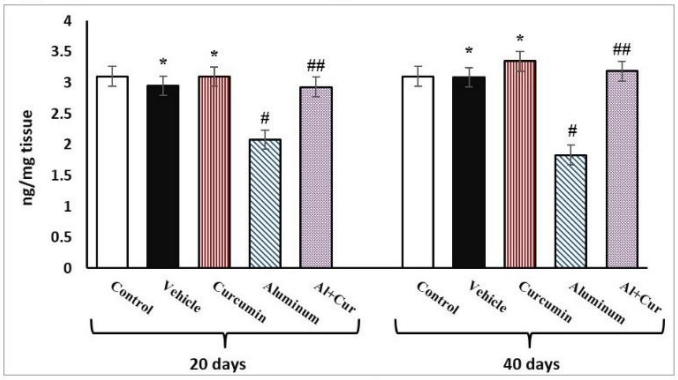

Figure 6. Concentration of dopamine in the hippocampus of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly Significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

Determination of AchE activity in the brain hippocampus

The tissue homogenates of brain hippocampus of the AL-treated group exhibited a significant ($p < 0.05$) increase in the activity of AchE as measured by mean± SE values of (1.2382 ± 0.0187 nmol mg^{-1} tissue and 1.5144 ± 0.0487 nmol mg^{-1} tissue) after 20 and 40 days of administration, when compared with the control group (Figure 8). In addition, AL+Cur treated group exhibited a significant ($p < 0.05$) decrease in the mean±SE value by (0.9018 ± 0.0099 nmol mg^{-1} tissue and 0.763 ± 0.0224 nmol mg^{-1} tissue) after 20 and 40 days of administration, respectively compared to treated group (Figure 8).

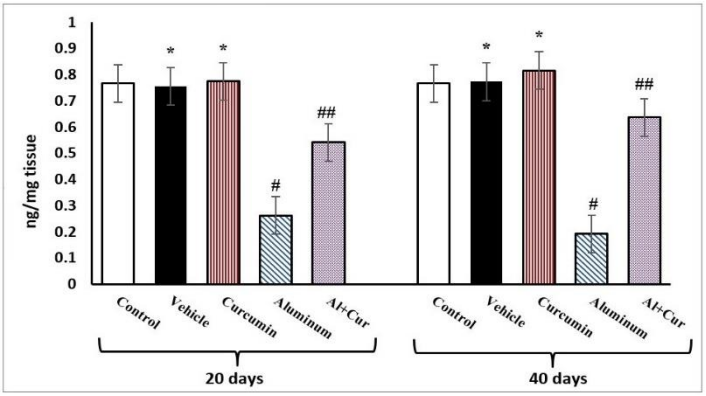

Figure 7. Concentration of serotonin in the hippocampus of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

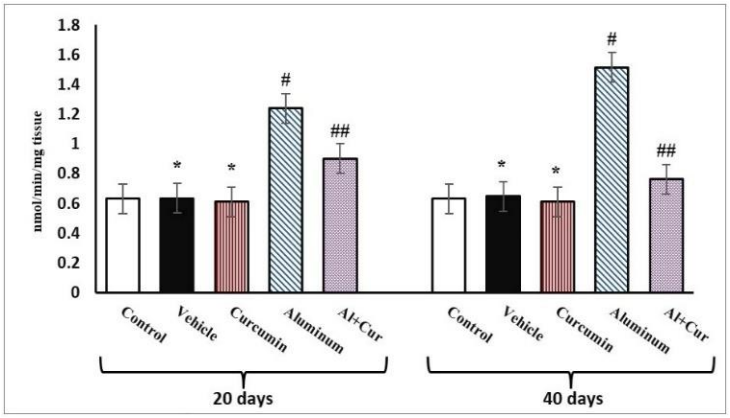

Figure 8. Activity of AchE in the hippocampus of treated rat groups (20 and 40 days). Values are means ± SE. of 6 animals in each group. *Non-significant compared with the control group ($p > 0.05$). #Highly significant compared with the control group ($p < 0.01$). ##Highly significant compared with aluminum group ($p < 0.01$).

Histopathological findings of the hippocampus among the various study groups

Microscopic examinations of sections in the hippocampus of control group (Figure 9a and b) showed different areas of the hippocampal structure where the hippocampus proper is formed of the Cornu Ammonis (CA) as CA1, CA2, CA3, and CA4 regions. Each of these regions consisted of five layers: stratum alveolus, the stratum oriens, stratum pyramidale, stratum radiatum, and stratum lacunosum-moleculare. The dentate gyrus is seen surrounding CA4 by its upper and lower limbs(Figure 9a).The stratum pyramidale of the CA1 region consisted of 4–5 compact layers of small pyramidal cells, most with vesicular nuclei and visible nucleoli. The neuroglia and the blood capillaries were observed in normal structure in both the stratum oriens and the stratum radiatumlayers (Figure 9b). Also, microscopic investigations of the hippocampal sections of DMSO-treated groups and curcumin-treated groups at 20 and 40 days (Figure 9c, d, e, and f respectively) showed a normal histological structure that was similar to that of the control group (Figure 9a and b). Administration of Aluminum induced several alterations in the hippocampus at 20 and 40 days (Figure 9g and h respectively). These alterations included karyomegaly of some neurons and atrophied, shrunken neuronal cells and proliferation of the glial cells (Figure 9g). Also, decreased thickness of the pyramidal cell layers and areas with neuronal loss were observed (Figure 9h). Interestingly, hippocampus of AL+Cur -treated groups at 20 and 40 days (Figure 9i and k respectively) retained its normal structure and appeared similar to that of the control group to a large extent.

Discussion

Environmental heavy metal exposure has been linked to the development of neurodegenerative diseases (Tan, Yu, & Tan, 2014; Oyetayo, Abolaji, Fasae, & Aderibigbe, 2020). Aluminum accumulates in the hippocampus at a higher rate than in other brain areas, influencing biochemical processes linked to neurodevelopment (Baydar et al., 2003; Liang et al., 2012). Oxidative brain injury, induction of apoptosis and

neuronal death, neuropathic inflammatory reaction, and decreased neurotransmitter biosynthesis are all biochemical and neurochemical problems that $AlCl_3$ can cause (Singla & Dhawan, 2013; Kim et al., 2008).

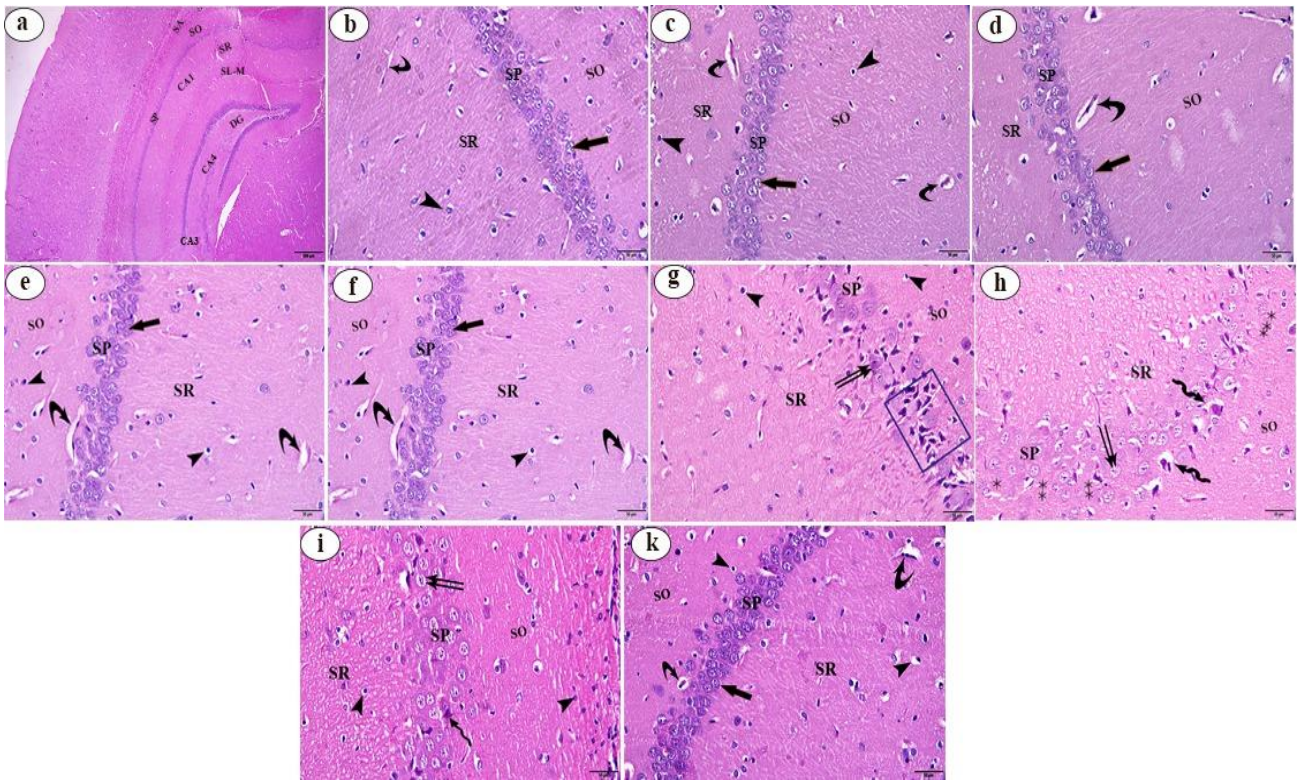

Figure 9. Photomicrographs hippocampal sections (a) to (K) stained with H&E stain. Hippocampus of control group (a & b). Section of hippocampus of DMSO- treated groups (c & d). Hippocampus sections of curcumin treated groups (e & f). Hippocampus sections of Aluminum treated groups (g & h). Sections of hippocampus of Aluminum+ Curcumin treated groups (i &k), all the previous sections in (20 days and 40 days, respectively). Cornu Ammonis regions (CA1, CA2, CA3, & CA4), Dentate gyrus (DG), Stratum alveolus (SA), Stratum oriens (SO), Stratum pyramidale (SP), Stratum radiatum (SR), Stratum lacunosum-moleculare (SL-M). Pyramidal cells of CA1 region(thick arrows), neuroglia (arrow head), blood capillaries ( ), Karyomegaly of some neurons (double arrows),Atrophied and shrunken cells (square), areas with neuronal loss (stars). (Original magnification,"a": X100 = 500 µm; "b-k": X400 = 50 µm).

Lipid oxidation products are one of the most common side effects of oxidative stress. The brain is the most vulnerable to oxidative stress due to its high lipid content and rapid oxygen consumption, (Kumar & Gill, 2014). AL caused an increase in lipid peroxidation levels in the current investigation. Hassan, Serage, and Gad (2015) published similar findings, suggesting that aluminum exposure causes an increase in lipid peroxidation. Although AL cannot modify its redox state, it exacerbates free radical damage and lipid peroxidation caused by iron, according to Said and Abd Rabo (2017). This is due to the fact that AL like iron, interacts with the iron regulatory protein (IRP), which regulates iron-binding protein expression. Auti and Kulkarni (2019) noted that lipid peroxidation is one of the main biomarkers of oxidative stress, which is consistent with the findings of this study. According to the present data, curcumin pre-treatment with aluminum resulted in a considerable reduction in LPO levels in the cerebral cortex. These findings were consistent with those of Kar, Hacioglu, Uslu, and Kanbak (2019), who found that combining curcumin with aluminum therapy reduced lipid peroxidation in curcumin-treated rats. These findings are in line with those of Sethi, Jyoti, Hussain, and Sharma (2009), who found that curcumin can control lipid peroxidation in chronic aluminum-intoxicated rats over time.

Antioxidant enzymes are thought to be the first line of defense for biological macromolecules against oxidative damage (Harsha & Anilakumar, 2013). SOD, CAT, and GPx levels were found to be lower after aluminum administration in this investigation. These findings are consistent with those of Laabbar, Elgot, Elhiba, and Gamrani, (2019); Liaquat et al. (2018), who found a significant decrease in antioxidant enzyme activity such as superoxide dismutase, catalase, and glutathione peroxidase. These enzymes are the body's first line of defense against oxygen free radicals, and a drop in their levels could indicate the onset of oxidative stress in the brain (Exley, 2004b). In the brain, Kakkar and Kaur (2011) found that superoxide dismutase (Kawahara et al., 2003) and catalase activities are inhibited, resulting in increased lipid peroxidation. The

absence of the endogenous antioxidant system causes a significant rise in phospholipid peroxidation in brain cells, resulting in membrane damage and neuronal death. Similarly, Azib et al. (2019) found that oral administration of $AlCl_3$ (100 ppm) for two months generated oxidative stress in mouse brains, as evidenced by a significant decrease in total thiol concentration as well as superoxide dismutase and catalase activity, as compared to the control group.

The present data discussed that curcumin reduces lipid peroxidation by keeping antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase active at higher levels. Curcumin appears to alleviate stress-induced essential organ disruption by regulating SOD activity and reversing the inhibitory effects of stress on CAT, resulting in normal GPx function, according to Samarghandian et al. (2017). These effects could be attributed to a reduction in lipid peroxidation, which would reduce the negative effects of chronic stress on tissue damage. The results are in accordance with Laabbar et al. (2021), who reported that curcumin reduced aluminum induced oxidative stress.

A surprising observation in our investigation was the finding that aluminum intoxication generated substantial DNA damage, as shown by increased DNA fragmentation in aluminum intoxicated rats. Kar et al. (2019) reported an increase in cytochrome C release caspase 3 activation and DNA fragmentation in $AlCl_3$-treated synaptosomes. Our findings are consistent with those of Liaquat et al. (2018), who found that exposure to Al resulted in DNA breakdown as evidenced by significant DNA fragmentation when compared to control rats. Furthermore, these findings are consistent with those of Kumar et al. (2009), who found that free radicals produced in response to AL exposure target DNA and cause oxidative damage. According to our findings, curcumin has a protective impact by lowering the increase in DNA fragmentation caused by aluminum treatment. These findings are consistent with those of Kar et al. (2019), who said that curcumin administration resulted in a significant reduction in apoptotic, genotoxic, and oxidative stress-related indicators (Kumar, Sasmal, & Sharma, 2018; Nariya et al., 2018). According to Ahmed, Goel, and Banerjee (2018), curcumin may also protect DNA from damage by decreasing oxidative stress and apoptosis.

Biogenic amines have a vital role in the biological system. They regulate a variety of physiological and pharmacological processes. The goal of this study was to see if chronic aluminum exposure causes alterations in the dopaminergic and serotoninergic systems, and if these changes were time (20 and 40 days) dependent. According to our data curcumin's neuroprotective impact on aluminum induced neurotoxicity in male albino rats was also investigated. Chronic injection of AL resulted in large decreases in dopamine and serotonin concentrations as well as a considerable rise in AchE activity in the brain. According to Khalil and Hussein (2015), there was a significant depletion in the brain monoaminergic system (5-HT, DA, and NE) levels, which were adversely linked with brain aluminum levels, which could explain the current findings.

According to our findings, curcumin pre-treatment with aluminum enhanced dopamine and serotonin levels in the cerebral hippocampus. These findings correspond with those of Mohamed, Khadrawy, El-Sherbini, and Amer (2019), who found that daily treatment of a rat model of depression with curcumin nano particles (CNPs) for 7 days restored cortical and hippocampal serotonin and DA levels to control-like levels, but failed to restore NE levels, despite a significant increase in comparison to reserpinized rats. The findings of this study are consistent with those of Abbaoui and Gamrani (2018), who found that curcumin had promising effects in preserving the DAergic system and astrocytes in damaged areas following subchronic Cu intoxication. According to Chang, Wang, Li, and Wu (2016), curcumin has also been proven to promote monoamine neurotransmitter levels by suppressing MAO activity. As a result, the restored monoamines generated by Cur-IONPs could be related to curcumin's inhibitory effect on MAO, which could prevent monoamine neurotransmitter catabolism and oxidative stress. These findings show that when depressed rats were treated with Cur-IONPs, their monoamine levels improved.

The findings of this study reveal that following aluminum exposure, AchE synthesis may be increased. However, co-administration of curcumin with aluminum restored AchE activity levels to normal levels. Auti and Kulkarni (2019) provided supporting evidence by observing that aluminum's neurotoxic effect increases AchE activity, which is responsible for acetylcholine hydrolysis. Similarly, Liaquat et al. (2018) discovered that Al is a potent AchE activator. Furthermore, Said and Abd Rabo (2017) discovered that sub-chronic aluminum injection dramatically increased brain AchE activity, which is consistent with the current findings. Aluminum chloride causes a considerable increase in AchE activity in brain tissue, according to Harsha and Anilakumar (2013). Curcumin has the ability to reverse the increase in AchE activity caused by aluminum, according to the findings of this study. Curcumin decreased AchE activity considerably, according to Oyetayo

et al. (2020). Several studies found that curcumin's inhibitory influence on AchE activity may enhance acetylcholine levels in synaptic clefts, resulting in efficient cholinergic system functioning in flies (Abolaji, Fasae, Iwezor, Aschner, & Farombi, 2020; Akinyemi et al., 2018; Shen & Ji, 2012).

The brains of experimental rats given aluminum showed extensive histological modifications in most sections of the CA1 subfield of the hippocampus. These modifications included the stratum pyramidale, stratum oriens, and stratum radiatum layers. These modifications may be due to pyramidal cell disarray karyomegaly in certain neurons, and atrophied and undersized neurons due to glial cell proliferation. These findings are consistent with those of Lahouel, Kharoubi, Boussadia, Bekkouche, and Aoues (2020), who observed pyknosis of pyramidal cells, a decrease in the number of cellular units, and pyramidal cell necrosis in the CA1 area of the hippocampus in the Al-treated group. In addition, Khalil, Salama, Al-Mokaddem, Aljuaydi, and Edris (2020) found that in the aluminum-treated group, the hippocampus had deteriorated neurons with the presence of microglia and decreased cellular density in some locations.

According to the current findings, curcumin's neuroprotective effects against brain disorders could be related to a variety of pathways. Curcumin has been shown to promote biological activity, perhaps enhancing developmental and hippocampus neurogenesis and neural repair, according to the researchers (Xu et al., 2007; Dong et al., 2012). Curcumin's ability to improve the oxidant/antioxidant status of brain tissues is due to a direct reduction of ROS formation and release, as well as scavenging of free radicals and subsequent suppression of oxygenation processes (Edrees, Galal, Abdel Monaem, Beheiry, & Metwally, 2018). Curcumin has also been shown in numerous studies to prevent neuronal damage-induced glial activation (Tripanichkul & Jaroensuppaperch, 2012; Yang et al., 2008).

Conclusion

Our findings shed light on the processes by which chronic aluminum exposure can cause neurotoxicity, as well as the protective impact of curcumin when given before aluminum. The current study demonstrated that aluminum causes considerable toxicity in the form of increased lipid peroxidation, large reductions in antioxidant enzyme activity, and significant increases in DNA fragmentation. Regressive changes in neurotransmitter concentrations demonstrated these effects. Curcumin has an antioxidant function, lowering LPO, DNA fragmentation, and AchE levels while increasing antioxidant enzyme activity and neurotransmitter levels. Curcumin's neuroprotective efficacy against neurotoxicity caused by long-term aluminum exposure has led us to believe that it is a natural medicine that protects the brain from neurotoxicity caused by heavy metals.

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