EFFECT OF CURCUMIN, EXELON AND THEIR COMBINATION ON HISTOPATHOLOGY OF BRAIN IN ALZHEIMER’S DISEASE -INDUCED RATS

Mohyel din Abdel Fattah, WailedFathy Khalil and Shahinda Mahmoud Mohamed Habib

1Department of Chemistry, Faculty of Science, Suez Canal University, Egypt
2Department of Pharmacology, Faculty of Veterinary, Suez Canal University, Egypt

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Abstract

Because of the continues rising in the number of patients who have Alzheimer’s disease (AD) throughout the twenty-first century, the looking for remedies increase by scientists. The treatment of AD remains a challenge due to an incomplete understanding of reasons that lead to the selective neurodegeneration typical of Alzheimer’s brains. Among treatment for AD, the renewed interest in curcumin is rise for its potential medication. As kind of natural product curcumin with anti-AD properties is very important for prevention and treatment. The aim of the present study was evaluated the activity of curcumin in the recession of AD induced in adult male albino rats. The results showed that treatment of AD groups with curcumin or rivastigmine experienced significant decreased in brain AChE, Aβ (1-42), and MAD levels with respect to untreated group associated with significant increase in brain GSH, SOD, and CAT activity. Further showed combination of curcumin with rivastigmine was more efficacious in treatment of AD as compared to their effect alone.

Keywords: Alzheimer’s disease, Oxidative stress, Curcumin, Aluminum chloride

INTRODUCTION

The aging is the most important demographic trends facing the world. By increasing life expectancy, the high prevalence of chronic disabilities represents one of the major causes of upward burden on the economy of Health Services, requiring clinical management on long-term of the affected subjects. Cognitive impairment leading to dementia remains the most invalidating one, due to deficiency of effective treatments and its hard effect on both healthcare workers and families. The main cause of dementia is Alzheimer's disease. People’s number with dementia will increase and triple by 2050 respect to current estimation predict (Alzheimer’s Association, 2016). For this reason, AD is a growing socio-economic problem worldwide and many researchers are focusing their efforts to get a treatment. AD is a neurodegenerative disease which clinically characterized by progressive loss of memory and selective neuronal damage in cerebral cortex and hippocampus in AD brain. There are lots of reasons for oxidative damage and the formation of free radicals such as exposure to chemicals, metals, irradiation and toxins causing to lipid peroxidation, which in turn affects the activities of protective enzymatic antioxidants that are greatly sensitive indicators of increased oxidation reactions (Bukowska, 2006). When free radicals attacked lipids, the chain reaction of lipid peroxidation proceed, and lead to broken chemical bonds, cross- linkages, and many bio molecular compounds have conformational changes. The main pathological hallmarks of AD are the deposition of extracellular Aβ plaques, the formation of intracellular neurofibrillary tangles (NFTs), and the selective loss of synapses and neuron, which lead to neural death in the hippocampus and cerebral cortex (Kawahara and Kato-Negishi, 2011). The neurotoxicity of aluminum is associated with various neurodegenerative diseases such as AD and Parkinsonism Disease.

The neurotoxicity of aluminum may affect through free radical production and peroxidation damage to lipids and proteins (Sethi et al., 2008). Chronic exposure of aluminum has ability to promote formation and aggregation of insoluble Aβ plaques and (NFTs) in Alzheimer brain, and associated with impairment of mitochondrial functions, in vivo and in vitro, as well as the antioxidant enzyme status decreases (Kumar et al., 2009; Lee and Wei, 2007). Also, the activity of enzyme engaged in metabolism of acetylcholine, aluminum can cause disturbance in it leading to cognitive impairment (Moshtaghie et al., 2007). Curcumin is a natural product derived from Curcuma longa (more commonly known as turmeric) (Ringman et al., 2005). Curcumin has anti-inflammation and antioxidant activities, so it can decrease inflammation, amyloid accumulation and oxidative stress which has ability to scavenge free radicals (Lee et al., 2013). Also, it has protective potent from lipid peroxidation, and scavenges nitric oxide (NO)-based radicals. Curcumin has ability to inhibit formation of Aβ plaques and lower soluble Aβ levels due to its metal chelation properties as it binds to redox-active metal ions such as iron and copper. These complexes may cause a net protective effect through decreased Aβ aggregation (Baum and Ng, 2004). Curcumin is safe product which large quantities can consumed without toxicity. These especial properties make curcumin valuable for drug development, and remain focus for several clinical trials (Goel et al., 2008).

MATERIALS AND METHODS

Adult albino rats weighing (220 g±10g), aged (16-18 weeks) used in this study supplied by theinimal house of faculty of veterinary medicine Zagazig University, Egypt. The rats were placed in special cages and classified to seven animals per cage (18 weeks). When free radicals attacked lipids, the chain reaction of lipid peroxidation proceed, and lead to broken chemical bonds, cross- linkages, and many bio molecular compounds have conformational changes. The main pathological hallmarks of AD are the deposition of extracellular Aβ plaques, the formation of intracellular neurofibrillary tangles (NFTs), and the selective loss of synapses and neuron, which lead to neural death in the hippocampus and cerebral cortex (Kawahara and Kato-Negishi, 2011). The neurotoxicity of aluminum is associated with various neurodegenerative diseases such as AD and Parkinsonism Disease.

*Corresponding Author: Mohyel din Abdel Fattah,
Department of Chemistry, Faculty of Science, Suez Canal University, Egypt.
standard laboratory diet and tap water. Sixty-three animals were classified into 9 main groups (7 rats for each group). Group 1: Normal healthy rats (receive vehicle for AlCl₃·6H₂O) served as Negative control group. Group 2: served as Positive control group (receive AlCl₃·6H₂O in dose 50 mg/kg b.w) orally daily for one month. Group 3: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of curcumin in dose (200 mg/kg b.w. O.P) every day for two months. Group 4: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of curcumin in dose (300 mg/kg b.w. O.P) every day for two months. Group 5: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of curcumin in dose (400 mg/kg b.w. O.P) every day for two months. Group 6: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of Rivastigmine (Exelon) in dose (0.3 mg/kg b.w. O.P) every day for two months. Group 7: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of Curcumin in dose (15 mg/kg b.w. i.P) every day for ten days. Group 8: receive AlCl₃·6H₂O in dose 50 mg/kg p.w orally daily for one month then treated by simultaneous administration of Curcumin in dose (300 mg/kg b.w. O.P) +Exelon in dose (0.3 mg/kg b.w. O.p) every day for two months. Group 9: (receive AlCl₃·6H₂O in dose 50 mg/kg b.w) orally +after one hour receive curcumin in dose (300 mg/kg b.w. O.p) every day for one month.

Tissue specimen collected from the brain of tested rats were fixed in formalin buffer (15%), and then the samples were routinely processed through: dehydrated in ascending conc of ethanol, cleared in xylene and fixed firmly in paraffin at 60°C in hot air oven for 24 hours. Paraffin bees wax tissue block were prepared for sectioning at 4µm thickness by sledge microtome. On glass slides the obtained tissue sections were collected, deparaffinized, stained by hematoxylin & eosin stain then examination was done through the light electric microscope.

Statistical analysis

In the present study, results were analyzed for 7 rats in each group using SPSS (ver. 25.0; IBM, Chicago, IL, USA). Quantitative data was displayed in the form of mean ± standard deviation (SD). Charts of different types were used to illustrate data and relations where appropriate. A probability value (P value) less than 0.05 was considered significant.

RESULTS

A cross-section taken from brain of normal control rats Figure 1 showing Dentageyirus contains three layers from the outer in: the molecular layer, the inner molecular layer and the granular layer. At aeraCA3 showing the following cell layer known as strata: lacunosum-molecular, radiatum, lucidum, pyramidal and oriens. CA2 and CA1 also have these layers except the lucidumstratum. Also CA4 known Hilus which considered part of the dentate gyrus contains mossy cells.

Figure 1. Photomicrograph section taken from Hippocampus of group 1(normal control group) showing the dentate gyrus contains three layers from the outer in: the molecular layer, the inner molecular layer and the granular layer. The following cell layers were shown in CA3 and known as strata: lacunosum-molecular, radiatum, lucidum, pyramidal and oriens. CA2 and CA1 also have these layers except the lucidumstratum. Also CA4 known Hilus which considered part of the dentate gyrus contains mossy cells.
CA4 also called Hilus which considered part of the dentate gyrus contains mossy cells. As shown in Figure 2, brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of (AD) model rats in group 2 (Rats inducted with 50 mg Al3Cl.6H2O) showing neuronal vacuolar degeneration, increase number of glial cells, neurofibrillary degeneration, separation and edema of vessels, congested capillaries, disorganization and areas of cell loss of contracted pyramidal cells of CA3 region, and many degenerated granule cells in the dentate gyrus with vacuolated cytoplasm, hemorrhage and pyknotic nucleus.

Figure 2. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of AD rats of group 2 (Rats inducted with 50 mg Al3Cl.6H2O) showing neuronal vacuolar degeneration, increase number of glial cells, neurofibrillary degeneration, separation and edema of vessels, congested capillaries, disorganization and areas of cell loss of contracted pyramidal cells of CA3 region, and many degenerated granule cells in the dentate gyrus with vacuolated cytoplasm, hemorrhage and pyknotic nucleus.

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Brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 3 (AD-induced rats treated with Cur.200 mg) Figure 3, showing decreased number of glial cells, moderate vacuolation of neurons, preservation of some pyramidal cells of CA1 region, few cells with pyknotic nuclei, few congested capillaries, preservation of some pyramidal cells of CA3 region, and preservation of granule cells of the dentate gyrus, and normal intact cell layer with mature cells appear big and rounded. Brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 4 (AD-induced rats treated with Cur.300 mg), showing mild vacuolation of neurons, mild number of glial cells, preservation of large pyramidal cells of CA1 region, few cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, and preservation of most of granule cells of the dentate gyrus, and normal intact cell layer with mature cells appear big and rounded. Brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 5 (AD-induced rats treated with Cur. 400 mg), showing mild neuronal degeneration, few glial cells, preservation of most of pyramidal cells of CA1 region, few cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, and preservation of the granule cells of dentate gyrus, and normal intact cell layer with mature cells appear big and rounded. Brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 6 (AD-induced rats treated with Exelon) Figure 6, showing few glial cells, increase number of mature cells, minimal neuronal degeneration, preservation of large pyramidal cells of CA1 region, few cells with pyknotic nuclei, some congested capillaries, preservation of large pyramidal cells of CA3 region, preservation of most of granule cells of the dentate gyrus. In Figure 7, brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 7 (AD-induced rats treated with Cur.15 mg i.p), showing mild vacuolation of neurons, some glial cells,
Figure 3. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 3 (AD-induced rats treated with Cur. 200 mg) showing moderate vacuolation of neurons, preservation of some pyramidal cells of CA1 region, mild number of glial cells, few cells with pyknotic nuclei, few congested capillaries, preservation of some pyramidal cells of CA3 region, and preservation of granule cells of the dentate gyrus.

Figure 4. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 4 (AD-induced rats treated with Cur. 300 mg) showing mild vacuolation of neurons, mild number of glial cells, preservation of large pyramidal cells of CA1 region, few cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, and preservation of most of granule cells of the dentate gyrus.
Figure 5. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 5 (AD-induced rats treated with Cur.400 mg) showing minimal neuronal degeneration, few glial cells, preservation of most of pyramidal cells of CA1 region, few cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, preservation of the granule cells of dentate gyrus, and normal intact cell layer with mature cells appear big and rounded.

Figure 6. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 6 (AD-induced rats treated with Exelon) showing mild neuronal degeneration, few glial cells, preservation of large pyramidal cells of CA1 region, few cells with pyknotic nuclei, some congested capillaries, preservation of large pyramidal cells of CA3 region, preservation of most of granule cells of the dentate gyrus, and normal intact cell layer with mature cells appear big and rounded.
Figure 7. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 7 (AD-induced rats treated with Cur. 15 mg i.p) showing moderate vacuolation of neurons, preservation of some of pyramidal cells of CA1 region, some cells with pyknotic nuclei, some congested capillaries, some glial cells, preservation of some of pyramidal cells of CA3 region, and preservation of some of granule cells of the dentate gyrus.

Figure 8. Photomicrograph section of Hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus) (magnification 400 X) taken from brain of rats of group 8 (AD-induced rats treated with Cur.300 + Exelon) showing minimal neuronal degeneration, minimal glial cells, preservation of most of pyramidal cells of CA1 region, minimal cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, preservation of most of the granule cells of dentate gyrus, and normal intact cell layer with mature cells appear big and rounded.
preservation of some of pyramidal cells of CA1 region, some cells with pyknotic nuclei, some congested capillaries, some glial cells, preservation of some of pyramidal cells of CA3 region, and preservation of some of granule cells of the dentate gyrus. Brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 8 (AD-induced rats treated with Cur.300 + Exelon) Figure 8, showing minimal neuronal degeneration, increase number of mature cells, minimal glial cells, preservation of most of pyramidal cells of CA1 region, minimal cells with pyknotic nuclei, few congested capillaries, preservation of large pyramidal cells of CA3 region, preservation of most of the granule cells of dentate gyrus, and normal intact cell layer with mature cells appear big and rounded. As shown in Figure 9, brain section taken from hippocampus (CA1, CA2, CA3, CA4, and dentate gyrus) of group 9 (Rats inducted with Cur.300 mg + 50 mg Al3Cl6H2O) showing neuronal vacuolar degeneration, apoptotic nuclei and cell, increase number of glial cells, congested capillaries, disorganization and areas of cell loss of contracted pyramidal cells of CA3 region, and many degenerated granule cells in the dentate gyrus with vacuolated cytoplasm, pyknotic nucleus and also showing some of normal intact cell layer with mature cells.

DISCUSSION
The human brain is a complicated organ, although it has 2% of body weight, it accounts for 20% of all the oxygen and 25% of all glucose consumed by the body. These characteristics make the human brain exhibit to aging and oxidative damage and that because of the high concentration of easily oxidizable polyunsaturated fatty acids (PUFAs) and also the high concentration of iron and metals (Huang et al., 2016). There is no escaping from stress as life experience that may attribute to oxidative stress leading to cognitive disturbances. There is a complex relationship between stressful situations, mind and body’s reaction to stress, and the onset of cognitive disturbances (Bhutani et al., 2009). Alzheimer’s disease (AD) is recognized by a progressive loss of memory and cognitive function and that happen by the presence of extracellular β-amyloid (Aβ) deposited as neurotic plaques (NP) and neurofibrillary tangles (NFT) made of abnormal and hyperphosphorylated tau protein with generating the neuronal damage that leads to cell death and cognitive failure through the generation of reactive oxygen species (ROS). In this study, the toxicity of AlCl3.6H2O was noticed by Morris Water Maze
test in group 2 (Rats inducted with 50 mg AlCI.6H2O) which there is significant increase in escape latency time to reach the hidden platform in acquisition trials and low number of platforms crossing in probe trial so that indicating toxicity of AlCI.6H2O caused a progressive deterioration in learning ability and spatial navigation task and that agreement with Sethi P and Ouafa R (Ouafa and Djebli, 2008; Sethi et al., 2008). These behavioral deteriorations in learning and memory seem to be because of the distribution of the hippocampal circuit and its extensive connections (Skutella and Nitsch, 2001). Treatment of AD groups with curcumin was observed neuroprotective effects of curcumin during the acquisition trial, by demonstration of the significantly decrease in escape latency time to reach the hidden platform, and observed more obviously during the probe trial, by demonstration of the significantly high number of platform crossings in the curcumin supplemented group. Behavioral tests of the present study showed that curcumin treatment improves aging induced cognitive impairment in rats that agree with (Khurana et al., 2012; Dong et al., 2012). In agree with (Yang et al., 2005) the antioxidant property of curcumin may be due to its nitric oxide scavenging ability, presence of two electrophilic α, β-unaturated carbonyl groups which react with nucleophiles, metal-chelating property, and has ability to inhibit various oxidases. The activity of AChE is implicated in cell proliferation and neurite outgrowth (Chacon et al., 2003). An important event that has been related to the pathogenesis and progression of a variety of CNS disorders AChE responds to it including oxidative stress (Chacon et al., 2003), so this enzyme is a target for the emerging therapeutic strategies to treat cognitive diseases like Alzheimer's disease (AD) (Shen et al., 2011). Our study found statistically significant difference between study groups in mean tissue acetyl cholinesterase and this finding suggests that alteration of AChE gene expression level by Aluminum administration could explain some possible molecular mechanism of Aluminum neurotoxicity in rats.

In agreement with another study (Akinwumi et al., 2016) which aimed to evaluate the effect of curcumin on cerebral cortex acetylcholinesterase (AChE) activity and their mRNA gene expression level in cadmium (Cd)-treated rats. The results showed that the decrease in mRNA expression levels of AChE by curcumin following Cd exposure maybe because of alterations in the transcriptional factors like SP1, c-fos and NF-kB which consequently deranges cell signaling and alters gene expression systems (Saritha et al., 2014). Aβ oligomers are considered the major killer form of the peptide (Bolmont et al., 2007), so it considered as a biomarker and a drug target for the therapy, being expected to ameliorate the accuracy of early diagnosis, and to investigate the influence of drugs on Aβ removal and aggregation. Our study found significant difference between study groups in mean Aβ1-42 as groups treated with the administration of curcumin, which have lower mean of Aβ1-42 compared to group 2 (Rats inducted with 50mg AlCI.6H2O). In agreement with a study reported that Cognitive decline in patients with AD is associated with elevated brain levels of Aβ, particularly neurotoxic Aβ1-42 (Zhang et al., 2015). Curcumin might restore memory and the learning ability impaired by Aβ in an AD model by activating the BDNF-ERK-CREB signaling in the hippocampus. Also, it was noticed that an intra-hippocampal infusion of ERK inhibitor could block the curcumin-induced cognitive improvement in Aβ-treated rats, so that ERK plays a critical role in hippocampus-dependent spatial memory (Zhang et al., 2015). Because of antioxidant effect of curcumin, so it has ability to scavenge free radicals, reduce the generation of ROS and act as strong inhibitor of lipid peroxidation (Abdollahi et al., 2016). In our study there were statistically significant differences between study groups in mean MAD, GSH, CAT and SOD as groups treated with curcumin had significantly higher means of GSH, CAT and SOD than group 2 (Rats inducted with 50 mg AlCI.6H2O). While groups treated with curcumin had significantly lower mean of MAD than group 2 which exposed to aluminum. In agreement with a recent study (Alizadeh and Kheirouri, 2019) showed that curcumin was effective in reducing MDA and in increasing levels of antioxidants. A large amount of in vivo, experimental and human evidence has suggested that curcumin can act as a free radical scavenger and an inhibitor of MDA production. The sirtuins (SIRT) are a group of proteins which act as intracellular regulatory proteins, and are involved in multiple cellular processes including aging, resistance to stress, metabolic regulation and transcription.

The activation or inhibition of sirtuins of 1, 2 and 3 by curcumin may be involved in reducing malondialdehyde and elevating the levels of antioxidants. Various studies suggest that SIRT1 and SIRT3 inhibit oxidative stress in cells (Miao et al., 2016), whereas SIRT 2 triggers it (Nie et al., 2014). Curcumin has been suggested to act as an activator of SIRT1 and SIRT3, but as an inhibitor of SIRT2. Oral administration of AlCl3 has neurodegenerative effects on the histology of hippocampus of rats as shown in group 2 (Rats inducted with 50mg AlCI.6H2O), and that in agreement with Ouafa R and Djebli who demonstrated that administration of AlCl3 at a dose of 50 mg/kg/day through drinking water for duration of three months causes a massive cellular depletion in the hippocampal formation with neurofibrillar degeneration and showed numerous ghosts like neurons with cytoplasmic and nuclear vaculations, which were thought to be due to the accumulation of Al in these regions which resulting in behavioral modification leading to cognitive impairment and could induce brain damage. Treatment by curcumin in groups showing different rates in improvement on histology of hippocampus of rats, and the results in agreement with Faheem and ElAskary, (2017). In our present study and according to our results we found that combination between Curcumin and Rivastigmine is more effective in inhibition of AChE activity, reduction of Aβ1-42 concentration, increase the bioactivity of antioxidant (GSH, SOD, and CAT), and inhibition of lipid peroxidation than their effect alone.

Conclusion

The results obtained from the present study revealed that curcumin can be effective in various types of oxidative associated Alzheimer’s disease and encouraged further in vitro studies to realize the accurate bioefficacy and bioavailability pathways of curcumin. Regarding the above-mentioned results which demonstrated the biological activities of curcumin in either protecting or treating brain, it is highly recommended to estimate curcumin as a safe and effective natural product for oxidative associated Alzheimer’s diseases. According to these results, curcumin as a dietary supplement has a protective role against the beginning of Alzheimer’s diseases. The intake of a significant content of curcumin in the daily regimen or as dietary supplementation along with specific therapeutic options can provide perfect prevention and treatment for Alzheimer’s diseases.
Abbreviations

BDNF → Brain-derived neurotrophic factor
CA → Cornu Ammonis
cFos → Proto-oncogene
CREB → cAMP response element binding protein
ELT → Escape latency time
ERK → Extracellular signal-regulated kinase
NFT → Neurofibrillar tangles
NF-kB → Nuclear factor kappa-light-chain-enhancer of activated B cells a protein complex that controls transcription of DNA
NO → Nitric oxide
NP → Neurotic plaques
PUFAs → polyunsaturated fatty acids
ROS → Reactive oxygen species
SP1 → Specificity Protein 1 containing a zinc finger protein motif

REFERENCES


