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Effect of Curcumin, Exelon and their Combination on Brain in Alzheimer's Disease-Induced Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WFK and SMM managed the analyses of the study. Author SM managed the literature searches. All authors read and approved the final manuscript.

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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.

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Keywords: Alzheimer's disease; oxidative stress; curcumin; aluminum chloride.

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 : Neurofibrillary tangles
NF-κB :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

1. 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 [1]. 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 [2]. 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 extracellula 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 [3].

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 [4]. 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 [5,6]. Also, the activity of enzyme engaged in metabolism of acetylcholine, aluminum can cause disturbance in it leading to cognitive impairment [7].

Curcumin is a natural product derived from Curcuma longa (more commonly known as turmeric) [8]. Curcumin has anti-inflammation and antioxidant activities, so it can decrease inflammation, amyloid accumulation and oxidative stress which has ability to scavenge free radicals [9]. 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 [10]. Curcumin is safe product which large quantities can consumed without toxicity.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult albino rats weighing (220 g ± 10 g), aged (16-18 weeks) used in this study supplied by the animal house of faculty of veterinary medicine Zagazig University, Egypt. The rats were placed in special cages and classified to seven animals per cage and maintained laboratory conditions, temperature (25±2), with dark and light cycle (12/12h). The rats were adapted to laboratory conditions for a week before starting of experiment. All procedures of experiment were carried out between 9-11 am. Rats were individually housed with ad-libitum access to

standard laboratory diet and tap water.

2.2 Experimental Design

Sixty-three animals were classified in to 9 main groups (7 rats for each group). Group1: Normal healthy rats (receive vehicle for $AlCl_3.6.H_2O$) served as Negative control group. Group2: served as Positive control group (receive $AlCl_3.6.H_2O$ in dose 50 mg/kg b.w) orally daily for one month. Group3: receive $AlCl_3.6.H_2O$ 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 properties make curcumin valuable for drug development, and remain focus for several clinical trials [11]. $AlCl_3.6.H_2O$ 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. Group5**:** receive $AlCl_3.6.H_2O$ 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. Group6: receive $AlCl_3.6.H_2O$ 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 wo months. Group 7: receive $AlCl_3.6.H_2O$ 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. Group8: receive $AlCl_3.6.H_2O$ 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. Group9: (receive $AlCl_3.6.H_2O$ 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. $AlCl_3.6.H_2O$ was obtained from Bio diagnostic company in Giza, Egypt dissolved in distilled water. Curcumin was obtained from Sigma Aldrich dissolved in 0.5M NaOH then diluted with phosphate.

2.3 Sample Collection

When the experimental period ended for each group the rats were sacrificed by cervical decapitation and the whole brain of each rat was rapidly dissected, thoroughly washed with isotonic saline, dried, and then weighed. Then each brain was individually sagitally into two portions. One half of each brain was homogenized immediately to give 10%(w/v) homogenate in ice-cold medium containing 50mM Tris- Hcl (pH 7.4) and 300 mM sucrose and then centrifuged at 3000 rpm for 10 min at 4 C.The supernatant was used to determine the biochemical analysis including AChE, Aβ (1-42), GSH, SOD, CAT, MAD. The other half of brain was fixed in (10%) formalin buffer solution for histopathological examination.

2.4 Behavioral Studies

Morris Mater Maze (MWM) was described by Morris (1981) to investigate the spatial learning and memory in laboratory rats. The MWM is a circular, galvanized steel tank with 150 cm diameter and 60 cm in depth, filled with water and was made opaque by nontoxic black paint. The temperature of water was maintained at 25 ± 2°C. Imaginarily the surface area of the tank was buffer solution to obtain suitable concentrations for oral administration. Riastigmine (Exelon) was obtained from Novarits Company Cairo, Egypt dissolved in distilled water. Divided into four quadrants such as (Q1), (Q2), (Q3) and (Q4). A hidden square escape platform was placed 2 cm below the water surface. It was located into the Q3 region and fixed during the training period. In the initial acquisition phase, the rats were trained to swim to visible platform in circular pool located in a test room. The MWM is based on a principle which rats can escape from swimming by climbing onto platform which placed in a pool 2 cm above the water level and over time the rats apparently learn the confirmed location of the platform from any position at the circumference of the pool. In maze acquisition phase (training), the platform was placed in a pool 2 cm below the water level and the water was made opaque by nontoxic paint. The position of the platform remained unchanged throughout the study. The water maze duty was executed for four consecutive days from the last four days of each group period. The rat received four consecutive daily training trials in the following 4 days, with each trial having a certain time of 90 s. For each trial and at one of four starting positions, each rat was gently placed in the water the position which being selected randomly and allowed 90 s to locate submerged platform. Then, it was within 90 s, it was guided gently onto platform and allowed to remain there for 20s. Each rat was subjected to four trials on each day. A rest period of 1 h was allowed in between each trial. Per day, four trials were carried out and repeated for

four days. In each trial there were changed in starting position to attitude four acquisition trials as illustrated below and Q3 was maintained as target quadrant in all acquisition trials.Day1 Q1 Q2 Q3 Q4,Day2 Q2 Q3 Q4 Q1,Day3 Q3 Q4 Q1 Q2,Day4 Q4 Q1 Q2 Q3.Mean escape latency time (ELT) calculated for each day during acquisition trials was used as an index of acquisition. In maze retention phase (probe trial) After Twenty-four hours for the last training session (On fifth day), a probe trial was performed to assess memory. During the probe trial, the hidden platform was removed from the tank and the rats were allowed to swim freely for 90s, and number of platform crossings in search of missing platform in target quadrant provided an index of retention.

2.5 Biochemical Analyses

Rat AChE was assayed by Elisa technique using AChE assay kit (Elisa for quantitative detection allowed to stay on the platform for 20 s. If animal failed to find the platform of AChE) purchased from My BioSource Company, USA. Rat Aβ 1-42 was assayed by Elisa technique using Aβ 1-42 assay kit (Elisa for quantitative detection of Aβ 1-42) purchased from Elabscience Company, USA. Colorimetric assay for the determination of GSH was estimated according to the method of Beutler,1963 [12] by using the kit purchased from Biodiagnostic Company, Dokki, Giza, Egypt. Colorimetric assay for the determination of SOD was estimated according to the method of Nishikimi et al., 1972 [13] by using the kit purchased from Bio diagnostic Company, Dokki, Giza, Egypt. Colorimetric assay for the determination of CAT was estimated according to the method of Aebi, 1984 [14] by using the kit purchased from Biodiagnostic Company, Dokki, Giza, Egypt. Colorimetric assay for the determination of Lipid peroxidation (MAD) was estimated according to the method of Satoh, 1979 [15] by using the kit purchased from Bio diagnostic Company, Dokki, Giza, Egypt.

2.6 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 0.01 was considered significant, Post hoc test to assess significance between each two groups.

3. RESULTS

3.1 Effect of Curcumin, Exelon and their Combination on AD-induced Rats by Behavioral Studies (Morris Water Maze) Test

The change in the escape latency time to reach the hidden platform was observed in the training/acquisition trials. There was a downward trend in escape latency time (ELT) in water-maze training session for four days, and study groups showed statistical significance differences Fig. 1. At group 6 (AD-induced rats treated with Exelon) had a significant shortened escape latency time compared to group 2 (Rats inducted with 50mg $AlCl_3.6.H_2O$), indicating improvement in memory performance. At group 3 (AD- induced rats treated with Cur.200 mg), group 4 (AD-induced rats treated with Cur.300 mg), group 5 (AD-induced rats treated with Cur.400 mg), and group 7 (AD- induced rats treated with Cur.15 mg i.p) showed statistical significance differences but all were significantly shortened escape latency time compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$), indicating improvement in memory performance. At group 8 (AD- induced rats treated with Cur.300 + Exelon) had a significant shortened escape latency time compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$), indicating improvement in memory performance and returned nearly to the normal control group. Further combination of curcumin with Exelon showed significant improvement in learning performance as compared to their effects alone. At group 9 (Rats inducted with Cur.300 mg + 50mg $AlCl_3.6.H_2O$) had slightly shortened escape latency time compared to group 2 (Rats inducted with 50mg $AlCl_3.6.H_2O$), indicating a poorer learning performance. On fifth day platform was removed to estimate the retention of memory, and study groups showed statistical significance differences in No. of platform crossing at 90s, indicating improvement in cognitive performance. In group 8 (AD-induced rats treated with Cur. + Exelon) showed the highest No. of platform crossing at 90s as the normal control group and that indicating improvement in cognitive performance compared to their effects alone Fig. 2.

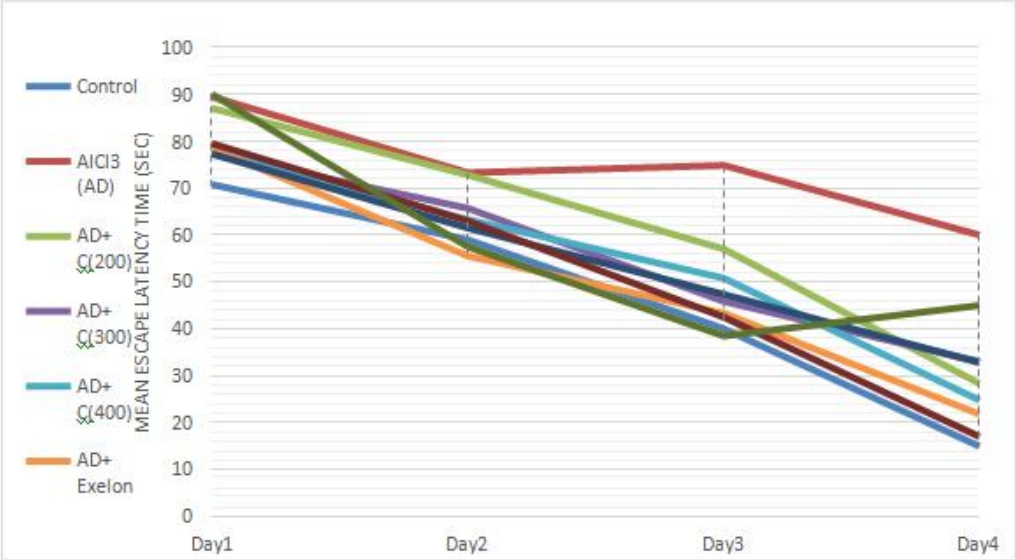

Fig. 1. Effects of curcumin, exelon and their combination on escape latency time in Morris water maze

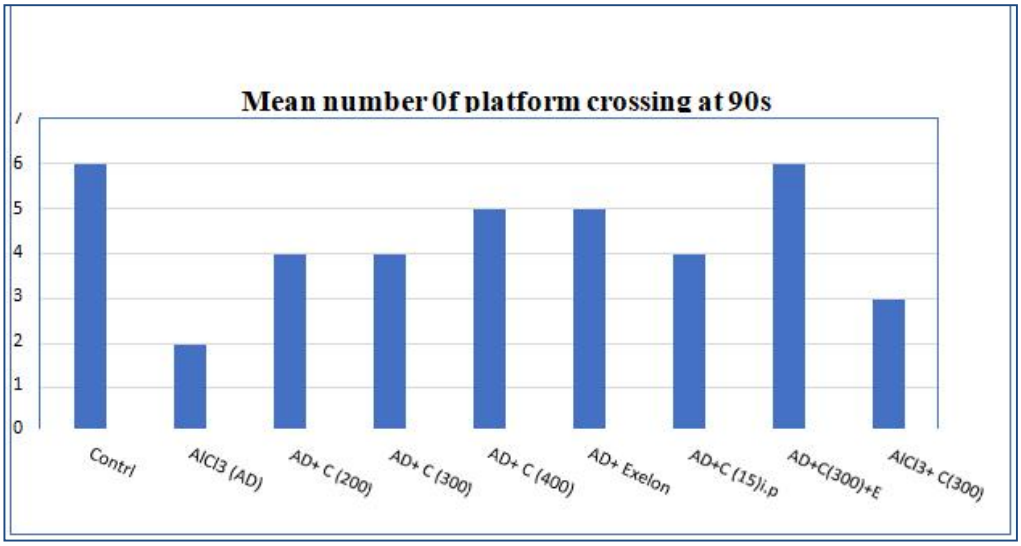

Fig. 2. Effects of curcumin, exelon and their combination on no. of platform crossing in Morris water maze

3.2 Effect of Curcumin, Exelon and their Combination on Alzheimer Disease Markers in AD-induced Rats

3.2.1 Effect on acetyl cholinesterase (AChE) activity

As shown in Table 1, Fig. 3, group 2 (Rats inducted with 50mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period showed significant increase in AChE activity compared to normal control group (724.50 ± 8.18 *vs.* 557.63 ± 18.25). At group 3 (AD-induced rats treated with Cur.200 mg), group 4 (AD-induced rats treated with Cur.300 mg), group 5 (AD-induced rats treated with Cur.400 mg), group 6 (AD-induced rats treated with Exelon), and group 7 (AD-induced rats treated with Cur.15 mg i.p) in the previously mentioned dose and period showed statistical significance differences but all

were significantly decreased in ACh E activity compared to group 2 (Rats inducted with 50mg $AlCl_3.6.H_2O$) as following (655.64 ± 15.31, 646.33 ±8.96, 681.66 ± 8.60, 600.88 ± 8.94, 682.06 ± 12.18 *vs.* 724.50 ±8.18). Also showed that low dose of Cur. (200 mg) was more effective than high dose of Cur. (400 mg) and returned nearly to group 6 (AD- induced rats treated with Exelon) as following (655.64 ± 15.31 *vs.* 600.88± 8.94). At group 8 (AD-induced rats treated with Cur. + Exelon) in the previously mentioned dose and period had a significant decreased in AChE activity compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (587.53 ±15.85 *vs.* 724.50 ± 8.18), and returned nearly to the normal control group (587.53 ± 15.85 *vs.* 557.63 ± 18.25). Further combination of Curcumin with Exelon was more effective as compared to their effects alone. At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly significant decreased in AChE activity compared to group 2(Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (703.89 ± 13.83 vs. 724.50 ± 8.18).

3.2.2Effect on beta amyloid Aβ (1-42) concentration

As shown in Table 1, Fig. 4, group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period showed significant increase in Aβ (1-42) conc. compared to normal control group (42.14 ± 5.22 *vs.* 12.32 ± 0.71). At group 3 (AD-induced rats treated with Cur.200 mg), group 4 (AD- induced rats treated with Cur.300 mg), group 5 (AD-induced rats treated with Cur.400 mg), group 6 (AD-induced rats treated with Exelon), and group 7 (AD-induced rats treated with Cur.15 mg i.p) in the previously mentioned dose and period showed statistical significance differences but all were significantly decreased in Aβ (1-42) conc. as following (16.54 ± 0.51, 16.20 ±0.75, 20.61 ± 1.49, 14.14 ± 0.31, 23.71 ± 5.16 *vs.* 42.14 ± 5.22), respectively compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$). Also showed that low dose of Cur. (200 mg) was more effective than high dose of Cur. (400 mg) and returned nearly to group 6 (AD-induced rats treated with Exelon) as following (16.54 ±0.51 *vs.* 14.14 ± 0.31). At group 8 (AD-induced rats treated with Cur.300 + Exelon) in the previously mentioned dose and period had a significant decreased in Aβ (1-42) conc. compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (13.59 ± 0.77 *vs.* 42.14 ±5.22), and returned nearly to the normal control group (13.59 ±0.77 *vs.* 12.32 ± 0.71). Further combination of curcumin with Exelon was more effective as compared to their effects alone. At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly significant decreased in Aβ (1-42) conc. compared to group 2(Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (32.67 ± 3.23 *vs.* 42.14 ± 5.22).

3.3 Effect of Curcumin, Exelon and their Combination on Antioxidant Parameters in AD-induced Rats

3.3.1 Effect on glutathione (GSH) levels

The results in Table 2 and graphically illustrated in Fig. 5 showed that the oral administration of $AlCl_3.6H_2O$ in group 2 in the previously mentioned dose and period caused significant decreased in GSH level (1.99 ± 0.18 *vs.*3.62 ± 0.05 respectively) with respect to normal control group. Interestingly, the treatment of group 3 (AD-induced rats treated with Cur.200mg), group4(AD-induced rats treated with Cur.300mg), group 5 (AD-induced rats treated with Cur.400 mg),group 6 (AD-induced rats treated with Exelon), group 7 (AD-induced rats treated with Cur.15 mg i.p), and group 8 (AD-induced rats treated with Cur.300 + Exelon)in the previously mentioned dose and period caused marked improvement in GSH level especially group 5 (AD- induced rats treated with Cur.400 mg) as following (3.50 ± 0.05, 3.93 ±0.09, 4.24 ± 0.31, 3.74 ± 0.05, 3.22 ±0.09, 3.97 ± 0.20 *vs.* 1.99 ± 0.18), respectively compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$). At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly marked improvement in GSH level compared to group 2(Rats inducted with 50mg $AlCl_3.6.H_2O$) as following (2.77 ± 0.24 *vs.* 1.99 ± 0.18).

3.3.2Effect on Superoxide dismutase (SOD) activity

As shown in Table 2, Fig. 6, group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period showed significant decrease in SOD activity compared to normal control group (241.3 ± 17.61 *vs.* 363 ±

8.18). The activity of SOD of group 3 (AD-induced rats treated with Cur.200 mg), group4(AD-induced rats treated with Cur.300 mg), group 5 (AD- induced rats treated with Cur.400 mg),group 6 (AD-induced rats treated with Exelon), group 7 (AD-induced rats treated with Cur.15 mg i.p), and group 8 (AD-induced rats treated with Cur.300 + Exelon)in the previously mentioned dose and period showed significant increase as following (339 ± 9.00, 344 ± 12.52, 327 ± 9.16, 350.3 ± 11.01, 314.6 ±9.29 , 351 ± 10.53 *vs*.241.3 ± 17.61), respectively compared to group 2 (Rats inducted with 50mg $AlCl_3.6.H_2O$).Further combination of curcumin with Exelon had similar effect on SOD activity as effect of Exelon alone. At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly significant increase in SOD activity compared to group 2(Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (291.6 ± 5.68 *vs.* 241.3 ± 17.61).

Table 1. Effect of curcumin, exelon and their combination on acetylcholinesterase (AChE) activity and beta amyloid Aβ (1-42) concentration (n=7)

Biomarkers	AChE (umol/g tissue)			Aβ (1-42) (pg/g tissue)		
Groups	**Mean**	**Std. Dev.**	**Std. error**	**Mean**	**Std. Dev.**	**Std. error**
Control	557.63	18.25	10.53	12.32	0.71	0.41
$AlCl_3$ (AD)	724.50	8.18	4.72	42.14	5.22	3.01
AD + C (200)	655.64	15.31	8.84	16.54	0.51	0.29
AD + C (300)	646.33	8.96	5.17	16.20	0.75	0.43
AD + C (400)	681.66	8.60	4.96	20.61	1.49	0.86
AD + Exelon	600.88	8.94	5.16	14.14	0.31	0.18
AD + C (15) i.p	682.06	12.18	7.03	23.71	5.16	2.98
AD + C (300) + E	587.53	15.85	9.15	13.59	0.77	0.44
$AlCl_3$ + C (300)	703.89	13.83	7.98	32.67	3.23	1.86
P-value	<0.001*[1]					

*Data presented as of mean ± standard deviation (SD) ANOVA test used;*statistically significant at p< 0.01*

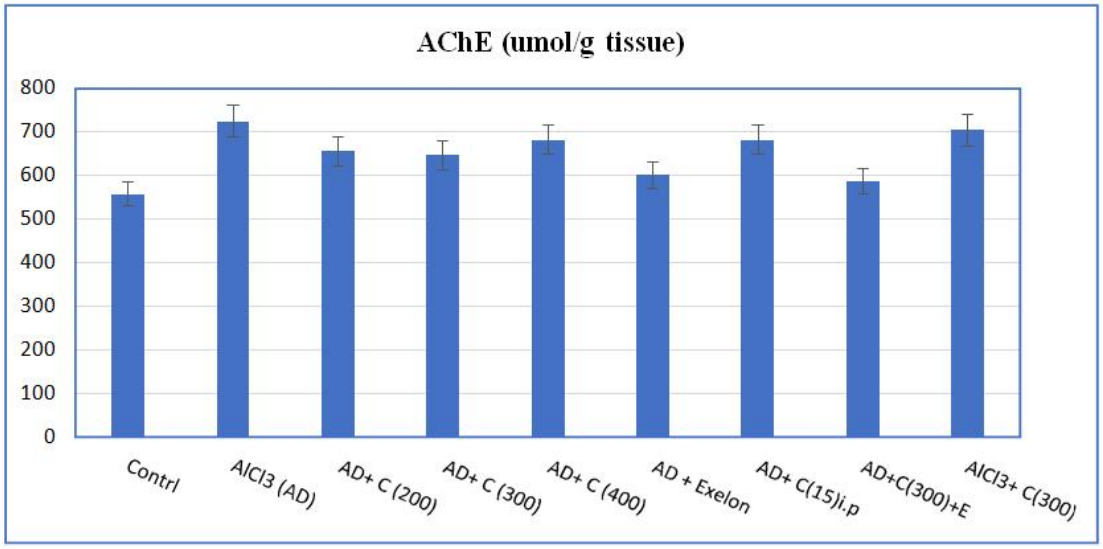

Fig. 3. Effect of curcumin, exelon and their combination on acetylcholinesterase (AChE) activity

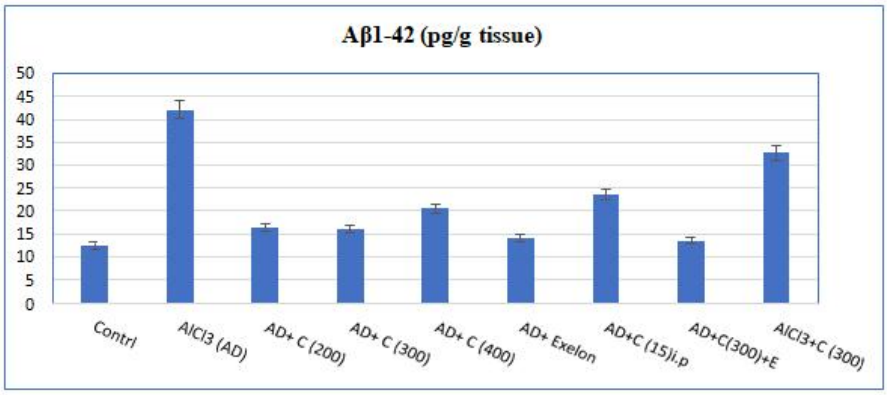

Fig. 4. Effect of curcumin, exelon and their combination on beta amyloid Aβ (1-42) Concentration

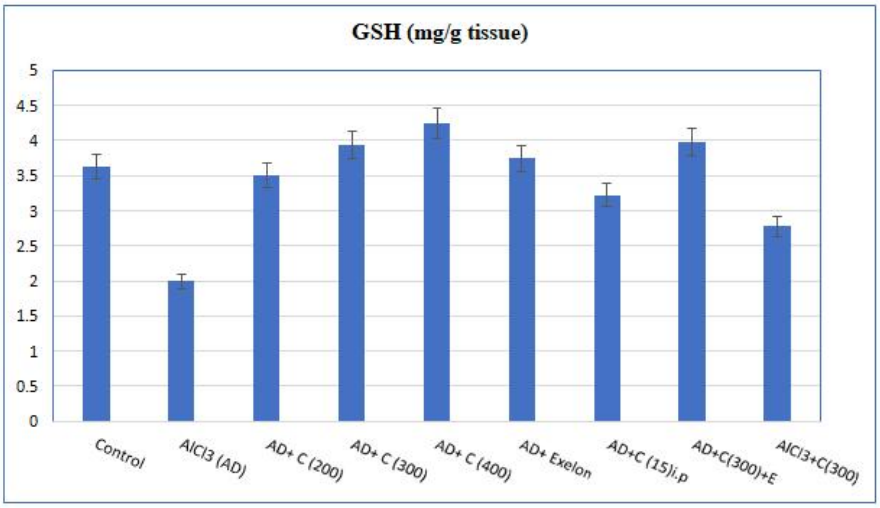

Fig. 5. Effect of curcumin, exelon and their combination on glutathione (GSH) concentration

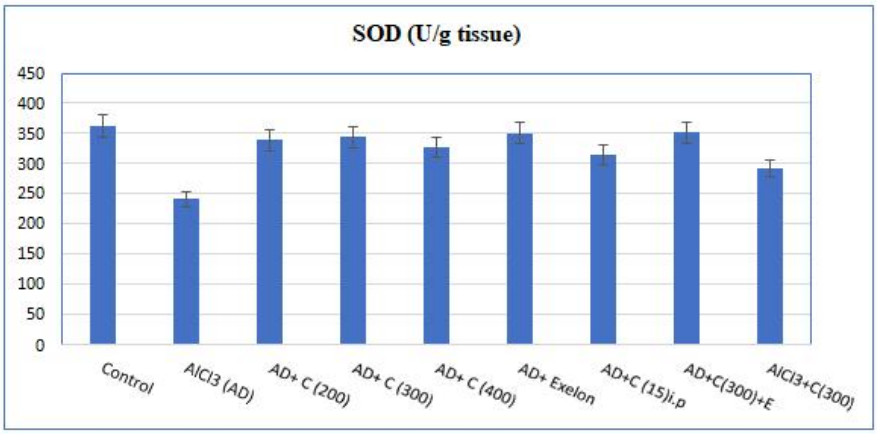

Fig. 6. Effect of curcumin, exelon and their combination on superoxide dismutase (SOD) activity

Table 2. Effect of curcumin, exelon and their combination on glutathione (GSH) conc., superoxide dismutase (SOD) activity, catalase (CAT) activity, and lipid peroxide conc. (Malondialdehyde, MAD) (n=7)

Antioxidants	GSH (mg/g tissue)			SOD (U/g tissue)			CAT (U/g tissue)			MAD (nmol/g tissue)		
Groups	**Mean**	**Std. Dev.**	**Std. error**	**Mean**	**Std. Dev.**	**Std. error**	**Mean**	**Std. Dev.**	**Std. error**	**Mean**	**Std. Dev.**	**Std. error**
Control	3.62	0.05	0.03	363	8.18	4.72	1.97	0.07	0.04	11.21	0.46	0.26
$AlCl_3$ (AD)	1.99	0.18	0.10	241.3	17.61	10.17	0.86	0.07	0.04	51.44	2.57	1.48
AD + C (200)	3.50	0.05	0.02	339	9.00	5.19	1.16	0.06	0.03	22.70	0.88	0.51
AD + C (300)	3.93	0.09	0.05	344	12.52	7.23	1.26	0.03	0.02	19.93	0.75	0.43
AD + C (400)	4.24	0.31	0.17	327	9.16	5.29	1.14	0.16	0.08	28.26	0.92	0.53
AD + Exelon	3.74	0.05	0.02	350.3	11.01	6.35	1.32	0.15	0.08	19.20	0.52	0.30
AD + C (15) i.p	3.22	0.09	0.05	314.6	9.29	5.36	1.11	0.19	0.11	30.26	2.66	1.53
AD + C (300) + E	3.97	0.20	0.11	351	10.53	6.08	1.59	0.41	0.24	19.46	2.62	1.51
$AlCl_3$ + C (300)	2.77	0.24	0.13	291.6	5.68	3.28	0.97	0.08	0.05	38.83	1.45	0.84
P-value	$<0.001^{*1}$											

*Data presented as of mean ± standard deviation (SD) ANOVA test used;*statistically significant at p< 0.01*

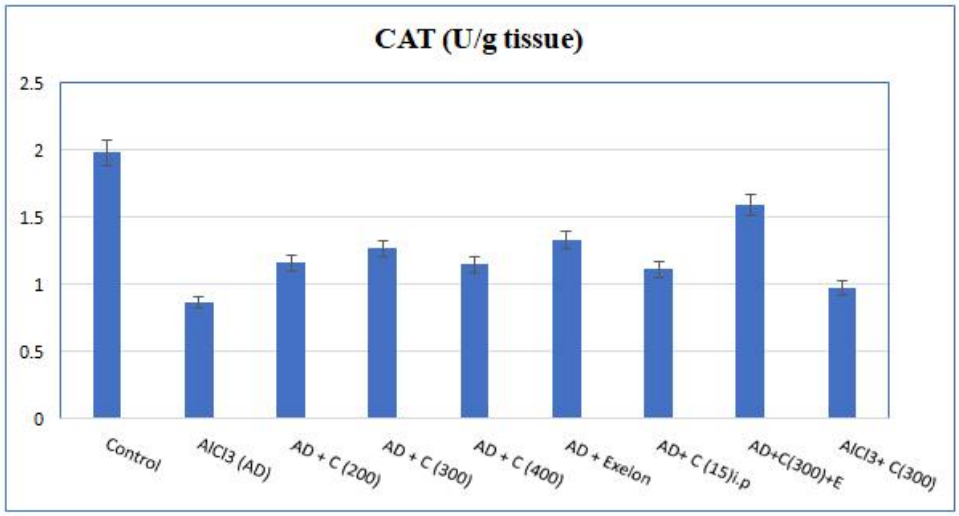

Fig. 7. Effect of curcumin, exelon and their combination on Catalase (CAT) activity

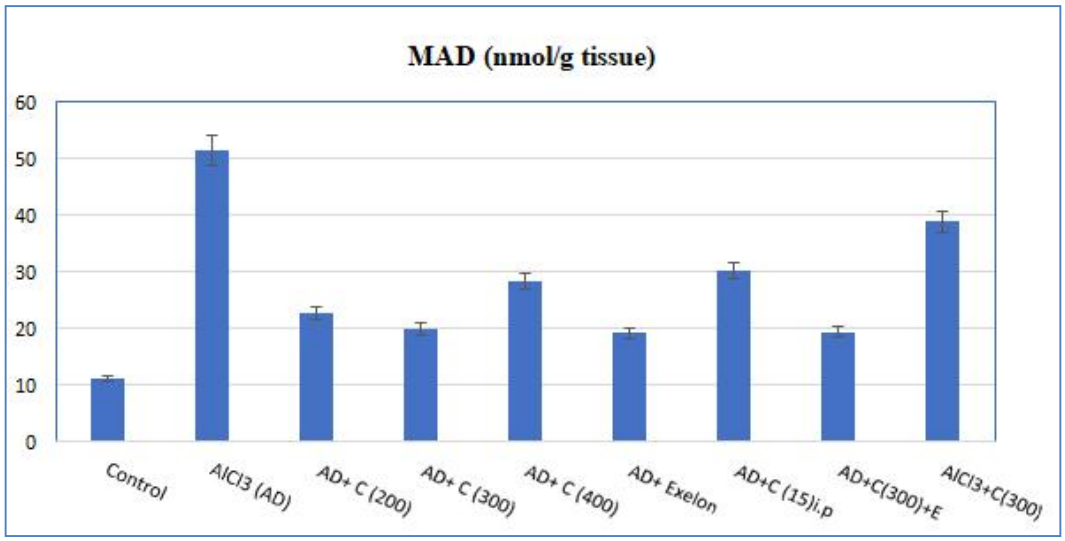

Fig. 8. Effect of curcumin, exelon and their combination on lipid peroxide conc. (Malondialdehyde, MAD)

3.3.3 Effect on catalase (CAT) activity

The results in Table 2 and graphically illustrated in Fig. 7 showed that the oral administration of $AlCl_3.6H_2O$ in group 2 in the previously mentioned dose and period caused significant decreased in CAT activity (1.97 ± 0.07 *vs.* 0.86 ±0.07 respectively) with respect to normal control group. Treatment of group 3 (AD-induced rats treated with Cur.200 mg), group 4 (AD- induced rats treated with Cur.300 mg), group 5 (AD-induced rats treated with Cur.400 mg),group 6 (AD-induced rats treated with Exelon), group 7 (AD-induced rats treated with Cur.15 mg i.p), and group 8 (AD-induced rats treated with Cur.300 + Exelon)in the previously mentioned dose and period caused marked improvement in CAT activity as following (1.16 ±0.06, 1.26 ± 0.03, 1.14 ± 1.16 , 1.32 ± 0.15, 1.11 ± 0.19, 1.59 ± 0.41 *vs.* 0.86 ± 0.07), respectively compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$). At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly significant increase in CAT activity compared to group 2 (Rats

inducted with 50mg $AlCl_3.6.H_2O$) as following (0.97 ± 0.08 *vs.* 0.86 ± 0.07).

3.3.4Effect on Lipid Peroxide Conc. (Malondialdehyde, MAD)

As listed in Table 2 and graphically illustrated in Fig. 8, group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period showed significant increase in level of MAD conc. compared to normal control group (51.44 ± 2.57 *vs.* 11.21 ± 0.46). At group 3 (AD- induced rats treated with Cur.200 mg), group 4 (AD-induced rats treated with Cur.300 mg), group 5 (AD- induced rats treated with Cur.400 mg),group 6 (AD-induced rats treated with Exelon), group 7 (AD-induced rats treated with Cur.15 mg i.p), and group 8 (AD-induced rats treated with Cur.300 + Exelon) in the previously mentioned dose and period showed significant decrease in level of MAD conc. as following (22.70 ± 0.88, 19.93 ± 0.75, 28.26 ± 0.92, 19.20 ± 0.52, 30.26 ± 2.66, 19.46 ± 2.62 *vs.* 51.44 ± 2.57), respectively compared to group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$). And also showed that group 8 (AD-induced rats treated with Cur.300 + Exelon), group 6 (AD-induced rats treated with Exelon), andgroup4(AD-induced rats treated with Cur.300 mg) had similar effect on MAD level. At group 9 (Rats inducted with Cur.300 mg + 50 mg $AlCl_3.6.H_2O$) in the previously mentioned dose and period had slightly significant decrease in MAD level compared to group 2(Rats inducted with 50 mg $AlCl_3.6.H_2O$) as following (38.83 ± 1.45 *vs.* 51.44 ± 2.57).

4. 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 oxidable polyunsaturated fatty acids (PUFAs) and also the high concentration of iron and metals [16]. 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 [17]. 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 $AlCl_3.6H_2O$ was noticed by Morris Water Maze test in group 2 (Rats inducted with 50 mg $AlCl_3.6.H_2O$) 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 $AlCl_3.6H_2O$ caused a progressive deterioration in learning ability and spatial navigation task and that agreement with Sethi P and [18]. These behavioral deteriorations in learning and memory seem to be because of the distribution of the hippocampal circuit and its extensive connections [19]. 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 [20,21]. In agree with [22] the antioxidant property of curcumin may be due to its nitricoxide scavenging ability, presence of two electrophilic α, β-unsaturated 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 [23]. 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 [23], so this enzyme is a target for the emerging therapeutic strategies to treat cognitive diseases like Alzheimer's disease (AD) [24]**.** Our study found statistically significant difference between study groups in mean tissue acetylcholinesterase 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 [25] which aimed to evaluate the effect of curcumin on cerebral cortex acetyl cholinesterase (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, cfos and NF-κB which consequently deranges cell signaling and alters gene expression systems [26]. Aβ oligomers are considered the major killer form of the peptide [27], 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 50 mg $AlCl_3.6.H_2O$). 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 [28]. 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 [28]. 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 [29]. 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 $AlCl_3.6.H_2O$). While groups treated with curcumin had significantly lower mean of MAD than group 2 which exposed to aluminum. In agreement with a recent study [30] 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 [31], whereas SIRT 2 triggers it [32]. Curcumin has been suggested to act as an activator of SIRT1 and SIRT3, but as an inhibitor of SIRT2.

5. 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 bio efficacy 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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