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Potential role of some nutraceuticals in neurotoxicity induced by aluminum oxide in experimental animal model

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Abstract

The present study aimed to evaluate neurorestorative potential of lecithin (L) and/or gallic acid (GA) in alleviating aluminum (Al)-induced neurotoxicity in rat brain. Rats were classified into seven groups: Group (1): served as control, Groups (2)&(3): received lecithin or gallic acid in a dose of 100 mg/kg body weight. Group (4): received Al in a dose of 300 mg/kg body weight, Group (5): received Al + lecithin, Group (6): received Al + gallic acid and Group (7): received Al + lecithin+ gallic acid. Rats treated orally for 28 days (5days/week). Al induced a significant increase in AChE activity, along with a significant decrease in dopamine (DA), norepinephrine (NE) and serotonin (5-HT) levels. Al treatment resulted in significant increase in malondialdehyde (MDA), nitric oxide (NO) and oxidized glutathione (GSSG) content. On the contrary, Al caused a significant decrease in glutathione (GSH) and GSH/GSSG ratio. As well as, it stimulates an apoptotic signaling program by increase of Bax, caspase-3 and Bax/Bcl-2 ratio accompanied with decrease of Bcl-2 in the tested brain areas. Histopathological examination confirms the biochemical results. Conclusively, treatment with lecithin and/or gallic acid can retrieve the effect of aluminum toxicities on the basis of cholinergic, dopaminergic, oxidative, and apoptotic status.

Keywords: Aluminum; Lecithin; Gallic acid; Antioxidants; Neurotransmitters; Apoptosis

Introduction

Aluminum (Al) is the third most abundant element and the most common metal in the earth's crust **(Farina *et al*., 2002).** A section of human population is routinely exposed to Al, the main food sources of aluminum are: hard cheese, grain products (flour), herbs and tea leaves, food preservatives, spices, water treatment reagents and number of pharmacological products (primarily antacids, analgesics, anti-diarrheal, astringents and as adjuvant for vaccines) **(McEvoy, 1990; Krewski *et al*., 2007 and Yokel *et al*., 2008)**.

Al has been reported to induce impairment in various neurotransmitter systems and can cause neurobehavioral dysfunctions in animals **(Yellamma *et al*., 2010 and Abu-Taweel *et al*., 2012).** Experimental studies have also shown that Al can cause neurochemical alterations and bring about similar neuropathological changes as observed in Alzheimer's patients **(Ribes *et al*., 2010; Walton, 2010; Kawahara and Kato Negishi, 2011)**. Al has been shown to accumulate in all regions of the rat

brain following chronic exposure. There is evidence of a relationship between the high levels of Al and increased risk of neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD) **(Kumar and Gill, 2009)**. Furthermore, chronic exposure to Al not only causes neurologic signs, which mimic progressive neurodegeneration, but also results in neurofilamentous changes in the hippocampus, cerebral cortex and biochemical changes **(Savory *et al*., 2006)**. It has also been suggested that Al can alter iron and calcium homeostasis in neurons, which leads to cytotoxic effects in different regions of brain **(Kim *et al*., 2007b and Walton 2012).** Al induces neurotoxicity primarily by triggering oxidative stress that affects a large number of signaling cascades and ultimately causes cellular death **(Verstraeten *et al*., 1997 and Sharma *et al*., 2013)**. $AlCl_3$ cause degeneration of cholinergic terminals in the cortical areas and cell depletion in the brain regions to induce impairment in learning, memory and cognition function (**Dumont, 2011)**. Although aluminum-containing over the counter oral products are considered safe in healthy individuals at recommended doses, some adverse effects have been observed following long-term use in some individuals **(Keith *et al*., 2008)**.

Natural antioxidants, which alleviate the oxidative stress or induce the cellular antioxidant milieu, would most probably achieve neuroprotection against Al poisoning, Lecithin supplementation is necessary for overall health and prevention of many conditions and diseases. **(Amjad&Umesalma, 2015)**. Lecithin, an important phospholipid is found in the major organs in our body such as the heart, the liver, and the kidneys **(Jimenez *et al*., 1990 and Iwata *et al*., 1993)**. Lecithin is sometimes used as a synonym for pure phosphatidylcholine (PC) a phospholipid that is the major component of its phosphatide fraction. It may be isolated either from egg yolk or soya beans **(Raj *et al*., 2011)**. PC builds nerve cells and neurotransmitters. In fact 17 percent of the brain is lecithin, so lecithin is a brain food. It relaxes the nerves due to improving their protective covering, reduces stress and it hydrates the skin from the inside **(Dixon, 2008)**. Extensive studies concluded the pivotal role of PC ameliorating the neurotoxicity through its antioxidant, anti-inflammatory, antifibrotic and anti-apoptotic properties **(Akin *et al.*, 2007; Kim *et al*., 2015; Zaki & Hassanin, 2016; Ko *et al*., 2016 and Khafaga, 2017),** PC has a role in maintaining memory, cholinergic signaling, where it is metabolized to choline, which is a precursor for neurotransmitter acetylcholine **(Chung *et al*., 1995)**. Also, it has ability to elevate the brain content of the neurotransmitter acetylcholine **(Khafaga, 2017)**.

Gallic acid (GA), a polyhydroxyphenolic compound (3,4,5 trihydroxybenzoic acid), is a naturally occurring plant phenol present in nutgalls, green tea, grapes, red wine, hops, oak bark, different berries, mango, coffee, and other plants **(Niu *et al*., 2004; Singh *et al*., 2004; Kim *et al*., 2009; and You *et al*., 2010)**. GA is a powerful natural antioxidant that possesses a number of biological and pharmacological activities including scavenging of free radicals, anti-inflammatory, anti-apoptotic, anti-microbial and anti-cancer **(Manach *et al*., 2004; Priscilla & Prince, 2009 and Sarkaki *et al*., 2014)**. Also, it plays an important role in the prevention of malignant transformation **(Sakaguchi *et al*., 1998)** likewise prevents amyloid ß-induced apoptotic neuronal death by interfering with the increase of Ca^{2+} levels and by inhibiting glutamate release and generation of ROS **(Ban *et al*., 2008)**. GA has strong anti-tyrosine's activity **(Kim, 2007)** and protects the brain by improving antioxidant capacity and reducing inflammation in a rat model of permanent brain hypoperfusion **(Mansouri *et al*., 2013)**. Hence, this study aimed to evaluate the protective role of lecithin and /or gallic acid against Al-induced neurotoxicity in cerebral cortex and hippocampus-striatum areas on the basis of cholinergic, dopaminergic, oxidative, and apoptotic status. Besides, histological study for the tested brain areas.

Materials and Methods

Chemicals and drugs

Aluminum oxide (Al_2O_3), BDH Chemicals Ltd England; **Lecithin,** from soybean was obtained from Lewis Laboratories International Ltd.(Westport, CT) and **Gallic Acid 1-hydrate**, 99% PS (Panreac). All other chemicals and reagents used were of analytical grade.

Animals

The present study was conducted on sixty three adult male rats *Rattus norvegicus* weighing from 110 to 150 g, obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were maintained in clean plastic cages in the laboratory animal room. Animals are feeding on a standard pellet diet, water *ad libitum* and normal illumination of daily light. The experimental work was performed with the approval of the Animal Care &

Experimental Committee, National Organization for Drug Control and Research, Cairo, Egypt. Animal protocol was performed in accordance with the slandered procedures laid down by OECD (1997) guidelines 424 and the criteria outlined in the “Guide for the Care and Use of Laboratory Animal”. After an acclimation period of one week, the animals were classified into seven groups (9 rats/group), and treated orally for 28 days (5days/week) as follows:

Group (1; Control): normal, healthy rats served as negative control.

Group (2; L): received lecithin in a dose of 100mg/kg body weight (**Lee *et al*., 2013**).

Group (3; G): received gallic acid in a dose of 100mg/kg body weight (**Sarkaki *et al*., 2014**).

Group (4; Al): received Al_2O_3 in a dose of 300mg/kg body weight (**Reinke *et al.,* 2003).**

Group (5; Al+L): received Al_2O_3 +lecithin with the same doses as mention pervious.

Group (6; Al+G): received Al_2O_3 +gallic acid with the same doses as mention pervious.

Group (7; Al+L+G): received Al_2O_3 +lecithin+ gallic acid in the same manner.

Brain tissue homogenate sampling and preparation

Rats of each group were decapitated, then the head moved onto the dry ice, the whole brain was rapidly dissected on an ice-cooled glass plate, thoroughly washed with isotonic saline, dried **(Glowinski & Iversen, 1966)**. Then brain areas (cerebral cortex and hippocampus-striatum) were separated immediately. The brain tissues of six rats from each group were homogenized with 0.1 M phosphate buffer saline at pH 7.4 to give a final concentration of 10 % w/v for the biochemical assay **(Abdel-Salam *et al*., 2016)**. The brain tissues of the three remaining rats were used for histopathological investigation.

Biochemical assays

AChE activity was determined by a modification of Ellman method as described by **Gorun *et al*. (1978)**. Brain neurotransmitters determination, Dopamine (DA), Norepinephrine (NE) and Serotonin (5-HT) were determined using HPLC coupled with UV detection according to **(Pagel *et al*., 2000)**.

Malondialdehyde (MDA) as a marker for lipid peroxidation (LPO) was estimated by HPLC according to the method described by **Karatepe (2004)**. Glutathione (GSH), oxidized glutathione (GSSG) and nitric oxide (NO) as (NO_2/NO_3) were assayed by HPLC according to **Y lmaz *et al*. (2009)** and **Papadoyannis *et al*. (1999)** respectively. Bcl-2, Bax and Caspase-3 were estimated by the kit using a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) (Kono Biotech Co., Ltd).

Histopathological examination

Samples were taken from the brain of three rats from each group and fixed in 10% formal saline for 24 hours. Washing was done in tap water then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56ºC in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 µm thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin (H&E) stain for routine examination through the light electric microscope **(Banchroft *et al*., 1996).**

Statistical analysis

The data obtained are represented in tables as Mean ± SE. The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Tukey at $P < 0.05$ and were carried out using the SPSS-PC computer software package version 17.

Results

From Table (1), it can be easily demonstrated that Al produced significant increase in AChE activity in cerebral cortex by (43.57%) and hippocampus-striatum by (60.81%) compared with control group at ($p<0.05$). Treatment of Al intoxicated rats with lecithin or gallic acid and their combination did not restore this elevation in cerebral cortex. Meanwhile, in hippocampus-striatum area all treated groups resulted in a significant inhibition of AChE activity with the priority to gallic acid with Al by (36.85%) from Al intoxicated group. These results revealed that AChE exhibiting area-specific response.

Table (1): Effect of lecithin and/or gallic acid on AChE activity (µMSH/hr/g fresh tissue) in cerebral cortex and hippocampus-striatum areas of aluminum intoxicated rat.

Groups	Cerebral cortex	Hippocampus-Striatum
Control	816.58±3.35	1019.15±26.97
Lecithin (L)	1194.49±49.48	1324.99±24.31
Gallic acid (G)	878.8±50.41	1212.61±41.32
Al	1172.34±81.21[a]	1638.88±8.66[ab]
Al+L	1377.64±43.34[a]	1433.43±10.96[ab]
Al+G	1257.93±67.51[a]	1034.9±15.33[b]
Al+L+G	1853.12±69.87[ab]	1189.24±44.01[ab]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey's test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Our results demonstrated that NE, DA and 5-HT levels in cerebral cortex were significantly decreased by (48.15%, 45.45% and 48.21% respectively) following Al treatment as compared to control group ($p<0.05$). Supplementation with lecithin alone or in combination with gallic acid significantly increased monoamine levels, for NE (57.14% and 82.14% respectively) for DA (55.95% and 73.81% respectively) and for 5-HT (44.83% and 55.17% respectively) when compared to Al intoxicated rats ($p<0.05$). Noteworthy that combined group showed a pronounced improvement than lecithin alone. In cerebral cortex non-insignificant change was detected in NE, DA and 5-HT levels in gallic acid group as compared with Al group ($p<0.05$) as shown in Table (2).

Table (2): Effect of lecithin and/or gallic acid on neurotransmitters in cerebral cortex of aluminum intoxicated rats.

Groups	NE µg/g tissue	DA µg/g tissue	5-HT µg/g tissue
Control	0.54±0.05	1.54±0.14	0.56±0.03
Lecithin (L)	0.55±0.03	1.64±0.09	0.53±0.04
Gallic acid (G)	0.47±0.04	1.36±0.1	0.43±0.02
Al	0.28±0.02[a]	0.84±0.06[a]	0.29±0.02[a]
Al+L	0.44±0.03[b]	1.31±0.09[b]	0.42±0.03[ab]
Al+G	0.31±0.03[a]	0.92±0.08[a]	0.37±0.03[a]
Al+L+G	0.51±0.01[b]	1.46±0.03[b]	0.45±0.01[b]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey's test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Data represented in Table (3) revealed that Al induced significant decrease in NE and DA levels of hippocampus-striatum area by (48.98% and 33.85% respectively) compared to normal control ($p<0.05$). Treatment with lecithin or gallic acid alone showed non-significant change compared to control group. Treatment with lecithin or gallic acid to Al intoxicated rats singly or in combination significantly elevated NE level (112%, 124% and 80% respectively) and DA level (101.67%, 86.62% and 57.53 respectively) as compared to Al group ($p<0.05$). On the other hand, Al intoxicated rats showed a non significant change in 5-HT of hippocampus-striatum area as compared with control group ($p<0.05$). Co-treatment with gallic acid to Al intoxicated rats revealed a significant increase in 5-HT level (54.55%) compared to Al group. However, the combined group showed significant increase in NE and DA levels near control levels than each one alone (Table 3).

Table (3): Effect of lecithin and/or gallic acid on neurotransmitters in hippocampus-striatum of aluminum intoxicated rats.

Groups	NE µg/g tissue	DA µg/g tissue	5-HT µg/g tissue
Control	0.49±0.07	4.52±0.40	0.30±0.02
Lecithin (L)	0.39±0.01	4.82±0.35	0.35±0.02
Gallic acid (G)	0.55±0.03	4.96±0.08	0.35±0.03
Al	0.25±0.03[a]	2.99±0.35[a]	0.22±0.01
Al+L	0.53±0.03[b]	6.03±0.33[ab]	0.30±0.02
Al+G	0.56±0.04[b]	5.58±0.28[b]	0.34±0.02[b]
Al+L+G	0.45±0.02[b]	4.71±0.37[b]	0.31±0.02

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey's test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Pointed to the cerebral cortex oxidative stress marker, Al produced significant increment in MDA, NO and GSSG level by (101.9%, 102.5% and 29.55% respectively) relative to control group ($p<0.05$). Treatment of Al intoxicated rats with gallic acid alone or in combination with lecithin showed significant improvement in MDA level (29.74% and 41.67% respectively) (Table 4). Treatment with lecithin alone or in combination with gallic acid decreased NO content in cerebral cortex significantly by (20.99% and 49.38% respectively) compared to Al treated animals ($p<0.05$). Meanwhile, treatment of Al intoxicated rats with gallic acid did not show any significant difference in NO content. Also, non-significant change was detected in GSSG among all treated groups compared to Al group ($p<0.05$) as shown in Table (4).

On the other hand, Al intoxicated rats showed a significant decrease in cerebral cortex GSH by (34.2%) and GSH/GSSG ratio by (48.68%) when compared to control group ($p<0.05$).

Regarding to GSH all groups showed improvement while treatment of Al intoxicated rats with lecithin showed significant increment (44.16%) towards the control level versus Al group ($p<0.05$). Table (4) demonstrated that all groups revealed improvement in GSH/GSSG ratio of cerebral cortex while in combined group significant increase was detected (56.39%). It is worth to be mentioned that combined group showed more improvement near the control values for MDA, NO as well as GSH/GSSG ratio.

Table (4): Effect of lecithin and/or gallic acid on oxidative stress markers in cerebral cortex of aluminum intoxicated rats.

Groups	MDA µmol/g tissue	NO µmol/g tissue	GSH µmol/g tissue	GSSG µmol/g tissue	GSH/GSSG ratio
Control	19.97±1.38	0.40±0.04	13.01±1.11	0.44±0.04	29.58±0.89
Lecithin (L)	22.21±1.44	0.41±0.03	12.81±0.77	0.55±0.01	23.26±1.15
Gallic acid (G)	14.14±1.05	0.40±0.01	10.62±0.85	0.54±0.03	19.75±0.88
Al	40.32±2.28[a]	0.81±0.06[a]	8.56±0.64[a]	0.57±0.01[a]	15.18±1.44[a]
Al+L	35.86±1.71[a]	0.64±0.03[ab]	12.34±0.68[b]	0.54±0.02	23.08±1.91
Al+G	28.33±1.88[ab]	0.74±0.04[a]	10.95±0.46	0.53±0.01	20.82±0.95[a]
Al+L+G	23.52±1.86[b]	0.41±0.01[b]	11.10±0.95	0.49±0.03	23.74±3.78[b]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey’s test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

It can be noticed from Table (5) that hippocampus-striatum area showed a significant increase in MDA, NO and GSSG level (96.1%, 97.92% and 68.57% respectively) after Al administration when compared to control (p<0.05). Whereas, co-administration of lecithin and gallic acid in combination revealed a significant decrease near the control level of MDA, NO and GSSG (40.8%, 49.47% and 33.9% respectively) compared to Al intoxicated rats (p<0.05). On the other hand Al administration shows non-significant decrease in GSH level. Concerning to GSH/GSSG ratio, Al shows significant decrement (54.48%) in comparison with control group. Lecithin and gallic acid each one alone increased the ratio non-significantly, whereas, their combination exert significant increase in this ratio by (84.98%) (p<0.05).

Table (5): Effect of lecithin and/or gallic acid on oxidative stress markers in hippocampus-striatum of aluminum intoxicated rats.

Groups	MDA µmol/g tissue	NO µmol/g tissue	GSH µmol/g tissue	GSSG µmol/g tissue	GSH/GSSG ratio
Control	18.71±1.41	0.48±0.04	9.43±0.84	0.35±0.03	26.91±0.48
Lecithin (L)	20.36±0.99	0.47±0.04	10.48±0.37	0.44±0.01	24.08±0.67
Gallic acid (G)	13.13±0.91	0.47±0.01	7.91±0.6	0.50±0.06	16.89±1.90
Al	36.69±1.83[a]	0.95±0.06[a]	7.16±0.65	0.59±0.05[a]	12.25±0.86[a]
Al+L	33.23±4.91[a]	0.74±0.04[ab]	8.90±0.44	0.44±0.02	20.84±1.82
Al+G	26.82±1.57	0.87±0.04[a]	8.13±1.32	0.41±0.01[b]	19.56±2.79
Al+L+G	21.72±1.43[b]	0.48±0.01[b]	8.32±0.73	0.39±0.03[b]	22.66±3.79[b]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey’s test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Al administration showed a significant decrease in Bcl-2, accompanied with, significant increase in Bax, caspase-3 and Bax/Bcl-2 ratio in cerebral cortex and hippocampus-striatum areas (Table 6&7) as compared to control group (p<0.05). Treatment with lecithin, gallic acid singly or their combination revealed a significant improvement in all groups in the two tested areas. Noteworthy that the combined group showed more pronounced effect than each one alone in cerebral cortex. In hippocampus-striatum area combined group showed more improvement near the control in Bcl-2, while, gallic acid alone showed more enhancement of Bax/Bcl-2 ratio.

Table (6): Effect of lecithin and/or gallic acid on Bcl-2, Bax and caspase-3 and Bax/Bcl-2 ratio in cerebral cortex of aluminum intoxicated rats.

Groups	Bcl-2 ng/ml	Bax pg/ml	Caspase-3 pg/ml	Bax/Bcl-2 ratio
Control	54.40±3.03	136.00±1.26	36.33±1.65	2.54±0.14
L	40.00±1.31	163.33±1.84	73.67±2.01	4.11±0.18
Gallic acid	42.70±1.04	161.00±1.59	61.00±2.92	3.78±0.06
Al	22.03±0.82[a]	271.33±1.80[a]	146.33±4.15[a]	12.41±0.53[a]
Al+L	30.57±1.63[ab]	191.00±2.56[ab]	89.33±2.43[ab]	6.33±0.30[ab]
Al+G	37.67±0.85[ab]	172.33±1.28[ab]	62.00±0.97[ab]	4.58±0.08[ab]
Al+L+G	44.23±0.63[ab]	169.67±2.38[ab]	60.00±2.39[ab]	3.84±0.09[ab]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey's test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Table (7): Effect of lecithin and/or gallic acid on Bcl-2, Bax and caspase-3 and Bax/Bcl-2 ratio in hippocampus-striatum of aluminum intoxicated rats.

Groups	Bcl-2 ng/ml	Bax pg/ml	Caspase-3 pg/ml	Bax/Bcl-2 ratio
Control	49.50±1.92	146.00±1.46	34.67±2.43	2.97±0.09
L	39.17±2.21	163.00±2.22	60.00±2.92	4.23±0.23
Gallic acid	44.40±2.48	158.00±1.32	73.67±2.14	3.62±0.21
Al	21.33±0.81[a]	288.33±2.69[a]	187.00±5.11[a]	13.59±0.40[a]
Al+L	31.07±1.40[ab]	181.67±0.92[ab]	114.00±2.28[ab]	5.92±0.31[ab]
Al+G	38.47±0.56[ab]	102.67±0.92[ab]	98.67±0.76[ab]	2.67±0.05[b]
Al+L+G	43.17±1.43[b]	75.33±4.46[ab]	68.67±4.02[ab]	1.85±0.03[ab]

- Data are expressed as mean ± SE, n=6.
- One way ANOVA was first applied. Tukey's test was used for multiple comparisons; *P* <0.05 is regarded significant.
- [a]: Significant difference vs. Control group.
- [b]: Significant difference vs. Al group.

Histopathological observations of tested brain areas in control and experimental groups of rats are shown in Figure (1). The sections of brain of control rats showed normal histological structure of the neurons in the cerebral cortex (a), striatum (b) and hippocampus (subiculum) (c). No sign of degeneration was observed in tested brain areas of rats treated with lecithin or gallic acid singly as compared to control. Meanwhile, Al intoxicated rats showed marked histological changes and exhibit nuclear pyknosis and degeneration in the neurons of cerebral cortex (d) striatum (e) and the neurons of the subiculum in the hippocampus (f). Treatment of Al intoxicated rats with lecithin showed nuclear pyknosis and degeneration in some neurons of fascia dentate in hippocampus (g).

Supplementation of gallic acid with Al showed marked reduction in number of damaged cells manifested by nuclear pyknosis and degeneration in few neurons of the subiculum in the hippocampus (h). Supplementation with the combined therapy (lecithin and gallic acid) to Al intoxicated rats alleviated neurodegenerative brain changes, resulted in almost normal histological features, where nuclear pyknosis and degeneration were observed in very few neurons in the subiculum of the hippocampus (i). No histopathological alterations in the cerebral cortex as well as in the striatum were observed in lecithin and/or gallic acid co-treatment to Al intoxicated rats.

Although Al showed significant changes in all tested biochemical parameters and histopathology, no visual observations were detected.

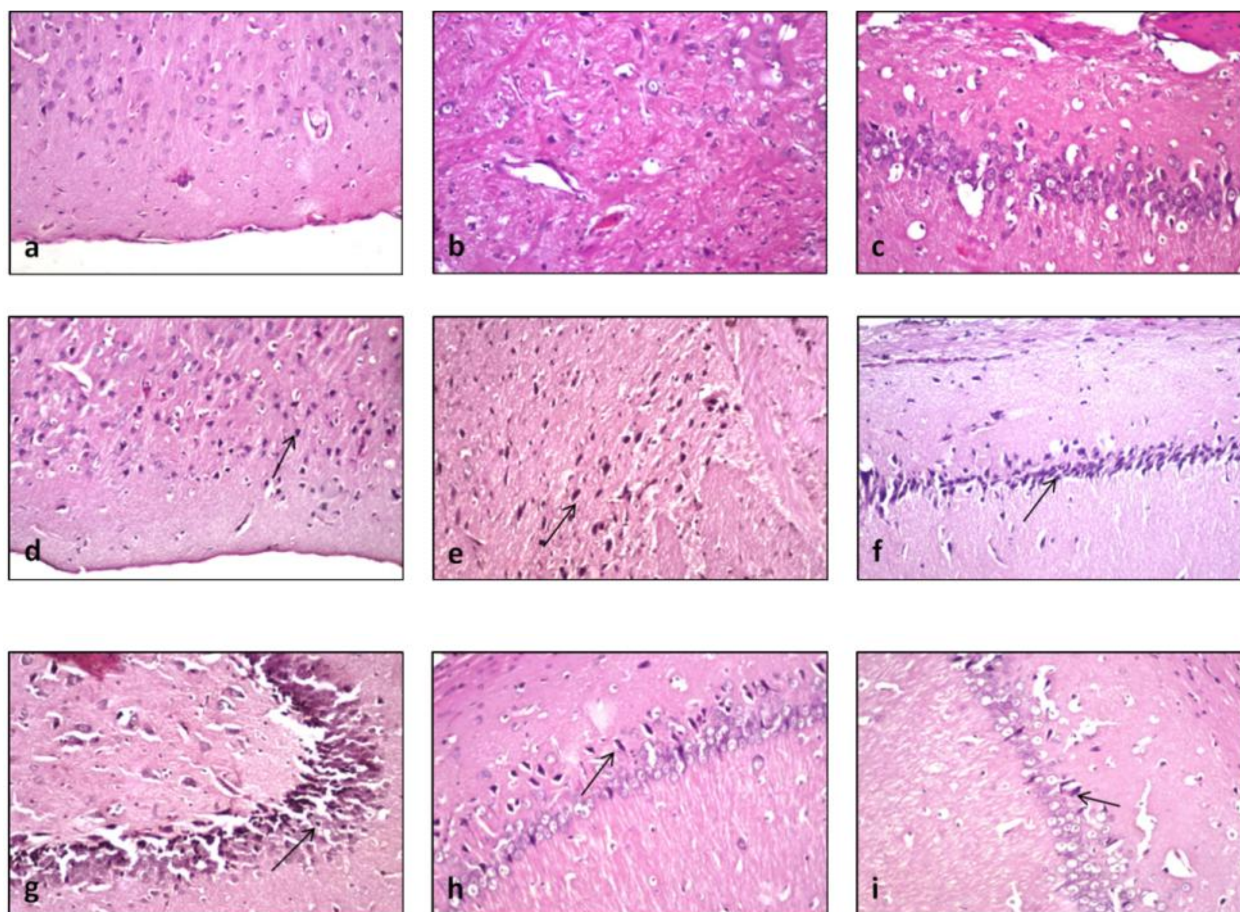

Fig.1: Histopathological observations of brain regions. Control rat brain sections showed normal histological structure of the neurons in the cerebral cortex (a) x200, striatum (b) x200 and hippocampus (subiculum) (c) x400. Al intoxicated rats showed nuclear pyknosis and degeneration in the neurons of cerebral cortex (d) x200 striatum (e) x200 and the subiculum in the hippocampus (f) x200. Treatment of Al intoxicated rats with lecithin showed nuclear pyknosis and degeneration in some neurons of foscia dentate in hippocampus (g) x400. Supplementation of gallic acid with Al showed nuclear pyknosis and degeneration in few neurons of the subiculum in the hippocampus (h) x200. The histopathological investigation of the brain sections of the rats supplemented with the combined therapy (lecithin and gallic acid) with Al showed nuclear pyknosis and degeneration in very few neurons in the subiculum of the hippocampus (i) x200. H&E.

Discussion

Aluminium interacts with the cholinergic system, acting as a cholinotoxin (neurotoxin) that provokes functional alterations in the cholinergic, dopaminergic and noradrenergic neurotransmission; therefore, it has the propensity to cause impaired cholinergic transmission by affecting the synthesis and release of neurotransmitters **(John *et al.*, 2015)**.

Our data showed that administration of Al induced cholinergic impairment in Al intoxicated rats represented by a marked significant increase of AChE activities in cerebral cortex and hippocampus-striatum areas as compared to control group. Previous studies concluded that acute, subacute, and chronic exposure to aluminum leads to its accumulation in different brain regions with subsequent elevation in cholinergic neurotransmitter and its associated enzymes (**Yellamma *et al.*, 2010 and John *et al.*, 2015).**

AChE is a specific cholinergic marker protein has received wide attention in the study of Al neurotoxicity. AChE is more responsible for acetylcholine metabolism whose alterations caused neurobehavioral changes especially memory, learning and cognitive functions therefore, the cholinergic toxic effect of aluminum may attribute to the erosion of cholinergic terminals in the cortex and hippocampus **(Platt *et al.*, 2001).** Impaired cholinergic transmission occurs in two ways: First, it occurs either due to decline in ACh release or due to decreased choline acetyltransferase activity, which results in the scarcity of ACh. Second, elevated AChE activity further adds to scarcity of ACh at the synapse by accelerating decomposition of available ACh **(Singh *et al.*, 2013)**. Excessive Al intake might lead to deposition of amyloid- (A) in central nerve cells and overexpression of -amyloid precursor protein (**Campbell *et al.*, 2000 and Exley, 2005**). The neurotoxicity of A is associated with oxidative stress **(Bush *et al.*, 1999)** and with the generation of reactive oxygen species that damage neuronal membrane, lipids, proteins, and nucleic acids. Acetylcholinesterase (AChE) has been found to co localize with A deposits and promotes the assembly of A into amyloid fibrils forming A -AChE complex that is more toxic than amyloid fibrils **(Holmquist *et al.*, 2007)**. It is well known that A induces elevations of intracellular free Ca^{2+} by increasing calcium entry through L-type voltage-dependent calcium channels (**Mattson, 2004**), and AchE release is a Ca^{2+}-dependent phenomenon (**Schweitzer, 1993**). Furthermore, oxidative stress, which is a key protagonist in AD, also plays a role in the enhancement of AChE activity induced by A peptide **(Melo *et al.*, 2003)**. The current decrease of biogenic amines in the selected brain areas in Al intoxicated rats run in parallel with **Mohamd *et al.* (2011); Mahdy *et al.* (2012), Borai *et al.* (2017) and Singla & Dhawan (2017)**. The significant decrease in dopamine (DA) level may be due to intervention of Al with the dopaminergic system or to its ability to induce oxidative stress. The current decreased level of DA and altered cholinergic function might also be attributed to increased monoamine oxidase (MAO) activity, that led to increased degradation of dopamine **(Singla & Dhawan, 2017)**. Also, **Crockett *et al.* (2008)** reported that oxidative stress and inflammation cause deficiency of several major neurotransmitters, including ACh and DA. In animal models of Al intoxication, decreased dopamine content in striatum by 40% was reported by **Ravi *et al.* (2000)**. Furthermore, Al can exert inhibiting activity on different levels; including inhibition of DA b-hydroxylase (responsible for the conversion of DA into norepinephrine) and inhibition of tryptophan decarboxylase activity (responsible for DA formation) as suggested by **Kim *et al.* (2007a)**.

In addition, there are many studies reported that Al decreased serotonin levels in different brain regions as cortex, hippocampus, septum, striatum, cerebellum and brain stem **(Ganrot, 1986; Beal *et al.*, 1989; Ravi *et al.*, 2000 and Kumar, 2002).** Serotonine is a key neurotransmitter involved in eating, sleeping, behavior and neuroendocrine functions. Low serotonine levels have been associated with cholinergic hypofunction. Al intoxication causes a decrease in serotonine levels and its deaminated derivative, 5-hydroxyindole acetic acid in brain cortex and hippocampus **(Kumar, 2002)**. These alterations could be due to the loss of cholinergic input which normally inhibits serotonine release **(Hörtnagl *et al.*, 1987)**, and/or to the activation of monoamine oxidase (MAO) enzyme, responsible for 5-hydroxyindole acetic acid generation, which is activated by Al intoxication **(Zatta *et al.*, 1998; 1999)**. Increased AChE activity in the current study was very much in favor of the suggestion of decreased 5-HT level consequent to loss of cholinergic input because the availability of acetylcholine would be reduced with increase in the activity of AChE **(Kumar, 2002)**.

Besides the fact that Al is a cholinotoxin agent, its neurotoxic effect could be exerted by additional mechanisms, such as induction of oxidative stress. Although Al is not a redox-active metal, there is

extensive experimental evidence on oxidative stress-mediated Al neurotoxicity **(Nayak, 2002 and Kumar & Gill, 2009)**. Oxidative stress has long been implicated in the initial and later stages of neuronal degeneration **(Melo *et al*., 2011)**.

In the present study elevation in MDA and depletion of reduced glutathione (GSH) levels combined with increase in GSSG level subsequently decreased GSH/GSSG ratio in cerebral cortex and hippocampus-striatum areas were recorded in Al-intoxicated rats. Thus, it can be hypothesized that oxidative stress could be one of the contributing factors for Al-induced central nervous system disorders which may be due to the effect of Al on GSH synthesis by decreasing the activity of glutathione synthase (GS), a rate-limiting step of whole reaction, that leading to reduced GSH content. Our hypothesis was in accordance with the previous studies by (**Christen, 2000; Zatta *et al*., 2002 and Ahmed *et al.*, 2011**). Moreover, it has been concluded that aluminum is able to inhibit NADPH-generating enzymes leading to inadequate supplementation of NADPH which is the main factor for GSH generation (**Zatta *et al.,* 2000**). A depletion of cellular GSH can impair cellular defenses against the toxic actions of reactive oxygen species (ROS) and other compounds that lead to cellular injury and death **(Wüllner *et al.*, 1999)**. Several reports have demonstrated increased lipid peroxidation levels and decreased GSH content in rat brain regions after long term oral administration of $AlCl_3$ **(Jyoti & Sharma, 2006; Nehru & Bhalla, 2006; Linardaki *et al.*, 2013, Oda, 2016 and Khafaga, 2017).**

Pointed to the current increase in NO level in the selected brain areas it can be deleterious to neuronal tissue, where nitric oxide released from activated glia inhibits neuronal respiration resulting in decreased ATP levels, glutamate release and excitotoxicity **(Bal-Price & Brown, 2001)**. Other mechanism by which nitric oxide causes oxidative changes to biomacromolecules (proteins, lipids and DNA) is ascribed to its ability to react with many other free radicals including the superoxide radical. This latter reaction results in the formation of the highly reactive peroxynitrite radical, capable of causing oxidative and/or nitrosative damage to tyrosine residues, thiols, DNA and unsaturated fatty-acid-containing phospholipids **(Szabó *et al.*, 2007)**. Recently **Abdel-Salam *et al.* (2016)** confirmed our results as he reported increased expression of iNOS in brain following AlCl3 treatment and suggesting that iNOS is responsible of increased brain nitric oxide following injection of $AlCl_3$.

Apoptosis is one of the mechanisms contributing to neuronal loss in Alzheimer's disease and Al induced neurodegeneration **(Singla & Dhawan, 2015)**. It has been shown that a wide variety of agents and conditions such as disturbance in calcium homeostasis, mitochondrial dysfunctioning, glutamate receptor stimulation and oxidative stress could also lead to apoptosis **(Rong & Distelhorst, 2008)**. Members of the bcl-2 family of proteins are considered as primary regulators of apoptosis. The bcl-2 family consists of various anti-apoptotic (bcl-2, bcl-xl, bcl-w, and mcl-1) and pro-apoptotic (bax, bak, bik, bad, and bcl-xs) members. The best characterized are Bcl-2 and Bax. Another kind of primary regulators of the cell death is a novel family of cysteinyl aspartate specific proteases, termed as caspases. Among them, Caspase-3, a primary executioner caspase, is the downstream regulator of many others including bcl-2 family proteins **(Cui *et al.*, 2007)**. In view of the obtained data, Al resulted in decreased Bcl-2 whereas increased Bax and caspase-3 subsequently increase in Bax/Bcl-2 ratio in cerebral cortex and hippocampus-striatum areas. This indicates the progression of apoptosis as a consequence of Al induced oxidative cellular stress **(Ghribi *et al.*, 2002; Niu *et al.*, 2005 and Singla & Dhawan, 2015)**. Additionally, **Liang *et al.*, (2004)** announced that the increase of caspase-3 activities is considered as one of the vital indicators during the process of neurodegeneration and reflects the alteration at the mRNA level. Similar results have been shown by **Johnson *et al.* (2004)** after Al maltolate treatment in neuro-2a cells. Induction of apoptosis is may be due to accumulation of Al in neurons following cell depolarization, which leads to inhibition of Na^{+}/Ca^{2+} exchange and thereby induces an excessive accumulation of mitochondrial Ca^{2+}. Further, increase in intra mitochondrial Ca^{2+} levels can lead to an opening of mitochondrial transmembrane potential, with release of cytochrome c and subsequent apoptosis resulting from activation of the caspase family of proteases **(Toninello *et al.*, 2000; Ghribi *et al.*, 2001 and Vasudevaraju *et al.*, 2008)**. In addition, Al induced oxidative DNA injury can also initiate the process of apoptosis in neurons **(Singla & Dhawan, 2015)**. The ratio of Bax/Bcl-2 plays an important role in determining whether cells will undergo apoptosis under experimental conditions that promote cell death. This ratio of Bax/Bcl-2 affects the levels of mitochondrial membrane potential in cells after exposure to inducer of apoptosis **(Merry & Korsmeyer, 1997)**. Other investigators **(Cory & Adams, 2002)** also reported that mitochondrial release of cytochrome c can be controlled by the ratio of Bax/Bcl-2 proteins and may be activated by

proteolytic cleavage and heterodimerization. Increase of Bax / Bcl-2 ratio, initiate mitochondrial dependent apoptotic pathway and ultimately causes activation of caspases. Furthermore, activated caspase-3 cleaves numerous nuclear enzymes leading to activation of caspase-activated DNAse (CAD) and cleavage of chromosomal DNA resulting in morphologic apoptosis (**Lukiw, 2010 and Zeng *et al.*, 2012**).

Lecithin is a major dietary source of choline and has important biological activities, in addition to its role in maintaining memory, cholinergic signaling, and as a precursor to important neurotransmitters mainly acetylcholine where it is metabolized to choline (**Chung *et al*., 1995**). Surprisingly, in the current study cerebral cortex AChE activity was significantly changed without noticeable improvement for lecithin. Similar result was concluded by **Zaki & Hassanin (2016)** where they reported that lecithin failed to restore serum cholinesterase activity against ifosfamide induced neurotoxicity. While, in hippocampus-striatum area lecithin showed significant inhibition in AChE activity, our results agreement with **Ahmed *et al*. (2011)**. Supplementation of lecithin to Al intoxicated rats increased DA, NE and 5-HT levels in two tested areas. Our results in agreement with previous study of **Zaki & Hassanin (2016)** who reported that lecithin attenuates ifosfamide neurotoxicity by improved DA and 5-HT levels in rat brain areas. Also, lecithin showed neuroprotective roles against ischemia/reperfusion insult in rat brain (**Aabdallah and Eid, 2004).** In the current study lecithin improved oxidative stress, through decreased MDA and NO levels and increased GSH which may be attributed to its strong antioxidant properties as previously demonstrated by **Navder *et al*. (2000); Lee *et al*. (2013) and Khafaga (2017)**.

Treatment of Al intoxicated rats with lecithin has been reported to regulate Bcl-2, Bax and caspase-3, as a consequence of this Bax/Bcl-2 ratio. Our results run in accordance with **Zaki & Hassanin (2016).** Furthermore, **Li & Ren, (2010)** reported that adding lecithin to diet is helpful to neural development, improvement of spatial learning, memory and decreases the neuronal apoptosis in cortex and hippocampus. Also, lecithin at a dose of 50 and 100 mg/ kg body weight prevented disruption of mitochondrial membrane, up regulation of Bax and down regulation of Bcl-2 mRNA levels in the liver of D-Galactosamine intoxicated rats (**Raj *et al*., 2011**).
Gallic acid supplementation clearly decreased AChE activity in hippocampus-striatum area, suggesting that GA may regulate cholinergic function in this area. Also, it improved NE, DA and 5-HT levels as well as attenuation of LPO. We can conclude that GA can pass through blood brain barrier and improve neuronal function and suppressing ROS generation. The current results run with (**Nabavi *et al*., 2011 and Borai *et al*., 2017)** and supported by previous studies by **Pervin *et al*. (2014)**, who suggested that polyphenols could offer AChE inhibitory activity and antioxidant effects, therefore ameliorating damaged membrane integrity. Also, **Yadav *et al*. (2017)** stated that gallic acid indicating protective effects in psychosis through reduced MDA level, increased glutathione level, restored dopamine level in brain mice and decreased AChE activity, thus showing the enhanced process of learning and memory. Additionally, **Kim (2007)** reported that treatment with GA lead to an increase in GSH levels and a reduction in oxidized glutathione (GSSG). The improvement in monoamine levels confirmed by (**Chhillar & Dhingra, 2013)** who announced that antidepressant-like effect shown by gallic acid may be attributed to inhibition of MAO-A and consequent increase in brain monoamine levels in both unstressed and stressed mice. Further, resveratrol as a polyphenol ameliorate NE, DA and 5-HT level in cerebrum biogenic amines in rat brain. Moreover, it decrease MDA level and concomitantly restored GSH content in different brain regions **(Pal & Sarkar, 2014)**.

Treatment of Al intoxicated rats with GA caused significant increase in Bcl-2 and decrease in Bax, caspase-3 as well as Bax/Bcl-2 ratio. Our results run in parallel with **Lo *et al.* (2010)** who propose the signaling pathway of GA induced apoptosis in A375.S2 cells and show that GA triggers apoptosis through the regulation of Fas/FasL, Bax and Bcl-2 and through activation of the caspase cascade (caspase-3, -8 and -9) or through the dysfunction of mitochondria leading to apoptosis. **Fernández-Fernández *et al*. (2012)** stated that polyphenols, as antioxidants, could lead to the inhibition of neuronal apoptosis induced by neurotoxicants (such as AlCl3) and to the promotion of neuronal survival and synaptic plasticity.

Histopathological investigation showed that Al causes neuronal damage and degeneration in the hippocampus and striatum areas. This result agrees with the previous report of **Ahmed *et al*. (2011).**

Oxidative stress has long been implicated in the initial and later stages of neuronal degeneration **(Melo *et al***.**, 2011)**. Excessive Al intake might lead to deposition of amyloid- (A) in CNS **(Campbell *et al*., 2000 and Exley, 2005)**. The neurotoxicity of A is associated with oxidative stress **(Bush *et al*., 1999)** and with the generation of reactive oxygen species that damage neuronal membrane. Further, apoptosis is one of the mechanisms contributing to neuronal loss in Al induced neurodegeneration **(Singla & Dhawan, 2015)**. Treatment of Al-intoxicated rats with gallic acid improved the structure of neuronal cells in the hippocampal region. These results are consistent with those of **Borai *et al*. (2017)** who indicates the strong potential of polyphenols to limit or delay neurodegeneration, and to reverse the Al-induced neurotoxicity**.** Furthermore, **Pervin *et al*. (2014)** suggested that polyphenols could offer AChE inhibitory activity and antioxidant effects, therefore ameliorating damaged membrane integrity. The combined therapy showed remarkable amelioration of brain architecture since the brain cells appeared more or less similar to the cells of control rats.

Our results showed that hippocampus is the most severely affected area, this may attributed to Al as a neurotoxicant potentially affecting ionic, cholinergic, and dopaminergic neurotransmission in the central nervous system and these alterations are known to be associated with learning ability. It accumulates in all most all the brain regions following chronic exposure and the maximum being in hippocampus, which is the site of memory and learning **(Yuan *et al*., 2012)**. In a study using electrothermal atomic absorption spectroscopy of the aluminium content of the arterial walls of eight arteries which supply the brain, it was found that aluminium concentration is far higher in the posterior cerebral artery (arteria cerebri posterior), which supplies the hippocampus, in late-stage AD patients **(Bhattacharjee *et al*., 2013)**. In addition, the same authors indicated the presence of biochemical mechanisms in the endothelial cells supplying the cerebral vasculature which enable the binding of aluminium to selected areas such as the hippocampus, known to play a major role in the pathogenesis of the illness.

It is worth to be mentioned that combined treatment showed more pronounced effect in all tested biochemical parameters. Therefore, we suggest that the synergistic effect of lecithin and gallic acid in the combined therapy results in marked neuroprotective action rather than the individual treatment with either one in reducing Al neurotoxcity. In addition, combined therapy counteracted NO overload in Al intoxicated brain areas. The above outcome may protect brain from oxidative injury through a nitric oxide dependent mechanism. Notably, combined therapy stabilized the reduced level of biogenic amines in tested areas of Al intoxicated rats. These effects of combined therapy further supported its neuroprotective action against Al toxicity.

Conclusion

The current study revealed that treatment of Al intoxicated rats with lecithin and/ or gallic acid can retrieve the effect of aluminum toxicities on the basis of cholinergic, dopaminergic, oxidative, and apoptotic status. Noteworthy, combined group revealed more pronounced modulatory effect in all tested biochemical parameters and histopathology.

Recommendations

Collectively, observations in the present study provide conclusive evidences that Al toxicity to human beings needs special attention for avoiding unnecessary exposure to environmental sources of aluminium salts. As Al cookware may be replaced by safer alternatives, while Al containing antiperspirants, potentially implicated in the rise of cases of breast cancer, may be replaced by non-aluminium versions. The use of Al salts in medical products is a more contentious issue. While antacids are available which do not contain Al salts, finally, the avoidance of vaccines which contain aluminium salts as adjuvants.

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