

Oxidative Stress in Brains of Male Rats Intoxicated With Aluminum and the Neuromodulating Effect of Some Forms of Sage (*Salvia officinalis*)

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Abstract: The present study was designed to investigate the role of oxidative stress and the status of antioxidant system in the management of aluminum chloride (AlCl₃) induced brain toxicity in rats and further to elucidate the potential role of three forms of *Salvia officinalis* (sage) in alleviating such negative effects.

The results revealed that the levels of thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) were significantly increased, however, the activities of superoxide dismutase (SOD) and catalase (CAT) as well as the reduced glutathione (GSH) content were significantly decreased in the cerebral cortex (Co) and hippocampus (Hip) of rats intoxicated with AlCl₃. In addition, the lipid profile, total lipids (TL), total cholesterol (TC) and triglycerides (TG) were significantly increased in serum and the mentioned brain regions, while phospholipids (PL), total protein (TP) and serum HDL-C were significantly decreased in AlCl₃ group. Additionally, serum and brain regions acetylcholinesterase (AChE) as well as alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were significantly increased. On the other hand, the results exhibited that, sage when given in any form along with AlCl₃ was able to regulate the mentioned parameters and the values returned close to the normal ones. It can be concluded that Al-induced neuronal oxidative stress and inhibition of the antioxidant system, accompanied with disturbed lipid profile, total protein and enzyme activities could be the cause of AlCl₃ neurotoxicity. In addition three different sage forms, by their antioxidant constituents, could be able to antagonize Al neurotoxicity perhaps by reducing the oxidative stress and improving the antioxidant status and particularly by inhibiting the acetylcholinesterase activity, thus may improve memory and other brain cognitive activities.

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1. Introduction:

Aluminum has been implicated in many human neurodegenerative diseases; various investigations have suggested that Alzheimer's disease (AD) is more common in areas where Al content in water supplies is the highest (Lynch *et al.*, 2000). Alzheimer's disease is a complex, multifactor, heterogeneous mental illness, which is characterized by an age-dependent loss of memory and an impairment of multiple cognitive functions (Mattson, 2004) and has been shown to be associated with both plaques and tangles in the brain. Indeed, the brain is a target of Al toxicity which can alter blood-brain barrier (BBB) mediating Al transport to the brain (Zatta *et al.*, 2002a) and gets deposited in the cortex (Platt *et al.*, 2001) and hippocampus (Struys-Ponsar *et al.*, 1997). This can be occurring by altering the physiological ligands present at these barriers in states (Yokel, 2001).

Possible mechanisms of Al induced neurotoxicity have been related to cell damage via free radical production and oxidative stress (Kumar *et al.*, 2009a, b). High aluminum levels exposure leads to increased central nervous system (CNS) Al

concentrations that altered CNS concentrations of the essential trace elements; iron and manganese and increased the susceptibility of CNS to lipid peroxidation (LPx) (Oteiza *et al.*, 1993).

Oxidative stress, caused by reactive oxygen species (ROS), is known to cause the oxidation of biomolecules leading to cellular damage. Increased lipid peroxidation (LPx) is the major consequence associated with oxidative stress. It is also speculated to be pathologically important in various neurodegenerative processes including cognitive deficits that occur during normal cerebral aging, Alzheimer's (AD), and Parkinson's diseases (Gray *et al.*, 2003). Alternatively, the most remarkable biochemical change in AD patients is a reduction of acetylcholine (ACh) levels in the hippocampus and cortex of the brain (Jaen *et al.*, 1996). Therefore, inhibition of acetylcholinesterase (AChE), the enzyme responsible for hydrolysis of ACh at the cholinergic synapse, is currently the most established approach to treating AD (Akhondzadeh *et al.*, 2003).

On the other hand, *Salvia officinalis* (sage) specially the oil had apparent dual cholinergic activity, as it was active on both, AChE and

butyrylcholinesterase (BuChE) (Savelev *et al.*, 2004). Besides the cholinergic activity, there has already been a wider range of activities reported for the genus *Salvia*, which may be relevant for CNS disorders. These include antioxidant (Celik and Isik, 2008 and Carla *et al.*, 2009), nicotinic activity (Wake *et al.*, 2000), anti-inflammatory properties (Moretti *et al.*, 1997), and glutamergic activities (Kuang and Xiang, 1994). The essential oils of the plant, also, tested for its memory-enhancing effect (Akhondzadeh *et al.*, 2003). Therefore, the main goal of the present study was to examine the possible mechanisms by which Al exposure could induce Alzheimer-like condition related alterations in brain of male rats, and extend to investigate the beneficial effects of sage in preventing or modulating these risks.

2. Materials and methods

Material and methods

Chemicals:

Aluminum Chloride (AlCl₃) was obtained from agents of Sigma Chemicals (St. Louis, MO, USA). *Salvia officinalis* (sage) oil was obtained from (NATURE'S ALCHEMY) distributed by LOTUS BRANDS, USA. Dried leaves of sage, for preparations of sage tea (water extract and ethanolic extract), were purchased from a local herb market. The taxonomic identity of the plant was confirmed by the botanist of the Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt. All other chemicals were purchased locally and were of analytical reagent grade.

Sage extracts preparation:

1- Sage water extract (sage tea): was routinely prepared by pouring 150 ml boiling water onto 2 g of dried grounded leaves and allowing it to steep for 5 min. (Lima *et al.*, 2005).

2- Sage ethanolic extract: was prepared according to the method described by Eidi *et al.* (2006). Dried grounded leaves of *Salvia officinalis* (60 g) were subjected to extraction with 300 ml of ethanol (80%) in a glass container for 72 h. The extract was decanted and filtered through Whatman No 1 filter paper into a clean flask. The same procedure was repeated a further two times. The solvent was evaporated using a rotary evaporator, and then the flask was weighed to determine dried weight of extract. The supernatant was reconstituted using 53% ethanol and assayed using serial dilutions and the dose was calculated according to Ghosh, (1971)

3- Sage oil: diluted 1:2 in sunflower oil according to Perry *et al.* (2002).

Experimental animals:

This study was carried out on 48 adult male albino rats weighing 130 ± 10 g b.w., supplied by The Urology & Nephrology Center; Mansoura University. The rats were maintained under controlled humidity; temperature (25 ± 2°C) and light (12h light/ 12h dark). They were fed standard commercial rodent pellet diet and water *ad libitum* (free access to water and food).

Experimental Protocol:

After one week of acclimatization, the rats were divided into 8 groups consisting of 6 animals each. All treatments were continued for 90 days as follows:

1. Normal control
2. Sage water extract (given instead of drinking water) according to Lima *et al.* (2005).
3. Sage ethanolic extract (given orally by stomach tube as 0.1 ml/kg b.w.) (Akhondzadeh *et al.*, 2003).
4. Sage oil group (given orally by stomach tube as 100 µl/ kg b.w.) every other day (Perry *et al.*, 2002).
5. Aluminum (Al) treated group (mixed with diet as 100mg AlCl₃/ kg b.w.) (Bilkei, 1993).
6. Al + sage water extract group (given as in groups 5&2 respectively).
7. Al + sage ethanolic extract group (given as in groups 5&3 respectively).
8. Al + sage oil group (given as in groups 5&4 respectively).

Sample preparation:

At the end of the experimental period, overnight-fasted animals were decapitated, blood samples were collected and sera were separated and stored at -20°C until biochemical assay. The brain was then gently removed; the cerebral cortex and hippocampus were separated on an ice-chilled glass plate as described elsewhere (Nayak and Chatterjee, 2001). The tissue samples were quickly frozen on dry ice, weighed, and stored at -80°C until biochemical assay. Cortex and hippocampus were chosen for the present study because; aluminum affects more severely the cortex and hippocampus regions than any other area of the central nervous system (Urano *et al.*, 1997). Also, these brain regions are known to be particularly susceptible in Alzheimer's disease, and have an important role in learning and memory functions (Bihaqi *et al.*, 2009).

Biochemical analysis:

Determination of lipid peroxidation product thiobarbituric acid reactive substances (TBARS) was carried out according to the method of Ohkawa *et al.*, (1982). Meanwhile, protein carbonyl was

measured spectrophotometrically according to the method of Smith *et al.*, (1991). On the other hand, superoxide dismutase (SOD) and catalase (CAT) activities were determined following the methods of Nishikimi *et al.*, (1972) and Bock *et al.*, (1980), respectively. Additionally, reduced glutathione (GSH) content was determined spectrophotometrically according to the method of Prins and Loose, (1969) as well as Acetylcholinesterase (AChE) activity was measured according to the method of Ellman *et al.*, (1961). Alkaline phosphatase (ALP), acid phosphatase (ACP), total protein (TP), total lipids (TL), phospholipids (PL), triglycerides (TG), total cholesterol (TC) in (serum, cortex and hippocampus) and serum HDL cholesterol were determined by using commercial kits from (Biodiagnostic, 29 Tahreer Str., Dokki, Giza, Egypt).

Statistical analysis:

Data were presented as means \pm standard error (SE). The statistical analysis was performed with one-way analysis of variance (ANOVA) using SPSS (version 17) software package for Windows followed by *Dunnett test*. A p-value of less than 0.05 was considered statistically significant.

3. Results:

Cortex and Hippocampus Lipid Peroxidation (LPx), Protein Carbonyl (PC) and Antioxidants:

As observed in table (1), the statistical analysis showed that the level of cortex and hippocampus TBARS and PC were significantly ($p < 0.05$) increased by Al intoxication in comparison with the control value.

Concerning sage only, there were significant ($p < 0.05$) decreases and increases in hippocampus TBARS level and catalase activity respectively in the groups administered (ethanolic extract and oil) comparing to the normal control group. Regarding the antioxidants, the data indicated that, Al group exhibited significant ($p < 0.05$) reduction in cortex and hippocampus SOD, CAT activities as well as GSH content compared to normal control group.

Sage administration to rats intoxicated with Al caused a significant ($p < 0.05$) reduction in the elevated cortex and hippocampus TBARS and PC concentrations compared to Al intoxicated group. The reduction in hippocampus TBARS reached levels below the normal ones and was significant ($p < 0.05$), only, in (Al + sage ethanolic extract) group. On the other hand, daily administration of sage preparations to Al intoxicated animals revised the decreases in the cortex and hippocampus SOD and CAT activities as well as GSH contents to marked increases compared to Al intoxicated group.

Indeed, the modulating effects of sage preparations on both the oxidative stress markers (LPx and PC) and the antioxidant system (SOD, CAT and GSH) arrived them to values within the normal ranges where non significant ($p < 0.05$) alterations were seen in comparison with the normal group except a significant ($p < 0.05$) reduction in Hip LPx of (Al + sage ethanolic extract) and a significant ($p < 0.05$) increase in Hip CAT of (Al + sage water extract) and (Al + sage oil) groups as well as in Hip GSH of (Al + sage oil) group.

Serum, Cortex and Hippocampus Total Lipids (TL), Total Cholesterol (TC), Triglycerides (TG) and Phospholipids (PL) Concentrations:

The data presented in table (2), exhibited that, serum, cortex and hippocampus total lipids (TL), total cholesterol (TC), and triglyceride (TG) contents showed significant ($p < 0.05$) increases, but phospholipids (PL) exhibited significant ($p < 0.05$) reductions in Al intoxicated animals in comparison with normal control.

Concerning sage only, cortex phospholipids content was significantly ($p < 0.05$) increased in the groups administered (sage water extract and oil) and in hippocampus of (sage ethanolic extract and oil) groups comparing to the normal control group. But, significant ($p < 0.05$) decreases were observed in TC of cortex in (sage water extract and ethanolic extract) as well as Hip TC and TG in the groups administered (sage oil). Such results referred to the benefits of sage preparations, specially the oil for reducing TC and increasing brain PL.

On the other hand, concomitant administration of sage (different preparations) with Al reduced the elevation of serum, cortex and hippocampus TL, TC and TG contents and enhanced serum, cortex and hippocampus PL approaching most of them to the normal values except in case of cortex PL only of (Al + sage water extract) which showed a non-significant ($p < 0.05$) increase compared to Al intoxicated animals indicating no protection.

However, the reduction in serum, cortex and Hip TL levels reached to values within normal levels, except Hip TL in Al + sage ethanolic extract which, still, exhibited a significant ($p < 0.05$) reduction compared to normal control group. Regarding TC, the reduction was, still, significant ($p < 0.05$) in both, cortex of Al + sage ethanolic extract and hippocampus of Al + sage oil groups comparing to control one. Concerning TG, the reduction arrived to values significantly ($p < 0.05$) lower than normal in serum TG of Al + sage ethanolic extract and Hip TG of Al + all sage preparations groups comparing to control one.

On the other hand, the enhancement in serum and cortex PL by sage was non-significantly ($p < 0.05$) changed in (Al + all sage preparations) compared to normal control. But in the Hip, PL reached to values that still significantly ($p < 0.05$) lower than normal in all Al + sage different preparations groups.

Serum HDL-C, Serum, Cortex and Hippocampus Total Protein (TP) Content:

As seen in table (3) serum, cortex and hippocampus total protein content and serum HDL-C showed highly significant ($p < 0.01$) decreases in Al intoxicated animals compared to the normal control group. Concerning sage only, total protein exhibited a significant ($p < 0.05$) increase in hippocampus of the groups administered all sage preparations compared to the normal control group.

Administration of different preparations of sage to Al intoxicated animals reversed the decrement in serum, cortex and hippocampus total protein contents and serum HDL-C to significant ($p < 0.05$) increases compared to Al group. But, there were non-significant ($p > 0.05$) elevations in serum HDL-C concentration of Al + all sage preparations except Al + sage water extract group which exhibited a non-significant ($p < 0.05$) reduction compared to normal control group. Interestingly, the elevation in TP arrived to the normal levels except hippocampus total protein in Al + sage water extract and Al + sage ethanolic extract which showed significant ($p < 0.05$) increases compared to the normal control group. These results indicated pronounced ameliorating effects of sage oil, followed by sage ethanolic extract, the water extract showed the lowest protective effect

Serum, Cortex and Hippocampus Acetylcholinesterase (AChE), Alkaline phosphatase (ALP) and Acid Phosphatase (ACP) Activities:

As shown in table (4), in Al intoxicated rats, there were significant ($p < 0.05$) elevations in serum, cortex and hippocampus AChE, ALP and ACP activities compared to normal control ones. Concerning sage only, ALP and ACP activities were significantly ($p < 0.05$) decreased in hippocampus of (sage oil) group only compared to normal control one.

On the other hand, significant ($p < 0.05$) reductions were seen in all Al + sage treated groups serum, cortex and hippocampus AChE, ALP and ACP activities comparing to Al intoxicated group, arriving the values within the normal levels, with the exception of AChE activity, which was still significantly ($p < 0.05$) higher than normal control in cortex of Al + sage ethanolic extract group and hippocampus in (Al + sage oil) group in comparison with normal control.

Regarding ALP activity, it was still significantly ($p < 0.05$) higher than the control activities in all Al + sage preparations groups except serum (Al + sage water extract) and cortex Al + sage ethanolic extract groups which showed non-significant ($p < 0.05$) elevations (within normal ranges) when compared to control.

Concerning serum, cortex and Hip ACP activities, marked ameliorations were seen, where non-significant ($p < 0.05$) decreases and increases were shown respectively compared to normal control animals except hippocampus ACP activity of Al + sage ethanolic extract and Al + sage oil groups which were still, significantly ($p < 0.05$) declined comparing to the normal control.

3. Discussion:

Aluminium has an association with the etiology of Alzheimer's disease and some other neurodegenerative diseases. It exerts its toxic effect on nervous system especially at high concentration, causing loss of memory, speech disturbances, dyspraxia, tremors, jerking movement's impaired muscular coordination and paralysis (Drago *et al.*, 2008). *Salvia officinalis* (common sage) is a medicinal plant that has strong antioxidant properties (Baricevic and Bartol, 2000). For that reason, the present study aimed to look into the antioxidant potential of various sage preparations against Al neurotoxicity.

In the present study, there were significant increases in the oxidative stress markers lipid peroxidation (LPx) and protein carbonyl (PC) contents following Al exposure for 90 days in both cerebral cortex and hippocampus regions of rats. Such results are in harmony with those obtained by Deloncle *et al.* (1999) and Johnson *et al.* (2005) who reported that the neurotoxicity of Al may be a result of LPx. Furthermore, Nehru and Anand (2005) reported a significant increase in brain thiobarbituric acid reactive substances in rats after stimulation by Al salts which was known to be bound by the Fe^{3+} carrying protein transferrin, thus reducing the binding of Fe^{2+} and increasing free intracellular Fe^{2+} that causes the peroxidation of membrane lipids and consequently membrane damage. Aluminum, being an inert metal, has been suggested to induce oxidative damage indirectly by potentiating the peroxidative effect of Fe^{2+} . It promotes reactive oxygen species (ROS) formation. ROS subsequently attack almost all cell components including membrane lipids thus producing lipid peroxidation (Christen, 2000). The findings of the present study, also, showed that the rise in LPx in Al treated rats was accompanied by concomitant decrease in the activity of some antioxidant enzymes involved in the detoxification of

ROS, namely SOD, CAT as well as the level of GSH in the cortex and hippocampus tissues comparing with the control declaring the prooxidant effect of Al. These findings agreed with the antecedent studies of Savory *et al.* (2003) and Johnson *et al.* (2005) whom showed that Al exposure enhanced the neuronal lipid peroxidative damage with concomitant alterations in the enzymatic antioxidant defense status, thus having serious bearing on the functional and structural development of the central nervous system (Dua and Gill, 2001). Similar data recorded a decrease in the antioxidants such as GSH (Wu and Cederbaum, 2003) and SOD activity (Yousef, 2004) in the brain of Al exposed rats (Chainy *et al.*, 1996) and human (Dua and Gill, 2001).

Moreover, such results are consistent with the studies indicated that Al intake produced an oxidative stress-related change, contributed to its neurotoxicity (Flora *et al.*, 2003). However, in rats, a significant relationship between Al exposure and the presence of oxidative stress was established also by Gómez *et al.* (2005). This could be caused by inflicting damage to membrane lipids, proteins and antioxidative enzyme defense system (Jyoti *et al.*, 2007).

The elevation of LPx in the cortex and hippocampus in the present study and other ones (Dua and Gill, 2001) suggested participation of free-radical-induced oxidative cell injury in mediating neurotoxicity of Al. Lipid peroxidation of biological membranes results in the loss of membrane fluidity, changes in membrane potential, an increase in membrane permeability and alterations in receptor functions (Nehru and Anand, 2005 and Albendea *et al.*, 2007).

However, the increased Al concentration could deleteriously affect the neurons, leading to depletion of antioxidants and metal ions (Kumar *et al.*, 2008) through the induction of free radicals, that exhausting SOD and CAT which function as blockers of free radical processes. These results are in accordance with (Nehru and Anand, 2005) who recorded a significant decrease in the activities of SOD and CAT in brain of rats after Al treatment. Alternatively, the decreased enzyme activities could be related to a reduced synthesis of the enzyme proteins as a result of higher intracellular concentrations of Al (Albendea *et al.*, 2007).

The data obtained by the present study illustrated, further, that administration of water, ethanolic extracts of *S. officinalis* as well as sage essential oil to Al treated rats caused a significant decrease in the level of TBARS and protein carbonyl in the cerebral cortex and hippocampus and elevated the SOD and CAT enzymes activities and GSH contents when compared with Al intoxicated rats.

Moreover, the plant extracts and oil significantly, improved or restored the normal activities of the antioxidant enzymes (SOD and CAT) and GSH in both of the cortex and hippocampus regions as compared to normal control.

Generally, the antioxidant effects of sage extracts have often been attributed to phenolic and monoterpenic compounds (Ren *et al.*, 2003). Flavonoids are a diverse group of polyphenols (Havsteen, 2002) rosmarinic acid being the most representative that possess several modulatory effects, either inducing or decreasing the expression of SOD and CAT enzymes depending on structure, concentration, and assay conditions. Rosmarinic acid is the predominant phenolic compound in sage (Lima *et al.*, 2005) and its effects was attributed to the compound's antioxidant properties acting as scavenger of reactive oxygen species (Zheng *et al.*, 2004).

In point of fact, all of the tested forms of sage have previously shown to potently suppress hydroxyl radical formation (Kosar *et al.*, 2005). Additionally, the protection of cell viability conferred by sage extracts seemed to be due, mainly, to their ability to prevent GSH depletion by their main phenolic compounds, rosmarinic acid and luteolin-7-glucoside. Nevertheless, unknown compounds other than phenolics also seem to contribute to the antioxidant effects of sage on basal GSH levels (Lima *et al.*, 2007). However, the later authors (Lima *et al.*, 2007) besides other ones Brandstetter *et al.* (2009) showed the ability of sage (mainly the methanolic extract) to increase basal GSH levels, probably by the induction of glutathione synthesis. However, sage ethanolic extract strongly decreased the level of lipid peroxidation compared to Al intoxicated rats, such effect which may be due to its free radical scavenging potential induced by ethanol where various species of *Salvia* has inhibitory and quenching impact on lipid peroxidation along with enhancement of antioxidant defense system in brain tissue of rats treated with Aluminum (Zupkó *et al.*, 2001).

In fact, the glutathione peroxidase system consists of several components, including GSH that effectively remove (hydrogen peroxide) and serves as a cofactor for glutathione transferase, which helps remove certain drugs and chemicals and other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell. So, GSH is probably the most important antioxidant present in cells. *Salvia officinalis* had a potent increasing effect on GSH content in brain compared to Al treated rats. Also, the enzymatic antioxidant defense system including SOD

and CAT which can decompose superoxide and hydrogen peroxide in the cells are the main defense against oxidative injuries. The decreased level of these biomolecules may lead to increased severity of Al toxicities in the brain (Tripathi *et al.*, 2009). Most likely, the sage tea effects observed, herein, was a result of interactions and synergisms among the different compounds and metabolites present, which makes it difficult to attribute them to any particular compound or family of compounds (Lima *et al.*, 2005).

2- Lipid profiles

The present data indicated that serum, cortex and hippocampus total lipids (TL), total cholesterol, (TC) and triglycerides, (TG) were significantly increased by aluminum ingestion, while phospholipids (PL) and serum HDL-C levels were decreased; such results are in accordance with the results reported by Yousef (2004). Similarly, Wilhelm *et al.* (1996) suggested that long-term exposure to Al specifically altered the brain lipid/phospholipid metabolism and/or their transfer to various membrane systems and resulted in significant changes in phospholipid classes and in cholesterol contents of the rat brain. Alternatively, studies in monkeys revealed the chronic effects of Al exposure on brain physiology, including alteration of the lipid composition and the activities of various membrane-bound enzymes; Al was found to decrease significantly the total lipid, glycolipid, and phospholipid concentrations in the primate brain (Sarin *et al.*, 1997). In addition, cholesterol and cholesterol/phospholipid ratios were shown to be remarkably increased, indicating a relevant loss of membrane integrity, and consequently a strong effect of Al on the activity/functionality of various membrane-bound enzymes, including AChE (Atack *et al.*, 1983). Similarly, the long-term exposure to AlCl₃ was shown to result in a 60 % decrease in the total phospholipids content while total cholesterol content increased by 55 %. It is possible that this altered lipid /phospholipid content and composition could affect the insulation properties of the myelin. The finding may thus have some bearing on loss of short-term memory in Alzheimer's disease.

The increase in serum cholesterol and total lipids due to Al administration indicated, also, a loss of membrane integrity (Sarin *et al.*, 1997). This was further confirmed when Al was found to have a significant effect on the various membrane-bound enzymes (Newairy *et al.*, 2009). One possible way to explain the relatively more intense lipid peroxidation due to Al is that the susceptibility to catalyze oxidative cascades which is much easier for lipids than it is for proteins. Moreover, Al exhibited high

affinity for phosphate groups and binds to the phospholipid head groups through electrostatic forces, which may disturb the order as well as the other dynamic parameters of the lipid bilayer (Martin, 1986). From the foregoing results it is clear that Al resulted in significant reduction in the phospholipid content accompanied by major compositional changes, which is consistent with membrane hypothesis of AD. According to this hypothesis, in order to make up for the choline deficiency, the neurons try to extract choline from choline containing phospholipids. These results leads to the disruption of cell membranes and ultimately to neuronal cell death (Roth *et al.*, 1995). Such elevations in TL, TC together with the reduction of HDL-C following Al intoxication shown herein, represent risk factors for atherosclerosis and decreased blood flow to the brain (ischemia) which may be added to the mechanisms involved in Al-induced neurotoxicity.

On the other hand, the present study showed that treatment of rats with AlCl₃ plus different preparations of sage decreased serum and brain total lipids, total cholesterol and triglycerides and enhanced phospholipids and serum HDL-C levels compared to AlCl₃ intoxicated group. These results are in agreement with Ninomiya *et al.* (2004) and Carla *et al.* (2009) who found that oral administration of sage significantly lowered total cholesterol, triglycerides in serum of rats; and increased serum levels of HDL-C. Also, Akram and Maryam (2009) showed that oral administration of sage water extract significantly decreased serum cholesterol and triglycerides. These results suggested that *S. officinalis* tea consumption is accountable for the improvement of the lipid profile inducing an increase in the HDL-C particles, contributing, therefore, positively to the control of the dyslipidaemia observed in Type 2 diabetes but also related to other diseases (Nesto, 2005). However, sage modulating results may attributed to several sage natural components have been shown to act on cholesterol metabolism by reducing its absorption or its synthesis, such as catechins (Plana *et al.*, 2008). This is in addition to the polyphenols, especially, phenolic rosmarinic acid in sage which has potent antioxidant effects protecting membrane lipids of fatty acids and phospholipids from oxidative stress (Lima *et al.*, 2005).

3-Total protein content

The data of the present work showed that Al intoxication caused a significant decrease in the protein contents of serum, cortex and hippocampus protein. These results are in accordance with those

obtained by Nayak *et al.* (2006) and Newairy *et al.* (2009).

Thus, the observed alterations could be attributed to direct or indirect effects of aluminum on protein synthesis and breakdown and interaction with neurotransmitter synthesis and degradation, through a series of reactions that depends on many enzymatic pathways and regulatory mechanisms (Goncalves and Silva, 2007).

The decline in the levels of protein in Al-treated rats is in agreement with Chinoy and Memon (2001) and might be due to changes in protein synthesis and/or metabolism and could be, also, attributed on one hand to an under nutrition and on the other hand to a reduction of the protein synthesis in the liver resulted from Al intoxication as well as to reduced enzymes of protein synthesis as a result of higher intracellular concentration of Al (Tripathi *et al.*, 2009).

Alternatively, since GSH has been reported to be involved in protein and DNA biosynthesis so, the reduction in its content and in the antioxidant enzymes (SOD and CAT) resulted from Al intoxication may partly explain the decline in the total protein content. Additionally, Al induced reactive oxygen species (ROS) formation and promoted oxidative stress (Exley, 2004 and Kumar *et al.*, 2009 a,b) enhancing peroxidative damage to lipids and proteins of the cellular membranes (Julka and Jill, 1996) is another suggestion for protein decline. Such an explanation, which was confirmed by Jyoti *et al.* (2007), indicated that Al exposure caused oxidative stress inflicting damage to membrane lipids, proteins and antioxidant enzyme defense system. Exposure of proteins to free radicals leads to gross structural and functional modifications including protein fragmentation, formation of cross-links and aggregates, protein peroxides generation, and enzymatic oxidation and degradation or clearance (Albendea *et al.*, 2007).

On the other side, the results, herein, indicated that all sage preparations enhanced the protein contents in serum and cerebral cortex and hippocampus of Al intoxicated rats reached them within or near the normal levels comparing to the control group. Regarding the protective mechanisms of sage, it has been speculated that antioxidative properties of sage components may be primarily involved, since changes related to the oxidative stress, where lipid peroxidation and oxidative DNA damage, were shown to be eliminated by sage tea consumption; possibly due, in part, to scavenging the nitrogen oxide or their radical derivatives (Lima *et al.*, 2005). Since the antioxidants play an important role in the regulation and maintenance of metabolism in the body against oxidative stress. So, sage

constituents with their antioxidant properties overcame the lower in the total protein content perhaps by preventing oxidative stress and protein breakdown and enhancing protein synthesis and antioxidant system. Not only phenolic (Durling and Catchpole, 2007) or other flavonoids (Wang *et al.*, 2001), but all other sage components known to be participating in the series of reactions, hence the observed improvement in the present results may be due to all those components.

4- Acetylcholine system:

Cholinesterases are a large family of enzymatic proteins widely distributed throughout both neuronal and non-neuronal tissues. In Alzheimer's disease (AD), analytical as well as epidemiological studies suggested an implication of an abnormal focal accumulation of Al in the brain (Zatta *et al.*, 2002b). In this devastating disease, Al may interfere with various biochemical processes including acetylcholine metabolism, and can thus act as a possible etiopathogenic cofactor. Aluminum is known to interfere with cholinergic (Amador *et al.*, 2001), glutamatergic and gamma-aminobutyric acid neurotransmission. A disturbance in the enzyme activities involved in the acetylcholine metabolism has, also, been reported following Al exposure (Cordeiro *et al.*, 2003).

In the present work, the data obtained showed that Al intoxication caused significant activation of AChE in the serum, cortex and hippocampus. Prior studies have reported the influence of Al on the metabolism of acetylcholine (Jankowska *et al.*, 2000). They exhibited that, specifically, there was a selective loss of acetylcholine releasing neurons in the basal forebrain, hippocampus and cortex. However, impaired cholinergic function in AD has been correlated with loss of memory.

Generally, there have been some hypotheses to explain pathogenesis of the disease such as "cholinergic hypothesis" and "amyloid formation hypothesis". Nowadays, the most accepted treatment strategy in AD has been accepted as "cholinesterase inhibitors" that can inhibit acetylcholinesterase (AChE) enzyme in order to increase acetylcholine level in the brain (Akhondzadeh *et al.*, 2003). In fact, the most remarkable biochemical change in AD patients is a reduction of acetylcholine (ACh) levels in the hippocampus and cortex of the brain. Therefore, the inhibition of AChE (the enzyme responsible for hydrolysis of AChE at the cholinergic synapse), is currently the most established approach to treating AD (Tariot *et al.*, 2000). Al interacts with the cholinergic system, acting as a cholinotoxin. According to (Kaizer *et al.*, 2005) the alterations in

the lipid membrane could be a decisive factor in changing the conformational state of the AChE molecule. However, another explanation for increased AChE activity following Al exposure could be the allosteric interaction between the cation and the peripheric anionic site of the enzyme (Gulya *et al.*, 1990).

On the other hand, the results of the present study showed that sage administration alone or to Al-intoxicated rats led to AChE inhibition. However, it has been reported that *Salvia officinalis* has CNS cholinergic receptor binding activities that may be relevant to enhance or restore mental functions including memory (Wake *et al.*, 2000). Similar effects are also observed *in vivo* (Howes *et al.*, 2003), at least for AChE, suggesting that relevant components of *Salvia* can cross the blood– brain barrier and increase cholinergic transmission via cholinesterase inhibition (Perry *et al.*, 2002). Similarly, up to date, a number of studies on AChE inhibitory activity of several *Salvia* species have been reported. Among these, the essential oil and ethanolic extract of *S. officinalis* have been shown to possess anti-cholinesterase activity (Perry *et al.*, 1996). This finding is consistent with recent reports established sage benefits to memory following administration of the essential oil in healthy young adults (Andrew *et al.*, 2008). Moreover, the essential oil as well as its major components, α -pinene, 1, 8-cineole, and camphor were determined to have uncompetitive and reversible acetylcholinesterase inhibitory activity (Perry *et al.*, 2000). Additionally, the constituents contained within *Salvia* oil may combine non-linearly to produce cholinesterase inhibition. A combination of the major monoterpene constituents (camphor, 1,8-cineole, borneol, α -pinene and β -pinene) reconstituted in a naturally occurring ratio was significantly less potent than that of the whole oil (Perry *et al.*, 2003 and Savelev *et al.*, 2004). The monoterpenoids may therefore act synergistically to inhibit AChE.

The activity of the essential oils was concluded mainly to be due to its monoterpenoids. The data indicated that the terpenoids, monoterpenes in particular, may have anticholinesterase activity (Orhan *et al.*, 2007). Alternatively, the ethanolic extract of *Salvia officinalis* potentiated memory retention and it has also, an interaction with muscarinic and nicotinic cholinergic systems involved in the memory retention process but to less extent than oil (Eidi *et al.*, 2006).

5- Alkaline and acid phosphatases:

The present study illustrated that Al ingestion led to significant elevation in alkaline

phosphatase (ALP) and acid phosphatase (ACP) activities in sera, cerebral cortex and hippocampus. Alkaline phosphatase is a membrane-associated enzyme, which predominantly concentrated in the vascular endothelium in the brain. There is a more or less continuous sheath of ALP covering all internal and external surfaces of the central nervous system including the spinal cord and thus it may functionally be part in the blood-brain barrier mechanism. On the other hand, intracellular ACP is largely confined to lysosomes, which primarily respond to cellular injury. Within the brain, the ACP is found to be concentrated in the gray matter, although it shows the activity in the white matter, also, to some extent. However, significant contribution by Al was observed to induce changes in ACP activity (Dasgupta and Ghosh, 1993).

The increased activity of ALP and ACP enzymes in serum & brain of animals treated with $AlCl_3$ are in accordance with the findings of Ochmanski and Barabasz (2000). Also, El – Demerdash (2004) found that the activities of these enzymes were increased in serum of mice fed on wheat containing Al residue of 0.2 g /kg b.w. The present results are further, in consistent with the recent findings of Esmaeili *et al.* (2009) who showed that chronic Al consumption caused significant increases in the activities of ALP and ACP enzymes which could be due to severe damage to tissue membranes.

In addition, the increase in the activity of ALP or ACP in blood might be due to the necrosis of liver, kidney and lung (Sallam *et al.*, 2005). Our own interpretation for increased levels of ALP and ACP is the disruption of the blood brain barrier and oxidative damage of tissue membranes, releasing membrane bound enzymes following Al intoxication which is also confirmed previously by Exley (2004) and recently by Esmaeili *et al.* (2009).

Moreover, regarding Al enhanced serum, cortex and hippocampus ACP activities of rats, herein, it was in agreement with the earlier observations recorded altered activities of specific lysosomal hydrolytic enzymes in neuronal tissues (Suzuki *et al.*, 1988) due to Al administration. From these observations it can be suggested that Al induced an increase in ACP activity of the brain may be an indication of lysosomal proliferation and increasing catabolic rate. The increased ACP activity may result in phosphate accumulation within the lysosomes, and this in turn may lead to decreased plasma inorganic phosphate concentration (Hussain *et al.*, 1990).

In the present work, administration of sage tea, ethanolic extract and oil caused marked reduction in the elevated activities of ALP and ACP in Al

treated rats. Such decrease could be due to the antioxidant properties of sage constituents as polyphenols (carnosol, carnosic acid, and rosmarinic acid) and flavonoids (apigenin) that protect cellular

membranes integrity from Al-induced oxidative damage and repair the antioxidant system (Carla *et al.*, 2009), consequently, improve brain structure and function against Al toxicity.

Table (1): Cortex and Hippocampus (Hip) Lipid Peroxidation (TBARS) Product Concentration and Protein Carbonyl (PC) Content ($\mu\text{mol/g}$ tissue), Superoxide Dismutase (SOD), Catalase (CAT) Activities and Reduced Glutathione (GSH) Content in Different Animal Groups

		Control	Sage water extract	Sage ethanolic extract	Sage oil	Aluminum (Al)	Al + Sage water extract	Al + Sage ethanolic extract	Al + Sage oil
TBARS	Cortex (n mol/g tissue)	322.77 \pm 1.51	321.25 \pm 1.93	323.59 \pm 0.98	345.65 \pm 8.54	426.3 \pm 2.01 ^a	356.7 \pm 13.04 ^b	365.18 \pm 16.29 ^b	361.64 \pm 14.90 ^b
	Hip (n mol/g tissue)	352.82 \pm 4.67	358.48 \pm 0.55	345.1 \pm 1.64 ^a	337.41 \pm 1.94 ^a	380.15 \pm 0.42 ^a	347.32 \pm 0.55 ^b	331.52 \pm 0.55 ^{a,b}	346.45 \pm 0.47 ^b
PC	Cortex ($\mu\text{mol/g}$ tissue)	0.16 \pm 0.02	0.17 \pm 0.01	0.14 \pm 0.03	0.15 \pm 0.02	0.45 \pm 0.04 ^a	0.26 \pm 0.02 ^b	0.24 \pm 0.04 ^b	0.19 \pm 0.04 ^b
	Hip ($\mu\text{mol/g}$ tissue)	0.29 \pm 0.01	0.31 \pm 0.02	0.29 \pm 0.02	0.29 \pm 0.01	0.84 \pm 0.01 ^a	0.32 \pm 0.02 ^b	0.29 \pm 0.02 ^b	0.30 \pm 0.02 ^b
SOD	Cortex (U/min/g wet tissue)	189.50 \pm 2.51	193.52 \pm 4.17	185.47 \pm 7.70	188.41 \pm 5.04	163.21 \pm 1.91 ^a	184.18 \pm 3.94 ^b	185.67 \pm 4.35 ^b	169.57 \pm 7.16 ^b
	Hip (U/min/g wet tissue)	171.29 \pm 9.57	194.54 \pm 2.26	195.48 \pm 0.12	176.21 \pm 12.01	137.81 \pm 7.88 ^a	191.97 \pm 5.53 ^b	175.06 \pm 5.69 ^b	169.01 \pm 1.50 ^b
CAT	Cortex ($\mu\text{mol/sec/g}$ wet tissue)	1.73 \pm 0.12	2.49 \pm 0.29	2.22 \pm 0.15	1.11 \pm 0.11	0.83 \pm 0.07 ^a	1.63 \pm 0.12 ^b	2.06 \pm 0.23 ^b	2.00 \pm 0.11 ^b
	Hip ($\mu\text{mol/sec/g}$ wet tissue)	2.08 \pm 0.14	3.08 \pm 0.06	4.92 \pm 0.72 ^a	5.59 \pm 0.26 ^a	0.62 \pm 0.02 ^a	3.98 \pm 0.18 ^{a,b}	3.07 \pm 0.21 ^b	5.35 \pm 0.41 ^{a,b}
GSH	Cortex (mg/g tissue)	0.35 \pm 0.01	0.30 \pm 0.02	0.33 \pm 0.01	0.33 \pm 0.02	0.10 \pm 0.02 ^a	0.29 \pm 0.26 ^b	0.28 \pm 0.22 ^b	0.33 \pm 0.02 ^b
	Hip (mg/g tissue)	0.36 \pm 0.02	0.37 \pm 0.02	0.38 \pm 0.02	0.43 \pm 0.02	0.12 \pm 0.01 ^a	0.41 \pm 0.04 ^b	0.39 \pm 0.02 ^b	0.49 \pm 0.04 ^{a,b}

Values were expressed as means \pm SE of six animals. P<0.05 (significant)

a = Significant difference comparing to the normal control group.

b = Significant difference comparing to the aluminum group.

Table (2): Serum, Cortex and Hippocampus (Hip) Total Lipids (TL), Total Cholesterol (TC), Triglycerides (TG) and Phospholipids (PL) Concentrations in Different Animal groups

		Control	Sage water extract	Sage ethanolic extract	Sage oil	Aluminum (Al)	Al + Sage water extract	Al + Sage ethanolic extract	Al + Sage oil
TL	Serum (mg/dl)	397.03±33.69	397.63±43.17	407.05±31.33	337.63±7.73	775.25±41.64 ^a	392.07±1.85 ^b	381.07±13.05 ^b	393.26±10.99 ^b
	Cortex (mg/g)	622.19±5.73	637.51±7.15	658.23±11.81	638.40±7.29	782.46±10.89 ^a	646.20±12.26 ^b	650.65±10.12 ^b	657.40±6.65 ^b
	Hip (mg/g)	361.39±9.26	355.60±1.81	349.20±10.69	361.38±9.90	625.77±7.65 ^a	332.65±11.11 ^b	212.77±0.10 ^{ab}	325.74±7.41 ^b
TC	Serum (mg/dl)	85.20 ±1.02	83.00 ±0.95	81.60 ±0.81	82.60 ±0.81	126.11 ±2.77 ^a	83.80 ±5.42 ^b	86.00 ±1.00 ^b	84.20 ±1.24 ^b
	Cortex (m g/g)	183.60 ±9.88	122.04 ±7.03 ^a	138.52 ±7.21 ^a	196.97 ±0.12	363.96 ±10.28 ^a	196.14 ±3.71 ^b	142.27±2.01 ^{ab}	162.93±0.10 ^b
	Hip (m g/g)	89.86 ±0.84	92.06±0.18	53.14±5.13 ^a	52.45 ±0.27 ^a	198.53±0.98 ^a	93.70±2.35 ^b	81.20 ±2.01 ^b	73.20±3.71 ^{ab}
TG	Serum (mg/dl)	67.28 ±4.78	59.38±5.29	59.59±0.06	56.11 ±1.33	93.19±8.13 ^a	52.52±3.13 ^b	47.19±2.70 ^{ab}	49.45±2.55 ^b
	Cortex (m g/g)	142.24±4.77	135.44 ±0.53	160.80±3.89	162.32±3.49	182.00±4.14 ^a	127.73 ±6.31 ^b	142.20±4.63 ^b	143.90±4.35 ^b
	Hip (m g/g)	110.34±0.01	106.82±0.89	82.36±2.75 ^a	80.75±0.31 ^a	143.31±6.55 ^a	87.35±2.91 ^{ab}	57.14±4.58 ^{ab}	63.93±0.17 ^{ab}
PL	Serum (mg/dl)	142.64±8.12	143.31±20.28	109.53±1.94	131.52±6.61	102.39±0.56 ^a	142.00±1.84 ^b	141.21±1.02 ^b	142.42±2.87 ^b
	Cortex (m g/g)	149.40±1.53	232.91±13.04 ^a	155.41±6.22	368.21±9.30 ^a	87.29 ±2.70 ^a	123.84±1.98	143.15±21.97 ^b	147.71±6.96 ^b
	Hip (mg/g)	150.55±1.18	149.84±0.59	172.23±0.03 ^a	167.27±3.46 ^a	65.52±0.05 ^a	119.88±0.01 ^{ab}	94.49±0.41 ^{ab}	117.65±2.08 ^{ab}

Values were expressed as means ±SE of six animals. P<0.05 (significant)

a = Significant difference comparing to the normal control group.

b = Significant difference comparing to the aluminum group.

Table (3): Serum High Density Lipoproteins Cholesterol (HDL-C), Serum Cortex and Hippocampus Total Protein (TP) Content in Different Animal Groups

		Control	Sage water extract	Sage ethanolic extract	Sage oil	Aluminum (Al)	Al + Sage water extract	Al + Sage ethanolic extract	Al + Sage oil
HDL-C	Serum (mg/dl)	72.75±3.73	75.90±5.64	78.27±7.12	80.20±2.46	48.67±2.22 ^a	70.86±3.37 ^b	84.67±4.65 ^b	80.20±7.20 ^b
	Cortex (m g/g)	4.65±0.12	5.01±0.30	4.93±0.56	5.88±0.27	2.89±0.24 ^a	6.19±0.46 ^b	4.54±0.39 ^b	5.94±0.32 ^b
TP	Serum (mg/dl)	5.99±0.12	4.81±0.24	5.68±0.13	5.39±0.34	3.91±0.32 ^a	5.52±0.37 ^b	5.38±0.23 ^b	5.23±0.41 ^b
	Hip (mg/g)	2.30±0.06	2.70±0.02 ^a	2.60±0.04 ^a	2.53±0.02 ^a	2.07±0.03 ^a	2.79±0.11 ^{ab}	2.62±0.01 ^{ab}	2.52±0.01 ^b

Values were expressed as means ±SE of six animals. P<0.05 (significant)

a = Significant difference comparing to the normal control group.

b = Significant difference comparing to the aluminum group.

Table (4): Serum, Cortex and Hippocampus (Hip), Acetylcholinesterase (AChE), Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) Activities in Different Animal Groups

		Control	Sage water extract	Sage ethanolic extract	Sage oil	Aluminum (Al)	Al + Sage water extract	Al + Sage ethanolic extract	Al + Sage oil
AChE	Serum ($\mu\text{mol SH}/0.1\text{ml}/\text{min}$)	2.39 \pm 0.19	2.43 \pm 0.05	1.61 \pm 0.01	2.13 \pm 0.46	5.70 \pm 0.11 ^a	3.22 \pm 0.37 ^b	3.27 \pm 0.24 ^b	2.45 \pm 0.07 ^b
	Cortex ($\mu\text{mol SH}/\text{g}/\text{min}$)	3.64 \pm 0.11	3.39 \pm 0.12	3.75 \pm 0.14	3.94 \pm 0.22	13.17 \pm 0.065 ^a	4.03 \pm 0.08 ^b	4.37 \pm 0.19 ^{ab}	4.28 \pm 0.23 ^b
	Hip ($\mu\text{mol SH}/\text{g}/\text{min}$)	1.67 \pm 0.15	1.46 \pm 0.14	1.37 \pm 0.04	1.66 \pm 0.04	7.49 \pm 0.01 ^a	2.82 \pm 0.19 ^{ab}	2.45 \pm 0.28 ^b	2.69 \pm 0.28 ^{ab}
ALP	Serum (U/L)	78.54 \pm 2.94	81.72 \pm 1.90	84.13 \pm 0.21	74.37 \pm 4.56	189.53 \pm 1.77 ^a	84.03 \pm 7.56 ^b	103.02 \pm 0.56 ^{ab}	150.69 \pm 8.90 ^{ab}
	Cortex (K.A.U./g tissue)	241.18 \pm 3.68	263.55 \pm 7.70	241.31 \pm 10.99	246.99 \pm 4.93	376.95 \pm 7.46 ^a	292.27 \pm 6.33 ^{ab}	276.73 \pm 11.48 ^b	333.05 \pm 12.78 ^{ab}
	Hip (K.A.U./g tissue)	134.94 \pm 0.55	142.59 \pm 4.38	142.59 \pm 4.38	93.46 \pm 6.28 ^a	173.20 \pm 0.79 ^a	150.66 \pm 2.43 ^{ab}	150.86 \pm 0.29 ^{ab}	152.27 \pm 2.35 ^{ab}
ACP	Serum (U/L)	32.18 \pm 0.44	31.74 \pm 1.02	31.67 \pm 1.52	24.97 \pm 0.95	49.31 \pm 3.71 ^a	30.11 \pm 2.18 ^b	28.23 \pm 2.65 ^b	31.66 \pm 2.06 ^b
	Cortex (K.A.U./g tissue)	134.94 \pm 5.13	136.39 \pm 5.61	131.36 \pm 4.85	128.14 \pm 9.37	228.10 \pm 5.46 ^a	155.85 \pm 14.23 ^b	156.11 \pm 7.22 ^b	154.67 \pm 7.19 ^b
	Hip (K.A.U./g tissue)	68.88 \pm 0.56	71.56 \pm 0.14	47.04 \pm 0.26 ^a	56.46 \pm 3.89 ^a	78.62 \pm 0.41 ^a	66.58 \pm 0.15 ^b	32.68 \pm 1.60 ^{ab}	37.54 \pm 0.86 ^{ab}

Values were expressed as means \pm SE of six animals. $P < 0.05$ (significant)

a = Significant difference comparing to the normal control group. **b** = Significant difference comparing to the aluminum group.

4. Conclusion:

Aluminum plays a pivotal role in the neuropathology of many neurodegenerative diseases including AD and validate the fact that chronic exposure to aluminum causes oxidative damage to the membranes and neural cells affecting memory loss and other cognitive dysfunction and exhaust the antioxidant system. Moreover, it is clearly evident that sage (all forms) has a neuroprotective effect against neuronal structural dysfunction caused by aluminum which may be attributed, mainly, to their high ability to scavenge ROS and augment the repair of the antioxidant system as well as its anti-ChE activity, thus it has been suggested that sage has shown promise in the treatment of many neurodegenerative diseases including Alzheimer's disease.

No fixed pattern of protection was seen specific for the different preparations of sage, but, we can arrange them in the following order: sage oil >

ethanolic extract > water extract Future study may be designed and further warrants the need for molecular studies to elucidate the mechanisms underlying the protective effects of sage and its active components.

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