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ORIGINAL ARTICLES

Effect of some Nutraceutical Agents on Aluminum-Induced Functional Neurotoxicity in Senile Rats: II. Effect of L-Serine and Methionine

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ABSTRACT

Alzheimer's disease (AD) is an age related neurodegenerative disorder, comprising complex neurobiochemical and neuropathological events. Aluminum, a well-known neurotoxin, has been proposed to play a role in the development of Alzheimer's disease (AD), and produced clinical and pathological features which were strikingly similar to those seen in AD-brain. The present study aimed to elucidate the possible neuroprotective roles of the two amino acids methionine and serine (MS) in alleviating aluminum chloride ($AlCl_3$) - induced neurotoxicity in male senile rats. The animals were classified into 4 groups and treated orally for 3 months as follows: (1) control group, (2) rats received $AlCl_3$ alone (100mg/kg b.w. /day), (3) rats received methionine (100mg/kg b.w. /day) and serine (150mg/kg b.w. /day) and (4) rats intoxicated with $AlCl_3$ and were treated with methionine plus serine. At the end of the experimental period, the two brain areas, cortex and hippocampus as well as blood were taken for different biochemical determinations. In comparison with normal control group, $AlCl_3$ administration caused significant increases in cortical and hippocampal lipid peroxidation, nitric oxide, ionized calcium levels as well as acetylecholinesterase activity. Additionally, serum alanine aminotransferase, aspartate aminotransferase, nitric oxide, total calcium, urea, creatinine, tumor necrosis factor alpha and interleukin 1-ß levels were also elevated significantly due to $AlCl_3$ intoxication. On the other hand, total antioxidant capacity level and Na^+-K^+ ATPase activity were significantly reduced in brain cortex and hippocampus of $AlCl_3$-treated rats. Treatment with the selected amino acids improved the neurological damages induced by $AlCl_3$ as indicated by the modulations in most of the biochemical markers. In conclusion, methionine and serine amino acids may play a beneficial role in delaying the progression of neurodegenerative disorders.

Key words: Aluminium chloride, methionine, serine, brain aging, Alzheimer's disease

Introduction

Aging is a natural process in all living organisms (Zhao *et al.*, 2009**)**. Normal brain aging is associated with a varying degree of cognitive impairment. Although aging is a complex, multifactorial process, and no single process could explain the aging phenotype, a number of processes and homeostatic systems, due to their central roles in cellular physiology, have been identified as playing important roles in the process of normal aging (Toescu and Vreugdenhil, 2010).

Alzheimer's disease (AD) and Parkinson's disease (PD) are aging associated neurodegenerative diseases that can greatly impair quality of life. These diseases affect the brain, which is the control centre of our body, and lead to cognitive impairment and motor deficits (Zhao *et al*., 2009). AD is characterized by extracellular beta amyloid deposits, intraneuronal neurofibrillary, tangles and selective neuronal loss (Zhao *et al*., 2009).

Aluminum is a potent neurotoxin that plays a pivotal role in the neuropathology of AD, prolonged aluminum exposure induces cognitive dysfunction, related oxidative damage and increases the deposition of amyloid beta *in vivo* (Kumar *et al.,* 2009). It may affect several enzymes and other biomolecules related to neurotoxicity and AD (Garcia *et al.,* 2010). Exposure of aluminum, led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration as cytoplasmic vacuolization, hemorrhage, ghost cells and gliosis as shown by the finding of Bihaki *et al*. (2009).

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the neuroprotective effects of natural compounds (Slameňová *et al.,* 2011). Amino acids are mostly supplied in the normal diet not as free amino acids but rather as protein constituents. Moreover, they have been taken as food additives in addition to normal dietary intake of protein (Tanrikulu-Kucuk and Ademoglu, 2012). The safety of amino acids consumed from the diet has not been of great concern because they

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are nutrients required for the synthesis of functional and structural components of the body and are consumed in large quantities from food as an essential part of the diet. In recent years, there has been growing interest in the safety of individual amino acids due to a large increase in the consumption of individual amino acids therapeutically or as dietary supplements for supporting pharmacological actions or enhancing health or physical performance (Tanrikulu-Kucuk and Ademoglu, 2012).

Methionine, one of the most easily oxidized amino acids, may have several important cellular functions (Butterfield and Boyd-Kimball, 2005). It is readily taken up by the hepatocytes for the direct synthesis of glutathione and, thus, acts as a precursor amino acid for this low molecular weight antioxidant. Glutathione protects the cells from oxidative damage and plays vital role in detoxification (Nandi *et al.,* 2005).

L-Serine plays an important role in various cellular reactions. In the brain, astrocytes play an essential role in synthesizing L-serine from glucose and in this way these cells have an extensive role in neuronal survival and activity, not only through the conversion of L-serine to the neuromodulators D-serine and glycine but also via the synthesis of neuronal membrane lipids (Tabatabaie *et al.,* 2010).

This study might help in throwing light on the possible neuroprotective roles of two important amino acids (methionine and serine) in ameliorating $AlCl_3$-induced cognitive dysfunction and oxidative damage in two different brain areas (hippocampus and cerebral cortex) as well as in bood of senile rats.

Materials and Methods

Materials:

(i) Amino acids: DL- serine was obtained from BDH Biochemicals Co., England and DL- methionine from Central Drug House (P) LMT, New Delhi, India.
(ii) Aluminum chloride anhydrous LR (M.wt. 133334) was obtained from fine-chem. limited, Mumbai, India.

Experimental animals and treatment plan:

Adult senile male albino rats weighing 250-300 gm were used in this study. They were obtained from Animal House Colony of National Research Centre (NRC), Giza, Egypt. Animals were kept in a quiet place and were allowed free access to water and standard food pellets throughout the period of investigation. All animals received human care in compliance with the guidelines of the Animals Care and Use Committee of NRC. Experiments were performed after the approval of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC). Rats were then divided into four groups each of fourteen rats and treated for 3 months as follows. Group (1): served as normal control, Group (2): animals received aluminum chloride ($AlCl_3$) orally alone (100 mg/kg b.w. /day) (Sethi *et al*., 2009), Group (3): rats were orally provided with amino acids [methionine (100mg/kg b.w. /day) (Amanvermez *et al.,* 2008) and serine (150 mg/kg b.w. /day) (Kaneko *et al*., 2009)] orally and finally, Group (4): rats supplied with combined treatments of $AlCl_3$ and the two amino acids.

Samples preparation:

At the end of the experimental duration, fasting blood samples were collected from one half of the animals of each group. Blood samples were taken from the retro-orbital venous plexus under diethyl ether anesthesia (Schermer, 1967) in clean tubes and then centrifuged at 3000 rpm for 10 minutes. The separated sera were stored at -20^{o}C till examined for the detection of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, interleukin- 1ß (IL-1ß), tumor necrosis factor alpha (TNF- α), nitric oxide (NO), calcium, creatinine and urea levels.

The second half of animals was suddenly decapitated (to avoid the biochemical changes which may occur as a result of ischemia), brains were removed and each hippocampus and cerebral cortex were dissected out, weighed and thoroughly washed with isotonic saline. The individual hippocampus and cerebral cortex of each animal were homogenized immediately to give 20% (w/v) homogenate in ice-cold medium containing 50 mM tris-HCl and 300 mM sucrose (pH 7.4) (Tsakiris *et al.,* 2004). The homogenate was centrifuged at 3000 rpm for 10 minutes in a cooling centrifuge (Brinkman Instruments, Inc., Westbury, NY, USA) at 0^{o}C. The supernatant was examined for the detection of acetylecholinesterase (AChE) and Na^{+}-K^{+} ATPase activities as well as lipid peroxidation, total antioxidant capacity (TAC), NO and calcium levels.

Analytical Determinations:

Malondialdehyde (MDA) which is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation was evaluated in brain

hippocampus and cerebral cortex homogenates according to the method of Ruiz-Larnea *et al.* (1994). TAC was assayed in brain hippocampus and cerebral cortex homogenates using Biodiagnostic Kit, Egypt. Na^+/K^+ ATPase activity was evaluated chemically in brain hippocampus and cerebral cortex homogenates by measuring the released inorganic phosphorus from the hydrolysis of ATP colorimetrically, according to the modified method of Tsakiris *et al.* (2004). AChE in brain hippocampus and cerebral cortex homogenates was performed using AMS Kit, United Kingdome. Calcium and NO in serum, hippocampus and cerebral cortex were determined colorimetrically using Biodiagnostic Kit, Egypt. AST and ALT activities and creatinine level were determined in serum according to the manual instruction of Quimica Clinical Aplicada S.A.(QCA) kits, Spain, and the quantitative determination of urea was performed in serum using Intermedical Kit, Italy. Quantitative determination of IL-1ß in serum was estimated using Orgenium Laboratories Business Unit ELISA (Enzyme-Linked Immunosorbent Assay) kit, Finland and TNF-α in serum was estimated using RayBiotech, Inc. ELISA kit, USA.

Statistical analysis:

The obtained data were subjected to one way analysis of variance (ANOVA). The analysis was performed using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. Tukey test (Steel & Torrie, 1980) was used to evaluate the significance between the individual groups at $p<0.05$.

Results:

As shown in Table (1), normal animals received methionine and serine mixture (MS) recorded insignificant changes in hippocampal and cortical MDA, NO, serum NO and TAC levels. Animals orally intoxicated with $AlCl_3$ exhibited significant increases in MDA and NO levels in both brain areas as well as serum NO level compared to the control group. Administration of MS in combination with $AlCl_3$ resulted in significant reductions in MDA and NO levels in the brain areas as well as serum NO level when this group was compared to $AlCl_3$-intoxicated rats. On the contrary, $AlCl_3$ intoxication induced significant decrease in cortical and hippocampal TAC level in comparison to the normal control group. Co-administration of MS with $AlCl_3$ revealed significant increase in TAC level of brain cortex and hippocampus as compared to $AlCl_3$- treated group.

Table 1: Effects of methionine and serine mixture (MS) on some markers of oxidative stress in $AlCl_3$-intoxicated rats.

	Malondialdehyde		Nitric oxide			Total antioxidant capacity	
	Hippoca. (nmol/g)	Cortex (nmol/g)	Hippoca. (nmol/g)	Cortex (nmol/g)	Serum (nmol/L)	Hippoca. (µmol/g)	Cortex (µmol/g)
Control	726±9.1^{c}	917±20.1^{de}	29.8±0.4^{fg}	32.2±0.3^{gh}	16.1±0.2^{ef}	863±15.7^{a}	527±12.1^{ab}
$AlCl_3$	932±11.6^{a}	1129±24.8^{a}	40.3±0.5^{a}	41.1±0.5^{a}	20±0.4^{a}	304±15.9^{d}	417±8.8^{f}
MS	712±4.4^{cd}	900±12.6^{e}	31±0.3^{ef}	33.1±0.3^{fg}	16.2±0.1^{ef}	867±14.6^{a}	516±11.6^{ab}
$AlCl_3$+MS	892±3.1^{b}	1057±10.7^{b}	35.8±0.3^{bc}	36.6±0.3^{bc}	18.2±0.2^{bc}	665±20.8^{bc}	454±11de

Data are presented as mean±S.E. Mean values within each column sharing the same superscript letters are not significantly different.

The data in Table (2) show that treatment with $AlCl_3$ resulted in significant increases in AChE activity in brain cortex and hippocampus and calcium level in both brain areas and serum, whereas Na^+/K^+-ATPase activity in brain cortex and hippocampus was decreased significantly by $AlCl_3$ administration. Co-treatment with MS and $AlCl_3$ reversed the changes occurred in these parameters towards the normal values of the controls.

Insignificant changes in serum ALT and AST activities as well as urea and creatinine levels were observed in normal rats received MS. Significant increases were marked in these parameters due to treatment with $AlCl_3$ with respect to normal animals. Whereas, the presence of MS in combination with $AlCl_3$ reduced significantly serum ALT and AST activities as well as urea and creatinine levels in relative to $AlCl_3$-intoxicated group (Table 3).

Table 2: Effects of methionine and serine mixture (MS) on AChE, Na^+/K^+-ATPase and calcium in $AlCl_3$-intoxicated senile rats.

	AChE		Calcium			Na^+/K^+-ATPase	
	Hippoca. (U/g)	Cortex (U/g)	Hippoca. (mg/g)	Cortex (mg/g)	Serum (mg/dL)	Hippoca. (µmol pi/h/g)	Cortex (µmol pi/h/g)
Control	65.2±5.7^{b}	36.7±5.5^{c}	8.2±0.3^{d}	7.1±0.3^{d}	10.9±0.4^{d}	9.6±0.5^{a}	5.4±0.3^{a}
$AlCl_3$	91.3±5.2^{a}	110±5.3^{a}	14.3±0.4^{a}	13.3±0.5^{a}	17.4±0.4^{a}	4.1±0.5^{d}	1.6±0.1^{e}
MS	65.3±2.1^{b}	36.1±1.4^{c}	8.4±0.3^{d}	7.2±0.2^{d}	11.3±0.3^{d}	9.2±0.4^{ab}	5±0.2^{abc}
$AlCl_3$+MS	72±4.9^{b}	66.5±2.1^{b}	12.3±0.3b^{c}	11±0.3^{b}	14.5±0.4^{b}	7.2±0.2^{c}	4.3±0.2^{cd}

Data are presented as mean±S.E. Mean values within each column sharing the same superscript letters are not significantly different.

Serum TNF-α and IL-1ß levels were insignificantly changes in normal rats received MS. While, significant increases resulted from the treatment with $AlCl_3$ in serum TNF-α and IL-1ß levels when compared to the control group. The treatment with MS significantly declined serum TNF-α and IL-1ß levels that elevated by $AlCl_3$-intoxication (Table 4).

Table 3: Effects of methionine and serine mixture (MS) on some specific markers of liver and kidney functions in serum of ACl_3-intoxicated senile rats.

	ALT (U/l)	AST (U/l)	Urea (mg/dL)	Creatinine (mg/dL)
Control	25.8±0.4^{cd}	38.3±0.4^{de}	38.8±2.4^{b}	1.69±0.03^{e}
$AlCl_3$	30.4±0.5^{a}	46.3±0.6^{a}	50.6±0.7^{a}	2.52±0.05^{a}
MS	26±0.3^{cd}	39.1±0.4^{d}	38.9±0.6^{b}	1.7±0.03^{e}
$AlCl_3$+MS	28±0.4^{b}	42.5±0.4^{b}	41.7±0.7^{b}	2.11±0.04^{bc}

Data are presented as mean±S.E. Mean values within each column sharing the same superscript letters are not significantly different.

Table 4: Serum TNF-α and IL 1-ß levels in different studied groups.

	TNF-α (pg/ml)	IL 1-ß (pg/ml)
Control	38.1±0.8^{f}	715±2.1^{cd}
$AlCl_3$	92.6±3.9^{a}	772±4.9^{a}
MS	38.5±0.5^{f}	715±7.5^{cd}
$AlCl_3$+MS	65±0.4^{e}	724±4.2^{bcd}

Data are presented as mean±S.E. Mean values within each column sharing the same superscript letters are not significantly different.

Discussion:

It is well established that $AlCl_3$ is a neurotoxic agent that gradually accumulates in the cortical and limbic regions of susceptible subjects' brains, eventually producing hippocampal lesions consisting of dysfunctional microtubule, depleted pyramidal cells with damaged neuritie and synapse loss. These lesions expand overtime with the development of clinically overt dementia (Walton, 2006). Also, $AlCl_3$ neurodegenerative effects include elevated amyloid precursor protein (APP) gene expression in hippocampal and cortical tissue that results in the formation of amyloid plaques in the brain (Garcia *et al.,* 2010). Therefore, the focus of this work was to evaluate the effectiveness of the amino acids: methionine and serine in alleviating the induced neurotoxicity of $AlCl_3$ in male senile rats.

The present results revealed that aluminum promotes oxidative stress. This appeared from the significant reduction in TAC level concomitant with increased level of lipid peroxidation (LPO) in brain cortex and hippocampus as well as NO in both brain areas and serum. These results suggest participation of free radicals-induced oxidative cell injury in mediating the toxicity of $AlCl_3$ (Yousef, 2004; Shati *et al.,* 2011) which was attributed to electron leakage, enhanced mitochondrial activity and increased electron chain activity. Reactive oxygen species (ROS) subsequently attack almost all cell components including membrane lipids and producing lipid peroxidation (Flora *et al*., 2003). Therefore, it can be hypothesized that oxidative stress may be one of the contributing factors for aluminum-induced neurotoxicity (Yousef and Salama, 2009).

It was demonstrated that Al^{3+} ion can effectively aggravate Fe^{2+} –initiated lipid peroxidation. This effect is largely due to the high polarizing power of the Al^{3+} ion. The capacities of aluminum for instance promote oxidative damage. Brain membranes are not only based on the presence of polyunsaturated fatty acids and phospholipids components, but also that of molecules with a high content of lipid/protein ratio supporting the fact that ions without redox capacity can stimulate lipid peroxidation by promoting phase separation and membrane rigidification (Christen, 2000).

Other studies have shown that $AlCl_3$-enhanced peroxidation and neurodegenerative disorders may be related to aluminum-induced nitric oxide synthase (NOS) activity and increased NO products in rat brain tissue and microglial cells (Bondy *et al.,* 1998). NO is generated from L-arginine by the enzyme NOS, formed in a variety of tissues and involved in many physiological and pathological processes (Moncada *et al.,* 1991).

In the present study, $AlCl_3$ produced significant elevation in acetylcholine esterase (AChE) activity in brain cortex and hippocampus, whereas the activity of Na^+/K^+-ATPase was markedly inhibited in both brain regions as compared to the normal control. These findings are consistent with the previous study of Mohamad *et al.* (2011) who suggested that $AlCl_3$ exposure increased AChE activity via allosteric interaction between $AlCl_3$ and the peripheral anionic site of the enzyme molecule, leading to the etiology of AD pathological deterioration. $AlCl_3$ exerts cholinotoxic effects by blocking the provision of acetyle-CoA, which is required for acetylcholine synthesis or by impairing the activities of choline acetyle trasferase itself (Aly *et al.,* 2011).

In contrast to AChE, the activity Na^+/K^+-ATPase was found to be significantly declined in brain cortex and hippocampus of $AlCl_3$-intoxicated rats. On the other hand, calcium levels in serum as well as in brain cortex and hippocampus were elevated significantly due to neurotoxic effects of $AlCl_3$.The disruptive action of ROS resulted from $AlCl_3$ intoxication might lead to lipid peroxidation and cause alterations of the membrane structure and function, including fluidity, permeability, and activity of enzymes, channels, and receptors. It has

been shown that ROS could inhibit Na^+/K^+-ATPase and Ca^{2+}-ATPase of *in vitro* membrane ion transport systems, and the antioxidants might prevent the decrease in both enzyme activities (Cheng *et al*., 2006). Therefore, changes in its activity could be associated with alterations in neuronal action potential firing (Zhang *et al.,* 2004). In accordance to our study, $AlCl_3$-induced disruption of Na and K channel proteins has been reported *in vitro* by Sethi *et al.* (2008).

The inactivation of Na^+/K^+-ATPase leads to partial membrane depolarization allowing excessive Ca^{2+} entry inside neurons with resultant toxic events like excitotoxicity. This membrane bound enzyme requires phospholipid for its activity and is highly vulnerable to oxidative insult and the mechanism of inactivation under such conditions involves disruption of phospholipid microenvironment of the enzyme or direct damage to enzyme protein by reactive oxygen radicals or lipid peroxidation products (Stefanello *et al.,* 2011). Augmentation of calcium levels in serum and brain tissues as a result of $AlCl_3$ administration as shown in our study is in agreement with Wang *et al* (2010) who demonstrated that chronic $AlCl_3$ intake can result in aluminium accumulation in the brain and cause an impairment of memory of rats. $AlCl_3$ accumulation inhibits the formation of long-term potentiation, by affecting multiple signaling pathways due to the disruption of the Ca^{2+} metabolism in hippocampal cells, including protein kinase C (PKC) signaling pathway and Ca^{2+}–calmodulin kinase II - dependent protein kinase signal transduction system.

In the present study, the activities of AST and ALT were significantly increased in serum of rats treated with $AlCl_3$ and this is in agreement with Yousef (2004). This may be due to the leakage of these enzymes from the liver cytosol into the blood stream which resulted from liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place. (Bogdanovic *et al.,* 2008). As a matter of fact, the elevation in transaminases are encountered in conditions causing hepatocellular damage, loss of functional integrity of the cell membrane, and necrosis such as in chemically induced liver injury and elevation in enzymes (Ninh *et al.,* 2003).

Our obtained data showed that the elevation in serum urea and creatinine levels in $AlCl_3$-treated rats is considered as a significant marker of renal dysfunction, and this is in accordance with the findings of Szilagyi *et al* (1994), who reported that alterations in serum urea may be related to metabolic disturbances such as renal function and cation–anion balance. Rudenko *et al* (1998) reported that $AlCl_3$ intensifies the acid-secretory function of kidney and changes the transport of sodium. In addition, Katyal *et al* (1997) reported that $AlCl_3$ has been implicated in the pathogenesis of several clinical disorders, including renal dysfunction. $AlCl_3$ accumulation in kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity and affects cellular metabolism, promotes oxidative stress, and induces alterations in renal tubular p-aminohippuric acid transport and renal tubular phosphate reabsorption, together with impairment in sodium and water balance (Mahieu *et al.,* 2009).

Regarding IL-1β and TNF-α, our results exhibited significant increases in serum IL-1β and TNF-α level in $AlCl_3$ intoxicated rats. The obtained data are in agreement with Passos *et al* (2010) who mentioned that chronic inflammatory process might contribute to the neurodegeneration associated with $AlCl_3$, by overexpression of cytokines such as IL-1β and TNF-α in activated microglia surrounding amyloid plaque. Thus a direct correlation has been established between Aβ induced neurotoxicity and cytokines (Lee *et al.,* 2009).

In age-related neurodegenerative disorders, such as AD, enhancement of inflammatory processes is thought to significantly contribute to pathogenic events. The number of activated astrocytes is increased in AD and these are associated with senile plaques and with cerebral microvessels (Cullen, 1997). In the hippocampus of AD patients, there is an up-regulation of proinflammatory genes (Colangelo *et al.,* 2002), and levels of cytokines are elevated in the brain (Zhao *et al.,* 2003) as well as cerebrospinal fluid and plasma (Sun *et al.,* 2003) of AD patients. Such age-related increased neuroinflammation has adverse consequences in that the brain is sensitized to the effects of infection or stress (Bondy, 2010).

In the present study, treatment of $AlCl_3$-intoxicated rats with methionine and serine mixture displayed significant reduction in MDA and NO levels of brain cortex and hippocampus. Serum NO level was also decreased significantly. In contrast, TAC level was significantly elevated in brain cortex and hippocampus. Stawiarska-Pięta *et al*. (2012) concluded that methionine reduces the formation of free radicals and participates in restoring the antioxidant compounds in the organism. Halsted and Medici (2012) corroborated our results and reported that methionine is a known precursor of glutathione by its conversion to cysteine and can also serve as a free radical scavenger by scavenging superoxide ions, increasing reduced glutathione levels and maintaining the energy state of mitochondria. Glutathione was reported to protect the cells from oxidative damage and plays vital role in detoxification of xenobiotics (Caylak *et al.,* 2008).

Methionine plays an important role in antioxidant defense mechanism by reacting readily with oxidant to form methionine sulfoxide (Nandi *et al.,* 2005), which is the initial oxidation product that must be further oxidized to yield the sulfone product, a biologically unfavorable and irreversible reaction. Surface-resident methionine residues may serve as a shield against oxidative insult protecting vulnerable amino acids and possibly the function of proteins (Pascual *et al.,* 2010).

L-serine, one of the nonessential amino acids, is biosynthesized from an intermediate of the glycolytic system and is a precursor for the synthesis of other amino acids (glycine and L-cysteine), lipids (phospholipids and sphingolipids), and nucleotides (Kaneko *et al.,* 2009).

In view of our obtained data, manipulation with methionine and serine mixture in combination with $AlCl_3$ produced significant elevation in Na+/K+-ATPase activity. While calcium level as well as the activity of AChE were significantly reduced indicating AChE inhibitory effect of this amino acids mixture. Oxidation of methionine residues to methionine sulphoxide deprives respective proteins of their function as methyl donors and may be responsible for the loss of their biological activity. On the other hand, methionine residues can act as powerful antioxidants (Ciorba *et al.,* 1997). A similar function can presumably be fulfilled by free L-methionine, as documented in the present work. L-methionine not only partly protected against synaptosomal membrane damage and oxidative shift in the glutathione system, but also completely prevented decrease of the Na+/K+-ATPase activity. This protective effect was apparently due to an antioxidative mechanism, as manifested by a considerable reduction in the accumulation of lipid peroxidation products state (Slyshenkov *et al.,* 2002). Furthermore, the increased level of oxidative stress (shown by increase of LPO and NO concentrations) perhaps contributed to the suppressed ATPase activities induced by $AlCl_3$. Methionine which is a sulfur containing nucleophile, could attenuate all of these biochemical changes by preventing either the depletion of glutathione or the binding to protein sulfhydryl groups (Huang *et al.,* 2008).

A number of observations, however, indicate that exogenous D-serine in the plasma may play an immediate role in N-methyl-D-aspartate (NMDA) receptor function. NMDA receptor are selectively and differentially decreased in areas of brain with AD (Sze *et al.,* 2001), suggesting that AD might be associated with a loss of NMDA receptors in selected brain regions (Hashimoto *et al.,* 2004). Wolosker *et al*. (2002) suggest that D-serine in the blood may originate from the brain, and that reduced levels of serum D-serine among patients may reflect decreased levels of D-serine in the brain, resulting in the hypofunction of the NMDA receptor in patients with AD.

Imbalances in the NMDA receptor system are involved in many brain diseases, elevated AChE activity and Na^+/K^+-ATPase inhibition allowing excessive Ca^{2+} entry into neurons with resultant toxic events similar to excitotoxicity and implicated in the pathological and physiological abnormality or neurodegenerative diseases such as AD (Hynd *et al.,* 2004). Nagata *et al*. (1995, 1998) showed that NMDA receptors exist on acetylcholine- and noradrenaline-containing neurons in the hippocampus, and they modulate the release of acetylcholine and noradrenaline from respective terminals.

It is well accepted that activation of NMDA receptor in hippocampal neurons is necessary for some forms of synaptic plasticity and in brain ischemia by regulating Ca^{+2} influx. Thus, the serine phosphorylation may be a kind of cellular response which protects neurons during brain ischemia (Hao *et al*., 2005).

As shown from our results, the hepatic and renal toxicity of $AlCl_3$ which were observed by the significant elevations of serum ALT, AST, urea and creatinine values can be modulated by administration of the amino acids mixture in combination with $AlCl_3$.The liver plays a central role in methionine metabolism as nearly half the daily intake of methionine is metabolized there. The first step in methionine metabolism is the formation of S-adenosylmethionine (SAM) in a reaction catalyzed by methionine adenosyltransferase (Lu *et al.,* 2002). SAM has been used increasingly for the treatment of liver diseases. In animal models of alcoholic liver disease and carbon tetrachloride hepatotoxicity, exogenous administration of SAM prevented the depletion of SAM and GSH levels and significantly ameliorated liver injury, including fibrosis (Halsted *et al.,* 2012). These findings may explain the attenuation in serum ALT and AST resulted from methionine supplementation. Stawiarska-Pięta *et al*. (2012) concluded that the beneficial influence of methionine on the morphological picture of the liver and kidney may be attributed to the activity of enzymes associated with glutathione which suggest that methionine act as antioxidants that may be effective in reversing the toxic changes resulting from the exposure to $AlCl_3$.

Our emerging data elicited that methionine and serine amino acids mixture has positive antiinflammatory effect represented by inhibiting the release of proinflammatory mediators, including TNF-α and IL-1β. Confirming these results, Locatelli *et al.* (2013) mentioned that methionine/choline-deficient diet caused lobular inflammation and TNFα production; this might be due to higher NF-κB activity, whose activation modulates the expression of genes involved in development, plasticity, and inflammation. Another important protective mechanism for methionine is that the first step in methionine metabolism is the formation of SAM which mediated inhibition of TNF-α release from macrophages (Lu *et al.,* 2002). Kawamoto *et al*. (2012) reported that NF-κB activation, which was partially blocked by NMDA receptor coagonist leading to reduced TNF-α and IL-1β. This response suggests the modulatory action of NMDA receptor in rat hippocampus as an effect of serine supplementation.

In conclusion, methionine and serine amino acids may play a beneficial role in delaying the progression of neurodegenerative disorders associated with aging. This beneficial effect may be related to the antioxidant properties of these amino acids.

References

Aly, H.F., M. Fatheya, M. Metwally, and H.H. Ahmed, 2011. Neuroprotective effects of dehydroepiandrosterone (DHEA) in rat model of Alzheimer's disease. Actabiochemica Polonica, 58: 513-520.

Amanvermez, R., O.K. Tunçel, S. Demir, M. Kefeli, Y. Bek, and C. Celik, 2008. Protective effects of cysteine, methionine and vitamin C on the stomach in chronically alcohol treated rats. Appl. Toxicol., 28: 591-598.

Bihaki, S.W., M. Sharma, A.P. Singh, and M. Tiwari, 2009. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. Journal of Ethnopharmacology, 124: 409-415.

Bogdanovic, M., A.B. Janeva, P. Bulat, 2008. Histopathological changes in rat liver after a single high dose of aluminium. Arh. Hig. Rada. Toksikol. 59, 97–101.

Bondy, S.C., 2010. The neurotoxicity of environmental aluminum is still an issue. NeuroToxicology, 31: 575-581.

Bondy, S.C., D. Liu, and S. Guo-Ross, 1998. Aluminum treatment induces nitric oxide synthase in the rat brain. Neurochem. Int. 33: 51-54.

Butterfield, D.A. and D. Boyd-Kimball, 2005. The critical role of methionine 35 in Alzheimer's amyloid β-peptide (1–42)-induced oxidative stress and neurotoxicity. Biochimica et Biophysica Acta, 1703: 149-156.

Caylak, E., M. Aytekin, and I. Halifeoglu, 2008. Antioxidant effects of methionine, a-lipoic acid, N-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats. Experimental and Toxicologic Pathology, 60: 289-294.

Cheng, P., S. Liu, Y. Young, S. Lin-Shiau, 2006. D-Methionine attenuated cisplatin-induced vestibulotoxicity through altering ATPase activities and oxidative stress in guinea pigs. Toxicology and Applied Pharmacology, 215: 228–236.

Christen Y, 2000.. Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr., 71: 621–629.

Ciorba, M.A., S.H. Heinemann, H. Weissbach, N. Brot, and T. Hoshi, 1997. Modulation of potassium channel function by methionine oxidation and reduction, PNAS USA, 94: 9932-9937.

Colangelo, V., J. Schurr, M.J. Ball, R.P. Pelaez, N.G. Bazan, and W.J. Lukiw, 2002. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J. Neurosc. Res., 70: 462-473.

Cullen, KM. 1997. Perivascular astrocytes within Alzheimer's disease plaques. Neuroreport, 8: 1961-1966.

Flora, S.J.S., A. Mehta, K. Satsangi, G.M. Kannan, and M. Gupta, 2003. Aluminum induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA. Comp. Biochem. Physiol., 134 (Part C): 319-28.

Garcia, T. J.L. Esparza, M.B. Nogués, M. Romeu, J.L. Domingo, and M. Gomez, 2010. Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminum. Hippocampus, 20: 218-225.

Halsted, C.H., and V. Medici, 2012. Aberrant hepatic methionine metabolism and gene methylation in the pathogenesis and treatment of alcoholic steatohepatitis. International Journal of Hepatology, 46: 94-98.

Hao, Z., D. Pei, Q. Guan, and G. Zhang, 2005. Calcium/calmodulin-dependent protein kinase II (CaMKII), through NMDA receptors and L-Voltage-gated channels, modulates the serine phosphorylation of GluR6 during cerebral ischemia and early reperfusion period in rat hippocampus. Molecular Brain Research, 140: 55-62.

Hashimoto, A. and Y. Chiba, 2004. Effect of systemic administration of D-serine on the levels of D- and L-serine in several brain areas and periphery of rat. European Journal of Pharmacology, 495: 153-158.

Huang, C., C. Hsuc, S. Liu, and S. Lin-Shiau, 2008. Neurotoxicological mechanism of methylmercury induced by low-dose and long-term exposure in mice: Oxidative stress and down-regulated Na^+/K^+-ATPase involved. Toxicology Letters, 176: 188-197.

Hynd, M.R., H.L. Scott, and P.R. Dodd, 2004. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem. Int., 45: 583-95.

Kaneko, I., L. Han, T. Lui, J. Li, Y. Zhao, C. Li, Y. Yi, A. Liang, and K. Hayamizu, 2009. A 13-week subchronic oral toxicity study of L-serine in rats. Food and Chemical Toxicology, 47: 2356-2360.

Katyal, R., B. Desigan, C.P. Sodhi, and S. Ojha, 1997. Oral aluminum administration and oxidative injury. Biol. Trace. Elem. Res., 57: 125-130.

Kawamoto, E.M., L.S. Lima, C.D. Munhoz, L.M. Yshii, P.F. Kinoshita, F.G. Amara, R.R. Pestana, A.M. Orellana, J. Cipolla-Neto, L.R. Britto, M.C. Avellar, L.,V. Rossoni, C. Scavone, 2012. Influence of N-methyl-D-aspartate receptors on ouabain activation of nuclear factor-κB in the rat hippocampus. J. Neurosci. Res., 90: 213-228.

Kumar, A., S. Dogra, and A. Prakash, 2009. Protective effect of curcumin (Curcuma longa), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. Behavioural Brain Research, 205: 384–390.

Lee, K.S., J.H. Chung, T.K. Choi, S.Y. Suh, B.H. Oh, and C.H. Hong, 2009. Peripheral cytokines and chemokines in Alzheimer's disease. Dement. Geriatr. Cogn. Disord., 28: 281-287.

Locatelli, I., S. Sutti, M. Vacchiano, C. Bozzola, and E. Albano, 2013. NF-κB1 deficiency stimulates the progression of non-alcoholic steatohepatitis (NASH) in mice by promoting NKT-cell-mediated responses. Clin. Sci. (Lond)., 124: 279-287.

Lu, S.C., H. Tsukamoto, and J.M. Mato, 2002. Role of abnormal methionine metabolism in alcoholic liver injury. Alcohol, 27: 155-162.

Mahieu, S., M.C. Contini, M. Gonzalez, and N. Millen, 2009. Melatonin reduces oxidative damage induced by aluminium in rat kidney. Toxicology Letters, 190: 9-15.

Mohamad, E.M., H.H. Ahmed, S.F. Estefan, A.E. Farrag, and R.S. Salah, 2011. Windows into estradiol effects in Alzheimer's disease therapy. Europian Review for Medical and Pharmacological Sciences, 15: 1131-1140.

Moncada, S., R.M. Palmer, and E.A. Higgs, 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev., 43: 109-142.

Nagata, Y., M. Borghi, G.H. Fisher, and A. D'Aniello, 1995. Free D-serine concentration in normal and Alzheimer human brain. Brain Res. Bull., 38: 181-183.

Nagata, Y., T. Uehara, Y. Kitamura, Y. Nomura, and K. Horiike, 1998. D-serine content and D-[3H] serine binding in the brain regions of the senescence-accelerated mouse. Mechanisms of aging and development, 104: 115-124.

Nandi, D., R. Patra, and C.D. Swarup, 2005. Effect of cysteine, methionine, ascorbic acid and thiamine on arsenic-induced oxidative stress and biochemical alterations in rats. Toxicology, 211: 26-35.

Ninh, T., M.D. Nguyen, M.D.S. Braley, 2003. Comparison of postoperative hepatic function after laparoscopic versus open gastric bypass. Am. J. Surg., 186: 40–44.

Pascual, I., I.M. Larrayoz, M.M. Campos, and I.R. Rodriguez, 2010. Methionine sulfoxide reductase B2 is highly expressed in the retina and protects retinal pigmented epithelium cells from oxidative damage. Experimental Eye Research, 90: 420-428.

Passos, G.F, C.P. Figueiredo, R.D. Prediger, K.A. Silva, J.M. Siqueira, and F.S. Duarte, 2010. Involvement of phosphinositide 3-kinase gamma in the neuro-inflammatory response and cognitive impairment induced by beta amyloid 1–40 peptide in mice. Brain Behav. Immun., 24: 493-501.

Rudenko, S.S., B.M. Bodnar, O.L. Kukharchuk, V.M. Mahalias, M.M. Rybshchka, I.O. Ozerova, K.M. Chala, and M.V. Khalaturnik, 1998. Effect of selenium on the functional state of white rat kidney in aluminum cadmium poisoning. Ukr. Biokhim. Zh., 70: 98-105.

Ruiz-Larrea, M.B., M. Liza, M. Lacort, and H. DeGroot, 1994. Antioxidant effects of estradiol and 2 hydroxyestradiol on iron induced lipid peroxidation of rat liver microsome. Steroids, 59: 383-388.

Schermer, S. 1967. In: The blood morphology of laboratory animals. 3rd. Ed. Philadelphia F. A. Davi Co. P. 42.

Sethi, P., A. Jyoti, E. Hussain, and D. Sharma, 2009. Curcumin attenuates aluminium-induced functional neurotoxicity in rats. Pharmacology, Biochemistry and Behavior, 93: 31-39.

Shati, A.A., F.G. Elsaid, and E.E. Hafeza, 2011. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of *Crocus sativus L*. extraction and honey syrup. Neuroscience, 175: 66-74.

Slameňová, D., E. Horvathova, Z. Kovacikova, K. Kozics, and L. Hunakova, 2011. Essential rosemary oil protects testicular cells against DNA-damaging effects of H_2O_2 and DMNQ. Food Chemistry, 129: 64-70.

Slyshenkov, V.S., A.A. Shevalye, A.V. Liopo, and L.Wojtczak, 2002. Protective role of methionine against free radical damage of rat brain synaptosomes, Acta Biochim. Pol., 49: 907-916.

Stawiarska-Pięta, B., B. Bielec, K. Birkner, E. Birkner, 2012. The influence of vitamin E and methionine on the activity of enzymes and the morphological picture of liver of rats intoxicated with sodium fluoride. Food and Chemical Toxicology, 50: 972-978.

Steel, R.G. and G.H. Torrie, 1960. Principles and procedures of statistics and biometrical approach. 2nd ed. pp.71-117, McGraw-Hill Book Co., New York, Tronto and London.

Stefanello, F., M. Ferreira, A.G.k. Talita, C.B. Pereira, M.J. Cunha, C.D. Bonan, M.R. Bogo, A.T.S. Wyse, 2011. Acute and chronic hypermethioninemia alter Na^+,K^+-ATPase activity in rat hippocampus: prevention by antioxidants. Int. J. Devl. Neuroscience 29: 483-488.

Sun, Y.X., L. Minthon, A. Wallmark, S. Warkentin, K. Blennow, S. Janciauskiene, 2003. Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. Dement. Geriatr. Cogn. Disord., 16: 136-144.

Sze, C., H. Bi, B.K. Kleinschmidt-DeMasters, C.M. Filley, L.J. Martin, 2001. N-Methyl-D-aspartate receptor subunit proteins and their phosphorylation status are altered selectively in Alzheimer's disease. J. Neurol. Sci., 182: 151– 159.

Szilagyi, M., J. Bokori, S. Fekete, F. Vetesi, M. Albert, and I. Kadar, 1994. Effects of long-term aluminum exposure on certain serum constituents in broiler chickens. Eur. J. Clin. Chem. Clin. Biochem., 32: 485-486.

Tabatabaie, L., L.W. Klomp, R. Berger, and T.J. de Koning, 2010. L-Serine synthesis in the central nervous system: A review on serine deficiency disorders. Molecular Genetics and Metabolism, 99: 256-262.

Tanrikulu-Kucuk , S. and E. Ademoglu. 2012. Dietary restriction of amino acids other than methionine prevents oxidative damage during aging: Involvement of telomerase activity and telomere length. Life Sciences, 90: 924-928.

Toescu, C. and M. Vreugdenhil, 2010. Calcium and normal brain aging. Cell Calcium, 47: 158-164.

Tsakiris, S., K.H. Schulpis, K. Marinou, and P. Behrakis, 2004. Protective effect of L- cysteine and glutathione on the modulated suckling rat brain Na+-K+ ATPase and Mg+2-ATPase activities induced by the "in vitro" galactoseaemia. Pharmacological Research, 49: 475-479.

Walton, J. (2006): Aluminum in hippocampal neurons from humans with Alzheimer's disease, Neurotoxicology, 27: 385-394.

Wang, B., W. Xing, Y. Zhao, and X. Deng, 2010. Effects of chronic aluminum exposure on memory through multiple signal transduction pathways. Environmental Toxicology and Pharmacology, 29: 308-313.

Wolosker, H., R. Panizzutti, and J. De Miranda, 2002. Neurobiology through the looking-glass: D-serine as new glial-derived transmitter. Neurochem. Int., 41: 327-332.

Yousef, M. I. 2004. Aluminum induced changes in hematobiochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology, 199: 47-57.

Yousef, M.I. and A.F. Salama, 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food and Chemical Toxicology, 47: 1168-1175.

Zhang, B., A. Nie, W. Bai, and Z. Meng, 2004. Effects of aluminum chloride on sodium current, transient outward potassium current and delayed rectifier potassium current in acutely isolated rat hippocampal CA1 neurons. Food Chem. Toxicol., 42:1453–1462.

Zhao, H., Q. Li, Z. Zhang, X. Pei, J. Wang, and Y. Li, 2009. Long-term ginsenoside consumption prevents memory loss in aged SAMP8 mice by decreasing oxidative stress and up-regulating the plasticity-related proteins in hippocampus. Brain Res., 1256: 111-122.

Zhao, M., D.H. Cribbs, A.J. Anderson, B.J. Cummings, J.H. Su, A.J. Wasserman, 2003. The induction of the TNF alpha death domain signaling pathway in Alzheimer's disease brain. Neurochem. Res., 28: 307-318.