Scientific Research and Essays Vol. 8(4), pp. 168-177, 25 January, 2013
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1849
ISSN 1992-2248 ©2013 Academic Journals

Full Length Research Paper

Amelioration of aluminium induced toxicity by *Allium sativum*

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Accepted 22 January, 2013

Garlic (*Allium sativum* L., Liliaceae) is a nature's boon to mankind, which has played an important dietary and therapeutic medicinal role throughout the history of mankind. Aluminum (Al) is a potent neurotoxin and has been associated with Alzheimer's disease causality for decades. The study aims to demonstrate the ameliorative effect of garlic extract against aluminium-induced toxicity in rat model. Animals received aluminium nitrate 32.5 mg/kg (1/2 LD_{50} of Al $(NO_3)_3$ *i.p.*) once only. Different doses of garlic extract (25, 50 and 100 mg/kg) was administered for 3 days after 24 h of Al exposure. The activity of AChE was inhibited in all the parts of the brain, significant rise was observed in the activities of serum LDH, AST and ALT whereas serum protein was declined after toxicant exposure. The activity of δ-aminolevulinic acid dehydratase in blood and δ-aminolevulinic acid synthetase in brain depicted fall after Al exposure. Al provoked significant increase in the level of cholesterol, triglyceride, creatinine and urea level in serum. TBARS level, total and esterified cholesterol in liver, kidney and brain were significantly higher with concomitant fall in reduced glutathione content during toxicity. Garlic markedly attenuated oxidative stress by scavenging various free radicals significantly restored the deranged biochemical variables. Histopathological changes in liver, kidney and brain were also recouped and biochemical findings substantiated. Results of present study concluded that garlic can counteract the deleterious effects of aluminium.

Key words: Garlic extract, aluminium, oxidative stress, liver, kidney, brain.

INTRODUCTION

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones. Garlic (*Allium sativum*) is a commonly worldwide used food, and its medical properties have been well recognized since the ancient times (Craig and Beck, 1999). Many beneficial health properties of garlic are attributed to organosulfur compounds, particularly to sulfur-bearing compounds such as allicin, S-allyl-cysteines, diallyl-di-sulfide and Diallyl-sulfide (Koch and Lawson, 1996). Garlic is one of the well known plants with multiple beneficial effects such as antimicrobial (Johnson and Vaughn, 1969), hypolipidemic (Bordia et al., 1975), hypoglycemic (Jain and Vjas, 1975) anticarcinogenic (Hussain et al., 1990), antifungal (Amer et al., 1980), anti-atherosclerotic (Bordia and Verma, 1980) and also acts as antioxidant against free radicals (Banerjee et al., 2003). It has been shown to be significantly effective against lead**,** cadmium, nickel, chromium, arsenic and mercury poisoning (Massadeh, 2009; Das Gupta et al., 2009; 2008; Kalayarasan et al., 2008; Senapati et al., 2001; Tandon et al., 2001; Lee et al., 1999).

Aluminium (Al) is the most widely distributed metal in the environment and is extensively used in daily life that provides easy exposure to human beings (Kumar and Gill, 2009). It is present in many manufactured foods, medicines, cheese, tea, cosmetics and is also added to drinking water during purification purposes (Newairy et al., 2009; Yousef and Salama, 2009). Al is, a non-redox active metal, a pro-oxidant and will promote biological oxidation both *in vitro* and *in vivo* (Exley, 2004), which disrupts the pro-oxidant/antioxidant balance of tissues and leads to various biochemical and physiological dysfunction (Nehru and Bhalla, 2006). It may bind to

DNA, RNA and inhibit enzymes such as hexokinase, acid and alkaline phosphatases, phosphodiesterase and phosphooxydase (Ochmanski and Barabasz, 2000). Different forms of Al are environmental xenobiotics which accumulate in different organs and provoke free radical-mediated cardiotoxicity, hepatotoxicity nephrotoxicity, neurotoxicity and caused alterations in antioxidant enzymes, both *in vivo* and *in vitro* (Belaid-Nouira et al., 2012; Turkez et al., 2010; Kan et al., 2010; Yousef and Salama, 2009; Prakash and Kumar, 2009; Bihagi et al., 2009; Mahieu et al., 2009; Sushma and Rao, 2007; Reinke et al., 2003). The high Al diet led to inflammation neutrophilic and oxidative stress in welders of aircraft plant (Stark et al., 2009). Yousef (2004) and Newairy et al. (2009) reported that Al-induced changes in biochemical parameters, increased lipid peroxidation and decreased the activities of the antioxidant enzymes in plasma and different tissues of animals. The liver is a critical organ which contains most of the accumulated metals and where toxic effects can be expected (Kurutas et al., 2009). Several preventive agents such as melatonin (Garcia et al., 2009), boric acid (Turkez et al., 2010), lithium (Bhalla and Dhawan, 2009), vitamin C and E (Yousef and Salama, 2009; Newairy et al., 2009; Yousef et al., 2007) were studied to minimize Al toxicity but not found significantly effective. So, the aim of the study was to evaluate the antioxidant potential of hydroalcoholic garlic extract against Al induced toxicity in rat model.

MATERIALS AND METHODS

Experimental animals

Sprague Dawley female albino rats weighing 160 ± 10 g from department animal facility were selected where they received standard pellet diet (Pranav Agro Industries, New Delhi, India having metal contents in ppm dry weight Cu, 10; Mn, 33; Zn, 45; and Co, 5) and drinking water *ad libitum*. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, Ministry of Culture, Chennai.

Chemicals

Aluminium nitrate (Qualigens Fine Chemicals Pvt. Ltd., India, Product no 21255) and other analytical grade laboratory reagents were procured from Merck (Germany), HiMedia and Glaxo chemical (India).

Extract preparation

Garlic was procured from local market and authenticated by the senior taxonomist of Botany department. The cloves were dried in shade and powdered (250 g) were mix with 50% alcohol (1 L) and shaking /day for 15 days. The extract was filtered and evaporated to give yellow powder. An aqueous suspension of crude extract was administered to the animals orally according to their weight.

Experimental design

Group 1: Control.
Group 2: Aluminium nitrate (1/2LD_{50} *i.p.* once only)
Groups 3: Al+ Garlic extract (25 mg/Kg, *p.o*. for 3 days)
Groups 4: Al+ Garlic extract (50 mg/Kg, *p.o*. for 3 days)
Groups 5: Al+ Garlic extract (100 mg/Kg, *p.o*. for 3 days)

Animals of all the groups were sacrificed after 48 h of last treatment for biochemical analyses.

Evaluation of antioxidant properties

Free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). The antioxidant activities of the garlic extract 88 (100 to 500 µg/ml) and standard vitamin C (10 to 50 µg/ml) were assessed on the basis of the radical scavenging effect of the stable. DPPH free radical (Blois, 1958). Vitamin C was used as the positive reference.

Quantitative estimation of total phenolic contents

The total phenolic content of garlic extract was determined with Folin–Ciocalteu reagent in alkaline medium using tannic acid as standard (Slinkard and Singleton, 1977). The total phenolic content was expressed in µg of tannic acid equivalents/mg of extract.

Biochemical assays

Blood was by puncturing the retro-orbital venosus sinus (Riley, 1960) and serum was isolated for the estimation of AST and ALT (Reitman and Frankel, 1957), Lactate dehydrogenase (LDH), (Wrobleski and Due, 1955) and serum protein (Lowry et al., 1951). Serum cholesterol, triglyceride (TG), creatinine and urea (kit method) were estimated by autoanalyser. The activity of δ-aminolevulinic acid dehydratase (ALAD) (Berlin and Schaller, 1974) was determined in blood. The activity of acetylcholinesterase (AChE) was determined in different parts of brain (Ellman et al., 1961). Homogenate in an isotonic solution was processed for total and esterified cholesterol (Zlatkis et al., 1953). Hepatic, renal and cerebral LPO (Sharma and Krishnamurthi, 1968) and GSH (Brehe and Burch, 1976) was estimated. The activity of δ-aminolevulinic acid synthetase (ALAS) was also determination in brain (Maines, 1980).

Metal estimation

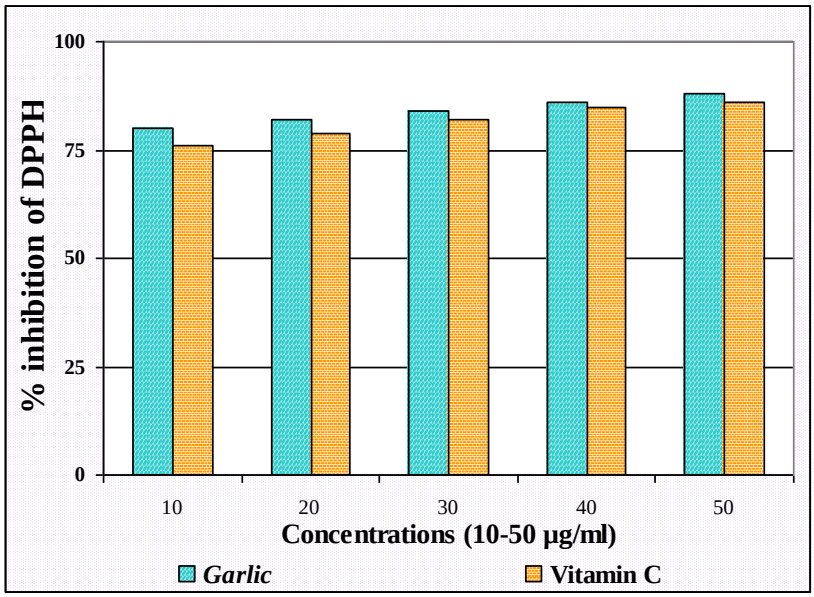
For atomic absorption studies, samples were prepared by dried digestion with HNO_3, diluted with water and read by the AAS (Boume et al., 1986), so as to estimate the concentration of the metal ion in liver, kidney, brain and serum.

Histopathological assays

Liver, kidney and brain were dissected out washed in saline and fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 µm and stained with haemotoxylin and eosin for examination by light microscopy.

Statistical analysis

P values at the level of ≤ 0.05 were evaluated by student's t test.



Graph 1. Demonstrates the antioxidant activity of garlic extract using DPPH dye.

Table 1. Total phenolic contents.

Concentration of extract	Tannic acid µg/mg of extract
Garlic extract, 1 mg	64.0 µg/mg

Significance of the difference among various groups was evaluated by one way analysis of variance (ANOVA) @ F=P≤0.05 (Snedecor and Cochran, 1994).

RESULTS

Antioxidant activity of garlic extract

DPPH assay is widely used as a free radical to evaluate the antioxidant activity of natural compounds. Graph 1 demonstrates the antioxidant activity of garlic extract using DPPH dye. Garlic extract at different doses, that is, 10 to 50 g/ml showed free radical scavenging activity in dose dependent manner. Maximum percentage inhibition of DPPH radicals by the ginger extract was about 88% at 50 µg/ml concentration. Standard drug ascorbic acid showed about 86% inhibition of the DPPH radicals at 50 µg/ml. The amount of phenolic components was calculated as a tannic acid equivalent as presented in Table 1 and was found to be 64 µg/mg in samples indicating considerable free radical scavenging activity.

Biochemical observations

The result demonstrates that the toxicity produced by Al was associated with a variety of biochemical abnormalities and these could usually be attributed to the release of intracellular constituents in to the circulation. Tables 1 and 2 depicts that administration of Al elevated the activity of serum AST, ALT and LDH where as activity of ALAD was found to be depleted (P≤0.05). Garlic extract therapy showed recovery pattern in a dose dependent manner. The serum profile of Al exposed rats reveals significant rise in triglyceride, cholesterol, creatinine and urea however induced depletion in the serum protein (P≤0.05), which was significantly restored with garlic extract therapy (Table 3). It reduced the AChE activity of cerebellum (hindbrain) when compared to controls. At the same time the midbrain and cortex (forebrain) enzyme activity was also reduced (P≤0.05). Toxicant also caused depletion in ALAS in brain. Treatment of garlic extract significantly restored all these parameters (Table 4). These results indicate a protective effect of extract (P≤0.05) on the membrane by virtue of its antioxidant property.

Further, we assessed oxidative stress and antioxidant activity in the liver and the kidney and the results are summarized in Table 5. Hepatic, renal and cerebral (brain) LPO was significantly increased after Al exposure whereas hepatic, renal and cerebral GSH was significantly decreased. Combination of garlic extract reduced TBARS concentration and restored the levels of GSH significantly (P≤0.05). Total cholesterol and esterified cholesterol in the liver, kidney and brain was increased after toxicant administration. Extract has a protective effect on the Al induced changes in the lipid levels (Table 6). Control rats had extremely small amount of Al in liver, kidney and brain. Exposure led to a pronounced increase in Al body burden in all the organs (P≤0.05). The accumulation of Al was in the order

Table 2 Effect of Garlic extract against Al treated rats in liver function test.

Treatment	ALAD(n mol/min/ ml erytrhocytes)	LDH (µ mole/min/L)	AST(IU /L)	ALT (IU /L)
Control	7.90±0.43	41.5±2.29	67.9±3.75	41.3±2.28
Al	3.30±0.18#	58.9±3.25#	159±8.78#	129±7.13#
Al + Garlic 25	6.06±0.33*	56.6±3.12	108±5.97*	64.0±3.53*
Al + Garlic 50	6.80±0.37*	52.3±2.89	107±5.91*	59.0±3.26*
Al + Garlic 100	7.22±0.39*	49.3±2.72*	102±5.63*	54.5±3.01*
Anova (F Value)	29.9@	6.73@	32.8@	79.9@

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group. ANOVA (F values) @ = Significant at 5 % level.

Table 3. Effect of Garlic extract against Al treated rats in liver and kidney function tests.

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Protein (mg/100 ml)
Control	19.8±1.09	0.22±0.01	68.9±3.80	47.2±2.60	37.3±2.06
Al	56.2±3.10#	0.69±0.03#	94.2±5.20#	75.9±4.19#	25.2±1.39#
Al + Garlic 25	44.6±2.46*	0.47±0.02*	76.0±4.20*	63.0±3.48	30.1±1.66*
Al + Garlic 50	38.2±2.11*	0.44±0.02*	73.0±4.03*	49.1±2.71*	33.1±1.82*
Al + Garlic 100	38.0±2.10*	0.43±0.02	72.7±4.01*	48.3±2.67*	35.1±1.95*
Anova (F Value)	40.5@	48.7@	6.49@	18.4@	8.27@

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group. ANOVA (F values) @ = Significant at 5 % level.

Table 4. Effect of Garlic extract against Al treated rats in brain function tests.

Treatment	Acetyl Cholinesterase (µ mole / min / mg protein)		ALAS (n mol/min/ mg Protein)	
	Fore Brain	Mid Brain	Hind Brain	Fore Brain
Control	41.4±2.28	21.4±1.18	40.5±2.23	11.9±0.65
Al	17.9±0.98#	9.48±0.52#	21.5±1.18#	4.1±0.22#
Al + Garlic 25	25.9±1.43*	12.9±0.71*	27.5±1.52*	9.50±0.52*
Al + Garlic 50	25.9±1.43*	13.5±0.74*	29.9±1.65*	9.88±0.54*
Al + Garlic 100	31.2±1.72*	15.5± 0.85*	32.1±1.77*	10.3±0.57*
Anova (F Value)	33.7@	33.2@	19.8@	38.1@

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group. ANOVA (F values) @ = Significant at 5 % level.

Table 5. Effectiveness of Garlic extract against aluminium treated rats in LPO and GSH in tissues

Treatment	Lipid peroxidation (n mole MDA / mg protein)			Glutathione (µ mole / g)		
	Liver	Kidney	Brain	Liver	Kidney	Brain
Control	0.39±0.02	0.68±0.03	1.02±0.05	7.18±0.39	7.10±0.39	7.20±0.39
Al	1.35±0.07#	1.99±0.11#	2.18±0.12#	5.03±0.27#	3.33±0.18#	4.90±0.27#
Al + Garlic 25	0.46±0.02*	1.10±0.06*	1.49±0.08*	6.40±0.35*	6.20±0.34*	6.12±0.33*
Al + Garlic 50	0.43±0.02*	1.09± 0.06*	1.23±0.07*	6.50±0.35*	6.50±0.35*	6.30±0.34*
Al + Garlic 100	0.43±0.02*	1.06±0.06*	1.15±0.06*	6.70±0.37*	6.90±0.38*	6.85±0.37*
Anova (F Value)	131@	57.8@	38.5@	6.17@	24.4@	7.61@

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group. ANOVA (F values) @ = Significant at 5 % level.

Table 6. Effect of Garlic extract against aluminium treated rats in total and esterified cholesterol in tissues.

Treatment	Total Cholesterol (mg /100 mg)			Esterified Cholesterol (mg /100 mg)		
	Liver	Kidney	Brain	Liver	Kidney	Brain
Control	0.12±0.006	0.11±0.006	2.08±0.11	0.06±0.003	0.04±0.002	0.17±0.009
Al	1.23±0.067#	0.76±0.042#	3.99±0.22#	0.20±0.011#	0.16±0.008#	0.66±0.036#
Al + Garlic $_{25}$	0.81±0.044*	0.38±0.021*	3.00±0.16*	0.12±0.006*	0.11±0.006*	0.49±0.027*
Al + Garlic $_{50}$	0.64±0.035*	0.38±0.021*	2.80±0.15*	0.11±0.006*	0.10±0.006*	0.46±0.025*
Al + Garlic $_{100}$	0.25±0.013*	0.32±0.017*	2.40±0.13*	0.10±0.006*	0.09±0.005*	0.45±0.024*
Anova (F Value)	145$^{@}$	108$^{@}$	23.83$^{@}$	64.7$^{@}$	56.9$^{@}$	54.3$^{@}$

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group.ANOVA (F values) @ = Significant at 5 % level.

Table 7. Effect of Garlic extract against aluminium treated animals in metal concentration.

Treatment	Liver	Kidney	Brain
Control	16.00±0.88	16.20 ±0.89	13.20 ±0.71
Al	33.22±1.83#	42.00 ±2.32 #	41.80 ±2.32 #
Al + Garlic $_{25}$	30.78±1.70	36.90 ±2.03	35.16 ±1.94*
Al + Garlic $_{50}$	29.82±1.64	31.00 ±1.71*	28.68 ±1.58*
Al + Garlic $_{100}$	23.13±1.27	29.17 ± 1.61*	26.42 ± 1.46*
Anova (F Value)	25.8$^{@}$	35.7$^{@}$	49.2$^{@}$

Values are mean ± S.E., N = 6. # P ≤ 0.05 vs control group, * P ≤ 0.05 versus Al administered group. ANOVA (F values) $^{@}$ = Significant at 5 % level.

liver>kidney>brain. Therapy of garlic extract was significantly effective in removing Al from all the organs significantly (P≤0.05) (Table 7). Extract treatment in Al intoxicated rats is effective on dose dependent manner.

Histological observations

Liver of control and garlic *per se* showed normal histoarchitecture. After Al exposure, disturbed chord arrangement, enlargement in sinusoidal spaces, hypertrophy of hepatocytes with hyperchromatic nuclei was observed. Kupffer cells were increased in number (Figure 1A). At places bile duct proliferation and inflammatory cells were seen. At 25 mg/kg dose of garlic therapy mild cytoplasmic vacuolation and granulation was seen in hepatocytes (Figure 1B). At 50 mg/kg dose of garlic therapy showed cuboidal hepatocytes with clear sinus (Figure 1C). However highest dose of garlic extract that is, 100 mg/kg showed hexagonal hepatocytes with clear nucleus (Figure 1D).

Kidney of control rat showed normal features. Al treatment showed higher degree of degeneration in cortex and medullary region. Bowman's capsules showed hypertrophy (Figure 2A). Disturbed endothelial lining was observed. Epithelial cells showed darkly stained nuclei. Cytoplasmic vacuolation was also noted in the renal tubules. Lumen of tubules was filled with debris. Improvement in Bowman's capsules was seen at 25 mg/kg dose of garlic treatment (Figure 2B). Renal tubules were maintained with compact glomeruli at 50 mg/kg garlic therapy (Figure 2C). However endothelial lining was maintained with higher most dose of garlic that is, 100 mg/kg (Figure 2D). Bowman's capsules and the glomeruli were also recouped. In some regions the epithelial cells of the uriniferous tubules showed apical position of nuclei.

Brain of control rats showed normal cerebral hemispheres, loose vascular supporting tissue called endoneurium, which was surrounded by a condensed layer of robust collagenous tissue \called the perineurium. Epineurium was condensed. Well formed astrocytes, oligodendrites, pyramidal cells, Schwann cells and fibroblasts were noted in the cortex region. After Al exposure neuronal degeneration and glial cell (microglia) proliferation was observed in brain, pyknotic neurons were noticed. Cerebellar atrophy was also observed (Figure 3A). Garlic extract of 25 mg/kg showed mild improvement in Purkinje cells. Astrocytes led to scattered neurons with reduced vacuolation in granule cell layer. The oligodendrites were reduced in size (Figure 3B). Astrocytes, oligodendrites and Purkinje cells were maintain at 50 mg/Kg dose of garlic extract but vacuoles in endoneurium were seen (Figure 3C). Treatment with higher most dose resulted in almost normal histoarchitecture depicting all the normal layers and

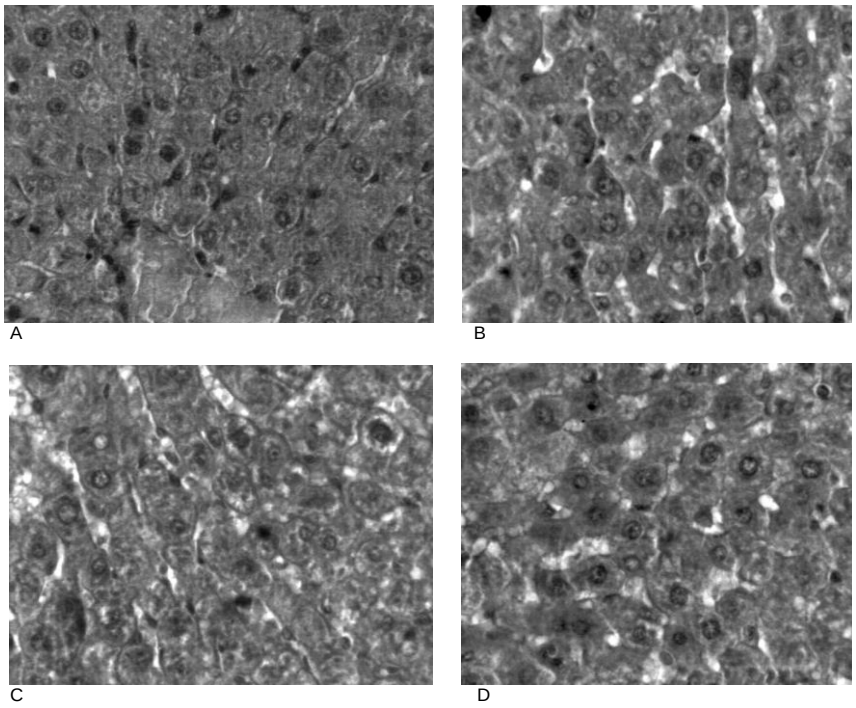


Figure 1. (A) Al administration shows enlargement in sinusoidal spaces with hyperchromatic nuclei(x400), (B) $Al(NO_3)_3$+ Garlic25 caused mild cytoplasmic vacuolation and granulation in hepatocytes (X400), (C) $Al(NO_3)_3$ + Garlic50 cuboidal hepatocytes were seen (x400). and (D) $Al(NO_3)_3$ + Garlic100 showed hexagonal hepatocytes and clear nucleus (x400).

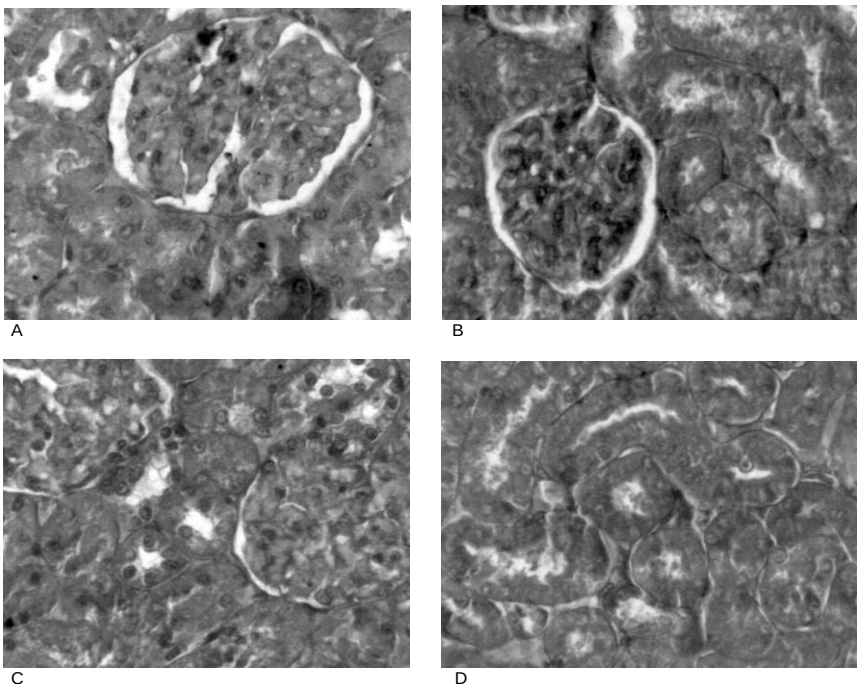


Figure 2. (A) Al exposure showed higher degree of degeneration in cortex and medullary region. Bowman's capsules showed hypertrophy (B) $Al(NO_3)_3$+ Garlic25 showed improvement in Bowman's capsules with glomeruli (x400) (C) $Al(NO_3)_3$ + Garlic50 showed compact glomeruli (x400) and (D) $Al(NO_3)_3$ + Garlic100 maintained compact glomeruli and well formed renal tubules(x400).

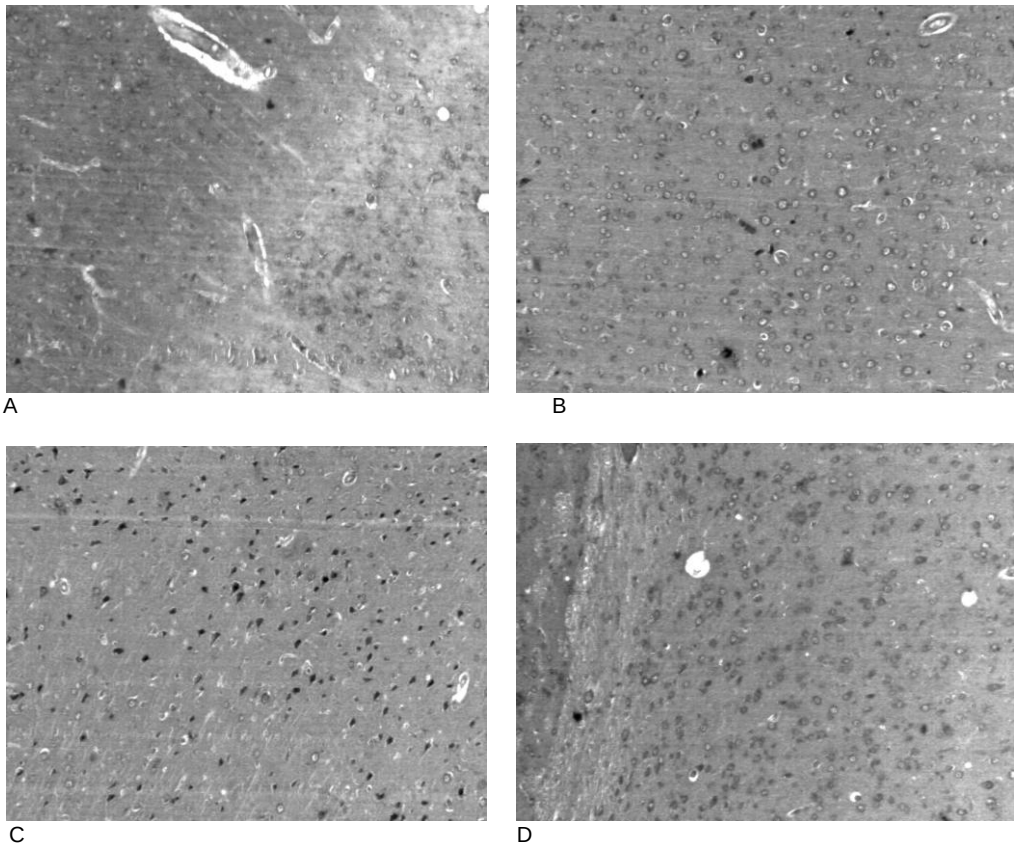


Figure 3. (A) Al exposure caused neuronal degeneration, glial cell proliferation with vacuolation(x100) (B) $Al(NO_3)_3$+ Garlic25 showed less recovery in brain cells (x100) (C) $Al(NO_3)_3$ + Garlic50 showed vacuoles in endoneurium (x100) and (D) $Al(NO_3)_3$ + Garlic100 showed well formed layers, epineurium, perineurium and endoneurium (x100).

Purkinje neurons. There was significant loss of vacuolization in neurons. Prominent nuclei were seen in Schwann cells. Fibroblasts and glial cells showed more or less normal structure (Figure 3D).

DISCUSSION

The present study was undertaken to determine whether garlic extract can prevent and/or reduce Al-induced oxidative stress by examining different biochemical parameters in serum, liver, kidney and brain in rats. Our results clearly showed significant changes in biochemical and histopathological studies after Al exposure suggesting strong prooxidant activity in spite of its non-redox status (Exley, 2004). Its toxicity may be mediated by free radical generation and alterations in antioxidant enzymes *in vivo* and *in vitro* (Tabaldi et al., 2009), which caused neurotoxicity (Wen et al., 2012; Kumar et al., 2009; Rodella et al., 2008), nephrotoxicity (Mahieu et al., 2009) and hepatotoxicity (Kutlubay et al., 2007; Abubakar et al., 2004).

Activities of serum AST, ALT and LDH were increased after toxicant administration may be due to the leakage of these enzymes from the liver cytosol into the blood stream. Supplementation of garlic extract prevented these abnormalities due to its role in stabilizing the cell membrane and protects the tissue from free radical mediated toxicity. Various authors reported similar findings after Al exposure (Belaid-Nouira et al., 2012; Wen et al., 2012; Turkez et al., 2010; Kumar and Gill, 2009; Mohamed and Awad, 2008; Nayak et al., 2006; Yousef, 2004). Al administration enhanced release of LDH significantly, might be due to increased by feed back mechanism as it is involved in glucose metabolism. It is used as marker of Al toxicity and similar finding was noted by El-Demardesh (2004), Yousef (2004) and Anane and Creppy (2001). These values were restored near to control after garlic extract may be due to its antioxidant nature.

The increase in plasma cholesterol and TG after Al administration may be due to hypoactivity of lipoprotein lipase in blood vessels which breaks triglycerides. Wilhelm et al. (1996) reported that Al exposure can result in Al accumulation in the liver and this may lead to disturbance of lipid metabolism and an elevation of serum

cholesterol (Wen et al., 2012; Kojima et al., 2004; Kantola et al., 1998). Fat lowering effect of garlic extract was found in the present study and may be due to inhibition of fatty acid synthesis (Nwanjo and Oze, 2007; Das Gupta et al., 2008). Previous studies also reported high cholesterol content in brain after Al intoxication (Pandya et al., 2004). Garlic can depress the hepatic activity of lipogenic, cholesterogenic enzymes such as malic enzymes, fatty acid synthase, glucose -6- phosphate dehydrogenase (Yu-Yan and Yeh, 2001; Jain and Koner, 1978) thus maintained lipid contents towards control.

The elevation in creatinine and urea levels in Al treated rats can be considered as a significant marker of renal dysfunction. This fact is well supported by the finding of Rudenko et al. (1998) who reported that Al intoxication intensifies acid secrete function of kidney and change the transport of sodium. In addition, Katyal et al. (1997) reported that Al has been implicated in the pathogenesis of several clinical disorders, such as renal dysfunction. The increase in urea concentrations in plasma of animals treated with Al may be due to its effect on liver function, as urea is the end-product of protein catabolism. Therapy of garlic extract decreased creatinine and urea level towards control and maintains normal physiology of kidney.

Our data point to the possible involvement of oxidative stress in Al induced hepatotoxicity. Elevation of LPO in liver, kidney and brain was evident by the increased production of TBARS, which suggests participation of free radical induced oxidative cell injury in mediating the toxicity of Al (Belaid-Nouira et al., 2012; Wen et al., 2012; Turkez et al., 2010; Kumar and Gill, 2009; Newairy, et al., 2009; Anane and Creppy, 2001). GSH play an important role in the detoxification and metabolism of many xenobiotic compounds. The decrease in SH group of GSH content was also found in the studies of Dua and Gill (2001) and Sharma and Mishra (2006). Therapy of garlic at 50 and 100 mg/kg doses provoked considerable recovery due to its antioxidant properties.

Earlier studies have already shown that ingested Al leads to the elevation of the brain content of Al in various brain regions (Deloncle et al., 1999; Kaur et al., 2003). Acetylcholinesterase (AChE) is an enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine, producing choline and an acetate group which is mainly found at neuromuscular junctions and cholinergic nervous system, where its activity serves to terminate synaptic transmission. A significant decrease was observed in the AChE of fore, mid and hind brain after Al exposure. Al may interfere with either synthesis of AChE or inhibit choline uptake by synaptosomes. The higher reduction in AChE activity of brain may suggest that this part of brain is much more susceptible to metal intoxication. Al also may bind with the active site of AChE hence, decreases the activity of AChE in fore, mid and hind brain. Wen et al. (2012), Kumar et al. (2009) and Moshtaghi et al. (1999) also reported significant decrease in AChE activity of brain after Al exposure. Treatment with garlic extract showed neuroprotection that may be by reducing Al accumulation in neurons, thus decreasing neuronal cell loss and reducing oxidative stress (Zatta et al., 1993; Suarez-Fernandez et al., 1999). The ALAS, is first and the rate-limiting enzyme of heme biosynthesis which is localized in mitochondria whereas, ALAD activity is second enzyme of the haem biosynthesis pathway. Activity of ALAD in blood and ALAS in brain was significantly decreased after Al exposure. The reduced level of hemoglobin can be associated with hemolysis or disturbances in heme biosynthesis as a result of inhibiting linking of iron with heme and drop in activity of ALAD and ALAS taking part in heme biosynthesis. Garlic extract may reduce the hemolysis, thus maintained normal level of ALAD and ALAS.

Garlic constitutes one of the first documented examples of plants used for its medicinal properties and / or antioxidant activity (Bozin et al., 2008). The efficiency of garlic is perhaps due to the presence of these sulfur-containing biologically active lipophilic compounds such as allicin, S-allyl-cysteine, diallyl disulfide and diallyl sulfide amino acids having free carboxyl (C=O) and amino (NH_2) groups in their structures. These biologically active compounds might have chelated Al by the formation of ionic bonds between sulfur containing compounds and enhanced its excretion from the body. These easily permeate through phospholipid membranes and reduce intracellular Al thus effective at fighting oxidative stress (Miron et al., 2000). It also protects histological structure of the liver, kidney and brain thereby depicting improvement at cellular level also. These results are in accordance with Banerjee et al. (2001) to some extent. Thus, present study concluded that garlic can be given as a dietary supplement to human populations, which are exposed to the environmental toxicants and its supplementation can provide protection against toxic effects without being appreciably harmful itself. The regular intake of garlic is suggested in reducing the toxic effects of heavy metals in the exposed population.

ACKNOWLEDGEMENTS

This work was supported by DST, New Delhi for financial assistance (SR/WOS-A/LS-153/2007). The author is thankful to Prof. Sangeeta Shukla, Dr. Monika Bhadauria and Jiwaji University, Gwalior (MP) for laboratory facility.

REFERENCES

Abubakar MG, Taylor A, Ferns GA (2004). The effects of aluminium and selenium supplementation on brain and liver antioxidant status in the rat. Afr. J. Biotechnol. 3(1):88-93.

Amer M, Taha M, Tosson Z (1980). The effect of aqueous garlic extract on the growth of dermatophytas. Int. Dermatol. 19:285-287.

Anane R, Creppy EE (2001). Lipid peroxidation as pathway of

aluminium cytotoxicity in human skin fibroblast cultures: Prevention by superoxide dismutase + catalase and vitamins E and C. Hum. Exp. Toxicol. 20:477-481.
Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK, Maulik SK (2001). Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem. Toxicol. 39(8):793-797.
Banerjee SK, Mukherjee PK, Maulik SK (2003). Garlic as an antioxidant: the good, the bad and the ugly. Phytother. Res. 17:97-106.
Belaid-Nouira Y, Bakhta H, Bouaziz M, Flehi-SlimI, Haouas Z, Ben Cheikh H (2012). Study of lipid profile and parieto-temporal lipid peroxidation in $AlCl_3$ mediated neurotoxicity modulatory effect of fenugreek seeds. Lipids Health Dis. 11:16-24.
Berlin A, Schaller AH (1974). European standarized method foor the determination of δ- aminolevulinic acid dehydratase activity in blood. Zeitschrif fur klinishe chemie und klinische biochemie 12:389-390.
Bhalla P, Dhawan DK (2009). Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. Cell Mol. Neurobiol. 29:513–521.
Bihagi SW, Sharma M, Singh AP, Tiwari M (2009). Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. J. Ethnopharmacol. 124(3):409-415.
Blois MS (1958). Antioxidant determination by the use of a stable free radical. Nature 181:1199–200.
Bogdanovic M, Janeva AB, Bulat P (2008). Histopathological changes in rat liver after a single high dose of aluminium. Arch. Ind. Hyg. Toxicol. 59(2):97-101.
Bordia A, Bansal HC, Arora SK, Singh SV (1975). Effect of essential oils of garlic and onion on alimentary hyperlipidemia. Atherosclerosis 21:15-19.
Bordia A, Verma SK (1980). Effect of garlic feeding on regression of experimental atherosclerosis in rabbits. Artery 7:428-437.
Boumen AA, Platenkamp AJ, Posma FD (1986). Determination of aluminum in human tissue in flame less atomic absorption spectroscopy and comparison of reference values. Ann. Clin. Biochem. 23:97-100.
Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R (2008). Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chem. 111:925–929
Brehe JE, Bruch HB (1976). Enzymatic assay for glutathione. Anal. Biochem. 74:189-197.
Craig W, Beck L (1999.) Phytochemicals: Health Protective Effects. Can. J. Diet. Pract. Res. 60:78-84.
Das GA, Das SN, Dhundasi SA, Das KK (2008). Effect of garlic (*Allium sativum*) on heavy metal (Nickel II and Chromium VI) induced alteration of serum lipid profile in male albino rats. IJERPH 5(3):147-151.
Das GA, Dhara PC, Dhundasi SA, Das KK (2009). Effect of garlic (*Allium sativum*) on nickel II or chromium VI induced alterations of glucose homeostasis and hepatic antioxidant status under sub-chronic exposure conditions. J. Basic Clin. Physiol. Pharmacol. 20(1):1-14.
Deloncle R, Huguet F, Babin P, Fernandez B, Quellard N, Guillard O (1999). Chronic administration of aluminum L-glutamate in young mature rats: Effects on iron levels and lipid peroxidation in selected brain areas. Toxicol. Lett. 104:65-73.
Dua R, Gill KD (2001). Aluminium phosphide exposure: Implications on rat brain lipid peroxidation and antioxidant defence system. J. Pharmacol. Toxicology 189:315-319.
El-Demardesh FM (2004). Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzymes activities and biochemical parameters in rats exposed to aluminium. J. Trace Elem. Med. Biol. 18:113-121.
Ellman GL, Courthey KD, Anders V, Feather S (1961). Determination of acetyl cholinesterase. Biochem. Pharmacol. 7:88-95.
Exley C (2004). The pro-oxidant activity of aluminium. Free Radic. Biol. Med. J. 36:380-387.
Garcia T, Ribes D, Colomina MT, Cabre M, Domingo JL, Gomez M (2009). Evaluation of the protective role of melatonin on the behavioral effects of aluminum in a mouse model of Alzheimer's disease. Toxicology 265:49–55.
Hussain SP, Jannu LN, Roa AR (1990). Chemopreventive action of garlic on methylcholanthrene induced carcinogenesis in the uterine cervix of mice. Cancer Lett. 49:175-180.
Jain RC, Koner DB (1978). Effect of garlic in experimental cholesterol atherosclerosis. Atherosclerosis 29:125-129.
Jain RC, Vjas CR (1975). Garlic in alloxan induced diabetic rabbits. Am. J. Clin. Nutr. 28:684-685.
Johnson MG, Vaughn RH (1969). Death of Salmonella typhimurium and Escherichia coli in the presence of freshly reconstituted dehydrated garlic and onion. Appl. Microbiol. 17:903-905.
Kalayarasan S, Sriram N, Sureshkumar A, Sudhandiran G (2008). Chromium (VI)-induced oxidative stress and apoptosis is reduced by garlic and its derivative S-allylcysteine through the activation of Nrf2 in the hepatocytes of Wistar rats. J. Appl. Toxicol. 28(7):908-919.
Kan WC, Chien CC, Wu CC, Su SB, Hwang JC, Wang HY (2010). Comparison of low-dose deferoxamine versus standard-dose deferoxamine for treatment of aluminium overloads among haemodialysis patients. Nephrol. Dial. Transplant 25(5):1604-1608.
Kantola T, Kivisto KT, Neuvonen PJ (1998). Grapefruits juice greatly increases serum concentration of lovastatin and lovastain acid. Clin. Pharmacol. Therapeut. 63:397-402.
Katyal R, Desigan B, Sodhi CP, Ojha S (1997). Oral aluminium administration and oxidative injury. Biol. Trace Elem. Res. 57:125-130.
Kaur J, Singh S, Sharma D, Singh R (2003). Neurostimualtory and antioxidative effects of L-deprenyl in aged rat brain regions. Biogerontology 4:105-111.
Koch HP, Lawson LD (1996). Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species, 2nd edition. Baltimore: Williams & Wilkins Publishing Co. Ltd.
Kojima M, Mausi T, Nemoto K, Degawa M (2004). Lead nitrate induced development hypercholesterolemia in rats; Sterol independent gene regulation of hepatic enzymes responsible for cholesterol homoeostasis. Toxicol. Lett. 154:35-44.
Kumar V, Gill KD (2009). Aluminium neurotoxicity: Neurobehavioural and oxidative aspects. Arch. Toxicol. 83:965–978.
Kumar A, Dogra S, Prakash A (2009). Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. Behav. Brain Res. 205(2):384-390.
Kurutas EB, Sahan A, Altun T (2009). Oxidative stress biomarkers in liver and gill tissues of spotted barb (*Capoeta barroisi* Lortet, 1894) living in the River Ceyhan, Adana, Turkey. Turk. J. Biol. 33:275–282.
Kutlubay R, Oguz EO, Guven C, Can B, Sinik Z, Euncav OL (2007). Histological and ultrasturactural evidence for protective effects on aluminium-induced kidney damage by intraperitoneal of alpha tocopherol. Int. J. Toxicol. 26(2):95-101.
Lee JH, Kang HS, Roh J (1999). Protective effects of Garlic juice against embryotoxicity of methylmercuryic chloride administered to pregnant fischer 344 rats. Yonsei Med. J. 40(5):483-489.
Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin's phenol reagent. J. Biol. Chem. 193:265-275.
Mahieu S, Contini MC, Gonzalez M, Millen N (2009). Melatonin reduces oxidative damage induced by aluminium in rat kidney. Toxicol. Lett 190 (1): 9-15.
Maines MD (1980). Regional distribution of the enzymes of heam synthesis and the inhibition of 5-aminoleveulinate synthase by manganese in the rat brain. J. Biochem. 190:315-321.
Massadeh Metwally MAA (2009). Effect of Garlic (*Allium sativum*) on Some Heavy Metal (Copper and Zinc) Induced Alteration in Serum Lipid Profile of *Oreochromis niloticus*. World J. Fish Mar. Sci. 1(1):1-6.
Miron T, Rabinkov A, Mirelman D, Wilchek M, Weiner L (2000). The mode of action of allicin: Its ready permeability through phospholipid membranes may contribute to its biological activity. Biochim. Biophys. Acta 1463:20-30.
Mohamed MA, Awad SM (2008). Effect of *Nigella sativa* Oil on Some Hematological values in Aluminium–treated rats. Austr. J. Basic Appl. Sci. 2(4):1157-1164.
Moshtaghi AA, Javadi I, Feghhi G (2003). Changes in the level of mitochondria and cytosolic aspartate aminotransferase activities in aluminium intoxified rat. J. Biomed. 7(4):167-171.
Moshtaghi AA, Rahimi S, Messripour, M (1999). Aluminium

administration on acetylcholinestrase activity of different regions of rat brain. MJIAS 12(4):105-108.
Nayak P, Das SK, Vasudavan DN (2006). Role of ethanol on aluminium induced biochemical changes on rat brain. Indian J. Clin. Biochem. 21(2):53-57.
Nehru B, Bhalla P (2006).Reversal of an aluminium induced alterationin redox status in different regions of rat brain by administration of centrophenoxine. Mol. Cell Biochem. 290:185–191.
Newairy AS, Salama AF, Hussien H M, Yousef M I (2009). Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem. Toxicol. 47(6):1093-1098.
Nwanjo HU, Oze GO (2007). Changes in serum lipid profiles and heart rate in rats treated with aqueous garlic extract. Int. J. Nutr. Wellness 4:1.
Ochmanski W, Barabasz W (2000). Aluminium-occurrence and toxicity for organisms. Przegl Lek 57:665–668.
Pandya JD, Dave KR, Katyare SS (2004).Effect of long-term aluminum feeding on lipid/phospholipid profiles of rat brain myelin. Lipids Health Dis. 3:13-22.
Prakash A, Kumar A (2009). Effect of N-acetyl cysteine against aluminium-induced cognitive dysfunction and oxidative damage in rats. Basic Clin. Pharmacol. Toxicol. 105(2):98-104.
Reitman S, Frankel AS (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28:56-63.
Reinke CM, Breitkreutz J, Leuenberger H (2003). Aluminium in over-the-counterdrugs: Risks outweigh benefits? Drug Saf. 26:1011–1025.
Riley V (1960). Adaptation of orbital bleeding technique to rapid serial blood studies. Proc. Soc. Exp. Biol. Med. 104:51.
Rodella LF, Ricci F, Borsani E, Stacchiotti A, Foglio E, Favero G, Rezzani G, Mariani C, Bianchi R (2008). Aluminium exposure induces Alzhemeir's disease-like histopathological alterations in mouse brain. Histol. Histopathol. 23:433-439.
Rudenko SS, Bodnar BM, Kukharchuk OL, Mahalias VM, Rybshchka MM, Ozerova IO, Chala KM, Khalaturnik MV (1998). Effect of selenium on the functional state of white rat kidney in aluminum cadmium poisoning. Ukr Biokhim Zh J. 70:98-105.
Senapati SK, Dey S, Dwivedi SK, Swarup D (2001). Effect of garlic (*Allium sativum* L.) extract on tissue lead level in rats. J. Ethnopharmacol. 76:229-232.
Sharma P, Mishra KP (2006). Aluminium induced maternal and developmental toxicity and oxidative stress in rat brain: Response to combined administration of Tiron and glutathione. Reprod. Toxicol. 21(3):313-321.
Sharma SK, Krishnamurthy CR (1968). Production of lipid peroxides of brain. J. Neurochem. 15:147–149.
Slinkard K, Singleton VL (1977). Total phenol analyses: Automation and comparison with manual methods. Am. J. Enol. Vitic 28:49-55.
Snedecor GW, Cochran WG (1994). Statistical method, 8th Edition. (Affiliated East-West Press, Ames, Iowa).
Stark M, Zubareb J, Jacovovitz R, Schwartz Y, Lerman Y, Grinberg N, Fireman E (2009). HO-1 and VEGF gene expressions are time dependant during exposure to welding fumes. Cytokine 46(2):290-295.
Suarez-Fernandez MB, Soldado AB, Sanz-Medal A, Vega JA, Novelli A, Fernandez-Sanchez MT (1999). Aluminiuminduced degeneration of astrocytes occur via apoptosis and results in neuronal death. Brain Res. 835:125-136.
Sushma NJ, Rao KJ (2007). Total ATPases activity in different tissues of albino mice exposed to aluminium acetate. J. Environ. Biol. 28:483–484.
Tabaldi LA, Cargnelutti D, Gonçalves JF, Pereira LB, Castro GY, Maldaner J, Rauber R, Rossato LV, Bisognin DA, Schetinger MR, Nicoloso FT (2009). Oxidative stress is an early symptom triggered by aluminum in Al-sensitive potato plantlets. Chemosphere 76(10):1402-1409.
Tandon SK, Singh S, Prasad S (2001). Influence of Garlic on the Disposition and Toxicity of Lead and Cadmium in the Rat. Pharmaceut. Biol. 39(6):450-454.
Turkez H, Yousef MI, Geyikoglu F (2010). Propolis prevents aluminium-induced genetic and hepatic damages in rat liver. Food Chem. Toxicol. 48:2741–2746.
Wen YF, Zhao JQ, Nirala SK, Bhadauria M (2012). Aluminium –induced toxicity and its response to combined treatment of HEDTA and Propolis in rats. Pol. J. Environ. Stud. 21(5):1437-1443.
Wilhelm M, Jaeger DE, Schull-Cablitz H, Hafner D, Idel H (1996). Hepatic clearance and retention of aluminium: Studies in the isolated perfused rat liver. Toxicol. Lett. 89:257–263.
Wroblewski F, Due L (1955). Colorimetric method for LDH. In: Microanalysis in Medical Biochemistry by I.D.P. Wootton 4th. Edn. J. and A. Churchill Ltd. 104, Gloucester Place pp. 115-118.
Yousef MI, Salama AF (2009). Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol. 47(6):1168-1175.
Yousef MI, Kamel IK, El-Guendi MI, El-Demerdash FM (2007). An *in vitro* study on reproductive toxicity of aluminium chloride on rabbit sperm: The protective role of some antioxidants. Toxicology 239:213–223.
Yousef MI (2004). Aluminium induced changes in hemato-biochemical parameters lipid peroxidation and enzyme activities of male rabbits: Protective role of ascorbic acid. Toxicology 199:47-57.
Yu-Yan Y, Yeh L (2001). Cholesterol-lowering effect of garlic extracts and organ sulfur compound: Human and animals studies. J. Nutr. 131:9895-9935.
Zatta P, Favarato M, Nicolini M (1993). Deposition of aluminium in brain tissues of rats exposed to inhalation of aluminium acetyl acetonate. Neuroreport 4:1119-1122.
Zlatkis A, Zak B, Boyle A J (1953). A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41(3):486-492.