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# Antioxidant Potential Properties of Mushroom Extract (*Agaricus bisporous*) against Aluminum-induced Neurotoxicity in Rat Brain

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**Abstract:** Aluminum (Al) is an environmental toxin that induces oxidative stress in neuronal cells. Mushroom cultivar extract (MCE) acted as a potent antioxidant agent and protects against cellular oxidative stress in human cultured neuronal cells. This study aimed to investigate the neuroprotective effect of MCE against Al-induced neurotoxicity in rat brain. Forty Sprague-Dawley rats were divided into 4 groups (10 rats per group), control group, MCE-fed group, Al-administered group and MCE/Al-treated group. Animals were continuously fed *ad-libitum* their specific diets for 4 weeks. At the end of the experiment, all rats were sacrificed and the brain tissues were homogenized and examined for biochemical measurements of neurocellular oxidative stress indices [glutathione (GSH), Total Antioxidant Capacity (TAC), antioxidant enzymes and oxidized dichlorofluoresceine (DCF)]. Al-administration caused inhibition of antioxidant enzymes and a significant decrease in GSH and TAC levels, meanwhile it positively increased cellular oxidized DCF level, as well as Al concentration in brain tissues. Feeding animals with MCE had completely offset the Al-induced oxidative stress and significantly restrict the Al accumulation in brain tissues of Al-administered rats. The results obtained suggest that MCE acted as a potent dietary antioxidant and protects against Al-mediated neurotoxicity, by abrogating neuronal oxidative stress.

**Key words:** Mushroom cultivar extract, rat brain, aluminum toxicity

## INTRODUCTION

Supply of adequate and safe food is essential for human health, as many studies have shown that the presence of heavy metals, which find their way into the food chain during food production, processing, packaging, transportation and storage (Burger *et al*., 2009). Aluminum (Al) is one of these heavy metals and human exposure to Al as a food contaminant occurs mainly during food processing and packaging in Al utensils, cans and foils (UNEP, FAO, WHO and GEMS, 1988; WHO, 1997; Exley *et al*., 1996). Many recent studies have reported the association between Al toxicity and the risk of many human diseases; including, fertility disorders, metabolic syndrome, neurodegenerative disease and neurodevelopmental disorders (Komatsu *et al*., 2011; Walton, 2007; Walton, 2012). Al is considered as a potent neurotoxic agent in human and experimental animals in a mechanism that is mediated by oxidative stress and impairing redox status of neuronal cells (Dua and Gill, 2011; Waly *et al*., 2012).

A lot of intention has been given recently to the biological activity of dietary supplementation with fruits and vegetables extracts in combating Al-mediated oxidative stress and its associated diseases such as cognitive deterioration and Alzheimer's disease; this is supported by many experimental studies (Kumar *et al*., 2009; Kumar *et al*., 2011; Flaten, 2001). Among this natural extracts, is mushroom cultivar, *Agaricus bisporous*, extract (MCE) which has antioxidant activity attributed to its high content of polyphenolic and flavonid contents and protects human neuronal cells against oxidative stress induced by various insults as evident by a recent *in-vitro* study model (Guizani and Waly, 2012).

The impact of MCE against neurotoxicity in experimental animal studies is scanty, therefore this study has been planned to evaluate the neuroprotective effect of MCE against oxidative stress indices in rats exposed to Al toxicant.

## MATERIALS AND METHODS

Experimental animals and study design: Forty male Sprague-Dawley rats (120±10 g) were used in this study. Rats were housed in galvanized iron cages in a temperature (23±1°C) and humidity-controlled animal

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facility with a 12 h light-dark photoperiod. They were randomly divided into 4 groups (10 rats per group), control group, MCE-fed group, Al-administered group and MCE/Al-treated group. MCE-fed group were fed 5 g of MCE/kg of experimental diet, meanwhile control groups was fed experimental diet without any MCE added. Al-administered rats received daily oral doses of aluminum chloride, $AlCl_3$, at a dose of 172.5 mg $kg^{-1}$ animal body weight. Solution of $AlCl_3$ was placed into a syringe that was inserted orally with the help of a tube directly into the oeso-pharyngeal region and adjusted so that each rat would receive a volume of 0.6 mL. The animals were supplied with their specific diets and water *ad-libitum* for four weeks; thereafter all rats were sacrificed by decapitation under diethyl ether anesthesia after an overnight fast and the brain of each animal was removed and washed with 0.9% cold saline and subsequently weighed, then immediately homogenized in 5 mL of cold 100 mM potassium phosphate buffer (pH 7.2) by a glass-teflon homogenizer and centrifuged at 100,000 g at 4°C for 1 h. The resulting supernatant was the soluble cytosolic fraction and used for biochemical measurements.

The study was approved by the Sultan Qaboos university animal ethical committee and was conducted in accordance to international laws and policies (EEC Council directives 86/609, OJL 358, 1 December, 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). All chemicals and supplies, unless otherwise specified, were purchased from Sigma Chemical Co (St Louis, MI, USA).

**Experimental diet:** The experimental diet was prepared according to AIN-93M formulation (Reeves *et al*., 1993). The diet contained (in g $kg^{-1}$ diet), 140 g protein (casein fat and vitamin free), 40 g dietary fat (corn oil), 620 g corn starch, 100 g sucrose, 50 g fiber (á-cellulose), 35 g mineral mixture (AIN-93M-MX), 10 g vitamin mixture (AIN-93-VX), 2 g choline bitartrate and 3 g DL-methionine (Table 1). The modifications in MCE-fed diet group was made by adding pure MCE to the experimental diet before mixing its ingredients.

Casein, dietary fat (corn oil), corn starch and sucrose were purchased from the national companies; AIN-93 mineral mixture and AIN-93 vitamin mixture were purchased from Dytes Inc., Bethelhem, PA, USA. The other dietary components (α-cellulose, choline bitartrate and DL-methionine) were purchased from Sigma Chemical Co., St. Louis, MI USA. All the dietary ingredients were added together, properly mixed, homogenized and sieved by the normal house hold sieve (25 mesh/$cm^2$). The resulting diet was homogenous and difficult to be sorted out for any preference. The processed diets were packed in opaque plastic containers and kept in cold storage at 4°C. Good care was taken to minimize the exposure to light during all operations.

Table 1: Composition of the experimental diet fed to rats

| Ingredients | Amount (g $kg^{-1}$ diet) |
|---|---|
| Casein | 140 |
| Starch | 620 |
| Sucrose | 100 |
| Dietary fat (corn oil) | 40 |
| Fiber (α-cellulose) | 50 |
| AIN-93 mineral mixture† | 35 |
| AIN-93 vitamin mixture | 10 |
| DL-methionine | 3 |
| Choline bitartarate | 2 |

†Calcium (35.7%), potassium (31.37%), sodium (7.4%), magnesium (2.4%), iron (0.606%), zinc (0.165%), manganese (0.063%), copper (0.03%), as supplemented by Dytes Inc., Bethelhem, PA, USA

**MCE preparation:** Fresh samples of mushroom (*Agaricus bisporous*) cultivar were bought from Gulf Mushroom Products Co, Muscat, Sultanate of Oman and transported to the Food Science and Nutrition laboratory on the day of harvesting. Mushroom samples were freeze dried in a freeze dryer for 3 days at -40°C. Dried samples were ground to a fine powder using a mechanical grinder. Subsequently, the freeze dried powdered sample (5 g) was mixed with 100 mL of methanol, using a magnetic stirrer and incubated tat room temperature for 24 h. The extracts were then filtered and centrifuged at 6,000 g for 30 min at 4°C using a Sanyo, Harrier MSE centrifuge, 18/80 Rotor No. AO9R8326. After centrifugation, the supernatant was collected and concentrated under reduced pressure at 40°C for 3-4 h using a rotary evaporator to obtain the Mushroom Crude Extract (MCE) powder which was stored at -40°C until used.

**Oxidative stress biomarkers assays:** The following biochemical parameters were determined according to the manufactures' instructions: Glutathione (GSH) concentration with glutathione assay kit (K251, Biovision, Mountain View, CA, USA), total antioxidant capacity (TAC) using Randox assay kit (Randox Laboratories, Crumlin, UK). The assayed antioxidant enzymes were glutathione peroxidase (GPx) assay kit (Oxis International, Inc., Foster City, CA, USA), catalase (CAT) assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) and superoxide dismutase (SOD) assay kit (Cell Technology Inc., Mountain view, CA, USA).

**Dichlorofluorescein fluorescence (DCF) assay:** The dichlorofluoresceine fluorescence assay was used to measure cellular peroxide production and other reactive species (Perez-Severiano *et al*., 2004). Aliquots of brains were added to a medium containing Tris-HCl buffer

(0.01 mM, pH 7.4) and dichlorofluoresceine diacetate (7 μM). After the addition of dichlorofluoresceine diacetate, the medium was incubated in the dark for 1 h until the fluorescence measurement (excitation at 488 nm and emission at 525 nm, with both slit widths at 1.5 nm). Oxidized dichlorofluoresceine was determined using a standard curve of oxidized dichlorofluoresceine and results were expressed as ìmol of oxidized DCF/mg protein (Sarithakumari *et al*., 2013).

**Protein measurement:** The protein content of supernatants was measured by the method of Lowry using bovine serum albumin as a standard and protein contents were expressed as mg $mL^{-1}$ of sample (Lowry *et al*., 1951).

**Aluminum measurement in rat brain:** The Aluminum concentrations (μg $g^{-1}$) of brain samples were determined using an Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) method. Briefly, One gm of each sample was digested by analytical grades of 36% $HNO_3$ (50 mL) and 30% $H_2O_2$ (50 mL) at a temperature of 120°C for 2 h. Then the samples were cooled and filtered through Whattman Glass Fiber Filters 110 mm in diameter. The Standard solution of 1000 mg $L^{-1}$ Plasma/free stock was used to calibrate the analysis. The intermediate and working standards were prepared by serial dilutions immediately before use.

**Statistical analysis:** Statistical analysis was performed using Graph Pad Prism (version 5.03; Graph Pad Software Inc., San Diego, CA, USA). The results are expressed as means±standard deviation of means (SD). Comparisons of groups were performed using one-way analysis of variance, followed by Tukey's test. Results were considered statistically significant different at $p<0.05$.

## RESULTS AND DISCUSSION

Table 2 shows that oral exposure to Al resulted in GSH depletion, impairment of TAC, antioxidant enzymes (CAT, $GP_x$ and SOD) inhibition and increased oxidized DCF when compared to control group. Amelioration of the Al-induced oxidative stress was observed in animal groups fed MCE in combination with Al-administration ($p<0.05$). There was no significant difference between MCE and control groups with regard the assayed biochemical markers ($p>0.05$). Table 3 illustrates the effect of MCE on Al accumulation in brain samples. Combined treatment of MCE with Al restricted the accumulation of Al in the brain organ as compared to Al-administered group ($p< 0.05$).

Table 2: Effect of MCE treatment on Al-induced oxidative stress in rat brain

| | Treatment | | | |
|---|---|---|---|---|
| Parameter | Control | Al | MCE | Al+MCE |
| CAT (μmol $min^{-1}$ $mg^{-1}$ protein) | 77.6±4.3 | 40.5±1.3* | 75.6±6.4 | 57.9±7.3** |
| $GP_x$ (μmol $min^{-1}$ $mg^{-1}$ protein) | 10.3±2.4 | 5.2±1.1* | 10.4±1.5 | 8.5±2.3** |
| GSH (nmol $mg^{-1}$ protein) | 8.4±0.8 | 4.2±0.6* | 8.1±0.6 | 6.3±0.4** |
| SOD (μmol $min^{-1}$ $mg^{-1}$ protein) | 42.4±4.1 | 22.3±1.1* | 41.8±3.2 | 33.9±3.3** |
| TAC (nmol $mg^{-1}$ protein) | 116.4±8.3 | 49.8±4.8* | 115.7±6.1 | 86.8±4.4** |
| Oxidized DCF (μmol $mg^{-1}$ protein) | 170.3±14.3 | 430.3±15.7* | 170.6±11.8 | 206.8±16.7** |

Values are Means±SD of 10 animals per group (n = 10). CAT: Catalase, DCF: Dichlorodihydrofluorescein, GPx: Glutathione peroxidase, GSH: Glutathione, SOD: Superoxide dismutase, TAC: Total antioxidant capacity, *Significantly less than control group, $p<0.05$, **Significantly higher than Al group, $p<0.05$

Table 3: Measurement of Al concentration in rat brain

| Treatment | Al concentration (μg $g^{-1}$) |
|---|---|
| Control | 3.8±0.4 |
| Al | 9.1±0.7* |
| MCE | 3.7±0.3 |
| Al/MCE | 5.9±0.4** |

Values are Means±SD of 10 animals per group (n = 10), *Significantly higher than control group, $p<0.05$, **Significantly less than Al group, $p<0.05$

This *in-vivo* animal model demonstrates that Al exposure induced neurotoxicity that can account to cognitive deterioration in animals. The neuropathology of this animal model provides a mechanistic role for Al-mediated toxicity in relation to Alzheimer's disease in human subjects as evident by clinical studies (Bharathi *et al*., 2008; Campbell and Bondy, 2000). It is apparent from Al accumulation in brain that Al was efficiently absorbed and crossed the blood-brain barrier possibly by diffusion or by a specific mechanism (carrier, channel or receptor). It seems evident that Al exposure significantly enhanced neuronal oxidative damage by altering redox status of neuronal cells, hence causing dysfunctional action of the brain tissue. MCE significantly protects against the observed Al intoxication, in a mechanism that might be attributed to its potential antioxidant properties in augmenting the neuro-cellular redox status as reported by our research group (Guizani and Waly, 2012).

Further physiological, biochemical and histopathological investigations are required to explore the potential use of MCE as a natural agent against Al-mediated neurotoxicity.

## CONCLUSION

It is concluded that Al exposure caused a decline in the antioxidant defense system in rat brain which was

accompanied by a marked increase in Al concentrations in brain tissues. However, concomitant treatment of Al-administered rats with MCE decreased the observed Al-mediated cellular insult and restricted Al accumulation in brain tissues.

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## REFERENCES

Bharathi, P., P. Vasudevaraju, M. Govindaraju, A.P. Palanisamy, K. Sambamurti and K.S. Rao, 2008. Molecular toxicity of aluminium in relation to neurodegeneration. Indian J. Med. Res., 128: 545-556.

Burger, J., J. Fleischer, C. Jeitner and M. Gochfeld, 2009. Environmental concerns and diet in Singapore. J. Toxicol. Environ. Health A: Curr. Issues, 66: 1405-1419.

Campbell, A. and S.C. Bondy, 2000. Aluminum induced oxidative events and its relation to inflammation: A role for the metal in Alzheimer's disease. Cell. Mol. Boil., 46: 721-730.

Dua, R. and K.D. Gill, 2001. Aluminium phosphide exposure: Implications on rat brain lipid peroxidation and antioxidant defence system. Pharmacol. Toxicol., 89: 315-319.

Exley, C., E. Burgess, J.P. Day, E.H. Jeffery, S. Melethil and R.A. Yokel, 1996. Aluminum toxicokinetics. J. Toxicol. Environ. Health, 48: 569-584.

Flaten, T., 2001. Aluminium as a risk factor in Alzheimer's disease, with emphasis on drinking water. Brain Res. Bull., 55: 187-196.

Guizani, N. and M.I. Waly, 2012. Mushroom extract protects against hydrogen peroxide-induced toxicity in hepatic and neuronal human cultured cells. Pak. J. Biol. Sci., 15: 1069-1074.

Komatsu, F., Y. Kagawa, T. Kawabata, Y. Kaneko, U. Chimedregzen, B. Purvee and J. Otgon, 2011. A high accumulation of hair minerals in Mongolian people: 2nd report: Influence of manganese, iron, lead, cadmium and aluminum to oxidative stress, Parkinsonism and arthritis. Curr. Aging. Sci., 4: 42-56.

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

Kumar, A., A. Prakash and S. Dogra, 2011. Neuroprotective effect of carvedilol against aluminium induced toxicity: Possible behavioral and biochemical alterations in rats. Pharm. Rep., 63: 915-923.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Perez-Severiano, F., R. Salvatierra-Sanchez, M. Rodriguez-Perez, E.Y. Cuevas-Martinez and J. Guevara *et al*., 2004. S-allylcysteine prevents amyloid-â peptide-induced oxidative stress in rat hippocampus and ameliorates learning d e ficits. Eur. J. Pharmacol., 489: 197-202.

Reeves, P.G., F.H. Nielsen and G.C. Jr. Fahey, 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Sarithakumari, C.H., G.L. Renju and G.M. Kurup, 2013. Anti-inflammatory and antioxidant potential of alginic acid isolated from the marine algae, *Sargassum wightii* on adjuvant-induced arthritic rats. Inflammopharmacology, 21: 261-268.

UNEP, FAO, WHO and GEMS, 1988. Assessment of chemical contaminants in food: Report on the results of the UNEP/FAO/WHO programme on health-related environmental monitoring. United Nations Environment Programme, Nairobi, Kenya, pp: 1-104.

WHO, 1997. Aluminium (Environmental Health Criteria Series 194). World Health Organization of the United Nations, Geneva, Switzerland, ISBN-13: 9789241571944, Pages: 282.

Walton, J.R., 2007. A longitudinal study of rats chronically exposed to aluminum at human dietary levels. Neurosci. Lett., 412: 29-33.

Walton, J.R., 2012. Cognitive deterioration and associated pathology induced by chronic low-level aluminum ingestion in a translational rat model provides an explanation of Alzheimer's disease, tests for susceptibility and avenues for treatment. Int. J. Alzheimer's Dis., Vol. 2012 10.1155/2012/914947

Waly, M.I., N. Guizani, A. Ali and M.S. Rahman, 2012. Papaya epicarp extract protects against aluminum-induced neurotoxicity. Exp. Biol. Med., 237: 1018-1022.