ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Morphofunctional Condition of Bones and Hippocampus of White Rats at Experimental Intoxication with Aluminium Chloride.

Areshidze DA[1*], Timchenko LD[2], Rzhepakovsky IV[2], Kozlova MA[1], Syomin IA[1], and Kuznetsova IA[1].

[1]Laboratory of experimental Biology and Biotechnology, Moscow State Regional University, Moscow, Russian Federation.
[2]Institute of Applied Biotechnology, Department of the living systems of the North Caucasus Federal University, Stavropol, Russian Federation.

ABSTRACT

It is established that use of aluminium chloride in a dose of 100 mg/kg Bw brings essential changes in the CNS and skeletal system. So, there are essential changes in population of neurons of a hippocampus of rats, particularly, the significant increase of the quantity of chromatolyzed, hyperchromic and wrinkled neurons. Such changes in general are a characteristic symptom of Alzheimer's disease. At the same time, decrease in trabecular bone mineral density at Al-treated rats against the background of the pattern of change of the value BV/TV demonstrates that trabecular bone mineral density goes down due to reduction of salts of calcium in favor of what also increase in level of a calcaemia at animals of this group testifies. Thus, the influence of $AlCl_3$ leads to a decrease in trabecular bone mineral density, and, as a result, to development of hypercalcaemia.

Keywords. Neuron, aluminium, hypercalcaemia, Alzheimer's disease.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Aluminium is the most widespread metal in a lithosphere and its extending use demands studying of its influence on an organism of mammals. There is an opinion that aluminium belongs to the category of eco-toxicants influencing, in particular, at human in living conditions as it arrives in a human body with food, (much of it contains in soy milk and tea, salts of aluminium are present at drinking water), and it arrives additionally from packing, ware, nutritional supplements and drugs. Preparation, storage and the use of foodstuff are also often integrated to use of aluminium wares.

Opinions on influence of aluminium on an organism of mammals are ambiguous. While there are as yet, no unequivocal answers to this problem, there are procedures to follow to ascertain the nature of human exposure to aluminium. It is also important to recognise critical factors in exposure regimes and specifically that not all forms of aluminium are toxicologically equivalent and not all routes of exposure are equivalent in their delivery of aluminium to target sites [9].

Aluminium compounds influence on metabolism of phosphorus and carbon in a human body, on development of epithelial, connecting and bone tissues, worsen uptake of calcium, are the reason of neurologic disorders, including Alzheimer's disease. So, it is shown with researches of Chappard D [6,7], that the presence of these metal in the exostoses advocates for a disturbed metabolism of osteoblasts which can deposit these metals into the bone matrix, similar to which is observed in case of hemochromatosis.

Violations at bone system are one of the leading manifestations of aluminium intoxication. Against the background of high concentration of aluminium in blood serum occures an inclusion of aluminium to a bone tissue [19]. Histologically at patients with damage of a skeleton owing to intoxication with aluminium deposition of salts of calcium on osteoides decreases. Expressiveness of an osteomalacia closely correlates with the content of aluminium in a bone tissue. In places of deposition of the aluminium, which is forming structures from 20 to 100 nanometers, there is almost complete absence of active osteoblasts, and in the cells covering the surface of a bone endoplasmic reticulum is not observed. At aluminium intoxication the bone tissue looks inactive, characteristic with acute delay of processes of remodeling (new growth) of a bone tissue. Further accumulation of aluminium leads finally to development of an osteomalacia, organic acids significantly change biochemical influence of aluminium. The mechanism by means of which aluminium induces changes in a bone tissue isn't deciphered decisively. However the researches conducted with culture of a bone tissue have shown that aluminium and citrate through hydroxyl group forms the metal-citrate complex interfering growth of crystals of phosphate of calcium and oppressing a mineralization of an osteoid [10,13,14,21].

Aluminium in concentration of 54-135 μg/l is capable to inhibit growth of crystals of calcium phosphate. Citrate strengthens the inhibiting action: the inhibiting activity of a metal-citrate complex which is deposited on the surfaces of crystals, is many times higher. Further insufficient calcification of big mass of an osteoid conducts to a softening of bones, development of deformations and pathological changes. Some manifestations of the bone changes dependent on aluminium are generalized in tab. 3. Intoxication with aluminium leads to development of osteochondrosis, rachitis and other diseases of the locomotorium. According to some information, aluminium can cause or strengthen neoplasmas of bones [8,23,22,25].

Aluminium (Al), is considered potentially toxiferous metal and inhibits an osteogenesis. The transforming growth factors beta 1 (TGF beta 1) and osteal morphogenetic protein 2 (BMP-2) play an important role in a regulation of an osteogenesis. Particulary, at research studying an influence of Al on TGF-1 and BMP-2 at rats, the animals of the Wistar strain were in a random way parted on the Al-processed group and control group. The Al-processed rats were provided with the drinking water containing 100 mg/l of $AlCl_3$, and at control rats gave the distilled water within 30, 60 and 90 days, respectively. Ten rats in each group were euthanized every 30 days. The Al-processed rats showed lower body weight and higher levels of aluminium in blood serum and bone in comparison with control rats. Levels of an expression of TGF-1 and BMP-2 were also considerably decreased in the Al-processed rats. Serumal levels of bone gamma-carboxiglutamic acid protein (BGP), procollagen I carboxyterminal propeptide (PICP) and bone-specific alkaline phosphatase (B-ALP) were were significally lower in Al-proceeded groups, than in a control group. These results specified that Al inhibits an expression of TGF-1 and BMP-2 in a bone which inhibits activity of osteoblasts and reduces synthesis of the BGP, B-ALP and collagen, thereby inhibiting formation of a bone tissue. Besides, influence of salts of aluminum

on a morphofunctional condition of a CNS is described. In particular, there are data about the neurodegenerative changes in a cortex and subcortical structures of a brain which are followed by a series of disturbances of higher nervous activity [1,2,15,16,17,20,21,24].

MATHERIALS AND METHODS

Animals:

Male Wistar Albino rats of body weights ranging from 200 to 220 g were used in the study. Age of the animals was 8 months old. The animals were fed with standard pellet diet and water ad libitum. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Treatment design:

The experiment is executed on 40 males of Wistar Albino rats of body weights ranging from 170 g to 200 g. Animals have been divided into 2 equal groups. The first served as intact control, the second group got aluminum chloride with drink.

The animals were fed with standard pellet diet and water ad libitum. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Induction Method:

AD-like model was achieved in rats by the oral administration of $AlCl_3$ in a dose of 100 mg/kg Bw [11] daily for 90 days.

Biochemical analysis:

Plasma was separated from heparinized blood by centrifugation and analyzed for the estimation of sodium and potassium by flame photometry (Corning 410), and estimation of plasma calcium ion by ion selective electrode method Jenway (Ion Meter 3345)

Histological studies:

Brain tissues previously fixed in 96% ethanol for 24 h were washed under tap water for 20 min. Then, the serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4-mm-thick using sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stains for histological examination through the light microscope.

The sections were successively rehydrated with 100% alcohol, 95% alcohol, and distilled water. Subsequently, the sections were stained in 0.1% Cresyl violet (Sigma-Aldrich) solution. The sections were then differentiated in 95% ethyl alcohol, dehydrated in 100% alcohol, and rinsed in xylene. Finally, the sections were mounted and observed under a light microscope. The average quantity of neurons was calculated by randomly selecting five Nissl-stained sections at the same site from each rat.

Study of structure of a bone tissue:

For a study of structure of a bone tissue at white rats the X-ray Skyscan 1176 (Bruker) microtomograph with the main software was used [3].

ISSN: 0975-8585

Statistical Analysis:

Values are expressed as mean (± SD). The statistical analysis was performed using one-way analysis of variance (ANOVA). The statistical difference determined using repeated measures analysis of variance or paired Student t-tests. A p value of < 0.05 was considered statistically significant.

RESULTS

As a result of the research conducted by us it is established that aluminium chloride exerts the expressed destructive impact on this structure of CNS.

In experimental group decrease in number of unchanged cells of a hippocampus from 74.0±2.66% to 41.0±3.60% is noted. At the same time in experimental group the quantity the chromatolyzed cells from 7.06±1.12% increases to 19.21±3.76%, quantity of hyperchromic cells increases from 10.81±1.41 to 28.44±1.05%. Invariable is a quantity of shadow cells (4.69±0.97% in control and 4.89±1.05% in experimental group), and also quantity of cells in a condition of apoptosis (1.03±0.49% in a hippocampus of intact rats and 1.15±0.44% in an experiment). At the same time under the influence of aluminium chloride the amount of the wrinkled neurons from 2.38±0.91% increases to 4.69±0.94% (Fig. 1,2,3,4,5).

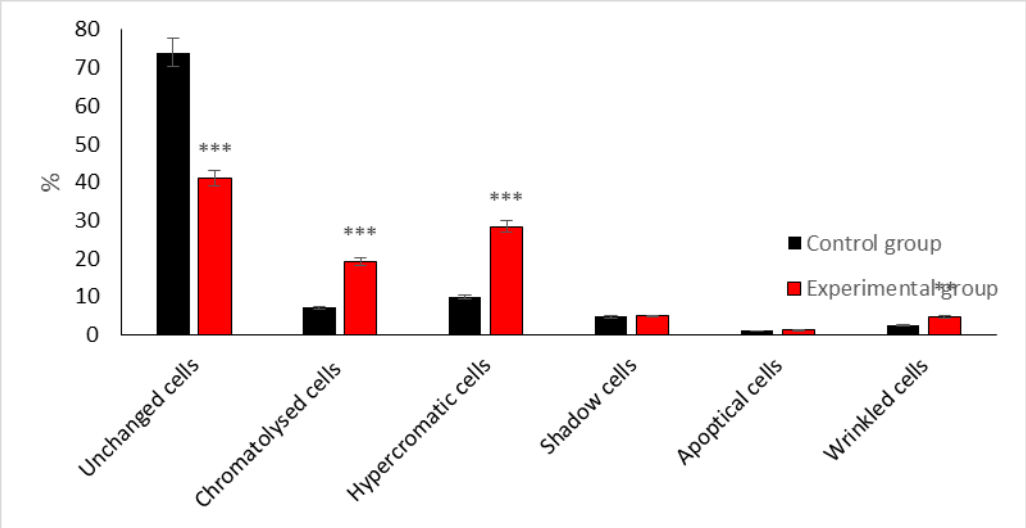

Fig. 1. Ratio of different types of cells in a hippocampus of rats.

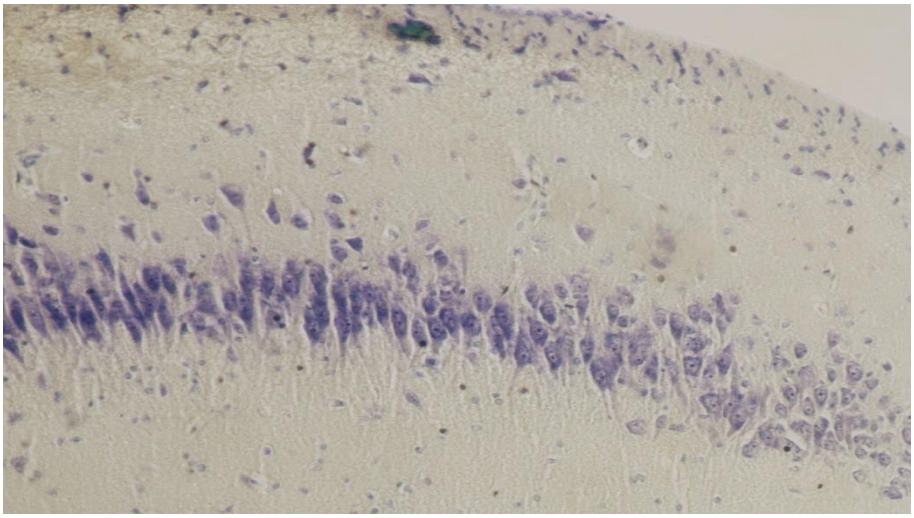

Fig. 2. Hippocampus of an intact rat. Nissl staining. ×200

ISSN: 0975-8585

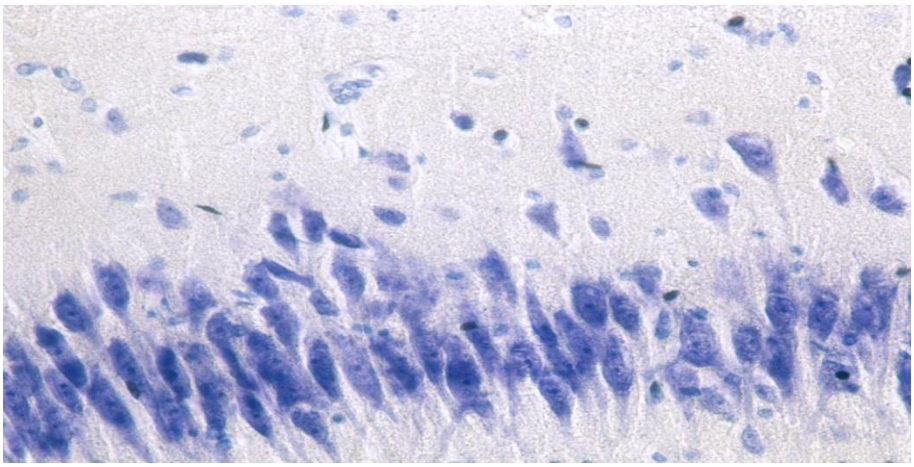

Fig. 3 Hippocampus of an intact rat. Nissl staining. ×400

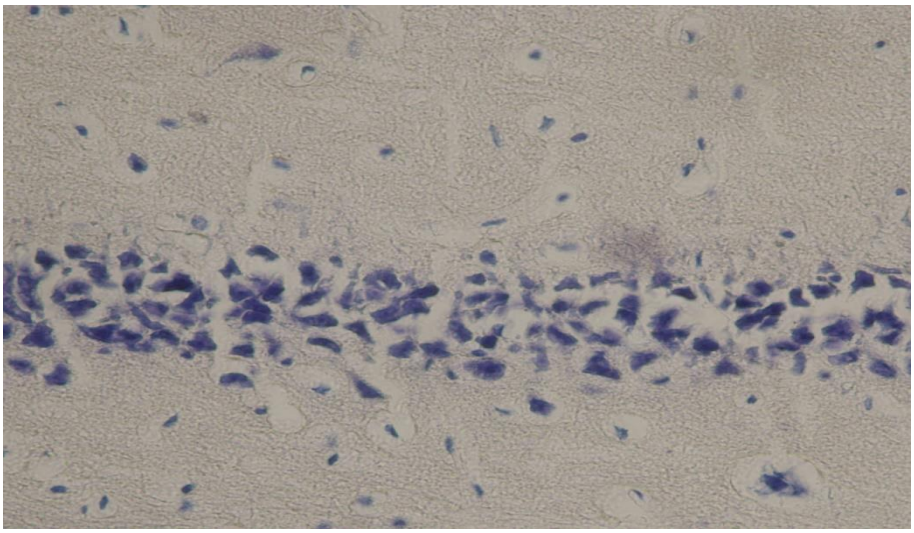

Fig. 4. Hippocampus of rat of experimental group. Nissl staining. ×200

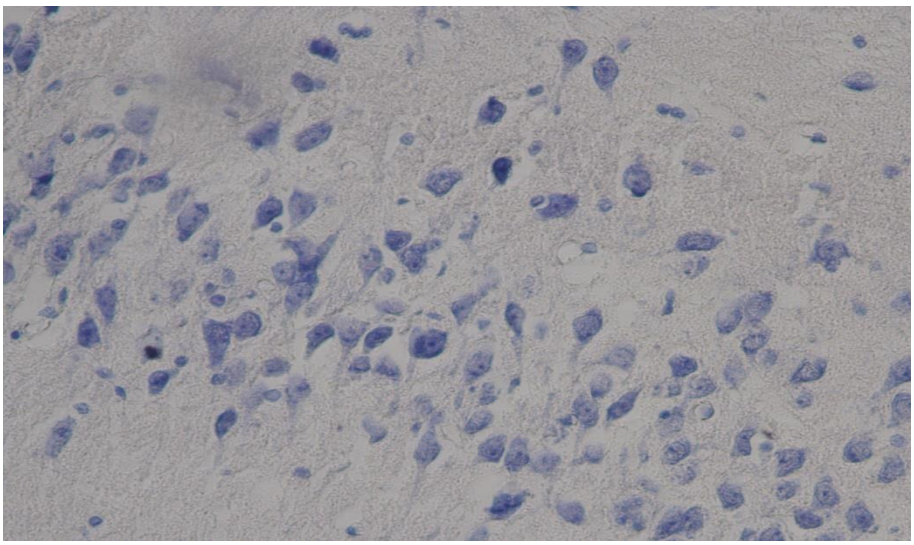

Fig. 5. Hippocampus of rat of experimental group. Nissl staining. ×400

ISSN: 0975-8585

By results of scanning with use of the Skyscan 1176 microtomograph it is established that the cortical bone mineral density (Cortical BMD) under the influence of $AlCl_3$ doesn't change, making 1.334±0.25 g/cm^3 at control animals and 1.350±0.15 g/cm^3 is at Al-proceeded animals (Fig. 6).

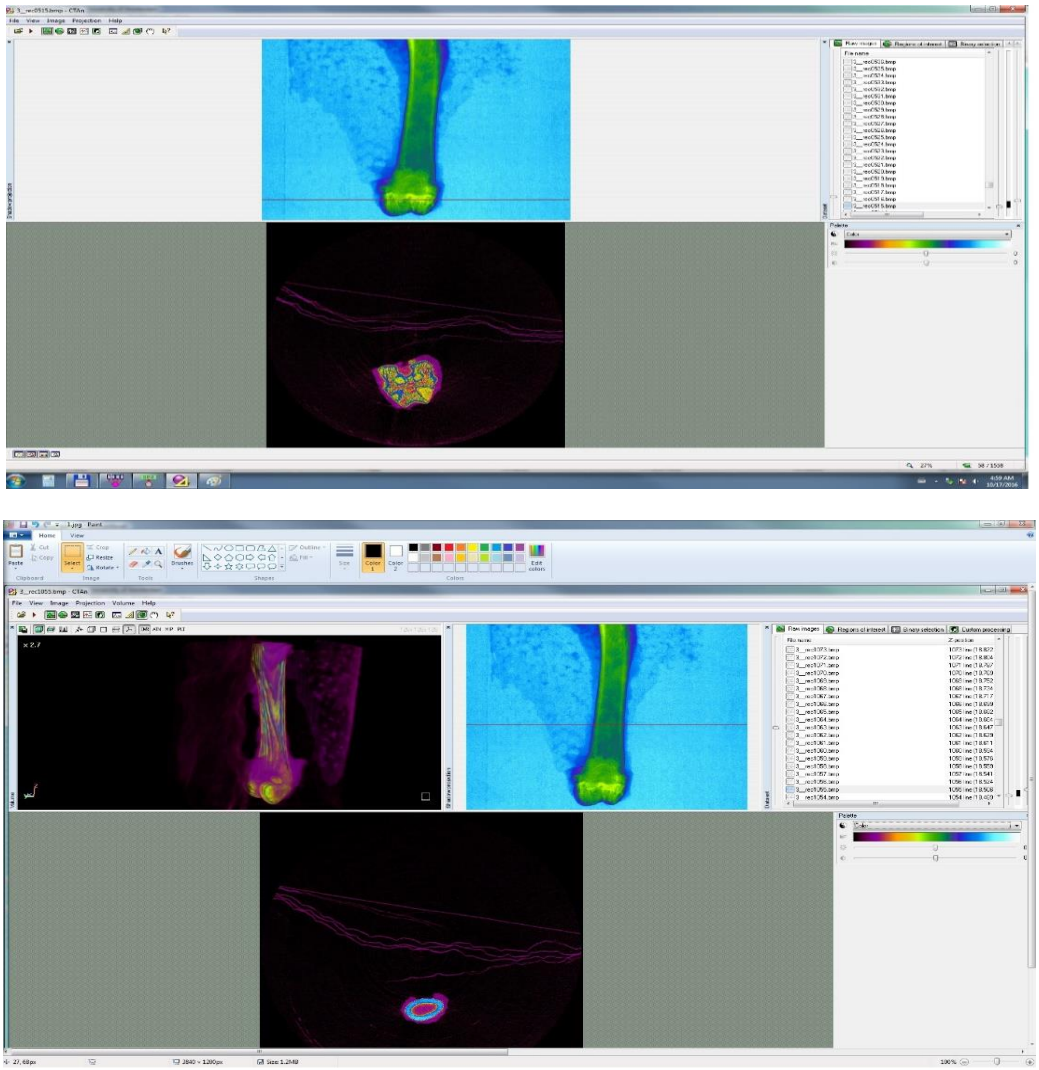

Fig. 6. Results of scanning with use of the Skyscan 1176 microtomograph.

At the same time, the trabecular bone mineral density (Trabecular BMD) in bones of rats of experimental group significantly decreases, making 0.518±0.10 g/cm^3 against 0.613±0.12 g/cm^3 in control.

The value of BV/TV in a femur of intact animals makes 52.33±1.21%, and in bones of control animals this index decreases to 48.17±2.1%.

Calcium level in blood serum of intact animals makes 2.36±0.01 mmol/l, and in blood of experimental animals this parameter reaches 2.71±0.1 mmol/l.

DISCUSSION AND CONCLUSION

As a result of the researches conducted by us it is established that use of aluminium chloride in a dose of 100 mg/kg Bw brings essential changes in the studied systems.

So, in population of neurons of a hippocampus of rats essential changes are noted. In particular, the quantity of chromatolyzed, hyperchromic and wrinkled neurons significantly increases. Such changes in general are a characteristic symptom of Alzheimer's disease.

At the same time, decrease in trabecular bone mineral density in experimental group against the background of the pattern of change of the value BV/TV demonstrates that trabecular BMD goes down due to reduction of salts of calcium in favor of what also increase in level of a calcaemia at animals of this group testifies.

Thus, under the influence of $AlCl_3$ there is a decrease in trabecular bone mineral density, and, as a result, a hypercalcaemia.

Besides, it is possible to assume that the hypercalcaemia can be the reason of neurodegenerative changes in a hippocampus though such assumption though is confirmed by literary data, but demands further studying [4,5,12,14,18,20].

ACKNOWLEDGEMENTS

The study was conducted under Task number 2014/2016 on the implementation of public works in the field of scientific activities of the base portion of the state task of the Ministry of Education and Science of the Russian Federa-tion. Financial support of research was carried out by Moscow State Regional University and by the Ministry of Education and Science of the Russian Federation, within performance of a basic unit of the state task (2014/2016).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

[1] Alishah S, Ullah F, Yoon G. Nanoscale. 2015. 7; 15225-15237.
[2] Aremu D. et al., Psychogeriatrics. 2002.2(4); 263-268.
[3] Banchroft JD, Stevens A, Turner DR. 4th ed. New York, London, San Francisco, Tokyo:Churchill Livingstone, 1996.
[4] Berridge MJ, Prion, (7)1; pp.2-13, 2013.
[5] Bezprozvanny I, Mattson MP. Trends in Neuroscience. 2008, 31; 454-463
[6] Chappard D et al., Morphologie. 2016. S1286-0115(15)00250-7. doi: 10.1016/j.morpho.2016.12.001.
[7] Chappard D et al., Journal of Inorganic Biochemistry. 2015 Nov;152:174-9. doi: 10.1016/j.jinorgbio.2015.09.008. Epub 2015 Sep 16.
[8] Chih-Hsiang H et al., Evidence-Based Complementary and Alternative Medicine Volume 2016 (2016),. doi:10.1155/2016/1939052.
[9] Exley C, Elsevier Science, Amsterdam, The Netherlands. 2001. 441p.
[10] Heaf JG et al., Scandinavian Journal of Urology and Nephrology. 1987;21(3):229-33.
[11] Krasovskii GN, Vasukovich LY, Chariev OG. Environmental Health Perspectives. 1979; 30: 47-51.
[12] LaFerla FM, Nature Reviews Neuroscience, 3(11); 862-872, 2002.
[13] Li P et al., Biological Trace Element Research. 2015 Nov 23. [Epub ahead of print]
[14] Magi S et al., BioMed Research International. Volume 2016 (2016), Article ID 6701324, 14 pages DOI: 10.1155/2016/6701324
[15] Mirza A et al., Journal of Alzheimer's Disease. 2016; 54(4):1333-1338.
[16] Perl DP, Environmental Health Perspectives. 1985. 63;149-153.
[17] Platt B et al., Brain Research Bulletin. 2001. 55 (2); 257-267.
[18] Reitz C, Mayeux R. Biochemical Pharmacology. 2014 Apr 15;88(4):640-51. doi: 10.1016/j.bcp.2013.12.024.
[19] Robinson RF. Journal of Toxicology. Clinical Toxicology. 2002, 5; 604.
[20] Sepulveda-Falla D et al., Journal of Clinical Investigation, vol. 124, no. 4, pp. 1552-1567, 2014
[21] Slanina P et al., Food and Chemical Toxicology. 1984, 22; 391
[22] Sun X et al., Food and Chemical Toxicology. 2015 Dec; 86:154-62. doi: 10.1016/j.fct.2015.10.005. Epub 2015 Oct 22.
[23] Tracey JA, Journal of Clinical Toxicology. 2001, 39(3); 240.
[24] Yokel RA. NeuroToxicology. 2000. 5(21); 813-828.
[25] Zhang Q et al., International journal of immunopathology and pharmacology. 2012. 25(1); 49-58.