Poll Res. 40 (4) : 1382-1389 (2021)
Copyright © EM International
ISSN 0257–8050

MERCURIC CHLORIDE INDUCED NEURODEGENERATIVE CHANGES: AN ASSESSMENT BASED ON NEURONAL CELL ARCHITECTURE IN ALBINO RAT

MADHURI YADAV[1] AND VISHNU SHARMA[2]

[1]Department of Zoology, D.B.S. College, Kanpur, U.P., India
[2]Toxicology Laboratory, Department of Zoology, School of Life Sciences, Khandari Campus, Dr. B. R. Ambedkar University, Agra 282 002, India

(Received 17 February, 2021; accepted 23 May, 2021)

ABSTRACT

Mercury is a heavy metal that has been reported to cause devastating health problems in developed and underdeveloped world. This work studies the effect of mercuric chloride on the histoarchitectural changes on the cerebrum of the adult albino rats. Twenty five female rats were divided into five groups of five rats each. Group I serves as control which receives normal water. Group II served as acute (1d) and received 10.3 mg/kg b.wt. of mercuric chloride orally, while group III, IV and V served as sub acute (7, 14 and 21ds) received 1.47, 0.73 and 0.49 mg/kg b.wt. of mercuric chloride. Animals were etherized and brains were dissected out after dismantling of cranium. The brains were fixed in Bouin's fluid and tissues were processed histologically using H and E stain. Histological observation of the cerebrum showed a normal architecture in group I while group II showed degenerative changes, necrosis, pyknosis in neurons and group III, IV and V exhibited hypertrophy in microglial cells, clumping of glial cells pyknosis and necrosis in neurons. It was concluded from the present study that mercuric chloride treatment has neurodegenerative effects on the cerebrum, a part of central nervous system pertaining to memory and learning.

KEY WORDS : Mercuric chloride, Cerebrum, Neurodegeneration, Hypertrophy, Albino rat

INTRODUCTION

Contamination of environment by heavy metals is a serious problem for developed and underdeveloped world. The three major heavy metals in this regard are Cadmium (Cd^{2+}), Lead (Pb^{2+}) and Mercury (Hg^{2+}) (Richetti *et al.,* 2011). Out of them mercury being highly toxic heavy metal, ubiquitous in the environment prevails in the environment on account of natural and anthropogenic activities. Occurrence of mercury in three different forms viz., elemental mercury (Hg^{0}), inorganic mercury salts (Hg^{2+}) and organic mercury in the environment is an established fact (Clarkson *et al.,* 2003).

Mercury has been used by humans since ancient time, Chinese and Romans first used mercury sulfide (cinnabar) as a printing material in the form of red dye and ink. The inorganic mercury became popular and used as dental amalgam since 1830, and calomel was used as teething powder (Mutter *et al.,* 2010) besides, thimerosal, a mercury product, used as a preservative for vaccines such as Hepatitis B, Diphtheria, pertussis and tetanus has also been a cause of mercury exposure to population (Clarkson *et al.,* 2003). Further, seaweeds also contribute to mercury poisoning, the best ever seen in Southeast Asia, in a particular aquatic macrophyte, water spinach (*Ipomea aquatic Forsk*.) (Mudawi *et al.,* 2014).

Though mercury is not essential to living cells and performs no known biological functions, but the toxicity of mercury is mainly associated with cationic state (Hg^{2+}); whereas absorption, distribution, biotransformation and elimination are significantly influenced by the valance state of this metal (Carpi, 2001). Inorganic mercury is poorly absorbed via gastrointestinal tract (<10%), however,

inhaled elemental mercury vapor is readily absorbed in the lungs and organic mercury (MeHg) gets completely absorbed (90-95%) (WHO, 1990-91).

The nervous system is the critical organ for toxic exposure to all forms of mercury. Mercury can react directly with important receptors in nervous system such as acetylcholine receptor in the nervous system (Vanithasri and Jagadeesan, 2013). Mercury a potent neurotoxic agent induces apoptosis in microglial cells by means of inducing Caspase-3 like activity in brain (Nishioku *et al.,* 2000), besides, low concentration of mercury also induces apoptosis in oligodendroglial cells (Issa *et al.,* 2003). Thimerosal, a mercury compound used for preserving the vaccines show strain dependent neurotoxicity in mouse and also hampered the brain of developing young children (Hornig *et al.,* 2004 and Aphosian, 2008).

The toxic effect of mercury and its relationship to Alzheimer's disease indicate that mercury biochemically mimics the observations seen in patients of Alzheimer's disease and play an important role in memory loss and as a co-factor in the development of Alzheimer's disease (Haley, 2007 and Mutter *et al.,* 2010). The small concentration of mercuric chloride and methyl mercury induces cholinergic neuromuscular transmission which resulted in enhanced amplitude of twitch contraction (Candura *et al.,* 1997).

The toxicity of mercury is dose dependent (Sheikh *et al.,* 2011and Sharma, 2014) and is also dependent on its chemical and physical form (Bernhoft, 2012). The toxicity of mercury is due to the thiol group (Nascimento *et al.,* 2008). Generally, inorganic mercury is less neurotoxic than organic mercury on account of its lower rate of transport comparison to organic mercury into CNS parenchyma. However, mercuric chloride acts as barrier toxicant, damaging blood-brain-barrier (Zheng *et al.,* 2003).

Motor and cognitive center gets hampered after accumulation of mercuric chloride in cerebral cortex and hippocampus in brain (Teixeira *et al.,* 2014). The toxicity of mercury in brain is facilitated by N-methyl-D-aspartate receptors which modulate the mercuric chloride induced neurotoxic insult (Xu *et al.,* 2012). The histopathological studies reveal that inorganic mercury has enough potential to damage the different parts of the brain including cerebellum, cerebrum, hippocampus and medulla oblongata (Verghese *et al.,* 1997; Hematian, 2013 and Ranjan *et al.,* 2014).

Further, organic mercury has been reported more toxic to animals, particularly to central nervous system, the brain. The selection of inorganic mercury (mercuric chloride) in the present study is to observe its toxicity and explore the possibility of similar toxicological manifestations induced in brain like organic mercury at histological level.

The emphasis has been laid to observe pathological changes, if any, in cerebral hemisphere (prosencephalon), site of memory and other related specificities.

MATERIALS AND METHODS

Experimental animal

The female albino rats (*Rattus norvegicus*) have been selected in the present investigation on account of their sensitivity to xenobiotic substances (OECD, 420). The albino rats were reared in the animal house as per directions of ethical committee of zoology department, Dr. B.R. Ambedker University, Agra.

The healthy and adult albino rats were 8-9 weeks old, weighing 90±20 g were kept in polypropylene cages of the sizes 45cm × 27 cm × 15 cm at temperature of 27±5 ^{o}C and relative humidity 57% with a photoperiod of 12 hours. The rats were provided standard pellet diet as food (Golden feed, New Delhi) and water *ad libitum*.

Experimental Chemical

Mercuric chloride (chemical formula-$HgCl_2$, CAS # No.-7487-97-7) procured from Merk India Ltd. (Mumbai, India) was dissolved in distilled water.

Experimental design

Twenty five experimental female rats were randomly divided into five different groups. The LD_{50} of $HgCl_2$ received per rat was 103 mg/kg body weight after Finney, 1971.

(1) Group I:- contained 5 female rats received normal water and served as control group.
(2) Group II:- served as acute (1d) and received mercuric chloride orally in a dose of 10.3 mg/kg b. wt. for once.
(3) Group III:- received mercuric chloride orally in a dose of 1.47 mg/kg b. wt. per day for 7 days.
(4) Group IV:- received mercuric chloride orally in a dose of 0.73 mg/kg b. wt. per day for 14 day.
(5) Group V:- received mercuric chloride orally in a dose of 0.49 mg/kg b. wt. per day for 21 day.

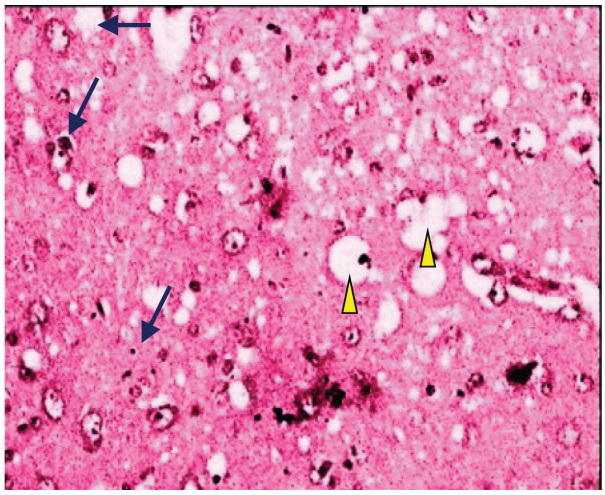
Histoarchitectural studies of brain

The animals were etherized and brain was dissected out from all the rats after dismantling cranium for the histopathological studies of brain. The brain of each of the rat was then washed in physiological saline (pH 7.4). It was then transferred to Bouin's fluid for fixation, washed in running tap water and proceeded for dehydration in ascending alcohol series and embedded in paraffin wax (M.P. 60 °C). 5µm thick sections were cut and stained with hematoxylin and eosin and mounted in Canada balsam (Humanson, 1979). The stained sections were then examined under Motic high power trinocular research microscope. The photographs were taken at different magnifications to observe the changes.

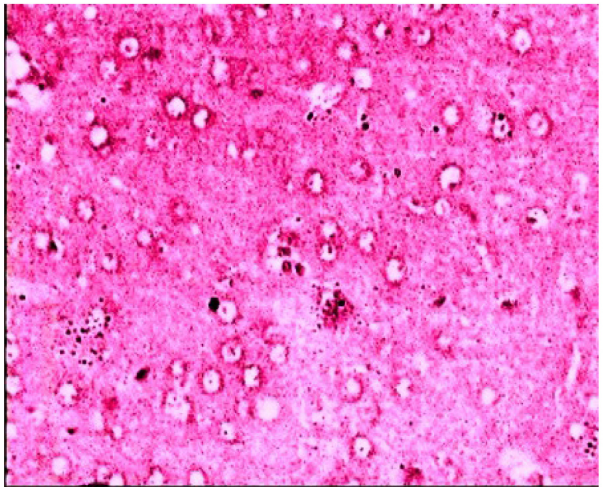
RESULTS

Histopathological findings

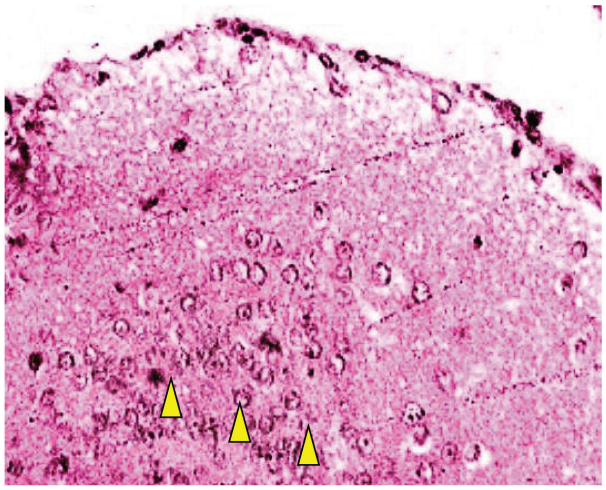
Histological studies of control brain (Fig. 1), exhibited different areas of cerebral hemisphere which include cingulate area, secondary motor area, prelimbic area and infralimbic area in control group, besides, normal morphology of pyramidal neurons, glial cells, stellate cells and microglial cells. The acute (1d, Fig. 2) treatment of mercuric chloride revealed spongiform appearance with vacuolization in cingulate and secondary motor area besides, pyknosis in cyton of pyramidal cells and stellate cells. The Sub-acute (7ds, Fig. 3) treatment of mercuric chloride exhibited aggregation of neuroglial cells in anterior cingulate area and prelimbic area with hypertrophy of microglial cells in prelimbic area and secondary motor area. The Sub-acute (14ds, Fig. 4) treatment of mercuric chloride showed Hypertrophy in microglia cells, gliosis, necrosis in neuron and increased intravascular spaces in anterior cingulate area and prelimbic area. The sub-acute (21ds, Fig. 5) treatment of mercuric chloride exhibited histopathological changes in form of focal gliosis and aggregation of neuroglial and stellate cells in anterior cingulate area besides, increased intravascular space and pyknosis in the cell body of neuron in secondary motor area.



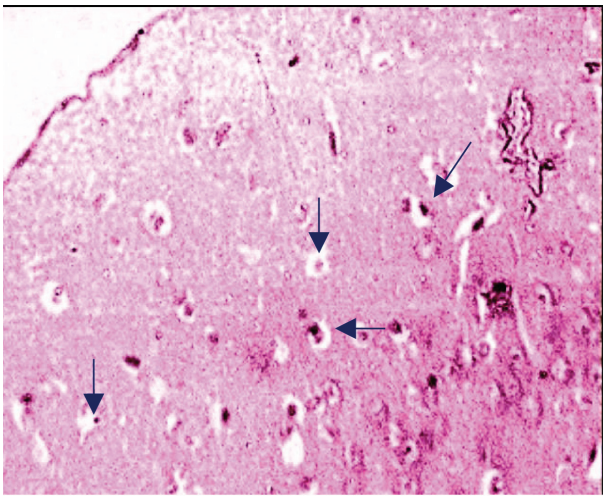
Photomicrograph of cerebral hemisphere of rat brain after acute (1d) intoxication of $HgCl_2$ showing vacuolation (yellow arrow head) and neurodegeneration in secondary motor area (blue arrow). 400X



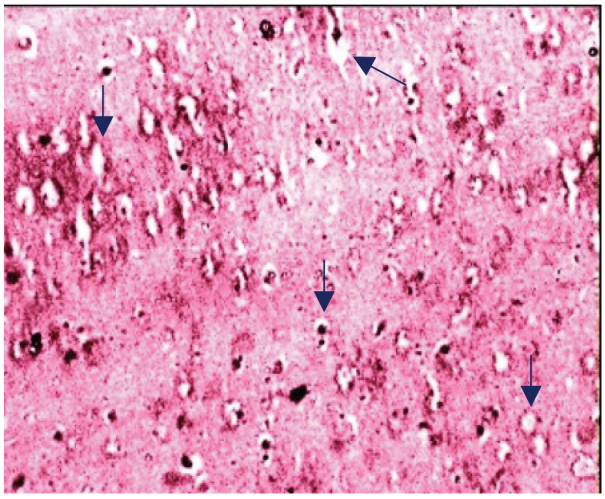
Photomicrograph of cerebral hemisphere of control rat showing normal organization of plexiform and external granular lamina in anterior cingulate area. 400X



Photomicrograph of cerebral hemisphere of rat brain after sub-acute (7ds) intoxication of $HgCl_2$ showing aggregation of neuroglial cells in secondary motor area (yellow arrow head). 400X



Photomicrograph of cerebral hemisphere of rat brain after sub-acute (14ds) intoxication of $HgCl_2$ showing gliosis and disorganization of external granular lamina in anterior cingulate area (blue arrow). 400X



Photomicrograph of cerebral hemisphere of rat brain after sub-acute (21ds) intoxication of $HgCl_2$ showing focal gliosis in external granular lamina of secondary motor area (blue arrow). 400X

Behavioral changes

Mercury also interferes with a number of body activities which are controlled by nervous system (central and peripheral nervous system). The histopathological changes observed *videsupra* also alter the behavior of animal. The behavioral responses include food avoidance, letharginess, pilling and scratching after mercuric chloride intoxication in all treated groups.

DISCUSSION

Heavy metals like, aluminum, cadmium and lead, mercury poses serious problems to the health of animals and affect different systems of the body, central nervous system, in particular. Environmental and occupational pollution leads to accumulation of mercury, responsible for serious health problems. Both organic and inorganic mercury compounds have proved their worth as potent toxic agents for wide range of animals.Severe effect of mercury poisoning target central nervous system (CNS), as all mercury compounds have avid affinity with –SH (thiol) group (Carpenter, 2001 and Vanithasri and Jagadeesan, 2013).

Brain is a heterogeneous conglomeration of many discrete "little organs" rather than one large organ, because cerebral cortex itself exhibits around 52 distinct Brodmann's areas (Choudhary *et al.*, 2013), and CNS exhibits so many pathological conditions of different neuronal cells (Garman, 2011).

The present investigation highlights the effect of mercuric chloride on the cerebral hemisphere (prosencephalon) in the brain of albino rats, which can be extrapolated to other higher mammalian species. Mercury is ubiquitous in the environment and affects the ecosystem at each tropic level. The brain seems to be main target of mercury intoxication because of being rich in lipids and proteins in the structural component of biomembranes and various cells present within. The inorganic mercury, mercuric chloride ($HgCl_2$), when comes in contact of meninges of the brain induces oxidative stress, by decreasing the endogenous antioxidant enzymes (SOD, CAT and GST) and non-enzymatic antioxidants (GSH and proteins), as a result of which the level of LPO get enhanced, which is highly destructive and induces plethora of the alterations in the structure and functions of cellular membrane, which could lead to cell injury (Patric, 2002; Neustadt and Pieczenic, 2007; Sharma *et al.*, 2011; Rastegar *et al.*, 2011 and Vanithasri and Jagadeesan, 2013).

Mercury binds to the thiol group of enzymes and proteins which help it to cross the Blood-Brain-Barrier easily and in turn get transported to glial cells and neurons by adopting procedure of molecular mimicry (Yokel, 2006).

Mercury accumulates in different parts of the CNS (Olfactory bulbs, cerebral hemisphere, cerebellum, medulla oblongata and spinal cord). Mercury also depletes mitochondrial enzymes leading to severe mitochondrial damage, it reduces CAT activity which is responsible for balancing the

production of H_2O_2 and superoxide radicals, as a result of which, level of H_2O_2 increases, which increases superoxide radical production and higher hydroxyl radical formation, which increases LPO level in brain and leads to mercury toxicity (Rao and Purohit, 2011).

In the present investigation acute treatment of mercuric chloride exhibits extreme vacuolization in the cerebral hemisphere, pyknosis in cell body of neuron and also in glial cells and enucleated cells, besides necrosis in the neuronal body and glial cells which is an indication of severe neurodegeneration and leads to memory dysfuctioning and impaired motor activities and is an affirmation to Kumar *et al.,* (2014).

The findings in the present study are further supplicated by $HgCl_2$ induced necrosis in neuron, evident by swelling and lysis with diffuse chromatin, cortical impairment and cell death due to mitochondrial dysfuctioning and impairment of cell membrane integrity (Issa *et al.,* 2003). Neuronal vacuolization and necrosis in cerebral cortex further supports the pathogenicity in neuron under aluminum exposure. Cerebrum (cerebral hemisphere) do play a key role in memory, attention, perceptual awareness, language and consciousness, which perhaps get affected under stressful conditions of heavy metals. Aluminum being a heavy metal has also been observed to hamper the functions within cerebrum *vide supra* and accelerates neurodegeneration (Buraimoh *et al.,* 2012[a]; Buraimoh *et al.,* 2012[b]; Douichene *et al.,* 2012 and Choudhary *et al.,* 2013).

Again, pyramidal cells manifest changes ranging from reduction in number to degeneration, besides vacuolization and distorted morphology of neuronal cells in the present investigation and is in affirmation to Sadeeq *et al.,* (2013). Neuronal degeneration and demyelination of neuron also observed to be due to mercuric chloride intoxication indicating target of cadmium and mercury to oligodendroglial cells of CNS (Shagirtha *et al.,* 2011 and Sheikh *et al.,* 2013).

The sub-acute studies conducted also reveal aggregation of neuroglial cells, neurophagia and hypertrophy of microglia cells, an indication that mercury poses severe effect on central nervous system even at low doses. Hussain and Mohey (2011) observed similar changes under stress of cadmium in the anatomy of brain.

Cadmium can critically damage the cerebral neuroarchitecture in cerebral cortex of wistar rat and vitamin C can protect from candmium toxicity in brain (Afifi and Embaby, 2015). Mercuric chloride induced clumping of cells, increased cellularity and increased hyperchromatic nuclei in the molecular layer of cerebrum observed in the present investigation, have also been the outcome of earlier experimentations on rat (Ibegbu *et al.,* 2013 and Ranjan *et al.,* 2014).

The study exhibited hypertrophy in microglia, increased intravascular space and gliosis at lower magnitude on day 14 compared to day 7. The present studies gain support by the findings of Nahla and Marwa (2011), who also observed focal gliosis, satellitosis and neurophagia in gray matter of brain under heavy metal intoxication. The activation of microglia is the sign of heavy metal intoxication (Kern *et al.,* 2012).

Further, changes observed after 21 ds interval include focal gliosis, cellular aggregation and increased intravascular space at higher magnitude than 7 and 14 ds of sub-acute treatment. However, hypertrophy of microglia could not be seen on day 21 following $HgCl_2$ intoxication. Lead induced edema with focal gliosis (Khalaf *et al.,* 2012) and aluminum induced gliosis are manifestations of CNS involvement under heavy metal intoxication and reveals typical similarities of toxicities of heavy metals (Thangarajan *et al.,* 2013). Heavy metal toxicity studies also showed that the metal ions did not accumulate in organs of the rats. The metal ion values were also found to be within tolerable value prescribed as safe levels. As a consequence, underexploited seaweeds studied were found to be safe for human consumption. Further, the use of 2/g upto 5/g on regular basis will not cause any toxic effect on humans was evident from the study (Ganeshan *et al.,* 2020).

The behavior of an animal describes its health status and is controlled by the central nervous system. Any damage in CNS (Prosen, mesen and rhombencephalon) alters the behavior of treated animals. The behavioral changes after mercuric chloride intoxication have also been observed in the present investigation including food avoidance, letharginess, pilling and scratching. Brain is the primary target of mercury toxicity, causes neurodegeneration which leads to pilling and scratching. Food avoidance as observed in present study results in physiological weakness leading to letharginess. The findings are in affirmation to the observations made by Teixeira *et al.,* (2014) who observed that mercuric chloride significantly

damage the motor and memory center of brain leads to impaired motor activities and learning behavior. The mercuric chloride intoxication also leads to and letharginess (Ibegbu *et al.,* 2013). The arsenic trioxide intoxication also hamper the central nervous system resulting in neuropathy which leads to piling and scratching (Saxena *et al.,* 2010). Histological Similar results have been obtained by Lazarus *et al.,* (2018) who observed histochemical assessements on the effect of ethanol fruit extract of *Phoenix dactylifer*a L. (Date Palm) on cerebral cortex of lead acetate treated wistar rats.

The possible mechanism that lies with mercury toxicity further reveals dose-related alterations in the cerebral architecture. Hypertrophy of microglial cells is indicative of neurotoxic insult. Since, these cells are the part of immune system of the brain and the main source of proinflammatory factors (TNF-α, PGE_2, INF-γ). The presence of mercury, thus, can be considered as an antigen and stimulates microglial reactivity. This reactivity is stimulated by neuron damage or death. In other words, neuron damage or death stimulates microglial cell reactivity and cycle goes on again and again, until a condition to which we call reactive microgliosis is achieved. The increasing number of microglial cells with increasing dose is an activation of mercury toxicity (Sugianto *et al.,* 2013). Albeit, inorganic mercury compounds are less neurotoxic than organic mercurial compounds, because their rate of transport into CNS parenchyma is lower than organic mercurials. Yet, some inorganic mercurials, such as, mercuric chloride acts directly on central nervous system (Zheng *et al.,* 2003).

CONCLUSION

In the light of the present investigation it is evident that mercuric chloride has profound effect on cell architecture of fore brain, the cerebral hemisphere in particular, which is the prime site of memory and motor activity. The study reveals that mercuric compounds should be at a distance from the human population as they are capable enough to discredit neuronal activities which are necessary and important for every moment of living being and the cells within, destined to perform various activities of life and in general activities. It is therefore concluded that mercury brings about brain dysfuctioning even at low-dosages (sub-acute treatments); the only difference lies in the magnitude of the toxicity. Inorganic mercury has a potential to damage cerebrum, a part of prosencephalon dedicated to cognition and memory setup. Hence, use of mercuric chloride should be restricted and minimized under legislative measures laid down by environmental laws.

REFERENCES

Afifi, O.K., Embaby, A.S. 2015. Histological Study on the Protective Role of Ascorbic Acid on Cadmium Induced Cerebral Cortical Neurotoxicity in Adult Male Albino Rats. *J. Microsc. Ultrastruct.* http://dx.doi.org/10.1016/j.jmau.2015.10.001

Aposhian, H.V. 2008. Mercuric mercury in the developing brains of young children exposed to thimerosal-containing vaccines. Aposhian supplement report, supplemental report in lieu of rebuttal testimony. U S federal court of claims. Vaccine Trial

Azevedo, B.F., Furieri, L.B., Pecanha, F.M., Wiggers, G.A., Vassallo, P.F., SimÕes, M.R., Fiorim, J., Batista de, P.R., Fioresi, M., Rossoni, L., Stefanon, I., Alonso, M.J., Salaices, M. and Vassallo, D.V. 2012. Toxic effects of mercury on the cardiovascular and central nervous systems. *Journal of Biomedicine and Biotechnology*: 1-11

Bernhoft, R.A. 2012. Mercury Toxicity and Treatment: A Review of the Literature. *J. Environ. Pub. Health.*

Bull, S. 2007. Inorganic mercury / elemental mercury: Toxicological overview. *Health Protection Agency*. Version 2: 1-13.

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. and Adebisi, S.S. 2012[a]. Effects of aluminium chloride on the histology of the cerebral cortex of adult wistar rats. *Journal of Biology and Life Science.* 3(1): 87-113

Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. and Adebisi, S.S. 2012[b]. Effects of aluminium chloride exposure on the cerebral cortex of adult wistar rats were not transferable to the offspring. *American International Journal of Contemporary Research.* 2 (8) : 294-303

Candura, S.M., D'Agostino, G., Castoldi, A.F., Messori, E., Liuzzi, M., Manzo, L. and Tonini, M. 1997. Effects of mercuric chloride and methylmercury on cholinergic neuromuscular transmission in the Guinea-Pig ileum.*Pharmacology and Toxicology.* 80 : 218-224

Carpenter, D.O. 2001. Effects of metals on the nervous system of humans and animals.*International Journal of Occupational Medicine and Environmental Health.* 14(3): 209-218.

Carpi, A. 2001. The toxicology of mercury. *National Science Foundation-Vision Learning,* New York

Choudhary, M., D.V. Joshi, S. Tripathi and Mahdi, A.A. 2013. Effect of aluminum on different parts of brainstem of old rats: Haematological, Biochemical and Morphological study. *Res. J. Pharmaceutical Sci.* 2(3) : 6-11.

Clarkson, T.W., L. Magos and Myers, G.J. 2003. The toxicology of mercury-Current exposures and clinical manifestations.*N. Engl. J. Med.* 349(18):

1731-1737.

Finney, D.J. 1971. Probit analysis (3rd Edi.), Cambridge University Press, London

Fisher, R.A. and Yates, F. 1950. *Statistical Methods for Research Workers* (11th Edi.), Oliver and Boyd Ltd., Edinberg, U.K.

Ganesan, A. R., Subramani, K. and Balasubramanian, B. 2020. Evaluation of *in vivo* sub-chronic and heavy metal toxicity of under-exploited seaweeds for food application *Journal of King Saud University - Science.* 32(1) : 1088-1095 https://doi.org/10.1016/j.jksus.2019.10.005

Garman, R.H. 2011. Histology of the central nervous system. *Toxicologic Pathology.* 39 : 22-35.

Haley, B.E. 2007. The relationship of the toxic effects of mercury to exacerbation of the medical condition classified as Alzheimer's disease. *Medical Veritas.* 4 : 1510-1524.

Hematian, A. 2013. Cerebellum alterations in offspring of mercury treated rats. *Bulgarian Journal of Veterinary Medicine.* 16(1) : 53-60.

Hornig, M., Chian, D. and Lipkin, W.I. 2004. Neurotoxic effects of postnatal thimerosal are mouse strain dependent. *Molecular Psychiatry.* 1-13.

Humason, G.L. 1979. *Animal Tissue Techniques.* (4th Edi.). W.H. Freeman and Company, San Francisco, U.S.A.

Hussein, A.K. aṇd Mohey, M.M. 2011. Effect of cadmium pollution on neuromorphology and function of brain in mice offspring. *Nature and Science.* 9(4) : 28-35.

Hyman, M. 2004. The impact of mercury on human health and the environment. *Alternative Therapies.* 10(6): 70-75.

Ibegbu, A.O., A.A. Animoku, M. Ayuba, D. Brosu, S.A. Adamu, P. Akpulu, W.O. Hamman, U.E. Umana and Musa, S.A. 2013. Effect of ascorbic acid on mercuric chloride-induced changes on the cerebral cortex of wistar rats. *African Journal of Cellular Pathology.* 1: 23-29.

Issa, Y., D.C. Watts, A.J. Duxbury, P.A. Brunton, M.B. Watson and Waters, C.M. 2003. *Biomaterials.* 24: 981-987.

Kern, J.K., D.A. Geier, T. Audhya, P.G. King, L.K. Sykes and Geier, M.R. 2012. Evidence of parallels between mercury intoxication and the brain pathology in autism. *Acta Neurobiol. Exp.* 72 : 113-153.

Khalaf, A.A., Moselhy, W.A. and Abdel-Hamed, M.I. 2012. The protective effect of green tea extract on lead induced oxidative and DNA damage on rat brain. *Neurotoxicology.* 33 : 280-289.

Kumar, G., A. Srivastava, S.K. Sharma and Gupta, Y.K. 2014. Safety evaluation of mercury based Ayurvedic formulation (*Sidh Makardhwaj*) on brain cerebrum, liver & kidney in rats. *Indian J. Med. Res.* 139 : 610-618.

Lazarus, S. S., Adebisi, S. S., Tanko, Y., Agbon, A. N. and Budaye, M. N. 2018. Histological and histochemical assessements on the effect of ethanol fruit extract of *Phoenix dactylifer*a L. (Date Palm) on cerebral cortex of lead acetate treated wistar rats. *Afr. J. Cell. Path.* 10(1) : 1-9.

Morken, T.S., U. Sonnewald, M. Aschner and Syversen, V. 2005. Effects of methylmercury on primary brain cells in mono- and co-culture. *Toxicological Sciences.* 87(1) : 169-175.

Mutter, J., A. Curth, J. Naumann, R. Deth and Walach, H. 2010. Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism. *Journal of Alzheimer's Disease.* 22 : 357-374.

Nascimento, J.L.M.do, K.R.M. Oliveira, M.E. Crespo-Lopez, B.M. Macchi, L.A.L. Maués, M.daConceição N. pinheiro, L.C.L. Silveira and Herculano, A.M. 2008. Methylmercury neurotoxicity & antioxidant defenses. *Indian J. Med. Res.* 128 : 373-382

Necib, Y., A. Bahi and Zerizer, S. 2013. Protective role of sodium selenite on mercuric chloride induced oxidative and renal stress in rats. *Journal of Stress Physiology & Biochemistry.* 9(2) : 159-172

Neustadt, J. and Pieczenik, S. 2007. Heavy-metal toxicity-with emphasis on mercury. *Integrative Medicine.* 6(2) : 26-32.

Nishioku, T., N. Takai, Ken-ichiro Miyamoto, K. Murao, C. Hara, K. Yamamoto and Nikanishi, H. 2000. Involvement of caspase 3-like protease in methylmercury-induced apoptosis of primary cultured rat cerebral microglial. *Brain Research.* 871 : 160-164

OECD 2001. Acute oral toxicity- Fixed dose procedure, Test Guideline No. 420, OECD guideline for testing of chemicals, OECD, Paris.

Oriquat, G.A., T.H. Saleem, R.R. Naik, S.Z. Moussa and Al-Gindy, R.M. 2012. A sub- chronic toxicity study of mercuric chloride in the rat. *Jordan J. Bio. Sci.* 5 (2): 141-146.

Pamphlett, R. and Kum Jew, S. 2013. Uptake of inorganic mercury by human locus ceruleus and corticomotor neurons: implications for amyotrophic lateral sclerosis. *Acta Neuropathologica Communications.* 1(13) : 1-11

Patrick, L., ND 2002. Mercury toxicity and antioxidants: Part I: Role of glutathione and alpha-lipoic-acid in the treatment of mercury toxicity. *Alt. Med. Rev.* 7(6): 456-470.

Pecze, L. and Papp, A. 2003. Neurotoxicity of lead and mercury in acute exposure. *ISIRR*: 103-108.

Ranjan, B., S.M.D. Husain and Yunus, S.M. 2014. Neurohistopathological effects of mercury on hippocampus of adult albino rat. *Global Journal for Research Analysis.* 3(7) : 237-238.

Rao, M.V. and Patel, T.A. 2013. Protective effect of curcumin on mercuric chloride induced oxidative stress in rats. *Indian Journal of Applied Research.* 3(11) : 541-543

Rao, M.V. and Purohit, A.N. 2011. Neuroprotection by melatonin on mercury induced toxicity in the rat brain. *Pharmacology and Pharmacy*. 2 : 375-385.

Rastegar, T., Parivar, K., Novakht, M., Shahbazi, A., Zendehrood, S.A. and Mehdizadeh, M. 2011[a]. Prenatal mercuric chloride exposure causes developmental deficits in Rat cortex. *Basic Clin. Neurosci*. 2(2) : 26-30.

Sadeeq, A.A., A.O. Ibegbu, M.G. Taura, J.A. Timbuak, L.H. Adamu and Kwanashie, H.O. 2013. Studies on the effects of mercury exposure on spatial learning and memory of adult wistar rats. *International Journal of Pharmaceutical Science Invention.* 2(12): 12-16.

Saxena, P.N., Rathor, S., Mahour, K., Saxena, N. and Bajaj, P. 2010. Alterations in hepatosomatic index and behaviour under stress of Arsenic Trioxide and their modulation by *Curcuma aromatica* plant extract in albino rats (*Berkenhout*). *Pharmacologyonline.* 1 : 243-251.

Shagirtha, K., Muthumani, M. and Prabu, S.M. 2011. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. *Eur. Rev. Med. Pharmacol. Sci.* 15 : 1039-1050.

Sharma, S., Sharma, V. Pracheta and Sharma, S. 2011. Therapeutic potential of hydromethanolic root extract of *Withaniasomnifera* on neurological parameters in swiss albino mice subjected to lead nitrate. *Int. J. Curr. Pharm. Res.* 3(2) : 52-56

Sheikh, T.J., Patel, B.J. and Joshi, D.V. 2011. Electrolytes alterations in plasma and urine after 28 days repeated oral dose toxicity of mercuric chloride in wistar rat. *Journal of Applied Pharmaceutical Science.* 1(10) : 150-153

Sheikh, T.J., Patel, B.J., Joshi, D.V., Patel, R.B. and Jegoda, M.D. 2013. Repeated dose oral toxicity of inorganic mercury in wistar rats: biochemical and morphological alterations. *Vet. World.* 6 (8) : 563-57.

Sugianto, P., Widodo, A.A. and Machfoed, M.H. 2013. Neurotoxic effect of mercury. *Journal of Applied Sciences Research.* 9(5) : 3391-3395

Teixeira, F.B., Fernandes, R.M., Farias Jr., P.M.A., N.M.M. Costa, L.P.M. Fernandes, L.N.S. Santana, A.F. Silva Jr., M.C.F. Silva, C.S.F. Maina and Lima, R.R. 2014. Evaluation of the effects of chronic intoxication with inorganic mercury on memory and motor control in rats. *Int. J. Environ. Res. Public Health.* 11 : 9171-9185.

Thangarajan, S., Chandrasekar, S., Varadharajan, M., Ramachandran, S., Manogaran, S., Arunachalam, V. and Krishnamoorthi, R. 2013. Oxidative stress in brains of male rats intoxicated with aluminium and neuromodulation effect of *Celastruspaniculatus* alcoholic seed extract. *Asian J. Pharm. Clin. Res.* 6(3) : 80-90.

Vanithasri, V. and Jagadeesan, G. 2013. Comparative beneficial effect of N-acetylcysteine and gallic acid on antioxidant and acetylcholine status of mercury intoxicated rat. *International Journal of Phytopharmacology*. 4 (5) : 293-300

Varghese, J., Shrilakshmi, K. and Rathore, H.S. 1997. Prevention of mercuric chloride induced cerebellar damage in mice with a multiherbal hepatic drug results and possibilities. *Indian journal of Occupational Health.* 1(40) : 1-6

Xu, F., S. Farkas, S. Kortbeek, Fang-Xiong Zhang, L. Chen, G.W. Zamponi and Syed, N.I. 2012. Mercury-induced toxicity of rat cortical neurons is mediated through N-methyl-D-Aspartate receptors. *Molecular Brain.* 5(30): 1-14.

Yokel, R.A. 2006. Blood-brain barrier flux of aluminium, manganese, iron and other metals suspected to contribute to the metal induced neurodegeneration. *Journal of Alzheimer's Disease.* 10 : 223-253.

Zahir, F., Rizwi, S.J., Haq, S.K. and Khan, R.H. 2005. Low dose mercury toxicity and human health. *Environ. Toxicol and Pharacol.*

Zalpus, R.K., Barfuss, D.W. and Lash, L.H. 1999. Disposition of inorganic mercury following biliary obstruction and chemically induced glutathione depletion: dispositional changes one hour after intravenous administration of mercuric Chloride. *Toxicology and Applied Pharmacology.* 154 : 135-144.

Zhao, Jun-Quan, Yi-Fei Wen, M. Bhadauria, S.K. Nirala, A. Sharma, S. Shrivastava, S. Shukla, O.P. Agrawal and Mathur, R. 2009. Protective effects of propolis on inorganic mercury induced oxidative stress in mice. *Indian Journal of Experimental Biology.* 47 : 264-269.

Zheng, W., Aschner, M. and Ghersi-Egea, J.F. 2003. Brain barrier systems: a new frontier in metal neurotoxicological research. *Toxicology and Applied Pharmacology.* 192 : 1-11.

Zimmermann, L.T., D.B. Santos, A.A. Naime, R.B. Leal, J.G. Dórea, F. Barbosa Jr., M. Aschner, J.B.T. Rocha and Farina, M. 2013. Comparative study on methyl- and ethylmercury-induced toxicity in C6 glioma cells and the potential role of LAT-1 in mediating mercurial-thiol complexes uptake. *Neurotoxicology.* 38 : 1-8.