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EFFECT OF *Rauvolfia vomitoria* ON MERCURY-INDUCED CHANGES ON THE FINE MOTOR COORDINATION AND HISTOLOGY OF THE CEREBELLUM IN WISTAR RATS

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ABSTRACT

The present study was aimed at evaluating the effect of the aqueous extract of *Rauvolfia vomitoria* (RV) on mercury-induced changes on the cerebellum of adult Wistar rats. Thirty male adult Wistar rats were divided into six groups of five animals each. The control was administered with distilled water for 35 days, mercury chloride ($HgCl_2$, 49.8mg/kg) body weight, RV (750mg/kg); $HgCl_2$ (49.8mg/kg) and low dose of RV (250mg/kg), $HgCl_2$ (49.8mg/kg) and medium dose of RV (500mg/kg) and $HgCl_2$ (49.8mg/kg) and high dose of RV (750mg/kg). The administration lasted for 35 days through oral route daily with $HgCl_2$ given for 21 days and RV was for 14 days for the treated groups. Walking track equipment was used to test for gait and motor coordination and the animals were humanely sacrificed after the animals were anaesthetised with chloroform while tissue samples were harvested by opening the mid sagittal suture for histological studies. The result of gait and motor coordination test showed decrease in stride length, increase in sway length and stance length after mercuric chloride administration but stride length increased while sway and stances length decreased after the administration of RV. Observation of the cerebellum showed normal histology in the control, while mercury control, RV control, mercuric chloride and low dose RV, mercuric chloride and medium dose RV, and mercuric chloride and high dose RV groups showed some degenerative and cellular changes. The administration of RV has shown to ameliorate the degenerative changes in the cerebellum caused by mercuric chloride toxicity in Wistar rats.

Key words: *Mercuric chloride, Cerebellum, Rauvolfia vomitoria, Wistar rats*

INTRODUCTION

Mercury and its compounds can be obtained from Industrial sources, fossils fuels power, mining co-operations, and natural forms such as mercury chloride that is found in higher densities in rocks and volcanic activities (FAO 1994; Park et al. 2000). Burning of fossil fuels such as petrol and gas, fumes, battery disposals, broken mercury thermometer and coal combustion are other high sources of emitting mercury and its compounds (Booth and Zeller 2005). Consumer products such as photographic plates and toners contain high amount of mercury chloride (Goyer 1986). Some cosmetics also contain mercury. Examples include creams, perfumes, soaps and mascara (Adepoju-Bello et al. 2012).

There are many routes of exposure to mercuric compounds but the evidence of exposure is dependent on the levels of toxicity (Vimercati and Pesola 2001; WHO 2005). These exposure routes include; oral exposure via consumption of food

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products and grains preserved with mercuric compounds (Vupputuri et al. 2005; WHO 2005).

Children and women within the reproductive age are more susceptible to mercury poisoning (Murphy et al. 1979). In children, mercury poisoning is known as acrodynia or pink disease. Mercury and its compounds have been shown to have effects on the respiratory, cardiovascular and reproductive systems, blood, hair, skin and enzymes, and many other organs and tissues (Olivieri et al. 2000; Valera et al. 2008; Rao and Chhunchha 2009).

Mercuric chloride was evaluated in toxicity and carcinogenicity studies because of its extensive use and its occurrence as an environmental pollutant (U.S. DHHS 1993). Neurological symptoms include mental retardation, seizures, vision and hearing loss, delayed development, language disorders and memory loss. In children, a syndrome characterized by red and painful extremities called acrodynia has been reported to result from chronic mercury exposure (WHO 2007).

Rauvolfia vomitoria (RV) is a medicinal plant widely distributed all over the world, especially in Asia and West African Countries. The constituents of the plant was first extracted by a Swiss chemist in 1952 becoming the first neuroleptic. The plant later become a source of a lot of drugs used in psychiatry (Ehiagbonare 2004). In traditional medicine the root and leaf of *R. vomitoria* are brewed as tea, and used in humans for the treatment of hypertention, insanity, snakebite and cholera (Erhenhi and Obadoni 2015). The common name is Swizzle stick (Fapojuwomi and Asinwa 2013) and its Nigerian names include Asofeyeje in Yoruba, Akanta in Igbo and Wada in Hausa (Etukudo 2003).

Research work devoted to phytochemical evaluation of *R. vomitoria* revealed the presence of alkaloids, tannins, saponins, and flavonoids (Akpanabiatu et al. 2006). The extract has also been found to exhibit antioxidant activity, significant hydrogen peroxide scavenging effect relative to ascorbic acid, nitric oxide scavenging effect, metal chelating activity and ferric reducing power relative to ascorbic acid (Okolie et al. 2011). The aim of the present study was to evaluate the effect of *R. vomitoria* on mercuric chloride-induced changes on the cerebellum of adult Wistar rats.

MATERIALS AND METHODS

Animal Handling

Thirty male adult Wistar rats of average weight of 195g were used for this study. They were acclimatized for two weeks and kept in the animal house of the Department of Human Anatomy, Faculty of Medicine Ahmadu Bello University Zaria. The rats were then divided into six groups of five rats per group for the experiment.

Chemicals/Extract Preparation

Mercuric chloride manufactured by May and Bakers Chemical Laboratory Limited Dagenham England. *R. vomitoria* root was obtained from Dakaci area of Zaria, Kaduna, Nigeria. The aqueous extraction *of R. vomitoria* root back was prepared according to Handa et al. (2008) in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Animal Experimentation

Thirty Wistar rats were divided into six groups of five animals each: Control was administered distilled water; mercury chloride ($HgCl_2$) group administered 49.8mg/kg body weight, corresponding to 30% of its lethal dose at 50% (LD_{50}) (Berlin et al. 2007); *R. vomitoria* (RV) group administered 750mg/kg body weight, equivalent to 4.29% of its LD50 (Amole et al. 2009); $HgCl_2$ (49.8mg/kg) and low dose of RV (250mg/kg) group; $HgCl_2$ (49.8mg/kg) and medium dose of RV (500mg/kg) group; and $HgCl_2$ (49.8 mg/kg) and high dose of RV (750mg/kg) group. The administration of $HgCl_2$ was for 21 days, after which RV was administered for 14 days. The administration was by oral route daily and lasted for 5 weeks while animal feed and drinking water was allowed *ad libitum*. The extract was diluted using distilled water. This experiment was approved by the Committee on Research and Postgraduate Studies of the Department of Human Anatomy, Ahmadu Bello University, Zaria.

Gait and Motor Co-ordination Assessment

Walking track equipment according Yu et al. (2001) and Paxinos and Watson (2005) was modified from previous studies for gait analysis and motor coordination in this study. The walking track apparatus consisted of a perplex glass chamber 80 (l) × 10 (w) × 12 (h) cm. The forepaw was painted red with ink and the hind paw painted black, thus permitting the recording of the prints of each forepaw

Figure 1: Picture of the Working Track

and hind paw on a reading sheet spread on the walking track as shown in Figure 1.

Animal Sacrifice

At the end of administration, the animals were weighed, humanely sacrificed after the animals were anaesthetized with chloroform in an anaesthesia box and incision was made through the skin and muscle of the skull. The skull was opened through mid sagittal suture incision and the brain was removed and fixed in Bouin's fluid for twenty four hours. The tissues were routinely processed and stained using haematoxylin and eosin method.

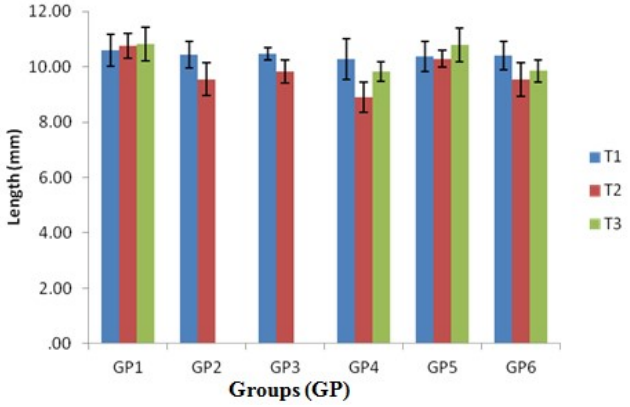

Figure 2: The mean training stride length (T1) and test stride length (T2) after $HgCl_2$ administration and after treatment with *R. vomitoria* (T3) in gait and motor co-ordination. No significant difference in mean training and test stride lengths.

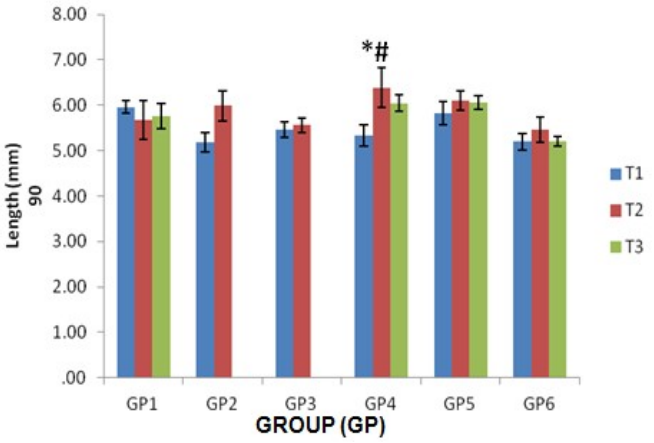

Figure 3: The mean training sway length (T1) and test sway length (T2) after $HgCl_2$ administration and after treatment with *R. vomitoria* (T3) in gait and motor co-ordination. *#p ≤ 0.001 in the $HgCl_2$ and low dose of RV group

Statistical Analysis

All data were presented as mean ± standard error of mean, and for establishing significant differences, data were analyzed by one-way analysis of variance, followed by Tukey post-hoc test. Values were considered significant if p value ≤ 0.05.

RESULTS

Physical Observation of the Animals

The result of physical observation of the animals showed that rats in control were active, while mercury chloride ($HgCl_2$) control, $HgCl_2$ and low dose of RV, $HgCl_2$ and medium dose of RV and $HgCl_2$ and high dose of RV animals exhibited increased activity and aggression, and drank more water and increased gnawing during $HgCl_2$ administration, RV only animals showed little gnawing, were calmer and passed watery stools, while $HgCl_2$ and low dose of RV animals exhibited restlessness and more gnawing during RV administration. $HgCl_2$ (49.8mg/kg) and medium dose of RV and $HgCl_2$ and high dose of RV animals showed least change in their physical activity compared to the control.

Gait and Motor Co-ordination Test Parameters

The motor co-ordination test used the narrow corridor to test for stride length, sway and stance of the animals.

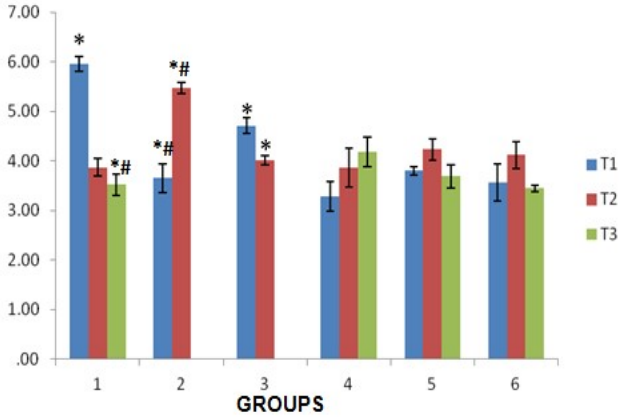

Figure 4: The mean training stance length (T1) and test stance length (T2) after $HgCl_2$ administration and after treatment with *R. vomitoria* (T3) in gait and motor coordination. *#p ≤ 0.001 in control and $HgCl_2$ groups; *p ≤ 0.01 in the *R. vomitoria* group

Stride Length

The results of the mean stride length during training (T1), after administration of $HgCl_2$ (T2) and after treatment with *R. vomitoria* (T3) are shown in Figure 2. The result showed decreased stride length across the groups after administration of $HgCl_2$ (T2) compared to the control group, but was not significant ($p > 0.05$). After the administration of *R. vomitoria* there was an increase in the stride length across the group although not significant.

Sway Length

The results of the mean Sway length of the rats during training (T1), after administration of $HgCl_2$ (T2) and after treatment with *R. vomitoria* (T3) are shown in Figure 3. The result showed increase in the sway length after administration of $HgCl_2$ (T2) with

statistically significant change in the Sway length in are $HgCl_2$ and low dose of RV ($p \leq 0.001$) when compared with mean training length (T1). The Sway length result after treatment *R. vomitoria* (T3) decreased but was not statistically significant.

Stance Length

The results of the mean stance length of the rats during training (T1), after administration of $HgCl_2$ (T2) and after treatment with *R. vomitoria* afzel (T3) are shown in Figure 4. The result showed significant decrease in the stance length in the control ($p \leq 0.01$), mercury chloride ($p \leq 0.001$) and RV ($p \leq 0.05$) groups compared to T1 and T2. The stance length result after treatment with *R. vomitoria* (T3) increased in are $HgCl_2$ and low dose of RV but decreased in other treated groups, however, this differenc- were not statistically significant. Also comp-aring mean stance len-gth of the animals dur-ing training (T1) show-ed significant decrease in control ($p \leq 0.001$) as the experiment progr-esses.

Histological Study of the Cerebellar Cortex

The histological exami-nation of the cerebellar cortex of the animals in the control administ-ered distilled water showed normal histo-morphology of the cerebellar cortical cells and layers as shown in Figure 5A. The cereb-ellum of animals in mercury chloride treat-ed group showed necr-osis with shrunked Purkinje cells and vac-uolation around some of the degenerating Purkinje cells, with lar-ge areas of absence of Purkinje cells, but rela-tively normal molecular and granular cell layers as shown in as shown in Figure 5B. The cere-bellum of animals in RV treated group showed relatively nor-mal Purkinje cells with disoriented Purkinje cells in the Purkinje cell layer and normal granular and molecular cell layers as shown in Figure 5C. The cerebe-llum of animals in $HgCl_2$ and low dose of RV showed fewer but normal Purkinje cells with some areas of loss of the cells, as shown in Figure 5D. Although there was loss of Purkinje cells in $HgCl_2$ and medium dose of RV group, the observed cells still appeared normal, with normal granular and molecular layers as shown in Figure 5E. The cerebellum of the $HgCl_2$ and high dose of RV showed better distribution of Purkinje cells, fewer necrosis and vacuolation around Purkinje cells in the

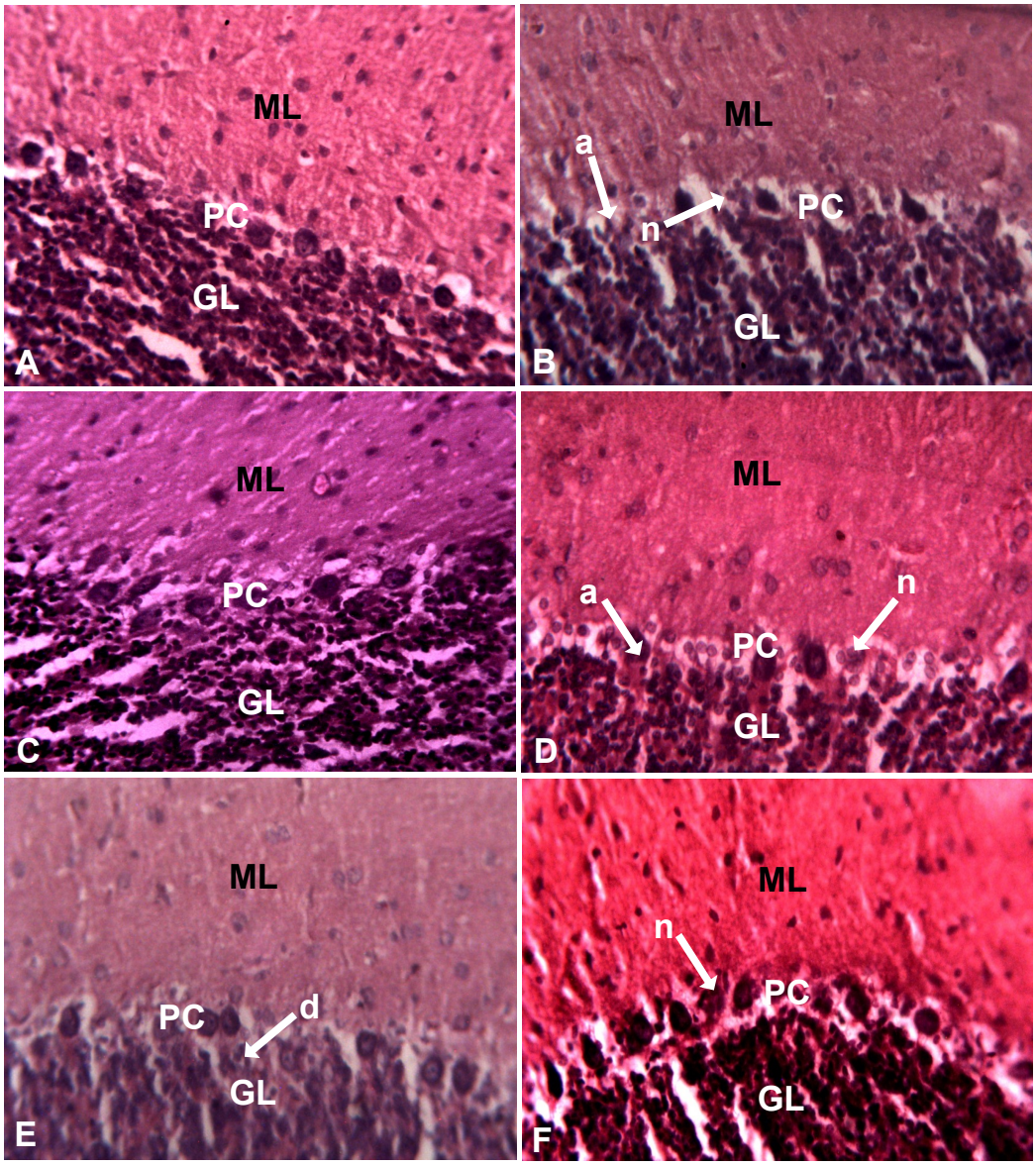

Figure 5: A. Section of the cerebellar cortex of the control group showing normal Purkinje cells (PC); B. Section of the cerebellar cortex of $HgCl_2$ group showing areas of absence (a) and necrotic cells (n); C. Section of the cerebellar cortex of *R. vomitoria* group showing shrunken Purkinje cells (PC); D. Section of the cerebellar cortex of $HgCl_2$ and low dose *R. vomitoria* group showing areas of absence of Purkinje cells (a); E. Section of the cerebellar cortex of $HgCl_2$ and medium dose *R. vomitoria* group showing displaced Purkinje cells (d); F. Section of the cerebellar cortex of $HgCl_2$ and high dose *R. vomitoria* group showing necrosis. Purkinje cells (PC), Molecular layer (ML} and Granular layer (GL)H & E X250

Purkinje cell layer with areas of complete absence of Purkinje cells, normal granular and molecular cell layers as shown shown in Figure 5F.

DISCUSSION

The walking track equipment determined gait and motor coordination of the animals. Tourtellotte and Milbrandt (1998) reported that abnormal gait is due to reduced muscular tone, impaired cerebellar motor or peripheral neurological function, and/or due to underlying musculoskeletal anomaly. The stride length of the control animals increase with age but not statistically significant. During administration of mercury chloride, the stride length of the animals decreased after treatment athough not statistically significant. The present result disagrees with the work of Carter et al. (1999), who stated that animals with motor deficit will display shorter stride length. Also, the sway length of the animals increased after administration of $HgCl_2$ and was statistically significant in the $HgCl_2$ and medium dose RV group, but decreased after treatment with RV, agreeing with the work of Reimold et al. (1996), who reported that sway length will increase resulting in a shorter, wider gait and motor abnormality. Furthermore, the stance length decreased significantly with age for the control but increased during administration of mercury chloride, which was significant in $HgCl_2$ control, but decreased after treatment with RV. This result is contrary to the work of Reimold et al. (1996), who reported decrease in stance length in gait and motor abnormalities.

Purkinje cells play a fundamental role in movement. They regulate and coordinate motor movement. So, any damage or loss would result in motor deficit and other neurological diseases. In this study, the cerebellum showed necrosis with shrinkage, karynorrhexis and vacuolation around Purkinje cells in $HgCl_2$ administration compared to the control group. This agree with the work of Ibegbu et al. (2014) and Ranjam et al. (2015), who reported degeneration and cellular necrosis with gliosis and increased cellularity of granular layer and molecular layer of the cerebellum after administration of mercury. Gray (2004) reported that mercury-induced toxicity in the cerebellum includes cerebellar atrophy involving predominant loss of granular cells and mild lose of Purkinje cells. These changes maybe transient but permanent abnormalities may be induced only by sustained exposure of these chemicals in an excessive quantity (Wolf et al. 2003). Gagalli et al. (2010) and Mello-Carpes et al. (2013), in their separate works reported decreased neural cell sizes and cell number in mice treated orally with inorganic mercury at high dose for a week. These researches were in agreement with the results of the present work. The present study has shown that RV has ameliorative effects on the Purkinje cells of the cerebellar cortex in mercury induced neurotoxicity. The present study has also shown slight effect of RV as observed in group 3 administered RV alone which shows some level of necrosis, shrinkage and loss in purkinje cells in the Purkinje cells layer. Similar effect was previously reported by Eluwa et al. (2009) and Ekong et al. (2013). This effect increase with volume of dosage even in groups co-administered mercury, indicating that there was a direct relationship between RV concentration and mercuric chloride concentrations and the level of neurodegeneration in the cerebellar cortex in the studied species of rats. In the work of Ekong et al. (2017) the co-administration of RV and *Gongronema latifolium* showed a protective effect of histopathology on cerebellum induced by RV. So, co-administration of RV should be a point of focus in its neuro-protective evaluation.

Conclusion

Mercury exposure induced neurodegeneration in the cerebellum of the adult Wistar rats and RV has shown to significantly ameliorate the neurotoxicity induced by mercuric chloride administration. As a such, the use of RV as a possible supplement for people exposed to mercury poison should be further investigated.

Conflict of Interest

None declared.

REFERENCES

Adepoju-Bello, A.A., Oguntibeju, O.O., Adebisi, R.A., Okpala, N. and Coker, H.A.B. (2012) Evaluation of the concentration of toxic metals in cosmetic products in Nigeria. African Journal of Biotechnology. 11(97):16360-16364.

Akpanabiatu, M.I., Otitoju, O., Edet E.E., Ndem J.I., Uwah, A.F. and Ufot, U. (2006) Vitamin E supplementation with Rauwolfia vomitoria root bark extract improves hematological indices. North American Journal of Medical Sciences. 4(2):86-89

Amole, O.O., Yemitan, O.K. and Oshikoya, K.A. (2009) Anticonvulsant activity of Rauvolfia vomitoria (Afzel). African Journal of Pharmacy and Pharmacology. 3(6):319-322.

Berlin, M., Zalups, R.K. and Fowler, B.A. (2007) Mercury Handbook on the Toxicology of Metals. In: Nordberg, G. F., Fowler, B. A., Nordberg, M. and Friberg, L. T, (ed.). 3rd edn. New York: Elsevier.

Booth, S. and Zeller, D. (2005) Mercury, food webs, and marine mammals: implications of diet and climate change for human health. Environmental Health Perspectives. 113:521-526.

Carter, R.J., Lione, L.A., Humby, T., Mangiarini, L., Mahal, A., Bates. G.P., Dunnett, S.B. and Morton, A.J. (1999) Characterization of progressive motor deficit

in mice transgenic for the human Huntington's disease mutation. Journal of Neuroscience. 19(8): 3248-3257.

Ehiagbonare, E.J. (2004) Regeneration of Rauwolfia vomitoria. African Journal of Biotechnology. 6(8) 979-981.

Ekong, M.B., Ekpene, U.U., Nwakanma, A.A. and Bello, E.F. (2017) The combination of the extract of Rauwolfia vomitorium and Gongonema latifolium show protective effect on the cerebellum. Synergy. 5(2017):29 -34.

Ekong, M.B., Peter. A.I., Davies, K., Bassey, E.I., Aquaisua, A.N., Akpanabiatu, M.I. and Ekanem, T.B. (2013) Gongronema latifolium ameliorates Rauwolfia vomitoria induced behavior, biochemicals, and histomorphology of the cerebral cortex. Journal of Neurochemistry. 125(Suppl. 1):369. doi:10.1111/jnc.12186

Eluwa, M.A., Idumesaro, N.B., Ekong, M.B., Akpantah, A.O. and Ekanem, T.B. (2009) Effect of aqueous extract of Rauwolfia vomitoria root bark on the cytoarchitecture of the cerebellum and neurobehaviour of adult male Wistar rats: Internet Journal of Alternative Medicine. 6(2).

Erhenhi A.H. and Obadoni B.O. (2015) Known medicinal and aphrodisiac plants of Urhorugbe forest reserve, Edo State Nigeria. Journal of Medicinal Plant Studies. 3(4):101-106.

Etukudo, I. (2003) Ethnobotany: Conventional and Traditional Uses of Plants. Uyo: Verdict Press. p.191.

FAO (1994) Food and Agriculture Organization of the United Nations. Manual of methods in aquatic environment research, part 10. Short term static bioassays, FAO Fisheries Technical Paper. 247: 92.

Fapojuwomi, O. A. and Asinwa, I. O. (2013) Assessment of medicinal values of Rauvolfia vomitoria (Afzel) in Ibadan municipality. Greener Journal of Medical Sciences. 3(2):37-41.

Gagalli, E., Kozlowski, H., Valensin D. and Valensin, G. (2010) Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chemical Review. 106:1995-2044.

Goyer, A.H. (1986) Toxic effects of metals: Casaret and Doull's (Ed.) Toxicology- The Basic Science of Poisons 3. New York: Macmillan Publishing. Pp. 582-609.

Gray, F., Girolami, U.D. and Poirier, J. (2004) Escoourolle and Poirier Manual of Basic Neuropathology. Philadelphia: Elsevier Inc. 4:211-212.

Handa, S.S., Khanuja, S.P.S., Longo, G. and Rakesh, D.D. (2008) Extraction Technologies for Medicinal and Aromatic Plants. 1st edn. No 66. United Nations Industrial Development Organization and International Centre for Science and High Technology: Trieste. Earth, Environment and Marine Science and Technology. Trieste, Italy. (http://wwworldcat.org/tittle/extraction-technologies-for-medicinal-and-aromatic-plants/oclc/740721851)

Ibegbu, A.O., Animoku, A.A., Ayuba, M., Brosu, D., Adamu, S.A., Akpulu, P., Hamman, W.O., Umana, U.E. and Musa, S A. (2014) The effect of ascorbic acid on mercury-induced changes on the histomorphlogy of the cerebellum of adult Wistar rats. African Journal of Cellular Pathology. 3:9-15.

Mello-Carpes, P.B., Barros, W., Borges, S., Alves, N., Rizzetti, D., Peçanha, F.M., Vassallo, D.V., Wiggers, G.A. and Izquierdo, I. (2013) Chronic exposure to low mercury chloride concentration induces object recognition and aversive memories deficits in rats. International Journal for Developmental Neurosciences. 31(7):468-472.

Murphy, M.J., Culliford, E.J. and Parsons, V. (1979) A case of poisoning with mercuric chloride. Resuscitation. 7:35-44.

Okolie, N. Israel, E. and Falodun, A. (2011) In-vitro evaluation of antioxidant potential of Rauwolfia vomitoria root extract and its inhibitory effect on lipid peroxidation as indication of aphrodisiac properties. Pharmaceutical Chemistry Journal. 45(8):476.

Olivieri, G. Brack, C. and Müller-Spahn, F. (2000) Mercury induces cell cytotoxicity and oxidative stress and increases β- amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. Journal of Neurochemistry. 74:231-236.

Park, S.H., Araki, S. and Nakata, A. (2000) Effects of occupational metallic mercury vapor exposure on suppressor-inducer (CD4 + CD45 RA+) T lymphocytes and CD57 + CD16 + natural killer cells. International Archives of Occupational and Environmental Health. 73:537-542.

Paxinos, G. and Watson, C. (2005) The Rat Brain in Stereotaxic Coordinates. Amsterdam: Elsevier Academic Press.

Ranjam, B., Husain, D.S.M., Kumar K. and Maheshwari T.K. (2015) Comparative study of histo-pathological effects of mercury on cerebrum, cerebellum and hippocampus of adult albino rats. Annals of International Medical and Dental Research. 1(1):21-24.

Rao, M.V. and Chhunchha, B. (2009) Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid. Food Chemical Toxicology. 48:7-10.

Reimold, A.M., Grusby, M.J., Kosaras, B., Fries, J.W., Mori, R., Maniwa, S., Cluass, I.M., Collins, T., Sidman, R.L., Glimcher, M.J. and Glimcher, L.H. (1996) Chondrodysplasia and neurological abnormalities in ATF-2-deficient mice. Nature. 379: 262-265.

Tourtellotte, W.G., Milbrandt, J. (1998) Sensory ataxia and muscle spindle agenesis in mice lacking the transcription factor Egr3. National Genetics. 20:87-91.

U.S. Department of Health and Human Services (1993) Toxicology and Carcinogenesis Studies of Mercuric Chloride in f344 rats and b6c3fl mice, National Toxicology Program Technical Report

Series no. 408 National Institute for Health Publication no. 93-3139.

Valera, B., Dewailly, E. and Poirier, P. (2008) Cardiac autonomic activity and blood pressure among Nunavik Inuit adults exposed to environmental mercury: a cross-sectional study. Environmental Health. 28:924.

Vimercati, L.L. and Pesola, S.G. (2001) Monocyte-macrophage system and polymorphonuclear leukocytes in workers exposed to low levels of metallic mercury. Science of the Total Environment. 270:157-163.

Vupputuri, S., Longnecker, M.P., Daniels, J.L., Guo, X. and Sandler, D.P. (2005) Blood mercury level and blood pressure among US women: results from the national health and nutrition examination. Environmental Research. 97:195-200.

WHO (2005) Policy paper: Mercury in health Care. World Health Organization(WHO/SDE/WSH/05.08). Geneva. http//www.who.int/water_sanitation_health/ medicalwaste/mercury/en/index.html.

WHO (2007) Policy paper: Mercury in health care exposure to mercury: a major public health concern. World Health Organization: Geneva. 1-4. (http://www.who.int/phe/news/mercury-flyer.pdf . (accessed 10 June 2014).

Wolf, U. Rapoport, M.J. and Schweizer, T.A. (2003) Evaluating the affective component of the cerebellar cognitive affective syndrome. Journal of Neuropsychiatry and Clinical Neuroscience. 21:245-53.

Yu, P., Matloub, H.S., Sanger, J.R. and Narini, P. (2001) Gait analysis in rats with peripheral nerve injury. Muscle Nerve. 24:231-239.