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Environmental Effects of Methylmercury on Anterior Pituitary Tissues: Evaluation of the Ameliorative Potential *Bryophyllum pinnatum*Compared with Succimer

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Abstract
Toxic metals such as mercury in the brain have been associated with the pathophysiology of several neurodegenerative disorders, and central nervous system as key brain cells are affected. This study explores the comparative effects of Bryophyllum pinnatum ethanolic leaf extract (BPELE) and succimer on methylmercury (MetHg) exposed Wistar rat.Neurotoxicity was induced in male Wistar rats by oral administration. Thirty-two male Wistar rats were grouped intonine: control (untreated); methylmercury (MetHg) 1mg/kg; MetHg (1mg/kg) + BPELE (100mg/kg); MetHg (1mg/kg) + BPELE (200mg/kg); BPELE (100mg/kg); BPELE (200mg/kg); MetHg (1mg/kg) + succimer and succimer for 42 days. Pathological changes in the anterior pituitary gland were evaluated histologically.Results showed that the mean concentration of the methyl mercury in wistar rat reproductive tissues indicated that rats treated with MetHg (1mg/kg) + Succimer (5mg/kg) has significantly lower mercury concentration compared with those treated with MetHg (1mg/kg) + BPELE (200mg/kg) and MetHg (1mg/kg) + BPELE (100mg/kg) ($p<0.05$). The mean concentrations of rats treated with MetHg (1mg/kg) + BPELE (100mg/kg) and MetHg (1mg/kg) + BPELE (100mg/kg) were also significantly lower than those treated with MetHg (1mg/kg) ($p<0.05$). Histology results indicated that MetHg(1mg/kg) methylmercury showed hypertrophy, overlapping of acidophils, pockets of chromophobes cells with pyknotic nucleus peripherally located and sinusoid within acidophilic cells and numerous shrinkage of sinusoids vacuolations are observed within the tissue. MetHg(1mg/kg)+ BPELE(200mg/kg) revealed fenestrated sinusoids with blood deposits and chromophobes observed distributed within the tissue with normal histological structures. This study findings suggest the potency of B. pinnatum as an ameliorative agent against methylmercury cytotoxicity.

Keyword*: Pituitary Gland, DNA Damage, Methylmercury, cytotoxicity*

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I. Introduction

Methylmercury is a highly neurotoxic short-chained alkyl mercury compound that poses a high risk of exposure to humans and animals through consumption of contaminated fish and seafood. Inorganic mercury enters the food chain in the aquatic environment and is converted to methylmercury by sulphate-reducing bacteria. Organic mercury compounds bioaccumulate and reach toxic levels in large fish.Organic mercury compounds are classified into three types: aryl ring, short chain alkyl, and long chain alkyl. While the aryl and long-chain forms of mercury rapidly convert to inorganic mercury, methylmercury has a short-chained form that is rapidly and almost completely absorbed from the gastrointestinal tract and can be distributed throughout the body while remaining in their organic forms (Broussard, Hammett-Stabler, Winecker, &Ropero-Miller, 2002; Erdemli- Köse, Yirün, Balci- Özyurt, &Erkekoğlu, 2022).

Methylmercury, administered orally or intraperitoneally, resulted in intracellular mercury accumulations in the lysosomes and granules of secretory cells (somatotrophs, thyrotrophs and corticotrophs).Mercury deposits were discovered in lysosomes of non-secretory cells (follicular cells and marginal layer cells).The number of mercury deposits increased significantly in orally treated rats up to day 21.Intracellular mercury accumulation increased continuously in rats exposed intraperitoneally.Aside from lysosome vacuolation, no structural damage was observed in mercury-containing cells. (Møller-Madsen & Thorlacius-Ussing, 1986).

Methylmercury is easily absorbed by the rodent anterior pituitary and is found in lysosomes and the secretory granules of somatotrophs.When exposed to as few as three dental amalgam fillings, the primate anterior pituitary absorbs mercury vapor; the mercury is then found in lysosomes and the secretory granules of

somatotrophs.Since mercury has been discovered in the anterior pituitary by numerous researchers, it is a preferred organ for mercury deposition in humans. However, it is unknown which hormone-producing pituitary cells were home to the mercury.The quantity of dental amalgam fillings is correlated with pituitary mercury concentrations(Ahlmark, 1948; Björkman et al., 2007; Danscher, Hørsted-Bindslev, & Rungby, 1990; Kosta, Byrne, & Zelenko, 1975; Pamphlett, Kum Jew, Doble, & Bishop, 2019; Rice, Walker Jr, Wu, Gillette, & Blough, 2014; Weiner & Nylander, 1993)

In folkloric medicine around the world, Bryophllum pinnatum is regarded as the miracle herb because it can be used to treat a wide range of illnesses (Garca-Pérez et al., 2021). It has not been investigated whether the plant, also known as Kalanchoe pinnata, can reduce the disruptive effects of methylmercury on testosterone, follicle stimulating hormone, and luteinizing hormone. contamination of the reproductive system with mercury. Testicular, sperm, or hormonal toxicity was not noted at low doses.

B. pinnatum has been fairly studied, with the majority of the folklore claims being justified, despite the fact that many herbal remedies' folklore claims have not yet been verified scientifically.

Due to this, more people are promoting B. pinnatum and other plants as complementary or alternative medicines. Furthermore, the use of herbal medicines like B. pinnatum leaf as alternatives, particularly in developing countries, has been prompted by the high cost of conventional medicines and the emergence of resistance to the majority of conventional chemotherapeutic agents. (Aprioku &Igbe, 2017; Osujih, 1993; Saad, Azaizeh, Abu-Hijleh, & Said, 2006). With the exception of calcium carbonate, chemomicroscopic analysis of Bryophyllum pinnatum revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain, and mucilage. Both aqueous and methanolic extracts contained phytochemicals such as alkaloids, phenols, flavonoids, saponins, tannins, carbohydrates, and triterpenes but not anthraquinones. A study has also suggested that the leaves of B. pinnatum have mild antioxidant potential (Namadina et al., 2020). Traditional medicines and food both use the leaves of Bryophyllum pinnatum (Lam.) Oken and the potentials of the aqueous extract and fractions of B. pinnatum leaves' antioxidant activity (reducing power, DPPH, ABTS, FRAP, H2O2 scavenging ability, and metal ion chelating), carbohydrate-digesting enzyme activity, and inhibitory activity of cholinergic enzyme have been studied (Ojo et al., 2018).

Chelators such as dimercaprol anddimercaptosuccinic acid (succimer) are used for the treatment of heavy metals poisoning, including mercury intoxication. While dimercaprol has been found to redistribute mercury to other soft tissues (Katzung, Masters, & Trevor, 2012), succimer and unithiol are reputed to be effective for the expulsion of mercury after intoxication. These drugs may be effective but there are no evidences that they completely address the damaging effects on the male reproductive and endocrine function of mammals (Adams, Frederick, Larkin, &Guillette Jr, 2009; Hintelmann, 2010; Jayasena, Frederick, & Larkin, 2011; Zimmermann et al., 2013). Succimer chelation for low level organic mercury exposure in children has limited efficacy (Cao et al., 2011). Although chelating agents administered for chronic intoxication may accelerate the excretion of heavy metals, their therapeutic efficacy in terms of decreased morbidity and mortality is largely unestablished. Recent investigations suggest that their use in such settings might be associated with deleterious effects. Potent mercury chelators, such as unithiol and succimer predominantly remove mercury from the kidney. Experiments have shown that they are inefficient in reducing the mercury content (Kosnett, 2010). The aim of this study is to determine the ameliorative effects of *B. pinnatum* ethanolic leaf extract on the disruptive effects of methylmercury on the reproductive hormones of male Wistar rats.

II. Materials and Methods

Plant material

Fresh leaves of *B. pinnatum* were collected from the botanical garden of the University of Port Harcourt, Port Harcourt. The leaves were washed and air dried, the dried powder were weighed and then extracted by maceration in hydroethanolic (70% Ethanol) medium at room temperature The extract was decanted and filtered using cotton handkerchief in a funnel and further filtered using Whatman No. 1 quantitative circle filter paper of 24.0 cm with cat. No. 1001 240. The filtrate was macerated twice using the same volume of solvent to exhaustively extract the leaves. The ethanol was then removed from the extract by evaporation under reduced pressure using a Rotary Evaporator RE-52A, E. Track Instruments England at 53°C to a constant volume. The extract was preserved in a desiccator. Water was used as diluent for the formulation of the doses (Dada &Ojo, 2018; Faleye& Dada, 2016).

Experimental animals and housing

Sixty-four (64) male Wistar rats of average weight of 201g, obtained from Priceless test animal farm, Badagary, Lagos, Nigeria, were used for the study. The animals were acclimatized for two weeks in the Animal house of Department of Animal and Environmental Biology, University of Port Harcourt and were given standard rodent feed and clean water ad libitum. The animals were kept in a well-ventilated room with a 12 hours light and dark cycle at room temperature. All animal were anesthetized using diethyl ether experimental

procedures were approved by the Animal Research Ethics Committee of the University, in accordance to the guide for care and use of laboratory animals (Rowsell, 1991)

Chemicals and Drugs

Methylmercury (CH_3Hg) and Succimer (meso-2,3-Dimercaptosuccinic acid) were both purchased from Sigma-Aldrich, Germany. A Sigma Due Diligence form was completed with a customer declaration of specific uses of controlled and voluntary monitored substances before these chemicals were shipped.

Experimental Design

Rats were divided randomly into eight groups as follows:

1) Control group (n = 8): Adult male rats administered distilled water daily by gavage for 42 days.
2) Methylmercury (MeHg) alone – 1mg.kg/bw was administered to the rats for 42 days
3) Methylmercury + *B. pinnatum* (100mg/kg/bw) – this group was first administered with MeHg for 14 days and then given the *B. pinnatum* leaf extract was administered together with mercury for another 28 days, making a total of 42 days.
4) Methylmercury + B. pinnatum (200mg/kg/bw) – this group was first administered with MeHg for 14 days and then given the *B. pinnatum* leaf extract was administered together with mercury for another 28 days, making a total of 42 days.
5) Methylmercury + succimer (5mg/kg/bw) – this group was first administered with MeHg for 14 days and then given the succimer for another 28 days, making a total of 42 days.
6) *B. pinnatum* (100mg/kg/bw) – this group was first administered with *B. pinnatum* leaf extract for 42 days.
7) *B. pinnatum* (100mg/kg/bw) – this group was first administered with B. pinnatum leaf extract for 42 days.
8) Succimer (5mg/kg/bw) – this group was first administered with B. pinnatum leaf extract for 42 days.

Determination of Methylmercury in the Pituitary

MethylMercury concentration was determined by the principle of Thermal Decomposition (Gold) Amalgamation Atomic Absorption Spectrophotometer (TDA/AAS) designed for direct mercury analysis (DMA-80, Milestone, Inc., Pittsburgh, PA) (Burke et al., 2006)

DNA Damage

The comet assay was done using the Silver staining techniques according to the standard method(Singh, 2016).

Histology

The anterior pituitary wascollected and preserved and later section using the microtome. Hematoxylin and eosin staining technique was used(Cheesbrough& McArthur, 1976)

Statistical analyses

Statistical analyses were performed by the standard method. All the results were expressed as mean ± standard deviation (S.D.). The mean of the all groups compared using One - way ANOVA by SPSS (Statistical Package for Social Sciences) and Tukey's post-hoc test. P -value of less than 0.05 was considered to represent statistically significant change (Abo-Allam, 2003; Levesque, 2005). Multivariate analysis from Minitab was used, showing cluster and dendrogram of the results.

III. Results

The concentration of methylmercury in the reproductive tissues in wistar rats indicated that rats treated with distilled water was significantly lower than the mean concentration of those treated with MeHg (1mg/kg) and MeHg (1mg/kg) + BPELE ($p<0.05$) but was not significantly different from those of MeHg (1mg/kg) + BPELE (100mg/kg); BPELE (100mg/kg), BPELE (200mg/kg), MeHg (1mg/kg) + Succimer (5mg/kg), and Succimer (5mg/kg) ($p>0.05$).

The mean concentration of wistar rats treated with MeHg (1mg/kg) + Succimer (5mg/kg) was significantly lower than those treated with MeHg (1mg/kg) and MeHg (1mg/kg) + BPELE (200mg/kg) ($p<0.05$) but insignificantly lower than in those treated with MeHg (1mg/kg) + BPELE (100mg/kg) ($p>0.05$). Those treated with MeHg (1mg/kg) + BPELE (200mg/kg) had higher methyl mercury concentration than in those treated with MeHg (1mg/kg) + BPELE (100mg/kg) and MeHg (1mg/kg) + Succimer (5mg/kg ($p<0.05$) as shown in Table 1.

Table 1: Concentrations of methylmercury in reproductive tissues of Wistar rats exposed to methylmercury and treated with BPELE and succimer (Ameliorative study).

Treatments	Brain µg/kg
Control Distilled Water	0.00 ± 0.00^{a}
MeHg (1mg/kg)	3.90 ± 0.45^{b}
MeHg (1mg/kg) + BPELE (100mg/kg)	0.91 ± 0.26^{a}
MeHg (1mg/kg) + BPELE (200mg/kg)	1.21 ± 0.26^{c}
BPELE (100mg/kg)	0.00 ± 0.00^{a}
BPELE (200mg/kg)	0.00 ± 0.00^{a}
MeHg (1mg/kg) + Succimer (5mg/kg)	0.82 ± 0.12^{a}
Succimer (5mg/kg)	0.00 ± 0.00^{a}

Means±SE of treatment parameters with the different alphabetic superscripts in the same column are significantly different from each other ($p>0.05$).
MeHg – Methylmercury
Succimer - (meso-2,3-Dimercaptosuccinic acid)
BPELE – *Bryophyllum pinnatum* ethanolic leaf extract
For the combination groups, MeHg was administered for 14days alone first, followed by BPELE and succimer for another 28 days till the 42nd day.

The mean concentration of the methyl mercury inwistar rat reproductive tissues indicated that rats treated withMeHg (1mg/kg) + Succimer (5mg/kg) has significantly lower mercury concentration compared with those treated with MeHg (1mg/kg) + BPELE (200mg/kg) and MeHg (1mg/kg) + BPELE (100mg/kg) ($p<0.05$). The mean concentrations of rats treated with MeHg (1mg/kg) + BPELE (100mg/kg) and MeHg (1mg/kg) + BPELE (100mg/kg) were also significantly lower than those treated with MeHg (1mg/kg) ($p<0.05$).

Table 2: DNA damage in testis and brain of Wistar ratsReproductive tissues exposed to methylmercury and treated with BPELE and succimer

Treatment	% DNA damage in tail
Control Distilled Water	4.60 ± 0.33^{a}
MeHg (1mg/kg)	22.00 ± 0.78^{b}
MeHg (1mg/kg) + BPELE (100mg/kg)	16.00 ± 0.50^{c}
MeHg (1mg/kg) + BPELE (200mg/kg)	15.60 ± 1.08^{c}
BPELE (100mg/kg)	10.3 ± 0.79^{d}
BPELE (200mg/kg)	9.00 ± 0.33^{e}
MeHg (1mg/kg) + Succimer (5mg/kg)	15.70 ± 0.88^{f}
Succimer (5mg/kg)	7.80 ± 0.32^{g}

Means±SEM of treatment parameters with the different alphabetic and symbol superscripts in the same column are significantly different from each other ($p>0.001$).
Keys.
MeHg – Methylmercury
Succimer - (meso-2,3-Dimercaptosuccinic acid)
BPELE – *Bryophyllum pinnatum* ethanolic leaf extract
For the combination groups, MeHg was administered for 14days alone first, followed by BPELE and succimer for another 28 days till the 42nd day.

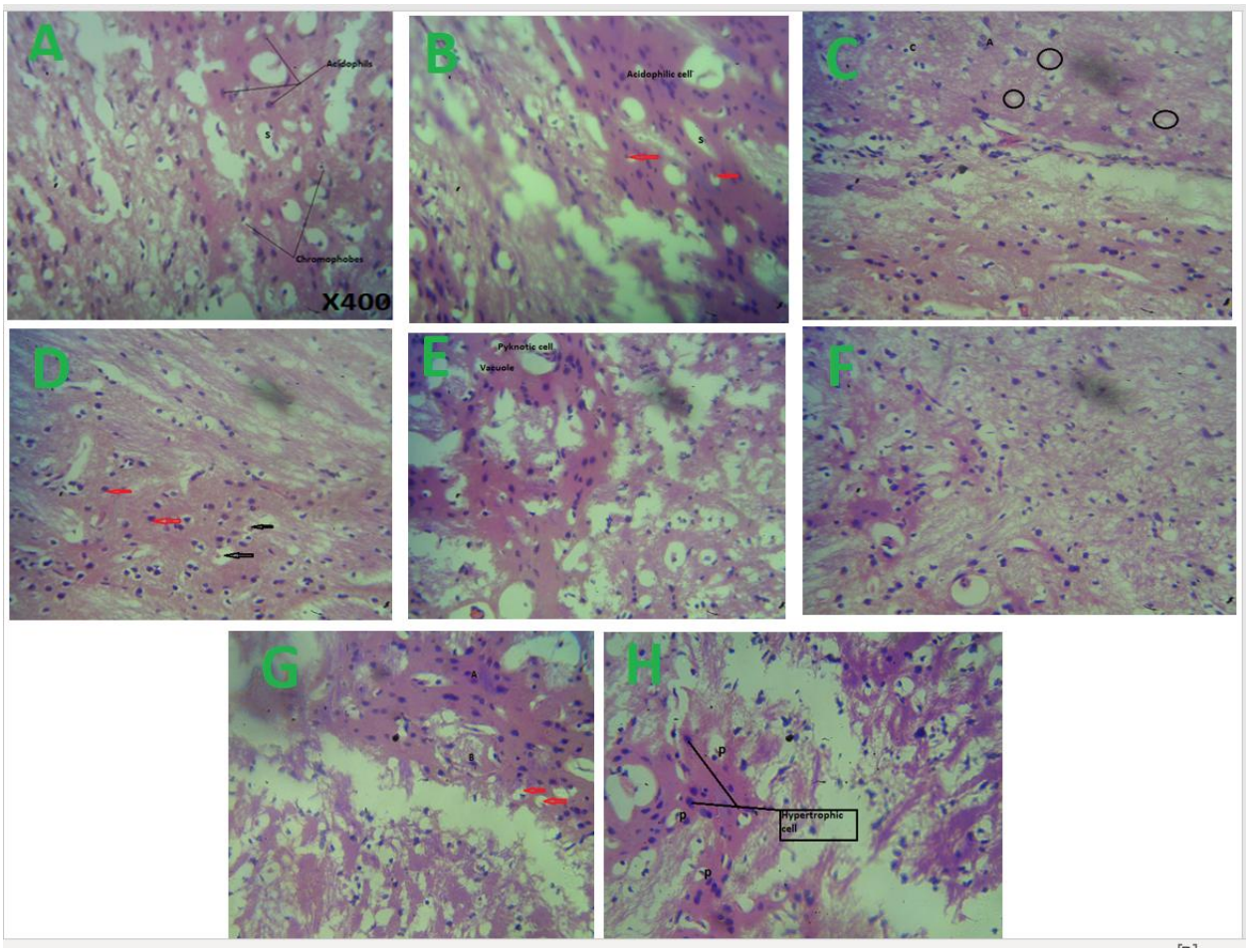

Plate 1. Photomicrograph of Anterior Pituitary Gland: **A**-control (Distilled Water); Fenestrated sinusoids with blood deposits and chromophobes observed distributed within the tissue with normal histological Structure. **B**-MeHg(1mg/kg); methylmercury group with hypertrophy, overlapping of acidophils. Pockets of chromophobes cells with pyknotic nucleus peripherally located (arrow) and sinusoid (S) within acidophilic cells and numerous shrinkage of sinusoids vacuolations (V) are observed within the tissue. **C**-MeHg(1mg/kg)+BPELE(200mg/kg); **D**-MeHg(1mg/kg)+ BPELE(200mg/kg); **E**-BPELE(100mg/kg); Fenestrated sinusoids with blood deposits and chromophobes observed distributed within the tissue with normal histological Structure **F**-BPELE(100mg/kg); **G**-MeHg (1mg/kg)+succimer(5mg/kg) indicating slight hyperplasia and hypertrophy of gonadotropic cells. Degeneration (pyknosis) are also observed, inflammatory acidophils, vacuolations (V) with numerous disorganised sinusoids. The overlapping acidophils have enlarge nucleus which is indicative of massive cellular inflammation; **H**-succimer(5mg/kg). The Anterior Pituitary Gland. Acidophil cells (red arrows) and basophils (blue arrows). Fenestrated sinusoids with blood deposits and chromophobes (C) observed distributed within the tissue with normal histological Structure. (H & E, X400).

IV. Discussion

Plants play a vital role in discoveries associated with new beneficial therapeutic agents and have received significant focus because of their bioactive substances. Plants have invariably been exemplary source of drugs and a number of currently available drugs happen to be derived directly or indirectly from them. Recent reports from preliminary studies on *B. pinnatum* leaves indicated the presence of bioactive compounds and an appreciable amount of polyphenolic compounds, particularly flavonoids, which exhibit significant in vitro antioxidant inhibitory properties (Ogidigo et al., 2021). In the present study, it was observed that methyl mercury concentration was significantly lower in the wistar rats treated with various doses of extracts of *B. pinnatum.* This showed that *B. pinnatum* conferred protective effects against methylmercury and thus the resultant toxicity effects of mercury. The decrease in the concentration of methyl mercury could be as a result of presence of antioxidants in the plant. Many bioactive compounds, like bryotoxin A, B, C, caffeic acid, and protocatechuic acid, have been reported from this plant (Aransiola et al., 2014) that exhibit various activities like antioxidant, antiulcer, analgesic activity (Ghasi et al., 2011); antiurolithiasis; and so forth (Mudi and Ibrahim, 2008). Despite the fact that the phytochemicals contain plenty of flavonoids and polyphenols like antioxidants,

they may also help ameliorate the heavy metals mediated toxicity in human and other animals. *B. pinnatum* has also been used to decontaminate heavy metals from soils (Odoemelam and Ukpe, 2008)

Also in the present study, the use of dimercaptosuccinic acid (succimer) or succimer as a chelating agent significantly reduced the amount of methylmercury concentration the tissues. This agrees with the findings of Bradberry and Vale (2009). Intestinal dysbiosis can limit oral administration of succimer. In humans succimer is broadly metabolized to mixed disulfides of cysteine. Ten to 25% of an orally administered dose of succimer is excreted in urine; the majority within 24 hours and most as succimer-cysteine disulfide conjugates. The remainder is largely eliminated in the faeces (Bradberry and Vale 2009; Sears, 2013). succimer increases urinary excretion of arsenic, cadmium, lead, methylmercury, and inorganic mercury, with removal from animals' brains of lead and methylmercury. Successful dialysis of methylmercury-succimer complexes has been reported (Sears, 2013).

Animal studies suggest that succimer is an effective chelator of soft tissue but it is unable to chelate lead from bones (Flora et al.., 1997). Succimer for being an antioxidant and a strong heavy metal chelator has been shown to significantly reduce lead concentration from hippocampus leading to recovery in the oxidative stress and apoptosis caused by lead (Zhang et al., 2004). One of the main disadvantage with the use of succimer is that it is principally a soft tissue heavy metal mobilizer and consequently incapable of removing these metals from hard tissues and intracellular sites (Flora, 2009). A rare side effect is mucocutaneous eruptions, gastrointestinal discomfort, skin reaction, mild neutropenia, elevated liver enzymes and toxic epidermal necrosis that resolves when the medication is stopped (Sears, 2013; Flora and Pachauri, 2010).

V. Conclusion

This study findings suggest the potency of *B. pinnatum* as an ameliorative agent against methylmercury cytotoxicity. Thus, the neuroprotective potential of *B. pinnatum* was established by inhibiting the concentration methylmercury in the reproductive tissues.

References

[1]. Aransiola,E. F. Daramola, M. O..Iwalewa,E. OSeluwa, A. M. and Olufowobi,O. O. "Antidiabetic effect of Bryophyllum pinnatum leaves," International Journal of Biological, Biomolecular,Agricultural, Food and Biotechnological Engineering, vol. 8, pp. 89–93, 2014.

[2]. Flora, S J.S and Pachauri V (2010). Chelation in Metal Intoxication. International Journal of Environmental Research and Public Health, 7:2745-2788

[3]. Zhang J, Wang XF, Lu ZB, Liu NO, Zhao BL. The effects of meso-2,3-dimercaptosuccinic acid and oligomeric procyanidins on acute lead neurotoxicity in rat hippocampus. Free Rad Biol Med, 2004, 37, 1037-1050.

[4]. Flora S.J.S. (2009). Metal Poisoning: Threat and Management Al Ameen Journal of Medical Sciences; 2 (2):4 -2 6

[5]. Odoemelam S. A. and Ukpe R. A.(2008). Heavy meal decontamination of polluted soils using Bryophyllum pinnatum.African Journal of Biotechnology; 7 (23):4301-4303

[6]. Sears, ME (2013). Chelation: Harnessing and Enhancing Heavy Metal Detoxification—A Review. The Scientific World Journal Volume; 219840

[7]. Flora SJS, Pant BP, Tripathi N, Kannan GM, Jaiswal DK. Distribution of Arsenic by Diesters of Meso 2, 3- Dimercaptosuccinic Acid during Sub-Chronic Intoxication in rats. J Occup Health, 1997, 39, 119-123.

[8]. S. Ghasi, C. Egwuibe, P. U. Achukwu, and J. C. Onyeanusi, "Assessment of the medical benefit in the folkloric use of Bryophyllum pinnatum leaf among the igbos of Nigeria for the treatment of hypertension," African Journal of Pharmacy andPharmacology, vol. 5, no. 1, pp. 83–92, 2011.

[9]. S. Mudi and H. Ibrahim, "Activity of Bryophyllum pinnatum Kurz extracts on respiratory tract pathogenic bacteria," Bayero Journal of Pure and Applied Sciences, vol. 1, pp. 43–48, 2008.

[10]. S. Bradberry and A. Vale, "Dimercaptosuccinic acid (succimer; DMSA) in inorganic lead poisoning," Clinical Toxicology, vol.47, no. 7, pp. 617–631, 2009.

[11]. Ahlmark, A. (1948). Poisoning by methyl mercury compounds. British journal of industrial medicine, 5(3), 117.

[12]. Björkman, L., Lundekvam, B. F., Lægreid, T., Bertelsen, B. I., Morild, I., Lilleng, P., . . . Vahter, M. (2007). Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study. Environmental health, 6(1), 1-14.

[13]. Burke, K., Cheng, Y., Li, B., Petrov, A., Joshi, P., Berman, R. F., . . . DiCicco-Bloom, E. (2006). Methylmercury elicits rapid inhibition of cell proliferation in the developing brain and decreases cell cycle regulator, cyclin E. Neurotoxicology, 27(6), 970-981.

[14]. Cheesbrough, M., & McArthur, J. (1976). A laboratory manual for rural tropical hospitals: a basis for training courses.

[15]. Danscher, G., Hørsted-Bindslev, P., & Rungby, J. (1990). Traces of mercury in organs from primates with amalgam fillings. Experimental and molecular pathology, 52(3), 291-299.

[16]. Kosta, L., Byrne, A. R., & Zelenko, V. (1975). Correlation between selenium and mercury in man following exposure to inorganic mercury. Nature, 254(5497), 238-239.

[17]. Møller-Madsen, B., & Thorlacius-Ussing, O. (1986). Accumulation of mercury in the anterior pituitary of rats following oral or intraperitoneal administration of methyl mercury. Virchows Archiv B, 51(1), 303-311.

[18]. Pamphlett, R., Kum Jew, S., Doble, P. A., & Bishop, D. P. (2019). Elemental analysis of aging human pituitary glands implicates mercury as a contributor to the somatopause. Frontiers in Endocrinology, 10, 419.

[19]. Rice, K. M., Walker Jr, E. M., Wu, M., Gillette, C., & Blough, E. R. (2014). Environmental mercury and its toxic effects. Journal of preventive medicine and public health, 47(2), 74.

[20]. Singh, N. P. (2016). The comet assay: Reflections on its development, evolution and applications. Mutation research/Reviews in mutation research, 767, 23-30.

[21]. Weiner, J. A., & Nylander, M. (1993). The relationship between mercury concentration in human organs and different predictor variables. Science of the total environment, 138(1-3), 101-115.