

Effect of Low Dose Lead (Pb) Administration on Tail Immersion Test and Formalin-induced Pain in Wistar Rats: Possible Modulatory Role of Cobalt (II) Chloride

*A. H. Umar, I. Suleiman and H. Muhammad

Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.

Summary: Lead (Pb) is cheap and there is a long tradition of its use, but its toxic effects have also been recognized. There is increased public health concern regarding the hazards of low dose Pb exposure to adults and children. Studies have shown the risks for hypertension, decrements in renal function, subtle decline in cognitive function, and adverse reproductive outcome at low blood Pb level. In this study, the possible modulatory role of cobalt (II) chloride (CoCl₂) on low level Pb exposure on tail immersion test and formalin induced pain was investigated. Twenty adult Wistar rats of both sexes (weight 150g to 200g) were used. The animals were divided into four groups (n = 5) and administered Pb (5mg/kg), Pb (5mg/kg) + CoCl₂ (50mg/kg) and CoCl₂ (50mg/kg) orally for twenty-eight days. The last group served as control and were given distilled water only. In the tail immersion test, there was no significant change in reaction time for all three groups when compared to the control. In the formalin-induced pain, pain score after five and forty-five minutes also do not show significant change for all the three groups when compared to control. This work suggested that exposure to 5mg/kg Pb for twenty-eight days do not significantly impair reaction time in tail immersion test and pain score in formalin induced pain in Wistar rats. Also, administration of 50mg/kg CoCl₂ do not improve performance of the animals in the experiments.

Keywords: Pain, Lead, Cobalt (II) chloride, Formalin, Tail immersion

©Physiological Society of Nigeria

*Address for correspondence: ahumar09@gmail.com

Manuscript Accepted: November, 2016

INTRODUCTION

Though lead (Pb) is recognized as an ecological hazard, it is a beneficial element in several industries and is brought in production of lead bullets and batteries, or in refinery industries to purify gold and silver (Fazli *et al.*, 2014). Lead is cheap and there is a long tradition of its use, but its toxic effects have also been recognized. There is increased public health concern regarding the hazards of low dose Pb exposure to adults and children. Studies have shown that even levels below 2µg/dL can cause significant, long-term effects. Government agencies have acted to lower the levels of blood lead that have been considered “safe” (Pokras and Kneeland, 2009).

Lead is a heavy metal that can be toxic when introduced into the human and animal bodies by ingestion or inhalation (Barbhuiya *et al.*, 2013). Research findings have heightened public health concern regarding the hazards of low dose lead exposure to adults and children. In adults, studies have established the potential for hypertension, decrements in renal function, subtle decline in cognitive function, and adverse reproductive outcome at blood lead levels less than 25µg/dL (Kosnett, 2009).

Acute and sub-acute effects of lead are caused by relatively large doses over a short period—days to months. The effects include sudden death, severe

abdominal cramps, anemia, ataxia, headaches, irritability and appetite loss. Smaller amounts of lead taken in over longer times—months to years—may cause effects such as lowered sex drive, decreased fertility, miscarriages and premature births, learning problems, hypertension, cardiovascular diseases, increased aggression and kidney problems (Pokras and Kneeland, 2009).

Lead is a well-known neurotoxicant and blood lead level (BLL) has been shown to be linked with malfunction of human cognition. Attention and intelligence rate in children can be negatively affected by lead. Lead has been shown to cause neurobehavioral problems or neurobiological defects such as schizophrenia. Studies have also verified the association between lead contamination and incidence of Alzheimer’s disease. It is anticipated that cardiovascular outcomes and mild mental retardation resulting from exposure to lead encompass almost 1% of the global burden of illness peaking in undeveloped regions (Fazli *et al.*, 2014).

Understanding the interaction between dietary protein deficits and neurotoxicants such as lead is critical since oxidative stress is a common denominator under such conditions (Lalith-Kumar-Venkareddy, 2015). In 1970, the United States Centre for Disease Control (CDC) issued guidance that

identified levels of lead in the blood of young children that were of concern with respect to public health intervention as 40 µg/dL. It fell to 30 µg/dL in 1975, 25 µg/dL in 1985, and 10 µg/dL in 1991 (CDC 1991). The main target for lead toxicity is the CNS. As such, the brain is the organ most studied in lead toxicity. Symptoms of lead poisoning include dullness, forgetfulness, irritability, poor attention span, headache, fatigue, impotence, dizziness, and depression (Waggas, 2012).

Knowledge of the general toxic effects of lead stretches back to many years. It has been used in medicines, paintings, pipes, ammunition and in alloys for welding chemical reagent storage. Human beings are also exposed to cadmium, lead and mercury from cigarette smoking. Thus, the concentration of lead in the environment and exposure to it have increased significantly during human history (Waggas, 2012). Lead-exposure occurs through the respiratory and gastrointestinal systems and absorbed lead is stored mainly in soft tissues and bones.

Administration of cobalt chloride may promote the delaying of cardiac preconditioning through selective activation of the hypoxia inducing factor (HIF) signalling. Cobalt is a relatively rare transition metal with properties similar to those of iron, chromium, and nickel. Cobalt chloride, a water-soluble compound traditionally used to treat anaemia in pregnant women, infants, and patients with chronic anaemia undergoing long term haemodialysis, is a well-established chemical inducer of hypoxia-like responses, such as erythropoiesis and angiogenesis in vivo. Hypoxia-like response probably involves increased DNA binding activity of HIF1a, as cobalt stabilizes HIF1a through generation of reactive oxygen species by a non-enzymatic, non-mitochondrial mechanism. The final result of this induction is enhanced erythropoietin production and more efficient stimulation of the erythropoietic response (Lippi *et al.*, 2005). Besides the relevant therapeutic benefits, especially in patients with acute myocardial ischaemia, cobalt chloride administration may have alternative and obscure potential applications.

The use of Cobalt chloride, which simulate erythropoiesis, may be of benefit in the management of lead induced neurotoxicity.

MATERIALS AND METHODS

Animals Grouping and Treatment

Twenty adult Wistar rats weighing 150g to 200g were used for each study. The animals were divided into four groups (n=5) and housed at the animal house of department of Human Physiology, ABU Zaria (each group in a separate cage). They were allowed food and water *ad libitum* and treated for 28 days (according to grouping) before commencement of the experimental protocols. Group I serve as control and were administered distilled water (1ml/Kg). Animals in

group II were orally administered 5mg/Kg of lead daily. Animals in group III were treated with lead (5mg/Kg, orally) plus optimum dose of CoCl₂ (50mg/Kg, orally) (Shrivastava *et al.*, 2008). Group IV animals received oral dose of CoCl₂ (50mg/Kg) only.

Experimental Protocols

Tail immersion test

In the tail immersion test, the tail of the rat was immersed in a hot water bath (53°C ± 1°C) until tail withdrawal (flicking response) or signs of struggle were observed (cut off 12 seconds). Shortening of the tail-withdrawal time indicates hyperalgesia (Umar *et al.*, 2015).

Formalin induced pain

It was carried out by the method described by Yerima *et al.*, (2009). 50µL of a freshly prepared 2.5% solution of formalin was injected subcutaneously on the plantar surface of the left hind paw of each rat. The rats were monitored after 5 min and then at the end of 45 min. The severity of pain response was recorded for each rat based on the following scale:

- 0- Rat walk or stand firmly on the injected paw
- 1- The injected paw is favoured or partially elevated
- 2- The injected paw is clearly lifted off the floor
- 3- The rat lick, chew or shake the injected paw.

Formalin-induced paw oedema

Fifty microliter (50µL) of a 2.5 % solution of formalin was injected subcutaneously on the plantar surface of the left hindpaw. An increase in the rats' hind paw diameter was used as the measure of acute inflammation (Winter *et al.*, 1963). The paw diameter was measured with the aid of a vernier caliper at 0, 1, 2, 3, 4, and 5h, after the injection of formalin. The difference between the readings at time 0h and different time interval was taken as the thickness of oedema (Tanko *et al.*, 2008).

% Inhibition=

$$\frac{\text{Mean paw diameter (control)} - \text{Mean paw diameter (treated)}}{\text{Mean paw diameter (control)}}$$

Statistical Analysis

Statistical analysis was performed using SPSS software for windows (version 16.0). Results for reaction time and paw oedema (Mean ± SEM) were analyzed using One way ANOVA followed by Tuckey's *post Hoc* test to identify significance between groups. Result for pain score were analyzed using Kruskal Wallis non-parametric test. The differences were considered significant at P < 0.05.

RESULTS

Tail immersion test

After the 28 days exposure, result of the tail immersion test shows no statistical significance in the groups (II, III and IV) when compared to the control group (group I). The mean reaction time (in sec) for the lead and cobalt

(II) chloride exposed groups were higher than the control, but are not statistically significant (fig. 1). The highest mean reaction time was observed in group IV (CoCl₂ exposed group), but the difference was not statistically significant.

Formalin induced pain

Five minutes after injection of 50µL of freshly prepared 2.5% formalin solution in the sub planter surfaces of left hind paw, the mean pain score for groups II, III and IV were found not to be statistically different when compared to the control groups (fig. 2). Result of the mean pain score after 45 minutes also shows no statistical significance in all the groups when compared to the control group (fig. 3).

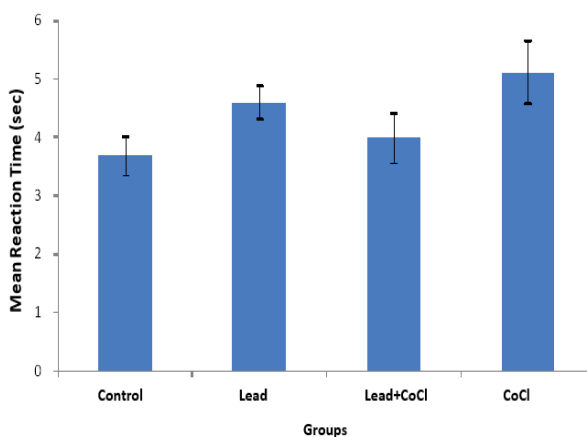


Figure 1: Effect of Cobalt chloride on mean reaction time during tail immersion test in lead treated rats.

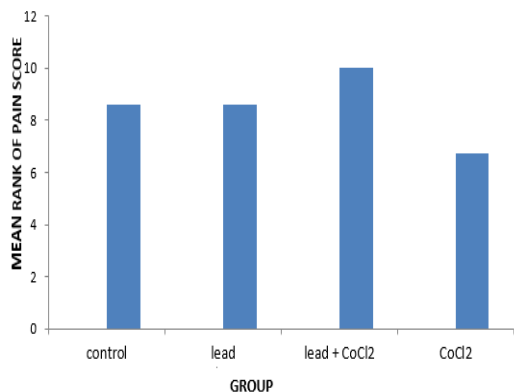


Figure 2: Effect of Cobalt chloride on mean rank of pain score at 5 min in lead treated rats.

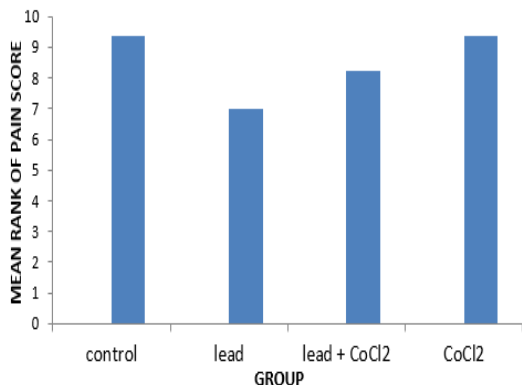


Figure 3: Effect of Cobalt chloride on mean rank of pain score at 45 min in lead treated rats.

Table 1: Result of formalin induced paw oedema showing paw diameter at 1hr, 2hr, 3hr and 4hr after formalin injection

Group	Time	Mean Paw Diameter (cm)			
		1 HR	2 HR	3 HR	4 HR
CONTROL		0.45 ± 0.03	0.3 ± 0.04	0.38 ± 0.06	0.40 ± 0.04
LEAD		0.34 ± 0.05	0.34 ± 0.05	0.36 ± 0.02	0.40 ± 0.05
LEAD + CoCl ₂		0.35 ± 0.05	0.32 ± 0.03	0.40 ± 0.00	0.43 ± 0.03
CoCl ₂		0.34 ± 0.05	0.44 ± 0.06	0.44 ± 0.05	0.42 ± 0.04

Formalin induced paw oedema

Injection of 50µL of freshly prepared 2.5% formalin solution in the sub planter surfaces of left hind paw causes paw swelling in all the groups. Differences in paw diameter were found not to be statistically different when compared to the control group (table 1).

DISCUSSION

Exposure to low level of lead has been associated with cognitive deficiencies in children, renal impairment, hypertension, cataracts, and reproductive problems such as miscarriage, stillbirth, and decreased fertility in men and women (Patrick, 2006). Lead has been shown to have a negative result on cognitive operations in children and its post-natal toxicity could be a causative factor in the pathogenesis of autism. Variation in gamma amino butyric acid(GABA), serotonin (5HT) and dopamine (DA) had been known to be responsible for chronic lead toxicity in autism. Some hypotheses had been proposed on the hypo function of the N-methyl-d-aspartate (NMDA) pathway throughout serious periods of growth as a connecting dot between early life exposure to lead and neurobiological outcomes associated with schizophrenia. Further, an inverse relationship between children’s neurodevelopment and BLL even in the ranges less than 10µg/dL had been reported in association with neurobehavioral outcomes (Fazli *et al.*, 2014).

Neurotoxic actions of lead include apoptosis, excitotoxicity affecting neurotransmitter storage and release and altering neurotransmitter receptors, mitochondria, second messengers, cerebrovascular endothelial cells, and both astroglia and oligodendroglia. Lead also causes toxic effects by oxidative stress and by either directly or indirectly causing lipid peroxidation (Waggas, 2012).

In this work, long term chronic administration of 5mg/kg lead acetate in Wistar rats do not significantly lower reaction time in tail immersion test. It also does not significantly affect pain score in formalin induced nociception. Results of formalin induced paw oedema also shows no significant differences when compared to the control. Oedema and pain induced by formaldehyde are mediated by substance P, bradykinin, histamine, serotonin and prostaglandins (Umar *et al.*, 2012).

Exposure to low-levels of Lead leads to the behavioural abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and is also reported in laboratory animals. Lead induced neurotoxicity was characterized by various potentially detrimental changes such as inhibition of NADH-cytochrome C reductase, SDH and cytochrome C oxidase activities (mitochondrial function); and decreased P-SH,

vitamin C and vitamin E; increased MDA and PCC levels (oxidative stress) (El-Masry *et al.*, 2011).

Biochemical and molecular mechanisms of Lead toxicity may be through inhibition of the calcium-pump, inactivation of P450 enzymes, nervous tissues demyelination, cholinergic dysfunction, glutamate receptor alteration, and enhanced oxidative stress (El-Masry *et al.*, 2011). Biochemical effect of lead on brain neurochemistry has been shown to be dependent on the degree and duration of lead exposure. Studies indicated that lead induced increase in spontaneous transmitter release is due to either an increase in intraneuronal ionized calcium or the stimulation by lead of Ca-activated molecules mediating transmitter release. Lead also exerts its neurotoxic effects by interfering with Ca²⁺calmoduline mediated neurotransmitter release that is eventually responsible for behavioural impairment. It also disrupts the activity of synaptotagmin. Lead intoxication induces an oxidative stress situation in the brain. It has been shown that oxidative stress because of decreased antioxidant function might be the main mechanism involved in brain neurotoxicity induced by lead exposure (Waggas, 2012).

Daily administration of lead at a dose of 5mg/kg orally for twenty-eight days do not significantly impair reaction time in tail immersion test and pain score in formalin induced pain in Wistar rats. Concomitant administration of 50mg/kg CoCl₂ do not also show significant change in performance of the animals in the experimental models used.

Acknowledgement

The authors acknowledge the assistance of Mallams Ya'u Bello and Umar Muhammed of the animal house section of the Department of Human Physiology, ABU, Zaria in taking care of the animals used for this study.

REFERENCES

Barbhuiya, S.S.K., Chakraborty, S. and Sengupta, M. (2013): Studies of lead toxicity on inflammatory damage and innate immune functions in testicular macrophages of male Swiss albino mice. *Modern Research in Inflammation* 2: 75-81

Centers for Disease Control and Prevention (CDC) (1991): Preventing Lead Poisoning in Young Children. CDC, Atlanta, Georgia, USA.

Chopra, K., Tiwari, V., Arora, V. and Kuhad, A. (2010): Sesamol Suppresses Neuro-Inflammatory Cascade in Experimental Model of Diabetic Neuropathy. *The Journal of Pain* 11(10):950-957

El-Masry, T.A., Emara, A.M. and El-Shitany, N.A. (2011): Possible protective effect of propolis against lead induced neurotoxicity in animal model. *Journal of Evolutionary Biology Research* 3(1): 4-11

Fazli, D., Malekirad, A.A., Mirzaee, M., Dastjerdi, H.A., Mostafalou, S., Karkhane, A., Bayrami, Z., and Abdollahi, M. (2014): Study on the Link between Lead Exposure and Hematological, Psychological, and Memorial Parameters in Automobile Repair Workers. *Health* 6:712-719.

Kosnett, M. J. (2009): Health effects of low dose lead

exposure in adults and children, and preventable risk posed by the consumption of game meat harvested with lead ammunition. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, USA.

Lalith-Kumar-Venkareddy, M. (2015): Potential of casein as a nutrient intervention to alleviate lead (Pb) acetate-mediated oxidative stress and neurotoxicity: First evidence in *Drosophila melanogaster*. *NeuroToxicology* 48: 142–151

Lippi, G., Franchini, M. and Guidi, G.C. (2005): Cobalt chloride administration in athletes: a new perspective in blood doping? *British Journal of Sports Medicine* 39:872–873.

Patrick, L. (2006): Lead toxicity, A review of the literature: Part I: exposure, evaluation, and treatment. *Alternative Medicine Review* 11:2–22.

Pokras, M. A., and Kneeland, M. R. (2009): Understanding lead uptake and effects across species lines: A conservation medicine approach. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, USA.

Shrivastava, K., Shukla, D., Bansal, A., Sairam, M., Banerjee, P.K., and Ilavazhagan, G. (2008): Neuroprotective effect of cobalt chloride on hypobaric hypoxia-induced oxidative stress. *Neurochemistry International* 52: 368–375

Tanko, Y., Mohammed, A., Okasha, M. A., Umar, A. H. and Magaji, R. A. (2008). Anti-nociceptive and anti-inflammatory activities of ethanol extract of *Syzygium aromaticum* flower bud in wistar rats and mice. *African Journal of Traditional, Complementary and Alternative Medicine* 5 (2): 209 – 212

Umar, A.H., Imam, M.I. And Anyanwu, P.I. (2015). Effect of restraint stress on tail immersion test and formalin induced Pain in wistar rats. *Nigerian Journal of Scientific Research* 14(2): 77 – 80

Umar, A.H., Mabrouk, M., Danjuma, N.M. and Yaro, A. (2012). Studies on the Analgesic and Anti-inflammatory Properties of Hydro-alcohol Extract of *Caralluma dalzielii* N.E. Br (Asclepiadaceae) in Rats and Mice. *British Journal of Pharmacology and Toxicology* 4(5): 169-175

Waggas, M.A. (2012): Grape Seed Extract (*Vitisvinifera*) Alleviate Neurotoxicity and Hepatotoxicity Induced by Lead Acetate in Male Albino Rats. *Journal of Behavioral and Brain Science* 2: 176-184.

Winter, E.A., and Nuss, G.V. (1963): Anti- Inflammatory and antipyretic activities of indomethacin. *Journal of Pharmacology and Experimental Therapeutics* 141: 369-376.

Yerima, M., Magaji, M.G., Yaro, A.H., Tanko, Y. and Mohammed, M.M. (2009): Analgesic and anti-inflammatory activities of the methanolic leaves extract of *Securine gavirosa* (euphorbiaceae). *Nigerian Journal of Pharmaceutical Sciences* 8(1): 47 – 53.