

Bioaccumulation of Heavy Metals in Liver of Albino Wistar Rats Exposed to Single and Heavy Metal Mixture

¹Okon, A.J., ²Aluko, O.O.,
³Tom, U.E. and
⁴Essien, N.E.

¹Department of Public Health,
University of Calabar,
Calabar, Nigeria

² Department of Community Health,
Obafemi Awolowo University,
Ile Ife

^{3,4}Department of Science
Technology, Akwa Ibom State
Polytechnic, Ikot Osurua

E-mail: aniekanjj@gmail.com

Corresponding Author:
Okon, A.J., as above

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Abstract

Heavy metals such as cadmium, lead and arsenic pose health hazards to animals and humans globally. These tend to bio-accumulate in body tissues and organs and interfere with the functioning of vital cellular components. This study was conducted to evaluate the bioaccumulation of these metals in the liver of albino Wistar rats following a sub-chronic exposure to single and heavy metal mixture. Twenty mature male Albino Wistar rats weighing 192 -200g were randomly assigned into five groups of four rats each. Groups 1, 2, 3 and 4 were treated for 12 weeks with 60mg/kg bodyweight of lead acetate, 22.5 mg/kg bodyweight of cadmium dichloride, 18.8mg/kg bodyweight of arsenic trioxide, and group 4 treated with a mixture of lead acetate (60mg/kg bodyweight) cadmium dichloride (22.5 mg/kg body weight) and arsenic trioxide (18.8 mg/kg bodyweight) while group 5 served as the control. Both treated groups of animals and the control group were fed with rat chow and water throughout the experimental period. At termination, there was significant accumulation of metals in the liver of animals in the intoxicated groups when compared with the control group. Lead accumulation in the liver was found to be $(41.41 \pm 33.72 \text{ kg/mg})$ in lead only group. Cadmium (Cd) accumulation in the liver was found to be $40.16 \pm 21.41 \text{ kg/mg}$ in the Cadmium alone group. Arsenic (As) accumulation in the liver was found to be $26.59 \pm 10.12 \text{ kg/mg}$. The co-metal exposure group had Arsenic (As) concentration of $51.89 \pm 11.12 \text{ kg/mg}$ which was significantly ($P_a < 0.05$) higher than that of As alone group and even other metals in the co-metal exposure group. There was a decrease in liver weight especially in As-exposed groups and a greater decrease in the co-exposure group. Similarly, change in body weight was higher in the mixed heavy metal group followed by the arsenic exposed group. The elevated levels of these metals in the liver of experimental suggests potential toxic effects. A greater bioaccumulation of the metals in the co-exposure group suggests a synergistic interaction which implies increased toxicity. There is need to limit human exposure to these heavy metals.

Une Bioaccumulation De Métaux Lourds Dans Le Foie De Rats 'Albino Wistar' Exposés À Un Mélange De Métal Unique Et Lourd

Abstract

Les métaux lourds tels que le cadmium, le plomb et l'arsenic présentent des risques pour la santé des animaux et des humains dans le monde. Ceux-ci

ont tendance à se bio-accumuler dans les tissus et les organes du corps et à interférer avec le fonctionnement des composants cellulaires vitaux. Cette étude a été menée pour évaluer la bioaccumulation de ces métaux dans le foie de rats albinos Wistar suite à une exposition subchronique à un mélange de métaux simples et lourds. Vingt rats mâles matures d'Albino Wistar pesant 192 à 200 g ont été assignés au hasard à cinq groupes de quatre animaux chacun. Les groupes 1, 2, 3 et 4 ont été traités pendant 12 semaines avec 60 mg / kg de poids corporel d'acétate de plomb, 22,5 mg / kg de poids corporel de dichlorure de cadmium, 18,8 mg / kg de poids corporel de trioxyde d'arsenic et le groupe 4 traité avec un mélange de plomb acétate (60 mg / kg de poids corporel), dichlorure de cadmium (22,5 mg / kg de poids corporel) et trioxyde d'arsenic (18,8 mg / kg de poids corporel) tandis que le groupe 5 a servi de témoin. Les deux groupes d'animaux traités et le groupe témoin ont été nourris avec de la nourriture pour rat et de l'eau pendant toute la période expérimentale. À la fin, il y avait une accumulation significative de métaux dans le foie des animaux dans les groupes intoxiqués par rapport au groupe témoin. L'accumulation de plomb dans le foie s'est avérée être (41,41 + 33,72 kg / mg) dans le groupe plomb uniquement. L'accumulation de cadmium (Cd) dans le foie était de 40,16 + 21,41 kg / mg dans le groupe Cadmium seul. L'accumulation d'arsenic (As) dans le foie était de 26,59 + 10,12 kg / mg. Le groupe d'exposition aux co-métaux avait une concentration d'arsenic (As) de 51,89 + 11,12 kg / mg, ce qui était significativement ($P < 0,05$) plus élevé que celui du groupe As seul et même d'autres métaux du groupe d'exposition aux co-métaux. Il y avait une diminution du poids du foie en particulier dans les groupes exposés à l'As et une plus grande diminution dans le groupe de co-exposition. De même, la variation du poids corporel était plus élevée dans le groupe des métaux lourds mixtes suivi du groupe exposé à l'arsenic. Les niveaux élevés de ces métaux dans le foie des tests expérimentaux suggèrent des effets toxiques potentiels. Une plus grande bioaccumulation des métaux dans le groupe de co-exposition suggère une interaction synergique qui implique une toxicité accrue. Il est nécessaire de limiter l'exposition humaine à ces métaux lourds.

Introduction

Heavy metals are natural constituents of the earth crust. They are high density elements occurring in minute quantities in the environment (Lu, Li, and Huang, 2017). Since they cannot be degraded or destroyed, they are persistent in all parts of the environment. With the rise in anthropogenic activities, heavy metal pollution has been on the increase. Some trace amounts of heavy metals such as zinc and copper are useful to the body, but other toxic metals can accumulate in large quantities and pose health problems. Multiple heavy metals may simultaneously enter the human body. Once inside the body, heavy metals are not easily metabolized or excreted, they build up quickly and bioaccumulate leading to an increase in their concentration (Cooper *et al.*, 2017).

Bioaccumulation describes the accumulation and enrichment of contaminants in organisms, relative to that in the environment (Borga, 2013).

Bioaccumulation of chemicals is the problem concerning all living organisms. This is the result of dynamic equilibrium between the uptake and elimination. The extent to which bioaccumulation occurs determines the toxic effects which are manifested. The knowledge of bioaccumulation enables the assessment of the risk related with the presence of various chemicals in the environment, food at workplace and to present quantitatively how to safely control the use and emissions of these chemicals (Chojnacka and Mikulewicz, 2014).

Heavy metals move into plant, animal and human tissues via inhalation of contaminated air, dermal contact with soil, air or water or through

ingestion, diet and handling. They subsequently bind to and interfere with the functioning of vital cellular components. Cadmium, lead and arsenic appear in the World Health Organization's list of 10 chemicals of major public health concern and according to the priority chemical list, they are all metals with great public health concern (ATSDR, 2013). Due to its toxicity and high frequency of occurrence, cadmium and arsenic and their respective compounds are classified as human carcinogens level 1, and inorganic lead compounds are classified as level 2A by the International Agency for Research on Cancer (IARC2020). Lead causes damage to the central nervous system and kidneys and affects hemesynthesis (Abadin, Ashizawa, Stevens, Llados and Diamond, 2007). Cadmium can induce disturbances in calcium metabolism, renal tubular dysfunction, osteoporosis and even lung cancer (Faroon, Ashizawa, Wright, Tucker, Jenkin, Ingeman and Rudisill, 2012). Cancers of the skin, lung, liver, lymph, nasal passage, kidney, bladder, prostate and haematopoietic systems of humans have been associated with inorganic arsenical toxicity. As induced cancer risks have been found among iron smelting workers and those engaged in the production and use of arsenical pesticides (Govind, Madhuri and Shrivastav, 2014). These metals are generally toxic since they accumulate in biological organisms over time.

The manifestation of toxicity of individual heavy metal differs depending on dose and duration of exposure, species, gender and environmental and nutritional factors. Major differences occur between the single exposure and exposure to a mixture of metals. The toxic effects usually associated with chronic exposure by pollutant heavy metals are mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor body condition and impaired reproduction. (Pandey and Madhuri, 2014). Living organisms are frequently exposed to a combination of heavy metals. The combined interactions between these metals are shown to produce increased toxicity compared to exposure to single metals (Singh, Gupta, Kumar, and Sharma, 2017). This research thus seeks to assess the level of bioaccumulation

of heavy metals in the liver following a period of exposure to single and a mixture of metals.

Materials and Methods

Experimental Design

Twenty male albino Wistar rats weighing between 192-200g were obtained from the animal house of Akwa Ibom State Polytechnic Ikot Osurua. The animals were housed in wooden cages with wire mesh and kept at constant room temperature with 12hrs of light/dark cycles. All animals received normal rat chow and water *ad libitum* during the acclimatization period and principles of animal care were duly followed.

Experimental Protocol

The animals were randomly shared into five groups with four animals per group. Lead-exposed groups received 60 mg/kg body weight of lead acetate; Cadmium-exposed groups received 22.5 mg/kg of body weight of cadmium chloride, while arsenic exposed groups received 18.8mg/kg body weight of arsenic trioxide. The co-exposure group received a mixture of lead acetate, (60 mg/kg) body weight, Cadmium chloride, (22.5 mg/kg) body weight, arsenic trioxide, (18.8 mg/kg) body weight. The control group was fed with normal rat chow and water only. All groups of rats were fed with normal ratchow and de-ionized water for the period of 12 weeks and all administrations were done through the oral route.

Preparation of Heavy Metals

Based on reported oral LD₅₀ for Wistar rats of 600, 225 and 188mg/kg body weight for lead acetate, Cadmium chloride and arsenic trioxide, respectively (Sujatha, Iswarga, Deepti and Shrikanya, 2011; Cheng and Roh, 2006), 10% of the oral LD₅₀ of each metal was administered. The respective dose of metal for each group was weighed out with respect to each group's mean body weight and dissolved in 1ml of de-ionized water and 0.2ml of each metal was then administered to individual rats in each group. None of the groups received more than 1 ml of each metal solution.

Determination of Body Weight

The body weight of each animal was determined using a weighing balance. The weight in grams(g) were recorded weekly throughout the experimental period. Change in body weight was determined as, final body weight - initial body weight

Tissue Preparation and Analysis

At the end of the experimental period, the rats were slaughtered under chloroform anesthesia. The liver was excised, weighed and dried in a hot air oven at 60°C for 3 hours after which a porcelain mortar was used to grind and homogenize the dry tissue samples before acid digestion.

Digestion and Determination of Metal Concentration in Liver

Metal levels in liver were estimated according to the method of (Ballantine and Barrford, 1997). To 0.2g of the homogenates of the liver, 10ml of nitric acid was added, followed by 20ml of perchloric acid. Each sample was then digested over a sand bath until the solution became clear and yellow in colour. The digest was made up to known volume with de-ionized water. The estimation of lead, cadmium and arsenic concentration was measured with hollow cathode lamps at wavelengths: 283.3, 228.80 and 193.70 respectively with air/acetylene gas using atomic absorption spectrophotometer (GBC SENSES AA).

Statistical Analysis

Data from the experiment was analysed using the IBM-SPSS, version 23 software. The summary of findings in the study were presented using one-way ANOVA with results expressed as mean + standard error of the mean. Significant differences among treatments were detected using the LSD test at $P_{\alpha} < 0.05$.

Results

Lead accumulation in the liver was found to be $(41.41 \pm 33.72 \text{ kg/mg})$ in lead only group. The co-metal exposure group had lead (Pb) concentration of $(6.35 \pm 3.69 \text{ kg/mg})$ which was non-significantly ($P_{\alpha} > 0.05$) lower than that of the lead alone group. Cadmium (Cd) accumulation in the liver was found to be $40.16 \pm 21.41 \text{ kg/mg}$ in the cadmium-alone group. The co-metal exposure group had a cadmium concentration of $18.59 \pm 2.18 \text{ kg/mg}$ which was significantly ($P_{\alpha} < 0.05$) lower than that of cadmium-alone group. Arsenic (As) accumulation in the liver was found to be $26.59 \pm 10.12 \text{ kg/mg}$. The co-metal exposure group had arsenic (As) concentration of $51.89 \pm 11.12 \text{ kg/mg}$ which was significantly ($P_{\alpha} < 0.05$) higher than that of As-alone group and even other metals in the co-metal exposure group. There was a decrease in liver weight especially in the As exposed groups and a greater decrease in the co-exposure group. A similar trend was observed with the body weight of the experimental animals where the change in body weight was higher in the mixed heavy metal group followed by the arsenic exposed group.

Table 1: Body Weight of Experimental Animals

Body Weight of Experimental Animals(g)					
	Lead group	Cadmium group	Arsenic group	Lead+cadmium + arsenic group	Control group
Week 1	231.30±29.24	251.52±13.90	247.04±32.22	235.86±9.88	233.32±35.19
Week 2	238.38±28.81	248.50±14.61	248.16±31.72	229.60±10.05	223.96±35.30
Week 3	244.30±25.97	252.72±12.73	242.68±29.86	242.24±13.02	231.32±34.95
Week 4	250.88±25.64	259.92±13.28	230.60±26.48	245.24±11.06	217.80±32.24
Week 5	258.12±21.97	270.82±13.03	255.12±23.86	239.50±9.715	264.02±31.15
Week 6	269.54±20.84	277.42±11.73	257.68±22.27	249.32±11.73	263.60±30.72
Week 7	276.40±18.86	273.96±10.93	273.60±21.36	256.84±12.74	278.34±29.78
Week 8	284.32±17.60	283.86±10.66	279.96±18.97	264.88±12.75	290.78±30.64
Week 9	286.44±16.54	280.46±12.59	288.12±17.91	271.26±13.62	291.52±30.94
Week 10	298.60±16.34	282.70±14.97	296.12±17.79	278.06±15.20	294.12±29.05
Week 11	295.40±16.63	286.32±14.94	282.42±19.42	278.84±15.30	289.68±29.49
Week 12	301.02±17.56	311.00±22.99	271.60±16.96	269.16±14.88	292.18±29.80
Change in body weight	69.72±20.10	59.48±31.04	24.56±16.99	33.30±9.85	58.86±10.57

Results are presented as mean± SEM, Significance = $P_{\alpha}<0.05$

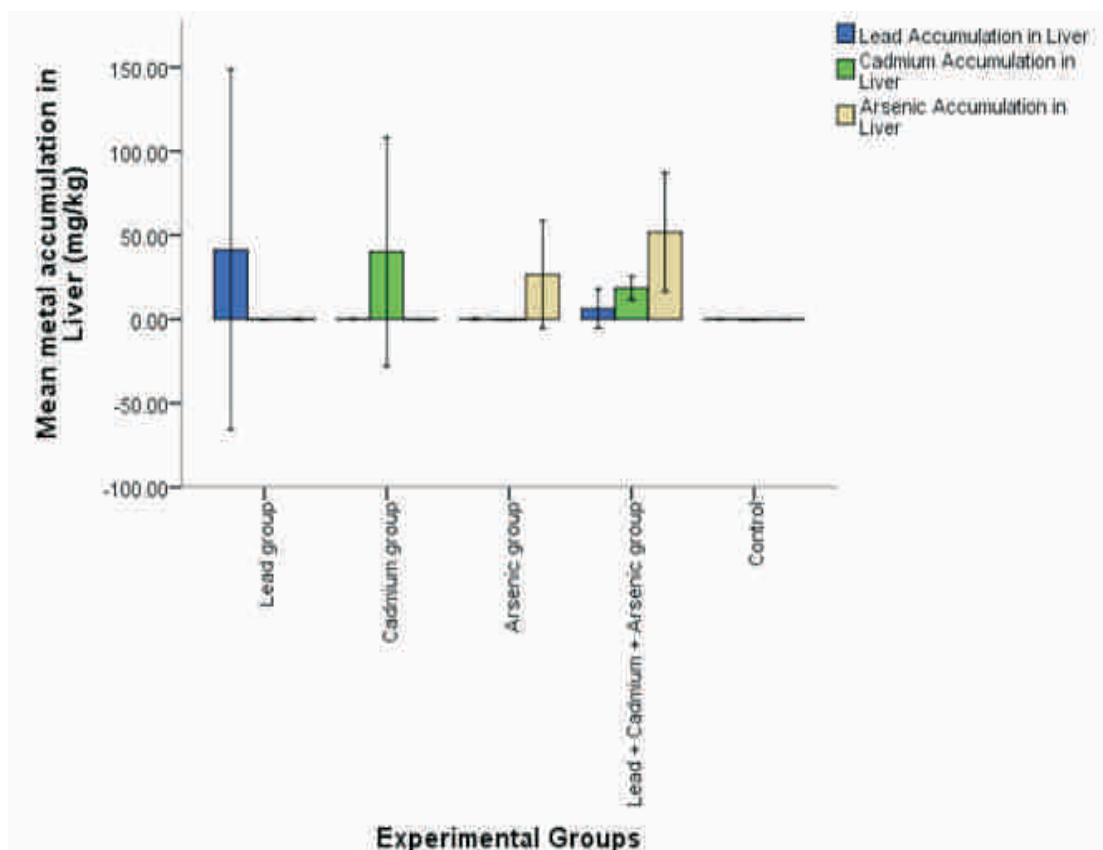


Figure. 1: Metal accumulation in the Liver of albino Wistar rats

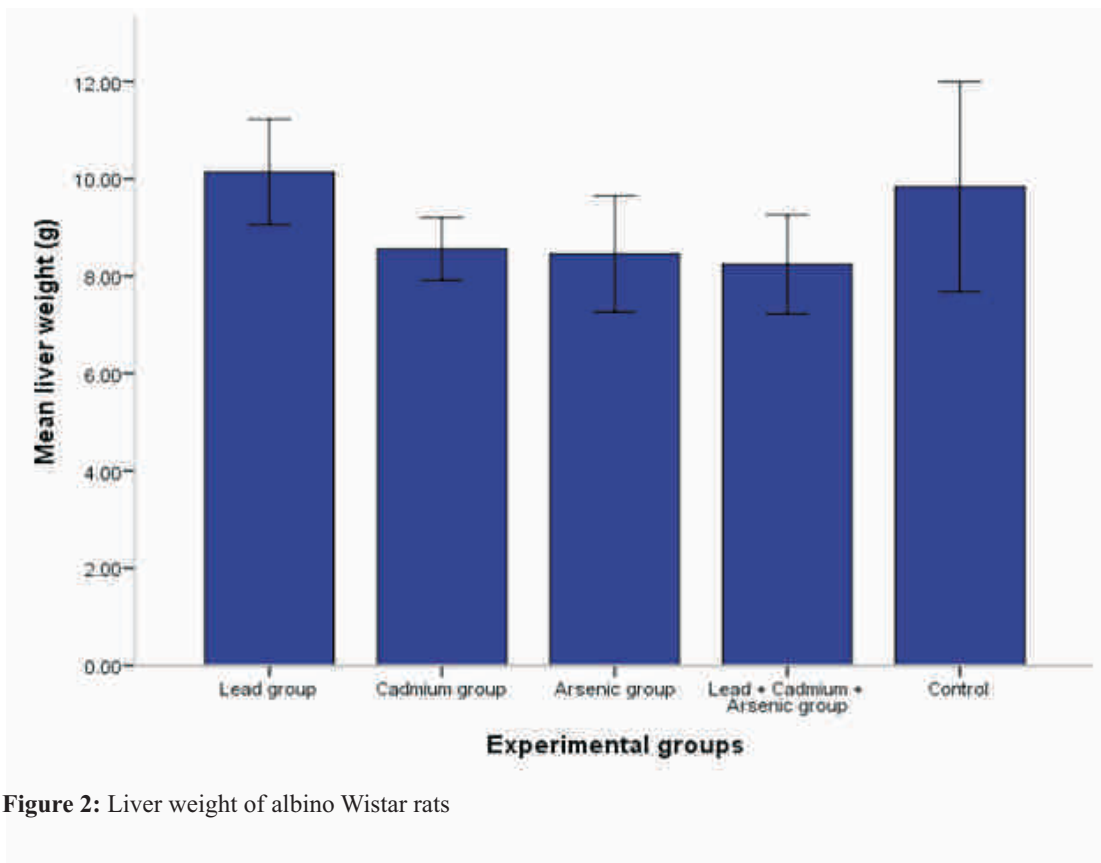


Figure 2: Liver weight of albino Wistar rats

Discussion

The accumulation of lead, cadmium (Cd), in the liver of albino Wistar rats following a sub-chronic exposure was investigated. Significantly high levels of metals (lead, cadmium and arsenic) were found in the liver. Exposure to lead, cadmium and arsenic mainly occurs through the respiratory and gastrointestinal system. Absorbed lead, cadmium and arsenic (Whether inhaled or ingested) are stored in soft tissues. These heavy metals were conjugated in the liver and passed to the kidney where a small quantity is excreted in urine and the rest accumulated in various body organs, affecting many biological activities at the molecular, cellular and intercellular levels. This results in morphological alterations that can remain even after levels have fallen (Sidhu and Nehru, 2004). Animals exposed to cadmium, lead and arsenic were observed to have aggressive behaviour during the 12 weeks of exposure. Colour of fur became light yellowish in both cadmium, arsenic and co-exposure groups. This might be due to the accumulation of cadmium

and arsenic in the fur. In the current study, a significant decrease ($p < 0.05$) in liver weights of animals intoxicated with Cd and As was observed. There was an exception with the lead (Pb) exposed group. The group exposed to the mixed metals experienced a greater decrease in liver weight. A similar trend was also observed in the body weight. Decreased body weight was previously observed by Allouche, *et al.* (2006) who administered 0.1% lead acetate to male rats for 11 months. Estimation of lead content in the liver showed a higher level of accumulation than cadmium. Similar findings were observed by Josthna, Geetharathan, Sujatha and Deepika (2012). In the lead-alone exposure group, the metal accumulation was higher compared to the combined exposure group. Co-exposure with Cd and/or As resulted in lower Pb concentrations in the liver. The lower level of Pb in the mixed metal group indicated an antagonistic interaction between Pb and the other metals especially (Cd). Divalent metals are absorbed in the body through similar mechanisms and accumulated in the same

tissues. Once in the body, they can alter each other's absorption, distribution and accumulation (Smith, Gancarz, Rofe, Kempson, Weber, and Juhasz, 2012). Smith *et al.* (2012) observed that Cd antagonizes Pb accumulation in the liver and kidney. This is in agreement with the findings of the present study. Approximately 90% of absorbed Pb is reported to be stored in the bone with a half-life of 600 - 3000 days. The remaining 10% is stored in soft tissues like kidney, liver and brain. Lead impairs learning, memory and audio-visual functions in children. Toxic effects of lead also include nephrotoxicity, hepatotoxicity and cardiovascular damages. Lead causes damage to the central nervous system, kidneys and affects heme synthesis. The carcinogenic effect of lead has received increasing attention. Research has shown that lead causes oxidative stress in the body by inducing the generation of free radicals thereby reducing the antioxidant defence system of the cells. Lead causes sterility in males by damaging the germinal epithelium and also spermatocytes (Abadin, Ashizawa, Stevens, Llados and Diamond, 2007).

The high level of Cd accumulation in the liver might be due to the involvement of this organ in metabolic activities (Klassen, Liu and Diwan, 2009). In the present study, cadmium accumulated in higher amount in the liver both in the cadmium-alone group and the combined exposure, but the cadmium-alone group was higher than the combined exposure. Similar findings were also observed by Anilkumar, Mahesh, Kishore and Vijayakumar (2018), who conducted a study on metallothionein in protection in cadmium toxicity. Cadmium accumulation in the liver was found enhanced in cadmium-alone group than in the combined exposure.

Cadmium derives its toxicological properties from its chemical similarity to Zn (an essential micronutrient for plants, animals and humans). The Cd once absorbed by an organism, remains in the system for many years (over decades for humans), though, it is eventually excreted. It is produced as an inevitable by-product of Zn (or occasionally Pb) refining since these metals occur naturally within the raw ore. But once

collected, the Cd is relatively easy to recycle. The Cd is mostly used in Ni/Cd batteries, rechargeable or secondary power sources exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. The coatings of Cd provide good corrosion resistance particularly in high-stress environments like marine and aerospace applications where high safety or reliability is required. The coating is preferentially corroded if damaged. It is also used as a pigment, stabilizer for PVC, in alloys and electronic compounds. As an impurity, it is present in several products, including phosphate fertilizers, detergents and refined petroleum products. Average daily intake of Cd for humans is 0.15 µg from the air and 1 µg from water. Cadmium can induce disturbances in calcium metabolism, renal tubular dysfunction, osteoporosis and even lung cancer (Faroon, Ashizawa, Wright, Tucker, Jenkin, Ingeman and Rudisill, 2012). Its high exposure may cause obstructive pulmonary disease and lung cancer. Bone defects (osteomalacia and osteoporosis) have also been reported in humans and animals. Besides, it can also cause increased blood pressure and myocardial disease in animals (Pandey and Madhuri, 2014).

Accumulation of Arsenic in the liver was found to be higher in the combined exposure group than the cadmium-alone exposure group. Anilkumar *et al.* (2018) observing a similar trend, concluded that coexistence of lead, cadmium and Arsenic showed a positive pharmacodynamic interaction. The atmospheric emissions from smelters, coal-fired power plants and arsenical herbicide sprays; water contaminated by mine tailings, smelter wastes and natural mineralization and diet, especially from consumption of marine biota all cause arsenic (As) toxicity. Generally, the inorganic As compounds are more toxic than organic compounds and trivalent As (arsenites, As⁺³) are more toxic than pentavalent As (arsenates, As⁺⁵). Cancers of the skin, lung, liver, lymph, nasal passage, kidney, bladder, prostate and haematopoietic systems of humans have been associated with inorganic arsenical toxicity. As induced cancer risks have been found among iron smelting workers and in those engaged in the production

and use of arsenical pesticides (Govind, *et al.*, 2014).

The liver is considered as one of the target organs affected by heavy metal toxicity owing to its storage in the liver after exposure. Also, the liver being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances, is of relevance in heavy metal poisoning (Herman & Geraldine, 2009). The present study observed significant changes in the mixed heavy metal exposure group than the individual metal exposure groups. Existing reports from several researches indicate that heavy metals in combination may lead to more severe impact on human health compared to their individual effects.

Wildermann, Weber, and Siciliano (2015), have reported that the synergistic effect of Pb and Hg are extremely neurotoxic and much worse than single exposure. Living organisms are frequently exposed to a combination of heavy metals. The combined interactions between these metals are shown to produce increased toxicity compared to exposure to single metals (Singh, Gupta, Kumar, and Sharma, 2017).

Conclusion

The present study revealed significantly high levels of metals (cadmium, lead and Arsenic) in the liver of albino Wistar rats following a 12-week exposure. The heavy metals assessed are classified as most toxic to humans. The elevated levels of these metals in the liver of experimental animals suggest potential toxic effects on different organs. The higher accumulation of the metals in the co-exposure group suggests a synergistic interaction which implies increased toxicity. Though this study was conducted on animal models a similar trend of accumulation and toxicological risk could occur in humans and this calls for a limit to exposure to sources of these heavy metals.

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