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Histopathological Changes Associated with Exposure to Some Toxic Heavy Metals in Kidneys of Albino Wistar Rats

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Abstract

Heavy metals are the most toxic substances of global concern. Since they are not biodegradable they tend to bioaccumulate in the environment producing toxic effects to both plants and animals even in low concentrations resulting in genomic instability. This study was therefore aimed at assessing the histopathological changes associated with exposure of some toxic heavy metals in kidneys of albino wistar rats. Thirty albino wistar rats were used for this study and divided into 6 groups of 5 rats each and were administered $\frac{1}{10}$ of the determined LD_{50} of the respective heavy metal salts of Lead acetate, Chromium sulphide, Cadmium chloride, Arsenic trioxide and Mercury chloride for groups 2,3,4,5 and 6 respectively daily. Group 1 served as the control was given commercial rat chow and water *ad libitum*. At the end of 45 days animals were sacrificed and kidneys collected for histological assessment using H & E staining technique. All the groups exposed to heavy metals showed histological alterations to include: degenerating tubules with vacuolated ductal and tubular shrinking nuclei, there were also hemorrhagic blood vessels within the renal cortical matrix. In conclusion heavy metal intoxication has been shown to cause histopathological changes in the kidney of experimental rat models.

Keywords: kidneys, heavy metals, histopathological changes, degenerating tubules

INTRODUCTION

Environmental pollution has become a concern in matters relating to human beings. Heavy metals are ubiquitous in various forms in the environment. Contaminated water, mining and battery recycling are some of the major contaminants primarily by means of consumption to human beings and animals. Heavy metals like Lead(Pb), Mercury(Hg), Chromium(Cr), Arsenic(Ar) and Cadmium(Cd) are major environmental and occupational hazard (Barbier et al. (2005). These non-essential metals are toxic at low levels with a very long biological half-life and are non-biodegradable making them very harmful when exposed to them.

These metals also have the ability to accumulate and reabsorb dia-valent metals when swallowed or inhaled, thereby affecting almost every organ and system in the body, it has been reported to affect the liver (Sharma & Street, 1980) as well as the kidney (Amah et al., 2014). In industries Lead has been one of the most useful elements but in the human body it has no useful function. Over the centuries Lead has posed major health challenges leading to deleterious effects like congentive impairment and behavioral deficits, high blood pressure(BP) and impaired renal function (Goyer, 1993; Munter et al., 2003).

Children are more susceptible to the effect of environmental Lead than adults resulting from increased gastrointestinal absorption and making them vulnerable because of their absorption rate which is about 5-10 times more effective than adults (Goyer & Mahaffey 1972). The prevalence of renal disease caused by exposure to Lead is dependent on the ability to detect the nephrotoxic damage at a stage when it can be reversed or not affecting or compromising with renal function. Research has identified early and sensitive markers that may be predictive and indicative of renal toxicity as a result of Lead exposure. Recent studies have shown indicators used for Lead induced nephrotoxicity (Rastogi, 2008). Chromium and Cadmium have been identified in autopsied human kidneys to be highly concentrated in the renal cortex and pathologically they have been found to trigger genotoxicity after oxidative stress and can increase the risk of chronic renal failure (Wilbur et al., 2012; Stohs et al., 2000; Nuyts, 1995; Tsai et al., 2017).

Chromium is found naturally in the earth's crust and is ubiquitously present in the environment due to anthropogenic sources. Cadmium is found in soil and rocks including coal mineral fertilizers are used in many products including batteries pigments, metal coatings and plastics and it is also found in cigarette smoke (Tsai et al., 2017).

Arsenic exposure is one of the major public health problems as many people are exposed to water level above the limit. Arsenic has been linked with tumor formation in skin, lungs, bladder, liver and kidneys (Ferreccio et al., 2013; Surdu et al., 2013). It has also been linked as a risk factor for cardiovascular disease (Chen et al., 2011), hypertension, peripherial atery disease and diabetes mellitus (Martin-Pardillos et al., 2013). Most recently renal diseases has been recognized as a result of Arsenic exposure though epidemiological data are still scares (Robles-Osono et al., 2015). Arsenic ingested in drinking water is absorbed by the intestine but other routes of entry into the body are by inhalation and dermal exposure and it induces synthesis of metallothioneins (He & Ma, 2009). Arsenic also uncouples oxidative phosphorylation causing reduction in Sodium-Phosphoshate and glucose transport resulting in Fanconi syndrome (Brazy et al., 1980).

Mercury exposure can be through inhalation of metallic Mercury, ingestion of both organic and inorganic forms of Mercury and its toxicity interferes with vital body systems which include the nervous, renal, cardiovascular, repiratory, gastrointestinal, heamatological, immune and reproductive systems (Ekawanti & Krisnayantic, 2015). Kidney damage usually result in an exposure to elemental Mercury and Methy-mercury, its exposure damages the secretory organs of erythropoietin (a hormone that stimulates erythrocyte synthesis in the

kidney and this declines the formation of the kidney thereby affecting red blood cell production (Limbong et al., 2003; Franko et al., 2005; Ribarow et al.,1983).

The exposure to heavy metals is harmful due to its ability to reabsorb and accumulate divalent metals, usually the kidney is always the first target organ of heavy metal toxicity and both acute and chronic exposure have been demonstrated to cause nephropathies, ranging from tubular dysfunction like acquired Fanconi syndrome to death all these damages depends on the nature, dose, route and duration of heavy metal exposure (Barbier et al., 2005).

METHODOLOGY

Thirty (30) male albino wistar rats were used for the study and randomly divided into six groups of five rats in each group. Group 1 served as the control and was administered commercial rat chow and distilled water only, Groups 2,3,4,5 and 6 were administered 10% of established LD_{50} of Lead acetate, Chromium sulphide, Cadmium chloride, Arsenic trioxide and Mercury chloride respectively. At the end of 45 days, rats were euthanized and kidney was collected in 10% buffered formalin solution, passed through ascending series of ethanol baths, cleared in toluene and embedded in paraffin wax. Tissues were sectioned at 4um and stained with Haematoxylin and Eosin (H&E). The sections were examined by light microscope (Sheeham & Hrapchak, 1980).

RESULTS

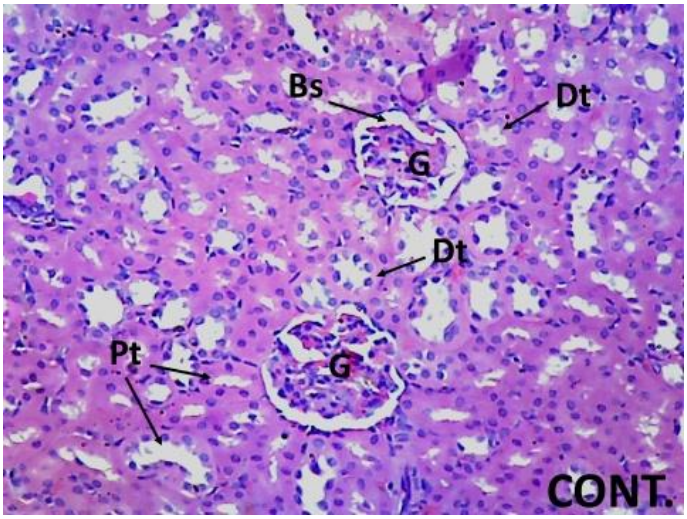

Plate 1:Photomicrograph of a transverse section of a control kidney tissue(Group1)

(H&E x100)..

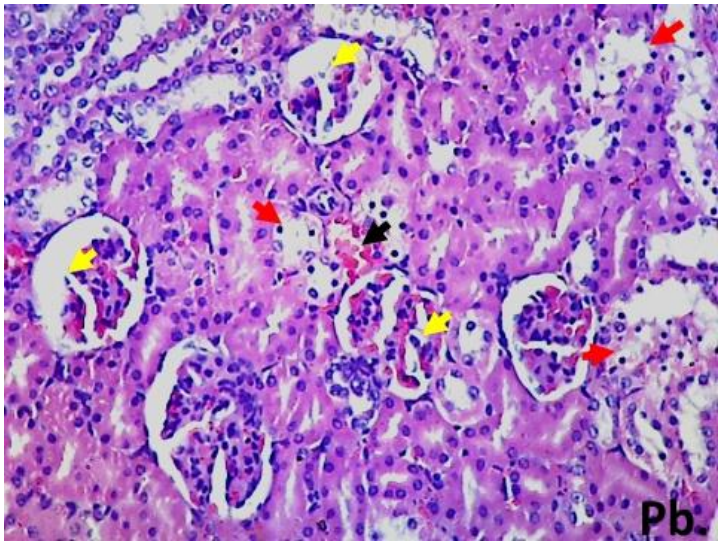

Plate2:Photomicrograph of a transverse section of a Lead acetate treated kidney tissue(.Group2)(H&E x100).

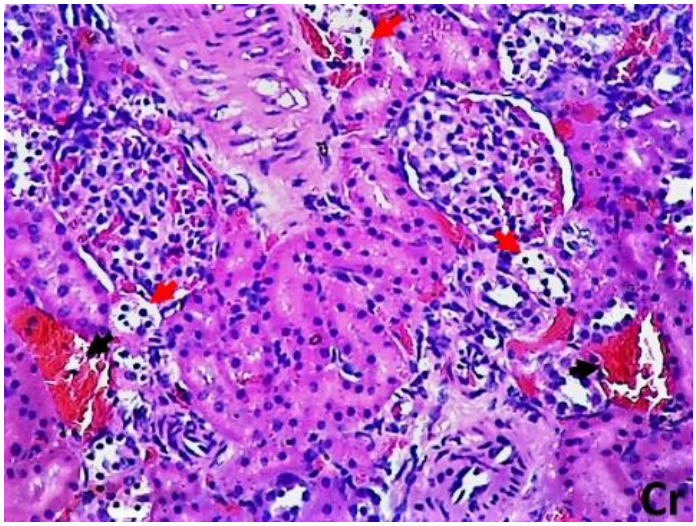

Plate 3:Photomicrograph of a transverse section of a Chromium sulphide exposed kidney tissue(Group3)(H&E x100).

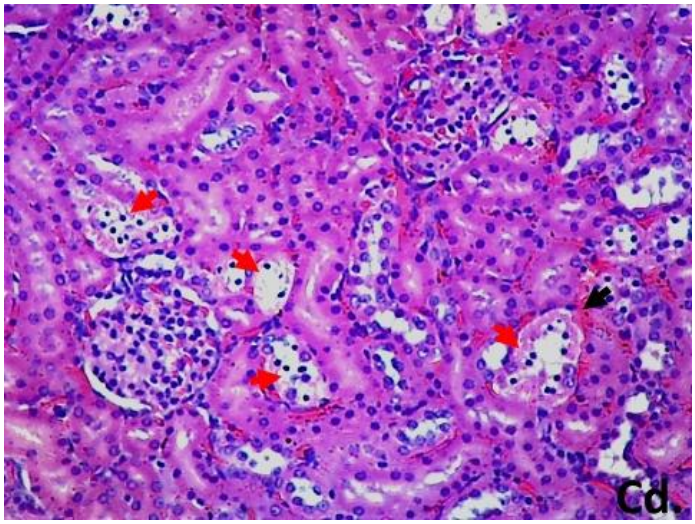

Plate4:Photomicrograph of a transverse section ofkidney tissue exposed to Cadmium chloride(Group4)(H&E x100).

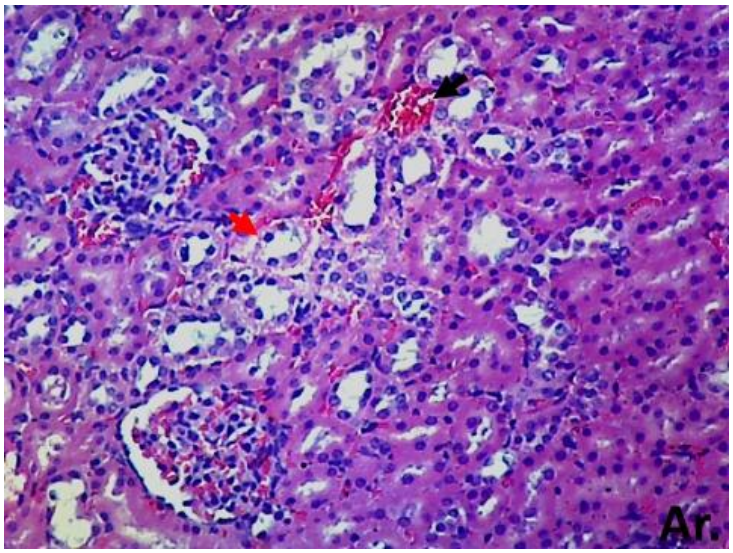

Plate5:Photomicrograph of a transverse section of kidney tissue exposed to Arsenic trioxide(,Group5)(H&E x100).

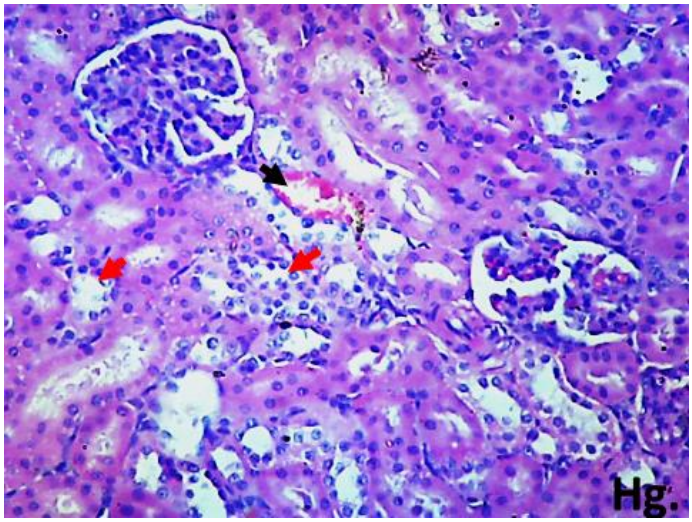

Plate6:Photomicrograph of a transverse section of a kidney tissue exposed to Mercury(Group6) (H&E x100).

DISCUSSION

Many studies have highlighted the toxicity of most heavy metals and humans are exposed to these metals via natural and anthropogenic activities and they get it through the food chain mostly through water, food and air. Heavy metals exposure has been linked to numerous challenges and health problems resulting in organ damage and accumulation in tissues and because these metals get into the body, the walls of the kidney are broken down and are altered. The concentration of various heavy metals such as Lead(Pb), Cadmium(Cd), Chromium(Cr), Arsenic(Ar) and Mercury(Hg) showed a decending and de-generational format when compared to the control. These heavy metals have the tendency of altering biological processes (Erden et al., 2016; Satarug et al., 2018).

The kidney is one of the principal organ involved in drug and xenobiotic metabolism and mot chemical toxicities is usually manifested in the kidney before other organs (Omar, 2013). Lead, Chromium, Cadmium, Arsenic and Mercury are extremely detrimental to human health. Significant heavy metal pathological changes in the kidney architecture were observed in the rats after 45 days of the heavy metal when compared to the control Group 1.

The histological findings in group 2 exposed to Lead acetate showed atrophying renal micro-architecture, having degenerating tubules with vacuolated ductal cells and tubular shrinking nuclie (red arrow), areas of degenerating glomerular cells (yellow arrow), and areas of hemorrhagic blood vessels (black arrow) within the renal cortical matrix. This study did not align with findings from Bergdahi and Skerfving (2021) who demonstrated that Lead accumulates in the bones and not the kidney, high exposure to Lead can result in intestinal nephritis and proximal tubular damage and can also affect renal function. Group 3 rats administered with Chromium sulphide demonstrated atrophying renal micro-architecture, having degenerating tubules with vacuolated ductal cells and tubular shrinking nuclie (red arrow), and areas of hemorrhagic blood vessels (black arrow) within the renal cortical matrix. This result was in agreement with Pan et al., (2012) who reported exposure of both hexavalent and trivalent Chromium to induce hepatotoxicity in female nude mice and histological changes showed fibrosis in portal areas Chromium toxicity is capable of causing cellular oxidative stress, autophagy, apoptosis, pyroptosis, endoplastic reticulum(ER) stress and inflammatory response giving rise to cellular damage as a result of the overproduction of reactive oxygen species (ROS) (Sharma et al., 2020). Group 4 rats exposed to Cadmium chloride showed

atrophying renal micro-architecture, having degenerating tubules with vacuolated ductal cells and tubular shrinking nuclie (red arrow), and areas of hemorrhagic blood vessels (black 2arrow) within the renal cortical matrix. This result was the same with findings of El-Refary and Eissa (2013); Garba (2007) who showed lesions in the cortex and medulla of the kidney. They further reported histological alterations that induce proteinaceous release inside channels, serve multifocal congestion, cystic dialation in the medulla and hemorrhage associated with intestinal mononuclear cellular infilteration.

The kidneys are the target organs of Cadmium toxicity and epidemiological studies have been shown relationship between Cadmium exposure and kidney damage Akesson et al., (2005). Cadmium has been reported to induce nephrotoxicity including oxidative stress which triggers (ROS) production by inhibiting antioxidant system in renal tubular epitheial cells, Cadmium also induce mitrochondrial dysfunction, genotoxicity, cell cycle arrest and apoptosis though there are limited data regarding the mode of action (Wang et al., 2017; Song et al., 2016; Ge et al., 2019).

Rats in group 5 administered with Arsenic trioxide demonstrated atrophying renal micro-architecture, having degenerating tubules with vacuolated ductal cells and tubular shrinking nuclie (red arrow), and areas of hemorrhagic blood vessels (black arrow) within the renal cortical matrix. This work was the same with the research of Norman et al. (2015) who observed degenerative changes in kidney tissue in Arsenic treated mice. Arsenic inhibit the vacuolar hydrogen ion ATPase and endocytosis, endocytosis of filtering protein may be impeded and vesicle mediated recycling of some specific membranes may be distributed (Harari et al., 2018).

Group 6 rats showed atrophying renal micro-architecture, having degenerating tubules with vacuolated ductal cells and tubular shrinking nuclei (red arrow) and areas of hemorrhagic blood vessels (black arrow) within the renal cortical matrix. This was also in line with Elinder and Barregard (2021) who reported membrane nephropathy with thickened glomemular basement membrane and immunoglobulin deposit. The mechanism of Mercury-induced kidney disease has not yet been understood but research suggests it may result from immune mechanism which induces glomerular diseases. Mercury has the tendency of combining with proteins to form haptens which produces antigen-antibody complexes as a result of immune reaction reactions and these complexes has the tendency of penetrating glomerular membrane thereby forming glomerular lesions (Polland et al., 2019; Qin et al., 2016). Mercury also impacts the immune system and promotes autoantibody production, inhibiting T lympohocyte function thereby inducing auto immune diseases (Clarkson et al., 2003; Sun et al., 2018). Mercury toxicity also results in vacuolation degeneration and necrosis of the tubular epithelial cells thereby causing kidney diseases. Mercury also binds sulfhydryl groups in-vivo giving rise to low levels of sulfhydryl-containing proteins (like Soidium ions and Pottasium ions, ATPase) and changes in the cell membrane. This increases free radicals outside the cell but decreases free radical scaverging system resulting in oxidative stress injury (Rana et al., 2018). Oxidative stress may also be the major source some heavy metal toxicity in mice. High exposure to Lead can result in intestinal nephritis and proximal tubular damage and can also affect renal function.

CONCLUSION

Most toxic heavy metals are emitted into the environment through natural and anthropogenic sources and the route which these metals get into our body vary. The result of this study demonstrated renal hemorrhagic blood vessels within the renal cortical matrix, degenerating tubules with vacuolated ductal cells and tubular shrinking nuclei in all the groups exposed to the different heavy metals. The kidney is a target organ in heavy metal toxicity because of its ability to filter, reabsorb and concentrate divalent ions and the extent of severity and fatality of the damage is dependent on the species of metals, dose and duration of exposure.

Refrences

Barbier, O., Jacquillet, G., Tauc, M., Cougnon, M., & Poujeol, P. (2005). Effect of heavy metals on and handling by the kidney. *Nephron Physiology* 99(4):105-110

Bergdahi,I.A & Serfving,A(2021).Lead binding to alpha-aminolevlinic acid dehydratase(ALAD) in human erythrocytes.Handbook on the Toxicology of Metals.Vol.11.

Brazy,P.C.,Balaban,R.S., & Gullans,S.R(1980). Inhibition of metabolism, Relative effects of Arsenic on Sodium Phosphate and glucose transport by the rabbit proximal tuble. *Journal Clin. Invest.*66:1211-1221.

Chen,Y.,Graziane,J.,Parvez,F.,Liu,M.,Slavkovich,V.,Kalra,T.,Argos,M.,Islam,T.,Ameh,A.,R akibuz-Zamam,M.,Hassan,R.,Levy,D and Ahsan,H(2011).Arsenic exposure from drinking water and mortality from cardiovascular disease in Banngladesh:Prospective Cohort Study.*BiochemicalMedical Journal*. 342:2431

Ekawanti,A and Krisnayanti,B.D.(2015).Effect of Mercury exposure on renal function and hematological parameters among Artis and small-scale Gold minners at Sekotong West Lombok,Indonesia.*Journal of Health Pollution5*(9):25-32

Elinder,C.G and Barregard,L(2021).Renal effects of exposure to metals.Handbook on the Toxicology of Metals.Vol.11

Erdem,O.,Yazihan,N., Kocak,M.K.,Sayal,A and Akil,E (2016)Influence of chronic Cadmium exposure on the tissue distribution of Copper and Zinc andoxidative stress parameters in rats.*Toxicology.Ind.Health* 32(8):1505-1514.

Faroon,D.,Ashizawa,A and Wright,S(2012).Toxicological profile for Cadmium Agency for Toxic Substance and Disease Registry(ATSDR) .Atlanta,Georgia.

Ferreco,C.,Smith,A.H.,Duran,V.,Barlano,T.,Benitez.H.,Vaides,R.,Aguirre,J and Moore,L.(2013).Case-control study of Arsenic in drinking water and kidney cancer in uniquely exposed Northen Chile.American *Journal of Epidemiology* 178(15):815-818.

Franko,A.,Budihha,M.V and Fikfak,M.D(2005).Long term effects of Elemental Mercury on renal function in miners of Idrija Mercury mines.*Ann.Occup.Hygiene* 49(6):521-527.

Goyer,R.A and Mahaffey,K.R(1977).Susceptibility to Lead toxicity.*Enviromental HealthPerspective* 2:73-80

Goyer,R.A(1993),Lead toxicity:Current cocerns,*Environmental health perspective* 100:177-187

Harari,F.,Sallsten,G.,Christensson,A.,Petkovic,B.,Hedbblad,N.,Fagard,O.,Melander,P.M.,Nill so,Y and Borne,G.(2018).Blood Lead levels and kidney function in a population-based cohort.*Ameriacn Journal ofKidney Disease* 72:381-389.

He,V and Ma,Q.(2009)Induction of metallothionein by Arsenic via metal-activated transcription factor 1:Critical role of C-terminal cysteine residue in Arsenic sensing.*Journal of BiologicalChemistry* 284(19):12609-12621.

Limbong,D.,Kumampung,J.,Rimper,T.,Arai,T and Miyazaki,N.(2003)Emmission and environmental implications of Mercury from artisanal gold mining in North Sulawesi,Indonesia .*Sci.Toatl.Enviromental* 302(1-3):222

Martin-Pardillos,A.,Sosa,C.,Sorribas.I(2013).Arsenic increase Pi-mediated vascular calcification and induces premature senescence in vascular smooth muscle cell.*Toxicology Science*131:641-653

Munter,P.,He,J.,Vupputur,S.,Coresh,J andBatuman,V(2003).Blood Lead and chronic kidney diseases in the general United States population:Results from NHANES111 .*Kidney International* 63:1044-1050.

Norman,A.S.M.,Dilruba,S.,Mohanto,N.,Rahman,L.,Khatun,Z.,Riad,W and Haque, A (2015) Arsenic-induced Histological Alteration in various organs of mice. *Journal of Cytology and Histology* 6(3):323

Nults,G.D.,Van-vlem,E. and Thys,J.(1995).New occupational risk factor for chronic renal failure..*Lancet* 346:7-11

Pari,T.L.,Wang,P.W.,Chen,C,C.,Fary,J.Y and Sintupisut,N(2012).Functional proteomics reveal hepatotoxicity and the molecular mechanisms of different forms of Chromium delivered by skin administration.*Proteomics* 12(3):477-489.

Parrish,A.R.,Zheng,X.H.,Turney,L.O.,Younnis,H.S and Gandolfi,A.J(1999).Enhanced transcription factor DNA binding and gene expression induced by Arsenite or Arsennte in renal slides.*Toxicology science* 50:98-105

Rastogi,S.K(2008), Renal effects of environmental and occupational Lead exposure.*IndianJournal Occupational Environmental Medicine* 12(30:103-106.

Ribarow,S.R.,Benow,L.C and Benchew,I.C(2015).The mechanism of Mercury induced hemolysis.*Gen.Physio.Biophys* 2(2):81-84

Robles-Osorio,M.,Sabath-Silva,T and Sabath,E(2015)Arsenic-mediated nephrotoxicity.*RenalFailure* 37(4)

Satary,S.,Nishijo,M.,Ujjin,P and Moore,M.R(2018)Chronic exposure to low-level Cadmium induced Zinc-Copper dysregulation. *Journal Trace Elem.Med,Biol* 46:32-38

Sheehan,D and Hrapchak,B (1980) Theory and Practice of Histotechnology(2nd edition) Batteke press Ohio.Pp 262-264.

Stohs,S.J.,Bagchi,D.,Hassoun,E and Bagchi,M.(2000).Oxidative mechanisms in the toxicity of Chromium and Cadmium ions.*Journal of Environmental Pathology Toxicology Onocology* 19:207-213

Surdu,S.,Edward,F.,Micheal,F.,Bloom,S.,Bosce,F.,Carpenta,D.,Haase,R.,Guizati,E.,Rudnal,P . ,Koppov,K and Tony,F(2013).Occupational exposure to Arsenic and risk of non-melanoma skin cancer in a multi-national Europeanian study.*International Journal of Cancer* 133(9):2182-2191

Tsai,T.,Kuo,C.,Pan,W.,ChenC.,Wu,T and Wang,S.(2017).The decline in kidney function with Chromium exposure is exacerbated with co-exposure to Lead and Cadmium .*ClinicalInvestigation* 92(8):710-720

Wilbur,S.,Abadin,H and Fay,M.(2012).Toxicological profile for Chromium .Agency for Toxic Substance and Disease Registry(ATSDR) .Atlanta,Georgia.