Original Research Article

EFFECT OF MINING ACTIVITIES ON SOME BIOCHEMICAL PARAMETERS OF OPANDA-UGYA INHABITANTS, TOTO LOCAL GOVERNMENT OF NIGERIA.

ABSTRACT

Mining activities are usually associated with environmental pollution and the infiltration oftoxic heavy metals into waterways which could be absorbed by plants or used by inhabitants. The aim of this research was to determine the effect of mining activities on some biochemical parameters of Opanda-Ugya inhabitants, Toto Local government of Nigeria. Four hundred and fifty (450) people were interviewed usingquestionnaires.Eighty (80) of them were carefully grouped into four: Group 1(20 Control), Group 2 (20inhabitants), Group 3 (20 indirect miners) Group 4 (20 direct miners). The Surveydiscovered some symptoms that could be associated with heavy metal toxicity among theinhabitants. Biochemical assay of blood samples revealed no significant (P >0.05) increase in Serum ALT, AST and Total bilirubin in groups 2, 3 and 4 when compared to control, but a decrease in TP. Serum creatinine levels increased significantly in group 3 when compared to the control. Both creatinine and urea levels elevated significantly in group 2 and group 4 when compared with the control. The result of Oxidative stress markers has indicated a significant increase in the levels of MDA with a concomitant decrease in the levels of GSH in groups 2, 3 and 4 compared to the control. The AChE activity in groups 2, 3 and 4 significantly increased when compared to the control. The levels of CRP increased significantly in groups 3 and 4 when compared to the control. We conclude that the neurological and other disorders observed in some participants could be attributed to chronic inflammation and oxidative stress induced by heavy metal toxicity, thus something should be done by the necessary authority to curtail the long-time effect of Mining activities in this community.

Keywords:Mining activities, Heavy metals, antioxidants; acetylcholinesterase; C-reactive protein.

1. INTRODUCTION

Mining is one of the most important industrial activities worldwide (1).Metals are found naturally in the earth's crust, the toxic ones among them are called heavy metals. Heavy metals include transition metals such as Copper, Lead, and Zinc (2) these metals constitute significant environmental pollutants, whose toxicity range from ecological, nutritional, evolutionary, and environmental health of both plants and animals (3). One of the major causes of heavy metal spillage to water bodies and human habitats is mining activities (3).Althoughheavy metalssuch as Fe, Cu, Zn, Mn, and Mg, are required for physiological activities, high concentrations can generate reactive oxygen species(ROS)which may lead to oxidative stress (OS).Excess ROS are usually handled by the body's antioxidant system, including enzymatic (SOD, GPx, GST, Catalase) and non-enzymatic (GSH, vitamin C etc.). However, when the system is overwhelmed with ROS due to heavy metal toxicity, the excess ROS reactswith biological molecules such as Lipids (to generate lipid peroxidation products like MDA),Proteins(affecting its function) DNA (causing cross linkages) and Carbohydrates.These collectively caused OS(4). OS has been shown to initiate many pathological disorders such as cancers, neurological disorders, kidney damage, endocrine abnormalities, etc. (5). Although OS is regarded as the initiator of inflammation as well as the consequence of inflammatory responses (6). Inflammation is also regulated by OS. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) proteins are transcription factors that play a key role in the regulation of inflammation and immunity (7).

2. MATERIALS AND METHODS

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2.1 Chemicals

The chemicals used in this research include disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) sodium chloride (NaCl), ethanol 95% and isoflurane were purchased from Marry Hallway multipurpose lab Ahmadu BelloUniversity Zaria, Kaduna State.

2.2 Experimental Design

The study was carried out in two phases. The first phase involved the use of questionnaires to obtain relevant information from the inhabitants of Opanda-Ugya. This provided an insight into the average quantity of effect of mining activities and also the reward-seeking behavior of some pathological symptoms.

The second stage involves the analysis of human blood samples.The subjectswere divided into four groups.GROUP1(consist of 20 control only), GROUP2 (consist of 20 direct laborers of the mining site)GROUP 3 consists of 20 non-direct laborers of the mining site)GROUP 4 (consist of 20 inhabitants living close by).The bloodsamples were taken to the laboratory for liver function, kidney function (compared with reference values), C-reactive protein a marker for chain inflammation, antioxidant parameter, and acetylcholinesterase enzyme activities

2.3 Sample Collection

Blood samples were collected into plain tubes using a 5ml syringe. Where 5ml blood sample was obtained. The blood samples were centrifuged at 1500rpm for 15 minutes to obtain 2ml serum. The serum was then used to run the different tests for liver function, kidney function, antioxidant, cognitive impairment and C-reactive protein analysis.

2.4 Biochemical Assays

The separated serum was analyzed for various biochemical parameters which are AST, ALT, ALP, Total protein, Total bilirubin and Serum urea, all the analyses were performed usinga standard diagnostic test kit (Randox).

2.4.1 Estimation of Alkaline Phosphate (ALP)

The activity of ALP was determined using the method of Bowers and McComb (8).

Kinetic determination of alkaline phosphate (ALP) based upon the reactions

Alkaline phosphatase, the absorbance was then read at 405 nm

2.4.2 Estimation of Aspatate Aminotransferase

The activity of AST was determined using the Reitman and Frankel method as described by(9) and the absorbance was then read at 340 nm

2.4.3 Estimation of Alanine Amino Transferase (**ALT**)

The activity of ALT is determined using the Reitman and Frankel method as described by(9)**.** The absorbance was then read at 340 nm

2.4.4 Determination of Total Protein (TP): Biuret Method.

The determination of total protein is carried out using the colorimetric method described by Gornall (10) the peptide bonds of proteins react with Cu^{2+} in an alkaline solution to form a coloured complex which,

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absorbance proportional to the concentration of total protein in the specimen, is measured at 550nm. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintained their solubility in an alkaline solution.

2.4.5 Determination of Total Bilirubin: Sulfanilic acid method.

The total bilirubin was determined by the reaction between bilirubin and diazotized sulfanilic acid which leads to a compound, azobilirubin, coloured in acidic or basic medium (11),modified the Malloy-Evelyn principle in an aqueous solution, only direct bilirubin reacts. To enable the assay of total bilirubin, it is necessary to break the link between unconjugated bilirubin and albumin, this step is done by adding dimethylsulfoxide (DMSO). The absorbance of azobilirubin thus produced is directly proportional to the concentration of bilirubin and is measured at 550nm.

2.5 Determination of Urea

Method: Urease Berthelot method was used to determine the urea. Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by berthelots reaction. Absorbance was recorded at 550nm (530-580) against blanks

2.6 Assessment of Antioxidant

2.6.1Superoxide dismutase(SOD)

Superoxide dismutase (SOD) was determined by the method described by Fridovich (12).

Principle: The ability of superoxide dismutase (SOD) to inhibit the auto-oxidation of adrenalineat pH 10.2 forms the basis of this assay. The Absorbance was measuredat480nm.

2.6.2 Assay of Glutathione Concentration

Reduced glutathione (GSH) concentration measurements are done according to the method described byEllmanas described by(13).

Glutathione (GSH). The absorbance was read at 412nm.The quantity of GSH was obtained from the graph of the GSH standard curve.

2.6.3 Lipid Peroxidation (MDA)

Lipid peroxidation is evidenced by the formation of TBARS measured by the modified method of (14).

Principle: Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a productthat reacts with thiobarbituric acid. The product of the reaction is a complex coloured which absorbed light at 535nm and can hence be measured.

2.6.4 Catalase activity (CAT)

Catalase (CAT) activity was measured using Abeis method (15). Exactly 10uL of serum was added to a test tube containing 2.8mL of 50mM potassium phosphate (buffer pH 7.0).The reaction was initiated by adding 0.1mL of freshly prepared 30mM H_20_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5minutes on a spectrophotometer.

2.7 Determination of blood acetylcholinesterasee (AchE) level:

The analysis was carried out using the assay kit where, AchE is a serine hydrolytic enzyme, which is widely found in various animal tissues and serum. AchE catalyzes the hydrolysis of Ach, which plays an

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important role in the regulation of nerve conduction. AchE catalyzes Ach hydrolysis to generate choline, and choline reacts with 5,5′-dithiobis(2-nitrobenzoic acid) to form 5-mercapto nitrobenzoic acid (TNB).TNB had an absorption peak at 412nm, and AchE activity was calculated by measuring the absorbance increasing rate at 412nm.

2.8 Determination of C-reactive protein

C-reactive protein levels increase rapidly as a response to inflammation. The method used to analyzethe C-reactive protein level is the fluorescence immunoassay for in vitro analysis. A C-reactive protein assay kit will bewasused for the analysis and the protocol by the manufacturers was followed. In normal individuals, it is trace protein, but when there is inflammation, it increased rapidly (16).

2.9 **Statistical analysis**

Statistical analysis was performed by the use of SPSS version l8 (Statistical Package for the Social Sciences). The differences between the groups would be tested for significanceby aone-wayANOVA test. Data would be expressed as the mean ± SD. P-values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 The demographics of respondents

The result in table 1 revealed the response of 300 inhabitants of Opanda-Ugya. 61.7% were males and 38.3% were females. 68.3% are betweenthe ages of 18-28 years and 31.7% were between the ages of 29 and above years. From the data obtained 65.3% stated the observation of Parkinson-like syndrome diseaseswhereas 34.7% stated the observation of CVD. From the data obtained 81% are more exposed to the mining site than the 19% who are regular miners.

Table 1 Demographics of Respondents

S/N	Variable	N	%
	Sex		
	Male	185	61.7%
	Female	115	38.3%
	Gender Total	300	
	Age Group		
	18-28 Year	205	68.3%
	29year- Above	95	31.7%
	Graduate	196	65.3%
	Not In School	104	34.7%
	Religion		
	Muslim	243	81%

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Christian		57	19%

Total No of Respondents n = 300, Percentage of respondents = %

3.1.2 Heavy Metal Content of Water Samples Collected fromThree Mining SitesofOpanda-Ugya Community

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The results of water analysis for heavy metal content indicatesa high amount of Zn, Pb, Ni, Mn, Fe, Mg, and Cd beyond the permissible limit while Cu was observed to be below the permissible limit as shown in table 2 below.

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Table 2: The result of the heavy metal content of water samples collected from three mining sites in Opanda-Ugya community:

Sample Location	Sample ID	Cu (mg/l)	Zn (mg/l)	Pb (mg/l)	N1 (mg/l)	Mn (mg/l)	Fe (mg/l)	Mg (mg/l)	Cd (mg/l)
Dan china	A	0.381	3.196	BDL	2.052	5.614	0.371	2.181	3.412
	B	0.425	8.182	4.601	3.801	8.021	2.142	0.612	1.291
	C	BDL	1.060	2.261	1.921	2.491	4.012	3.011	4.001
Upper mango tree	M	0.681	5.010	0.51	1.721	3.112	0.186	1.450	6.019
	N	1.091	2.901	BDL	0.110	2.611	8.02	BDL	2.912
Across	R	0.218	0.611	6.091	4.012	0.620	10.111	5.021	BDL
WHO L	X	1.0	3.0	0.05	0.02	0.05	0.30	0.001	0.003

BDL= Below Detection Limit WHOL: World health organization permissible limit.

Abbreviations: Cu (copper), Zn (zinc), Pb (lead), Ni (Nickel), Mn (Manganese), Fe (Iron), Mg (Magnesium), Cd (Cadmium.Values are expressed directly and compared with WHO permissible limits of heavy metals in drinking water.

3.1.3 Effect of Mining Activities on Liver Function

Table3 below shows the effect of mining activities on liver function parameters. The mean and standard deviation values of ALT, AST, T.Bil, D.Bil, and TP levels for miners indirect miners, inhabitants and control are presented in the table below. The results revealed no significant difference ($P<0.05$) in all the liver function parameters compared to the control.

Table 3: Effects of mining activities on Liver Function

Parameters	Group 2	Group 3	Group 4	Control
ALT (U/L)	31.27 ± 10.06^{a}	34.64 ± 13.31^{b}	34.36 ± 7.92^{b}	49

AST (U/L)	34.91±12.05[b]	43.85±13.25[c]	31.99 ± 7.89[a]	46
T.Bil (□mol/L)	12.51 ± 3.70[a]	17.01 ± 5.67[c]	15.82 ± 4.22[b]	21
D.Bil (□mol/L)	5.65 ± 1.71[b]	7.76 ± 2.48[c]	4.33 ± 1.08[a]	3.4
TP (g/L)	75.16 ± 12.21[c]	71.81 ± 14.32[a]	73.53 ± 7.37[b]	87

Abbreviations: ALT (Alanine aminotransferase); AST (Aspartate aminotransferase); T.Bil (Total bilirubin); D.Bil (Direct bilirubin); TP (Total protein). Values are expressed as Mean ± SD (n = 20 in each group). Group 2 (direct miners); Group 3 (Indirect miners); Group 4 (Inhabitants of Opanda-Ugya community).Control: healthy participants not close to mining sites. [a,b,c]Denotes statistical significance across different groups at 0.05 level.

3.1.4 Effect of Mining Activities on Renal Function

The result in Table4 below shows that, the concentration of urea and creatine are within the normal range and not Significantly (P<0.05) different from the control.

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Table 4: Effects of mining activities on Renal Function

Parameters	Group 2	Group 3	Group 4	Control
Creatinine (□mol/L)	85.65 ± 13.97[a]	90.47 ± 12.86[b]	111.04 ± 21.27[c]	133
Urea (□mol/L)	5.55 ± 1.29[a]	5.38 ± 1.34[a]	6.47 + 1.21[b]	8.5

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Values are expressed as mean ± SD (n = 20 in each group).Group 1 (control: healthy participant not close to mining sites.) Group 2 (direct miners); Group 3 (indirect miners); Group 4 (Inhabitants of Opanda-Ugya community) [a,b,c]Denotes statistical significance across different groups at 0.05 level.

3.1.5 Effect of Mining Activities on Some Antioxidant Parameters

The results in table 5 show an increment in both SOD, and GPx activities in groups 2,3, and 4 when compared to the control, However, the GSH level decreased significantly (P<0.05) in all the groupswhen compared to the control, Finally the MDA level in all the groups was elevated significantly (P<0.05) when compared to the control.

Table 5: Effects of mining activities on Antioxidants

Parameters	Group2	Group3	Group4	Group1

SOD (mmol/l)	28.67 ± 6.38[c]	22.63 ± 5.00[a]	16.04 ± 4.04[b]	10.96±1.29
GPx(mmol/l)	83.93± 10.94[b]	81.10 ± 4.52[b]	67.53 ± 10.93[b]	24.76±2.96
GSH (mmol/l)	30.98 ± 3.78[b]	32.65 ± 2.53[b]	29.40 ± 2.40[b]	40.10±4.18[a]
MDA(mmol/l)	37.70 ± 2.58[b]	40.67 ± 0.49[b]	39.09 ± 2.04[b]	11.25±0.98[a]

Abbreviations: SOD (Superoxide dismutase); GPx (Glutathione peroxidase); GSH (Reduced glutathione); MDA (Malondialdehyde).Values are expressed as Mean ± SD (n = 20 in each group). Group 1 (Control: healthy participants not close to mining sites.) Group 2 (direct miners); Group 3 (indirect miners); Group 4 (Inhabitants of Opanda-Ugya community) [a,b,c]Denotes statistical significance across different groups at 0.05 level.

3.1.6 Effects of Mining Activities on C-Reactive Protein (CRP) and Acetylcholinesterase (Ache) activities

The results in Table 6 below revealed a significant (P<0.05) increase in both the CRP and AchE in all the groups (2,3,4) when compared to the control.

Table 6: Effects of mining activities on C-Reactive Protein (CRP) and Acetylcholinesterase (AChE)

Parameters	**Group2**	**Group3**	**Group4**	**Group1**
CRP (mg/L)	3.84 ± 0.74[a]	2.83 ± 0.71[b]	2.30 ± 0.38[c]	1.39±0.47
AchE (u/mL)	74.49 ± 7.22[b]	58.01 ± 10.48[a]	52.14 ± 12.22[a]	28.13±5.14

Abbreviations: CRP (C-Reactive Protein); AchE (Acetylcholinesterase). Values are expressed as Mean ± SD (n = 20 in each group). Group 1 Control: healthy participant not close to mining sites.Group 2 (direct miners); Group 3 (indirect miners); Group 4 (Inhabitants of Opanda-Ugya community)

[a,b,c]Denotes statistical significance across different groups at 0.05 level.

3.2 Discussion

Mining activities bring out heavy metals to the earth surface leading to heavy metals contamination and environmental pollution (1). The main threats to human health from heavy metals includes Cd, Pb, Cu, Zn, Ni, Cr, As, Fe, etc. These metals can damage central nervous system, cardiovascular and gastrointestinal, endocrine glands, and bones (17). Although heavy metal such as Fe, Cu, Zn, Mn, and

Mg, are required for physiological activities, high concentration can generate reactive oxygen species (ROS) which may lead to oxidative stress (OS)(17). Excess ROS are usually handled by the antioxidant system in the body (4). However, when the system is overwhelmed with ROS due to heavy metal toxicity, the excess ROS react with biological molecules such as Lipid, Proteins, DNA, and Carbohydrate These collectively causes OS (4). OS has been showed to initiate many pathological disorders such as cancers, neurological disorder, kidney damage, endocrine abnormalities etc. (4). Although OS is regarded as the initiator of inflammation as well as the consequence of inflammatory responses (6).

Result of water analysis for heavy metal content indicate high amount of Zn, Pb, Ni, Mn, Fe, Mg, and Cd beyond permissible limit while Cu was observed to be below permissible limit as shown in table 2. High amount of zinc above permissible levels have been shown to induce oxidative stress (18). Zn^{2+} overload has been suggested to be closely linked to neurodegeneration in Alzheimer's disease (20). The neurodegenerative disease symptom seen in the occupant of Opanda-ugya could be attributed due to the accumulation of Zn^{2+}, Zn accumulation has seem shown to course neuronal death in animal model (21).

The low copper level observed is beneficial to health (22). Typically drinking water contains copper, which is naturally occurring ingredient. copper leaching can be accelerated by water characteristic such as increase acidity, temperature, acidic nature of water and decreased hardness (23).

An acute exposure to significant higher Cd and Fe levels can lead to a variety of negative health effects including: Diarrhea, Vomiting, Fever, Lungs damage, muscle pain (24). It continues intake apparently lead to diseases like kidney disorder, bone damage, reproductive problem and possibly cancer (25).

Parkinson-like syndrome seen, which include weakness, anorexia, muscle pain, apathy, slow speech monotonous tone of voice may be attributed to the high levels of Mg and lead (26).

The lead level observed indicated that Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects (27). Lead is known to interfere with a number of body functions and it is primarily affecting the central nervous, hematopoietic, hepatic and renal system producing serious disorders (28). These can be much more severe if not treated on time and this toxicity is characterized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma (27). Nervous system appears to be the most sensitive and chief target for lead induced toxicity which could be attributed to neurological disorder seen in the occupant of the host community (29). More severe manifestations occur at very high exposures which include lack of coordination, convulsions, paralysis, coma and ataxia (30).The mysterious death been observed within Opanda-Ugyay could be as a result of high level of lead, this is because Both chronic and acute lead poisoning causes cardiac and vascular damage with potentially lethal consequences including hypertension and cardiovascular disease (31).

From the result There were statistically insignificantly ($P<0.05$) changes in the mean values of liver parameters of all the groups (2,3 and 4) when compared to the control. The concentration of ALT is usually higher than that of AST In a normal individuals as well as most patients with liver disease, mainly because ALT is present in the cytoplasm which is released by minor injuries, and AST is usually cleared rapidly due to its shorter half-life (31). This maybe a contributing factor to the decline in AST level observed in group 4. Increased serum AST and ALT concentrations due to exposure to heavy metals may promote hepatocellular damage (32).but, it does not necessarily correlate with the extent of the damage (32). The increase in the level of direct bilirubin may be associated with hepatic and post-hepatic injury.The overall reduction in the total protein levels in both the Group 2 and group 4 of Opanda-Ugya community as presented in the study may be due to inhibition of protein synthesis as a result of exposure to heavy metals. It was reported that treatment with Cd can alter the biosynthesis of protein. The results of the present study indicated that both the group 2 and group 4 may be at risk for hepatocellular injury. The relationship between exposure to heavy metals and the resulting hepatocellular injury have been studied extensively in both human and animal subjects (33), and the results agreed with our findings. Although the mechanism of heavy metals hepatotoxicity is still not clear, The OS resulting from overproduction of ROS due to exposure to heavy metals is an important factor in the pathophysiology (34).This causes an imbalance between the synthesis and degradation of enzymatic proteins, thereby releasing liver enzymes in the blood stream because of hepatic necrosis (34).

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from table 4 above a significant decreased in the levels of urea in all groups (2,3,4) was observed compared to control, decreased urea concentration is seen associated with kidney diseases and congestive heart failure (35). The findings from the present study also suggested that inhabitants of Opanda-Ugya community may be associated with high risk of nephrotoxicity and the creatinine has not necessarily increased with time in the very elderly, even in those with mild azotemia (36). This may be due to long-term exposure to heavy metals through drinking water, inhalation of polluted air and malnutrition as a result of the mining activities within the community. A variety of heavy metals are nephrotoxic (37).((37),reported that the relationship between toxic metal exposure and kidney damage was historically characterized in occupational populations with high exposures and in communities exposed to pockets of high levels of naturally-occurring environmental metals. accidental or occupational exposure to several metals may cause renal toxicity and the magnitude of kidney impairment depends on the nature, the dose, and the time of exposure (38) These results are consistent with the findings in the present study muscle function and activity may also alter creatinine levels, This may be the possible reason for the decreased creatinine levels seen. Recent studies have recognized OS, apoptosis, and necrosis as common phenomena in the intracellular action of all toxic metals studied thus far (39). The exposure of individuals to the mining sites resulted in marked OS as indicated by significant increase in the level of MDA and concomitant decreased in the levels of GSH in all groups as compared to the control. Lipid peroxidation is used as a standard for metal-induced OS, and under pathological conditions such as atherosclerosis and ischemia, oxidation by means of ROS or other free radicals of polyunsaturated fatty acids is thought to be exacerbated by the presence of divalent metal ions (1). Upon interaction with copper, chromium, nickel, and cadmium, metals and their chelate complexes are believed to play an active role in lipid peroxidation and in the promotion of carcinogenesis (40). The reduction in GSH level could be as a result of interaction of MeHg with the thiol group of GSH which increases excretion of MeHg-GSH conjugate complex and it has been reported that an intimate association between decrease in the GSH level and occurrence of OS (40). Similarly, lead inactivates enzymes like δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GP_X) and glutathione-S-transferase, which further depresses the glutathione levels (41). However, the activity of SOD and GPx was significantly ($p<0.05$) increased in all the groups as compared to the control. This increase in the enzyme activity could be as a result of the presence of metals which serves as cofactors of the enzymes; since in humans superoxide dismutase exists in three isoforms, cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD); and Se-GPx (1). More so, it could be that absence of Pb at this mining site since antioxidant enzymes such as GPx, CAT, and SOD depend on trace elements and prosthetic groups to accomplish the enzymatic detoxification of ROS and they are potent targets to Pb toxicity by hampering the activity of antioxidant enzymes, which is implicated in Pb induced oxidative damage (41). It is well established that AChE inhibition is a useful biomarker for the effects of environmental contaminants on AChE activity (42). The enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine to acetate and choline at the cholinergic synapses, terminating nerve impulse transmission (43). There is evidence of the interaction between heavy metals as mercury and lead and the etiology of neurodegenerative diseases, since many of these metals can cross the blood brain barrier and accumulate in the brain, promoting the generation of OS and alterations in the metabolism of some proteins associated with the development of neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis (44)[. Studies have also shown that cadmium and zinc are toxic and possess an inhibitory effect on the acetylcholinesterase activity (45). This study demonstrated that exposed of all groups showed increased in the blood AChE activity as compared to control collected from the control individuals. This could be that the OS generated by this metal might have caused damage to the cholinergic system, with acetylcholine (ACh) which is involved in cognitive processes, through the activation of metabotropic muscarinic and ionotropic nicotinic cholinergic receptors (46). In this study CRP of both group 2 and group 3 elevated dramatically. The plasma half-life of CRP is constant under all conditions of health and disease (36).CRP levels increases with acute or chronic inflammation (47). In the absence of inflammation, it is not constitutively expressed and its level is undetectable (48). Elevated serum level of CRP is a strong independent predictor of cardiovascular disease in asymptomatic individuals (48). It is evident from the present study that the group 2 and group 3 may be at risk for inflammation. Inflammation is important in all phases of heart disease, including the early initiation of atherosclerotic plaques within the arteries, as well as the acute rupturing of these plaques that results in heart attack (48). Heavy metals such as cadmium, lead or mercury can trigger different diseases, especially CVDs(48). Although little is known about the

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relationship between CRP and heavy metals exposure, it may be a key mediator in the process (45). CRP levels increased in a continuum fashion with increasing OS and atherogenic factors (49), which established a relationship between inflammation, OS, and atherogenesis in human essential hypertension (49). Coupled with other findings from table 6, the elevated CRP levels support the indication of risk for cardiovascular diseases among group 2 and group 3. CRP has also been associated with various chronic inflammatory processes, such as certain rheumatologic conditions and cancer (49). Finally, it was also observed from the present study (table 6) that, The CRP may also contribute to atherosclerotic processes and can increase the expression of adhesion molecules such as vascular cell adhesion molecules and intercellular adhesion molecules in vascular endothelial cells including endothelial cells in human brain (50). Also the increase in CRP indicates that the individuals are at risk of inflammatory diseases (51). However, there was a non-significant ($p>0.05$) increase of CRP level in group 4 which is an indication that these individuals are at low risk of inflammatory diseases but can be affected over a long period of exposure.

REFERENCES

1. Arif, T. J., Mudsser, A., Kehkashan, S., Arif, A., Inho, C. and Qazi, M. R. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants, *International Journal of Molecular Sciences*, 16: 29592–29630.
2. Singh MR. Impurities-heavy metals: IR prespective. 2007. [Last cited on 2009 Aug 10]. Available from: http: //www .usp.o rg/p df/EN/me etings/asMeeti ngIndia/20 08Sessio n4track1 .pdf
3. Lambert M, Leven BA, Green RM. (2000). New methods of cleaning up heavy metal in soils and water. Environme ntal science and technolo gy briefs for citizens. Kansas State University, Manhattan, K S.
4. Muhammad, S., Shah, M.T., and Khan, S. (2015).Heavy metal concentrations in soil and wild plants growing around Pb-Zn sulfide terrain in the Kohistan region, northern Pakistan. *Microchem J*., 99, 67– 75.
5. Mudgal V., Madaan N., and Mudgal, A., Singh R, Mishra S. (2010)Effect of toxic metals on human health. *Open Nutraceut J*.. 3, 94– 99.
6. Guzik, T.J., and Touyz, R.M. (2017). Oxidative Stress, Inflammation, and Vascular Aging in Hypertension. Hypertension, 70, 660–667. [Google Scholar] [CrossRef]
7. Vallabhapurapu, S., and Karin, M. (2009). Regulation and function of NF-kappaB transcription factors in the immune system. Annu. Rev. Immunol., 27, 693–733. [Google Scholar] [CrossRef]
8. Bowers G, Mccomb R. (1975). Measurement of total alkaline phosphatase activity in human serum. DOI:10.21275/v5i4.nov162819.
9. Varley H, Gowenlock A.H, Bell m. (1980). Practical Clinical Biochemistry, Vol.1(5th ed.), pp.741-742, 892-908. W. Heinemann, London.
10. Gornall AG, Bardawill CJ, David MM. (1949). Determination of serum proteins by means of the biuret reaction. J Biol Chem; 177(2):751-66. PMID:18110453.
11. Walters M.I, Gerarde H.W (1970). An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma; https://doi.org/10.1016/0026-265X(70)90045-7.
12. Fridovich I. (1989). Superoxide dismutase's. J Biol Chem;264(14):7761-17764 DOI:10.1016/S0021-9258(18)83102-7
13. Rajagopalan R, Kode A, Penumatha SV, Kallikat NR, Venugopal PM (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. J. Pharm. Parmaceut.sci.83:2747-2752.
14. Niehaus, W.G and Samuelelsson, B. (1968) Formation of Malondialdehyde from phospholipid Arachidonate during Microsomal lipid peroxidation. European J.Biochem,6 126-130. https://doi.org/10.1111/j.1432-1033.tb00428.x
15. Aebi H. (1984). Catalase in vitro. Methods Enzymol; 105:121-6.DOI:10.1016/s0076-6879(84)05016-3.PMID:6727660.
16. Povoa P, Coelho L, Almeia E, FernandesA, Mealha P, Sabino H. (2005). C-reactivity protein as a marker of ventilator-associated pneumonia resolution: a pilot study. Eur Respir J;25(5): 804-12.doi:10.1183/09031936.05.00071704.PMID15863636.
17. Narjala R.J (2020) Heavy metal sources and their effects on human health., DOI:10.5772/intechopen.95370.
18. Flora, S.J.S. (2009). "Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure," *Oxidative Medicine and Cellular Longevity*, vol. 2(4), 191–206.

19. Yuan, Y., Niu, F., Liu, Y., and N. Lu, (2014). “Zinc and its effects on oxidative stress in Alzheimer’s disease,” *Neurological Sciences*, 35(6),. 923–928.
20. Clausen, A., McClanahan, T.S., Ji, G., and Weiss, J. H. (2013) “Mechanisms of rapid reactive oxygen species generation in response to cytosolic Ca^{2+} or Zn^{2+} loads in cortical neurons,” *PLoS One*, 8(12), article e83347.
21. Araya M, McGoldrick MC, Klevay LM, Strain JJ, Robson P, Nielsen F, Oliveres M, Pizarro F, Johnson L, Poirier KA (2001) Determination of an acute no-observed-adverse-effect level (NOAEL) for copper in water. Regul Toxicol Pharmacol 34(2):137-145.
22. Casper J (2017) copper toxicity and self-evaluation. Retrieved April 20,2017, from Nutritional Balancing.org:
23. Nordberg GF.(2004) Cadmium and health in the 21st century-historical remarks and trends for the future.Biometals 17(5)485-489.
24. Barbier O, Jacquillet G, Tauc M, Cougnon M, Poujeol O.(2005). Effect of heavy metals on and handling by the kidney. Nephron physiology 99,105-10.
25. Mergler D. et al.(1994). Nervous system dysfunction among workers with long term exposure to manganese. Environmental research, 64:151-180.
26. Pearce J.M.S (2007) Burtons line in lead poisoning, Eur Neurol;57: 118-119
27. Kalia, K., and Flora, S.J. (2005). Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. *J Occup Health.,*47:1–21.
28. Cory-Slechta, D.A. (1996). Legacy of lead exposure: consequences for the central nervous system. Otolaryngol Head Neck Surg. 114, 224–226.
29. Flora, S.J.S., Flora, G., and Saxena, G. (2006). Environmental occurrence, health effects and management of lead poisoning. In: José S. C, José S, editors. Lead. Amsterdam: Elsevier Science B.V. 158–228.
30. Navas-Acien, A., Guallar, E., Silbergeld, E.K., and Rothenberg, S.J. (2007). Lead exposure and cardiovascular disease--a systematic review. Environ Health Perspect.115, 472–482.
31. Mittal S, EL-Serag HB,Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. (2016) Hepatocellular carcinoma in the absence of cirrhosis in united states veterans is associated with nonalcoholic fatty liver disease. Jan(1):124-31
32. Abu-El-Zahab, Helal S.H, Reham Z.H, Metwally M.M, Magda M. EL., Wed a. AL-Harthi (2019) Antioxidant, antiapoptotic, antigenotoxic, and hepatic ameliorative effects of l-carnitine and selenium on cadmium-induce hepatotoxicity and alterations in liver cell structure in male mice; https://doi.org/10.1016/j.ecoenv.2019.02.041
33. Jagadeesan G and Pillai S.S (2007) Hepatoprotective effects of taurine against mercury induced toxicity in rats. 28(4)753-756.
34. Gwoda S, Desai PB, Kulkarni SS, Hull VV, Math AAK, Vernekar SN,(2010) Markers of renal function tests. North AmJ Med Sci;2:170-173
35. Fehrman-Ekholm I,Skeppholm L. (2004)Renal function in elderly(›70 years old) measured by means of iohexol clearance, serum creatinine, serum urea and estimated clearance. Scand J Urol Nephrol;38:73-7.
36. Emily C.M, Steven C, Alison P.S. (2018) Toxic metals and chronic kidney disease; DOI:10.1007/s40572-018-0212-1
37. Paolo angeli and Florence Wong (2016), new Diagnostic criteria and management of acute kidney injury ;So168-8278(16)30618-3
38. Sobolic I,Herak-Kramberger CM, Antolovic R, Brown D, (2006) Loss of basolateral invaginations in proximal tubules of cadmium-antoxicated rats is independent of microtubules and clathrin. 218:149-163.
39. Kaur, P., Aschner, M. and Syversen, T. (2006). Glutathione modulation influences methylmercury induced neurotoxicity in primary cell cultures of neurons and astrocytes. *Neurotoxicology*, 27, 492–500.
40. Ahamed, M. and Siddiqui, M. K. J. (2007). Low level lead exposure and oxidative stress: Current opinions. *Clinical Chemistry Acta*, 383: 57–64.
41. Frasco, M. F., Fournier, D., Carvalho, F. and Guilhermino, L. (2005). Do metals inhibit acetylcholinesterase (AChE)? Implementation of assay conditions for the use of AChE activity as a biomarker of metal toxicity. *Biomarkers,* 10(5), 360-375.
42. Bainy, A.C.D., Gennari de Medeiros, M.H., Di Mascio, P. and de AlmeidaIn E. A. (2006). vivo effects of metals on the acetylcholinesterase activity of the Perna perna mussel’s digestive gland. Revista Biotemas, 19 (1), 35-39.

43. Monnet-Tschudi, F., Zurich, M. G., Boschat, C., Corbaz, A. and Honegger P. (2006). Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Review in: Environmental Health,* 21(2), 105–117.
44. Sunita, R., Rajender K. G. and Jyoti, Y. (2017). Heavy metal induced alterations in acetylcholinesterase activity of Indian major carps. *Journal of Entomology and Zoology Studies*, 5(4): 818-82.
45. Richetti, S. K., Rosemberg, D. B., Ventura-Lima, J., Monserrat, J. M., Bogo, M. R. and Bonan, C. D. (2011). Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. *NeuroToxicology*, 32: 166-122.
46. Ost M, Nylen K, Csajbok L, Olsson Ohrfelt A, Tullberg M, Wikkelso C, Nellgard P, Rosengren L, Blennow K, Nellgard B, (2006). Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury.
https://doi.org/10.1212/01.wnl.0000242732.06714.0f
47. Chandrashekara Srikantiah (2014), C-reactive protein: An inflammatory marker with specific role in physiology, pathology, and diagnosis: DOI:10.1530/ijrci/v2iS1/117.
48. Rottenberg H., Hoek J.B. The path from mitochondrial ROS to aging runs through the mitochondrial permeability transition pore. Aging Cell. 2017;16:943–955.
49. Ferri, C., Croce, G., Cofini, V., De Berardinis, G., Grassi, D. and Casale, R. (2007). C-reactive protein: interaction with the vascular endothelium and possible role in human atherosclerosis. *Current Pharmacological Design*, 13, 1631–45.
50. Pepys, M. B. and Hirschfield, G. M. (2003). C-reactive protein: a critical update. *Journal of Clinical Investigation,* 11, 1805–12.