Banana peel ameliorated hepato-renal damage and exerted anti-inflammatory and anti-apoptotic effects in metal mixture mediated hepatic nephropathy by activation of Nrf2/ Hmox-1 and inhibition of Nfkb pathway

Boma F. Eddie-Amadi[1], Anthonet N. Ezejiofor[2], Chinna N. Orish[3], Joaquim Rovira[4,5], Theodore A. Allison[3] and Orish E. Orisakwe[1]*

1 World Bank Africa Centre of Excellence in Oilfield Chemicals Research (ACE-CEFOR), University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

2 African Centre of Excellence for Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

3 Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria.

4 Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain

5 Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain

*Correspondence author: Orish E. Orisakwe. African Centre of Excellence for Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, PMB, 5323 Port Harcourt, Choba, Nigeria. Email: orishebere@gmail; orish.orisakwe@uniport.edu.ng.

Abstract

This study evaluated the protective role of banana peel extract (BP) on heavy metal mixture (HMM) mediated hepatorenal toxicity using a rat model. Twenty-five female Wistar albino rats were weight-matched and divided into five groups of five female rats each. Group 1(control) received deionized water only. Group 2 received HMM only (20 $mg{\cdot}kg^{-1}$ of Pb, 0.40 $mg{\cdot}kg^{-1}$ of Hg, 0.56 $mg{\cdot}kg^{-1}$ of Mn and 35 $mg{\cdot}kg^{-1}$ of Al). Groups 3, 4 and 5 were co-administered with the same metal mixture with BP at 200, 400 and 800 $mg{\cdot}kg^{-1}$, respectively. Treatments were through oral gavage for 60 days; animals were sacrificed pentobarbital anesthesia and liver and kidney harvested for test. Thereafter, metal levels, malondialdehyde (MDA) and nitric oxide (NO), catalase (CAT), glutathione content (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx), interlukin-6 (IL-6) and tumor necrosis alpha (Tnf-α), caspase-3 (Cas-3), Nuclear factor kappa B (Nfkb), Nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (Hmox-1) were assayed. HMM group presented higher levels of metal, IL-6 and Tnf-α, MDA, NO, Nfkb and Hmox-1 in HMM group which were significantly reduced by BP. BP was protective against metal mixture induced histopathological distortions. HMM exposure significantly distorted hepatorenal functions and BP treatment reduced metal bioavailability and abrogated most of these alterations.

Key Words: *Banana*, metal mixture toxicity, hepatorenal functions, oxidative stress, inflammation, Programmed cell death.

1. INTRODUCTION

Civilization has come at a great cost with indiscriminate extraction, use and application of metals in diverse sectors of the economy like, mining, production industries, agriculture, healthcare, cosmetics, and domestic-sectors with accompanying environmental (soil, water, air, food) pollution and the inevitable health adverse effects. Oxidative stress is a principal cellular response characterized an imbalance in the redox system that has been linked to the macromolecular damages and activation of several cell survival and cell death pathways. There is overwhelming evidence (epidemiological and experimental) that have implicated oxidative stress-induced cellular response accompanying and heightened risk of cancer, neurodegeneration, metabolic disorders, male and female infertility, developmental disorders, renal failure, and cardiovascular disease, among others (Hallauer et al., 2016; Zhao et al., 2017; Yang et al., 2020).

Reactive oxygen species (ROS) generation is a common feature modulated by the cellular antioxidant system in normal body physiology. Enzymatic antioxidants such as, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and non-enzymatic antioxidants such as, glutathione (GHS), maintain redox homeostasis by repressing the generation of ROS. The enzymatic antioxidants SOD (SOD a metalloenzyme containing Cu/Zn or Mn/Fe., catalyzes the dismutation of superoxide radicals ($^{\cdot}O_2^-$) into oxygen (O_2) and hydrogen peroxide (H_2O_2)). CAT and GPx cause the decomposition of H_2O_2 to water and O_2. However, when ROS generation overwhelms the antioxidant system, there is heightened oxidative stress which lead to destruction and dysregulation of cellular macromolecules and cellular events, namely cell division, cell cycle, immune response, and an increase in cell death (Hrycay and Bandiera, 2015; Canli et al., 2017; Habtemariam, 2019; Elzagallaai et al., 2020). Exaggerated oxidative stress is a principal underlying phenomenon of the pathogenesis of many chronic diseases, such as cancer, diabetes, neurodegeneration, asthma, inflammation, development, reproductive and hepatorenal disorders (Wang et al., 2017c; Choudri and Charabi, 2019; Habtemariam, 2019). The disproportion in cellular redox homeostasis is one of the primary aftermaths of metal exposure and its associated toxicity (Tchounwou et al., 2012; Karri et al., 2016). There is an indication that ROS generated by metal exposure may be an upstream activator or a downstream effector of different cellular signaling (Paithankar et al. 2021). Understanding how metal-induced cellular response due

to the interaction of ROS and signal transduction have become the mainstay of research lately. Some previous studies in Nigeria have confirmed the presence of these metals lead (Pb), mercury (Hg), manganese (Mn) and aluminum (Al), among others in foods and beverages (Maduabuchi et al. 2008, Roberts and Orisakwe 2011). Furthermore, the metals of interest in this study have been predominant in electronic waste (e-waste), which has negatively impact on environment in Nigeria (Orisakwe et al. 2019, Frazzoli et al. 2022).

There is revived interest towards the identification and utilization of natural products for the prevention of diverse health problems. Banana (*Musa acuminata*) is a low-cost agricultural produce and an edible fruit usually consumed fresh when it is ripe or boiled as unripe in tropical countries where it grown as a common food and cash crop (Mohapatra et al., 2010). Around 35-40% of total weight is banana peel and usually is discarded as waste. Banana peel is rich dietary fiber and used in treating various diseases (Azarudeen and Nithya 2021). Low levels of toxic substances include hydrogen cyanide (1.3 mg/g) and oxalates (0.5 mg/g) are found in banana peels within the safety limits (Anhwange et al., 2009) suggest that banana peels are safe for human consumption and contain valuable functional ingredients.

The phenolic rich constituents of banana peel extract possess antioxidant capacity by scavenging of ROS due to the presence of an electron-rich aromatic ring system (Flôres et al., 2010; Mahieu et al., 2009b, Zhuang et al 2017). In addition, decrease oxidative stress in traumatic brain injury of mice through activation of Nrf2 to stimulate antioxidant enzymes (Ke et al., 2014; Onk et al., 2018).

In view of the foregoing, we hypothesized that banana peel could attenuate metal-induced hepatorenal toxicity through regulating oxidative stress and apoptosis, which is involved in activation of Nuclear factor erythroid 2-related factor 2 (Nrf2). In this study, the hepato-renal functions, oxidative stress markers, apoptosis key factor and signaling pathway crucial factors were evaluated to ascertain the protective effect of banana peel extract on metal-induced hepatic-nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Chemicals, reagents and banana peel extracts

Metals (Lead acetate, aluminum chloride, mercury chloride, and manganese dichloride) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tumor necrosis factor alpha

(Tnf-α), interleukin 6 (IL-6), Caspase-3 (Cas-3), nuclear factor erythriod 2- related factor 2 (Nfr2), Nuclear factor kappa B (NfkB) and Heme oxygynase-1 (Hmox-1) Rat ELISA Kit were purchased from Elab science Biotechnology Company, (Beijing, China). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Ripped banana fruits (*Musa Cavendish*) were purchased from International Institute of Tropical Agriculture (IITA) (Ibadan, Oyo State, Nigeria) and identified by IITA. Freshly ripe banana fruit peels were thoroughly washed and dried in room temperature, pulverized using laboratory blender and sieved. One hundred grams (100g) of this banana powder was weighed and 500mL of deionized water was added, mixed, and filtered with Whatman No.1 filter paper (GE GmbH, Freiburg, Germany) and stored frozen (Wohlt, et al., 2021).

2.2 Animals and treatments

A total number of 25 white female albino Wistar rats aged between 6-8 weeks were purchased from the Department of Pharmacology, Animal House, University of Port Harcourt, Rivers State. Animals were housed in standard polypropylene cages under room temperature 25±2 °C with a 12-h light/dark cycles throughout the duration of the experiment. Prior to the beginning of the study, the animals were acclimatized for two weeks. The experimental animals were weight matched and divided into five groups of five animals each. Treatments were through oral gavage for 60 days. First two groups were Group 1 (Control), with deionized water only and Group 2 (HMM) with a metal mixture (HMM) (20 $mg \cdot kg^{-1}$ body weight of Pb (Institoris et al., 2006); 0.40 $mg \cdot kg^{-1}$ of Hg (Institoris et al., 2006); 0.56 $mg \cdot kg^{-1}$ of Mn (Yuan et al., 2012); and 35 $mg \cdot kg^{-1}$; of Al (Su et al., 2017)). Group 3, 4 and 5 (HMM + low BP, HMM + medium BP; HMM + high BP, respectively) were the same than Group 2 (HMM) with 200, 400 and 800 $mg \cdot kg^{-1}$ body weight of banana peel extract (BP) (Akamine *et al.*, 2009). Rats were weighed weekly and daily feed and fluid intakes were recorded. After 60 days of treatment, animals in each group were euthanized under pentobarbital (50mg/kg), IP anesthesia. The liver and kidney of each rat were harvested, rinsed in cold saline water, weighed, and used for both biochemical parameters and heavy metal analyses.

The ethical approval was obtained from the University of Port Harcourt institutional Centre for Research Management and Development Animal Care and Use Research Ethics Committee (UPH/PUTOR/REC/12). The experiment was conducted in accordance with the

"Guide for the Care of Laboratory Animals" approved by the National Academy of Science (NAS). The animals received standard feed and deionized water *ad libitum*.

2.3 Metal analysis

Twenty milligrams of liver and kidney samples were digested separately using 2 ml of perchloric acid and 6 ml of nitric acid. Afterwards, the samples were shaken for 30 min at room temperature before heating at 105°C until digestion was completed. The solution was made up to 15 ml with deionized water. Solar thermo elemental flame Atomic Absorption Spectrometer (Model SG 71906) was used to determine lead (Pb), aluminum (Al), mercury (Hg) and manganese (Mn) concentrations (Okoye et al 2021). The analytical procedure was checked using spike recovery method (SRM). For this reason, a known standard of elements was spiked into already analyzed samples and reanalyzed. High purity multi-cathode lamp (1000 mg/kg) from Cambridge CB5 8BZ (UK) was used to obtain the calibration curves for each metal. The multi element calibration curves were verified with a multi-element certified material of 1000 mg/kg (Cambridge CB5 8BZ, UK). The percentages of recovery varied between 96.5 and 100%. The relative standard deviation between replicate analyses was less than 4%. The limits of detection (LoD) were 0.001 for Al, Hg and manganese Mn, and 0.01 mg/kg for Pb, while the limits of quantification (LoQ) were 0.0033 for Al, Hg and Mn concentrations and 0.033 mg/kg for Pb.

2.4 Biochemical, inflammatory and antioxidant analyses

Twenty milligrams of liver or kidney samples were homogenized separately in phosphate buffer saline (pH=7.4). The supernatants were collected after centrifugation (3000 rpm for 15mins at 4°C) for biochemical, inflammatory and antioxidant analyses. All experiments were conducted in triplicate.

For renal function test, kidney integrity markers sodium (Na), creatinine (Cr), chloride (Cl) and bicarbonate levels as well as potassium (K) and urea (Ur) levels were performed according to manufacturer's manual using Randox Kits from Randox Laboratories Limited (UK). To measure liver function, analysis of tissue activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), albumin level (ALB) and total protein (TP) were performed using Randox Kits from Randox Laboratories Limited (UK) according to manufacturer's specifications. To assess the oxidative stress (lipid peroxidation) in liver and kidney, the malondialdehyde (MDA) level

(lipid peroxidation marker) was assayed using the procedure of Ohkawa and Ohishi (1979). In this technique, MDA reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA) under acidic medium to produce a pink colored complex at 532 nm absorbance. Nitric oxide (NO) level was assayed according to the method described by Green et al., (1982).

The determination of the antioxidant capacity is described briefly below. Glutathione peroxidase (GPx) activity was assayed according to the method of Rotruck *et al.* (1973). Reduced glutathione (GSH) levels were assessed using the technique illustrated by Jollow *et al.* (1974). Catalase (CAT) activity was evaluated using the technique of Clairborne (1995) with slight modification. This technique is buttressed with the principle that catalase in the sample will split hydrogen peroxide which can be estimated at 240 nm using a spectrophotometer (Clairborne, 1995). Superoxide dismutase (SOD) activity was estimated with the technique previously illustrated by Misra and Fridovich (1972). This technique is based on the principle that at pH=10.2, SOD has the capacity to inhibit the autoxidation of epinephrine. Finally, **t**he content of the inflammatory cytokines including Tnf-α, IL-6, and Hmox-1, apoptotic marker (Cas-3), and transcription factors Nf-kB and Nfr2 in the homogenized renal cell supernatant was detected using commercially available ELISA kits following the manufacturer's instructions.

2.5 Histopathological examination of liver and kidney.

The liver and kidney tissues for histological studies were fixed in paraformaldehyde (4%) and embedded in paraffin. The tissues were sliced into 4-μm sections and subjected to hematoxylin and eosin (H&E) staining using standard procedures. The histology of the stained tissues was evaluated under a light microscope and the histopathological features recorded by pathologists who were blinded to the experimental groups.

2.6 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Microsoft Xlstat 2014 was used in performing Analysis of Variance and Tukey multiple comparison pairwise tests to check if the concentration of the biomarkers was significantly different between groups. Pandas was used in obtaining the descriptive statistical parameters (biomarkers and metals mean concentrations) for the various rat organs. Seaborn and Matplotlib were used in plotting all graphs. The data analysis involved performing descriptive statistics on the metals and biomarkers concentration before ANOVA was used to establish if there was significant

difference in the concentration of the heavy metals and biomarkers among groups. All significant differences were at a $p<0.05$. Pearson R correlation was used to understand the relationship among biomarkers.

3. RESULTS

3.1 Effect of banana peel extract on the body weight, absolute and relative weight of liver and kidney, feed and fluid intake

Table 1 shows the effect of banana peel extract on the body weight, absolute and relative weight of liver and kidney, feed and fluid intake in female albino rats exposed to heavy metal mixture. There were no significant changes in the absolute and relative weights of kidney, feed and fluid intakes between the control and the heavy metal mixture HMM only treated group. There were also no significant changes in these parameters when the HMM only treated group was compared with the banana peel treated groups. There were however significant differences ($p< 0.05$) in the absolute and relative weights of the liver between the control and HMM only treated group. HMM + high dose BPE showed significant difference in the absolute and relative weights of the kidney and liver when compared both the control and the HMM only treated groups. All groups showed increase in percent weight gain.

3.2 Metal accumulation in the liver and kidney.

There were no significant changes in the body weight gain, feed and fluid consumption, and relative weights of liver and kidney between the control and the HMM group plus banana peel treated groups at the end of sixty days of the study. There were significant changes in the body weight gain, feed and fluid consumption, and relative weights of liver and kidney between the control and the MM only group treated groups.

The concentrations of metals (Pb, Hg, Mn, and Al) in the liver and kidney (Figure 1) were markedly increased in metal mixture treated rats compared to the control, which presented metal levels below detection limit in liver and kidney. However, the co-administration with banana peel extracts significantly reduced the levels of these heavy metals in a dose dependent fashion with a more significant reduction of 79%, 74%, 52% and 65% observed in high dose (800 $mg \cdot kg^{-1}$) of banana peel extract for Hg, Mn, Pb and Al, respectively.

3.3 Liver and kidney function markers.

The hepatic AST and ALT levels were non-significantly ($p>0.05$) higher in heavy metal mixture HMM group in comparison to the control group. However, for all of three banana peel treatments, AST and ALT hepatic levels showed levels similar to the control group. The 800 $mg \cdot kg^{-1}$ body weight of banana peel extract (BP) group significantly ($p<0.05$) reduced the hepatic ALP level. Albumin and T-protein concentrations were not significantly ($p>0.05$) different between the groups. There were significant differences in AST and ALT (Fig 2a) between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant reduction in the HMM mediated increases in ALT, AST.

There were significant differences in Na and Cr (Fig 2b) between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant reduction in the HMM mediated increases in Na and Cr.

There were significant differences in conjugated bilirubin (CB) and Ur (Fig 2c) between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel. Banana peel extract caused significant reduction in the HMM mediated increases in CB and Ur. There was significant difference in K (Fig 2c) between the HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant reduction in the HMM mediated increases in K.

3.4 Antioxidants profile in liver and kidney

Figure 3 shows the antioxidant activities SOD, CAT, GSH and GPx in the liver and kidney of control and rats exposed to HMM alone or in combination with Banana peel extract. In Fig 3a banana peel extract caused significant increase in liver and kidney SOD following HMM only mediated decrease. There was significant difference in the liver and kidney catalase CAT (Fig 3b) between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant increase in the HMM mediated decrease in CAT. Banana peel extract caused significant reduction in the HMM mediated increases in

glutathione peroxidase GPx. There was significant difference in kidney GSH (Fig 3c) between the HMM only group and control, HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant increase in the HMM mediated decrease in kidney GSH. There was significant difference in liver and kidney GPx (Fig 3d) between the HMM only group and control, HMM only and medium banana peel, HMM only and high banana peel treated groups. Banana peel extract caused significant increase in the HMM mediated decrease in kidney GSH. Medium dose (400mg/kg) and high dose (800mg/kg) Banana peel extract significantly ($p<0.05$) increased the liver GPx (Fig 3d).

3.5 Lipid peroxidation and oxidative stress profile in liver and kidney

To assess the oxidative state of the liver, MDA and NO levels were evaluated. After the metal mixture exposure for sixty days, significant ($p<0.05$) increase in MDA levels in kidney and NO level in kidney and liver were observed of metal mixture-treated rats compared to the control (Figure 4). There was significant ($p<0.05$)difference in liver MDA (Fig 4 a) between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel. Banana peel extract caused significant ($p<0.05$) reduction in the HMM mediated increases in liver MDA. Conversely, rats co-treated with Banana peel extract at different doses revealed a significant ($p<0.05$) decrease in NO levels in liver and kidney when compared to the metal mixture exposed rats. MDA levels shown similar profile being however differences were only significant ($p<0.05$) in kidney between the HMM only group and control, HMM only and low banana peel, HMM only and medium banana peel, HMM only and high banana peel.

3.6 Apoptosis (Caspase 3 activation) in liver and kidney

The effect of Banana Peel extract on Cas-3, a biomarker of apoptosis, activity in the liver and kidney of rats exposed to metal mixture is shown in Figure 5. Compared with control group, exposure to metal mixtures resulted in an increase of Cas-3 activity in liver and kidney being only significant ($p<0.05$) in this last one. However, co-treatment with banana peel extract significantly ($p<0.05$) suppressed the activation of Cas-3 in both liver and kidney in a dose dependent manner.

3.7 Pro-Inflammatory markers (IL-6 and TNF–α) in liver and kidney

Figure 6 shows the effect of metal mixtures and the co-exposure with *Banana* peel extract on IL-6 and Tnf-α in the liver and kidney. HMM exposure resulted in significant ($p<0.05$) increase in Tnf –α and IL - 6 levels when compared to the control. Banana peel extract significantly ($p<0.05$) reduced IL-6 and Tnf-α..

3.8 Transcription factors (NfkB, and Nfr2) and Hmox-1 in kidney treated with BP extract following MM exposure.

Metal mixture without banana peel extract treatment shown significant ($p<0.05$) higher levels of transcription factors (NfkB, and Nfr2) and Hmox-1 in the liver and kidney compared with the control. These increases were significantly ($p<0.05$) reduced by banana peel extract co-exposure even at low (200 $mg \cdot kg^{-1}$) for Nfr2 in kidney, NfkB in kidney and liver and Hmox-1 in kidney or at mid (400 $mg \cdot kg^{-1}$) for Nfr2 and Hmox-1 in liver.

3.9 Relationship between biomarkers using Pearson R Correlation in the liver and kidney

Pearson's correlation was applied to determine the association between liver biomarkers of oxidative stress, pro-inflammatory indicators, apoptotic markers, and transcription factor (Table 1). In the same way, Pearson's correlation between the biomarkers of oxidative stress, pro-inflammatory markers, apoptotic marker and transcription factors in kidney (Table 2). In general way, as the oxidative stress markers (MDA, NO) increases there was significant reduction in the antioxidants (SOD, CAT, GSH, and GPx), ie the Pearson R correlation showed a significant negative relationship between oxidative stress and antioxidants. The oxidative stress also had a significant positive relationship with the pro-inflammatory markers (IL6 and TNF∝), increase in the oxidative stress markers led to an increase in the pro-inflammatory markers. Similarly, the oxidative stress and Hmox, and Nfkb showed a positive relationship i.e. increase in oxidative stress will result in an increase in Hmox, and Nfkb. Similarly, the result showed positive relationship between oxidative stress and apoptotic marker (Cas-3).

3.9 Histology of the liver and kidney

The photomicrographs of the rat liver from each group are shown in Figure 8. The Photomicrograph of the control rats showed normal liver hepatocytes (HP), sinusoids (SO) and central vein (CV) (Figure 8a), while the HMM only treated group had extensive damage

to the liver namely vacoulations (VO), damage central vein (CCV) with connective tissues fragmentation (CF) and lymphocytes infiltration and sinusoid expansions (Figure 8b). The groups co-treated with low, medium, and high dose of BP showed vacoulations (VO), necrosis (NC), damage Portal vessels connective tissues with lymphocytes infiltrations (Figure c), vacoulations (VO), nuclear pyknosis and some connective recovery (Figure 8d) and expansion and congestion of central vein (Figure 8e) respectively. Exposure to HMM caused extensive damage to the hepatocytes of the experimental rats and co-treatment with BP, considerably reversed the damaged histoarchitecture seen in the HMM only treated group showed vacoulations (VO), damage central vein (CCV) with connective tissues fragmentation (CF) and lymphocytes infiltration and sinusoid expansions.

The photomicrographs of kidney tissue of rats from each group are shown in Figure 9. The photomicrograph of the control rats showed normal glomerulus (NG), proximal tubules (PT) and distal tubules (DT) (Figure 9a), while the HMM only treated group showed glomerunephritis, with cellular and tissue damage, hazy swollen proximal and distal tubules (HST) (Figure 9b). The groups co-treated with low, medium and high dose of bannana peel extract showed damaged glomerular cells (DGC) with expanded urinary space in the glomerulus and damage tubular tissues (Figure 9c). In medium banana peel dose treatment shown recovered glomerular cells (NG) with less expanded urinary space and improved proximal and distal tubules cellular properties (DT) (Figure 9d). Finally, in high dose bannana peel extract group treatment, expansion of the urinary space in the glomerulus, myxoid proximal tubules (DPT), and damaged distal tubules (DT) with mesangial expansion were detected (Figure 9e). Exposure to HMM caused extensive damage to the kidney tissues of the experimental rats and co-treatment with banana peel, considerably repaired the lesions in the kidney, particularly at medium dose concentration of banana peel.

4. DISCUSSION

The present study has evaluated a possible beneficial role of banana peel extract BP in the management of metal mixture induced hepatorenal toxicity in a rat animal model. Understanding the toxicity of metals as mixtures is a true mimicry of real-life situation given the emergence of public health maladies associated with exposure to cocktail of metals. Chronic Kidney Disease (CKD) and Chronic Interstitial Nephritis in Agricultural

Communities (CINAC) (previously referred to as Chronic Kidney Disease of Unknown Etiology (CKDu)) seen in young adults in many agricultural communities (Gifford et al., 2017, Weaver et al., 2015), have been linked to a cocktail metals from cooking utensils and contaminated food among others.

The ubiquity of metals in the environment is a public health concern. Human exposure through food or drinking water led to an extensive organ internalization especially in liver and kidney. Metals such as Al, Pb, Hg or Mn have been implicated in both the potentiation and induction of oxidative stress and inflammatory responses, in tissue injury (Willhite et al., 2014). A relevant hepatorenal toxicity is reported after exposure to excessive doses of aluminum (Barceloux 1999, Krewski et al., 2007, ATSDR 2008, Lim et al., 2022). High aluminum concentrations have also been linked to immunologic alterations, genotoxicity, proinflammatory effect, protein denaturation and enzyme malfunction, metabolic derangement, membrane perturbation, iron dyshomeostasis, apoptosis, necrosis, and dysplasia (Wei et al., 2018, Igbokwe et al., 2019). Hepatic sequestration of divalent metals (eg., Pb) increase the breakdown of membrane bound molecules, distorted membrane permeability and integrity which culminate to tissue damage. Although Pb is a redox inactive metal since it does not participate in direct production of free oxygen radicals, however it is indirectly involved in lipid peroxidation (Hasanein et al., 2017). The covalent bonding of Pb with the sulfhydryl moieties of amino acid residues which is quite high predisposes the hepatocytes to diverse deleterious consequences. In the same vein, the binding of Pb with calmodulin by molecular mimicry of Ca leads to induction of plumbotoxicity (Singh et al., 2018). Furthermore, the hepatotoxicity of Pb is exacerbated by its biochemical cascade on the arachidonic acid pathway namely: upregulation the transcription process of the cyclooxygenase-2 gene, increased the production of prostaglandin E2 etc. These events tend to exaggerate the twin processes of hepatorenal oxidative stress and inflammation (Badary, 2017, (Dong et al., 2019).

In present study, exposure to HMM increased significantly hepatic and renal levels of metals from below detection limits to around 2 mg/kg in liver and kidney for Hg and Mn and around 4 mg/kg in liver and kidney for Pb and Al (Figure1). These tissue levels decreased across BP treatment groups but still higher than control treatment. Banana and other fruits peels are well known bio-adsorbents for metals and nanoparticle such Ag nanoparticles (Abdel-Khalek

et al., 2022; Tejada-Tovar et al. 2019). The metals levels in liver and kidney decrease in BP treated groups, strongly suggest a decrease of metal bioavailability effect due to BP.

Metabolic byproducts including urea and creatinine as well as electrolytes are primary indicators of renal injuries and renal integrity because they are often elevated and released in the blood following renal compromise (Nakhaee et al., 2019). In this study, significant elevation of the hepatorenal injury biomarkers were observed in the HMM exposed rats in comparison to the control (Figure 2bc). The elevation of hepatic injury biomarkers (AST, ALT, ALP, ALB and TP) and renal injury biomarker (creatinine and urea), even not significant, in MM treated group suggests possible hepatorenal damage. The observed increase in creatinine levels in this study is a clear manifestation of alteration in the glomerular filtration rate while the high urea level signifies diminished reabsorption at the renal epithelium in HMM-treated rats. Nakhaee et al., (2019) and Anyanwu et al., (2020) reported similar observations following exposure to metals. Numerous reports suggest that elevation of liver biomarkers (AST, ALT, ALP, ALB and TP) usually occur in hepatotoxicity (Saha et al., 2016, Lim, 2020). In the cell membrane, ALP assists in ionic movement across the membrane, and it linked to absorption and secretory processes of the cell (Grant et al., 2015). ALT is predominantly domiciled in the liver, the increase of these liver marker enzymes in the tissues has been employed as an indicator for cellular damage, distorted permeability of the membrane and distorted metabolism in hepatotoxicity (Dhir and Dhand, 2010). Co-treatment with banana peel extract caused a significant decrease in these liver function marker enzymes. The reversal towards normalcy of these liver marker enzymes by banana peel extract may be attributed to the decrease in of metal bioavailability and the presence of phytochemicals in this extract (Anjum et al., 2014). This finding agrees with the universally accepted perspective that concentration of liver function markers revert to normalcy when the hepatic parenchyma is repaired and the hepatocytes are regenerated (Grant et al., 2015). Remarkably, the apparent reduction in creatinine, urea and electrolytes levels observed in this study in rats co-treated with BP indicate the protective potential of BP in MM mediated renal damage in the experimental rats.

The cocktail of Pb, Al, Hg and Mn significantly reduced the activities of CAT and SOD. This observation is in consonance with recent reports from other studies (Seif et al., 2019; Goodarzi et al., 2020). These metals synergistically must have been involved in covalent

binding to the amino acid residues of these enzymes thus interfering with the enzymatic activities and functions (Matovi´c et al., 2015; Almenara et al., 2013). Similarly, the level and activities of glutathione (GSH) had significant decrease following heavy metal mixture exposure. Perhaps, accumulation of these heavy metals in the liver steered its binding to sulfhydryl groups of GSH therefore inhibiting the extensive reductive activities of GSH (Goodarzi et al., 2020). This would have also further destabilize the antioxidant defense system leading to overexpression of reactive oxygen species (ROS) (Al Omairi et al., 2019), which in turn provokes lipid peroxidation as witnessed with the elevation of MDA recorded in this study (Figure 4). According to the study done by Al Omairi et al. (2019) and Andjelkovic et al. (2019), MDA is usually expressed because of increase in ROS in tissues. Heavy metals are proponent for the increase of MDA in cells and tissues (Al Omairi et al., 2019; Andjelkovic et al., 2019). Interestingly, in this current study, banana peel extract abrogated all the oxidative dysregulations provoked by the HMM exposure in a dose dependent manner. Banana peel extract perhaps, by augmenting the activities and/or provoking the synthesis of endogenous antioxidant enzymes, further annulled the oxidative effects of the mixed metal toxicity in the Wistar rats. Similarly, the oxidized milieu precipitated by HMM by the decrease in the levels of reduced GSH and the increase in oxidized glutathione (GSSG) culminates to heightened programmed cell death or apoptosis as shown by increase in Cas-3 (Saha et al., 2016). Banana peel extract protects the hepatocytes from metal mixture induced activation of caspases (Figure 5).

Contemporary advances in oxidative stress and comprehension of its management entail a twin function of the antioxidants – free radical scavenging and metal chelation (Jomova and Valko, 2011; Sarpong-Kumankomah et al., 2018; Amadi et al., 2019). It is plausible to opine that banana peel could have exerted its beneficial effect via metal ion chelation sequel to lower hepatorenal metal (Pb, Al, Hg and Mn) levels and antioxidant mechanism. Oxidative damage and oxyradical production are effectively inhibited during metal ion chelation (Policegoudra et al., 2010). Further investigations will be required to appreciate the specific mechanism of action and the concentration of these metals (Pb, Al, Hg and Mn) in fecal matter and urine to determine their routes of elimination.

Chronic metal exposure is associated with inflammation of hepatic tissue (Algandaby et al., 2016) secondary to oxidative stress and generation of free radicals which of course

exacerbates release inflammatory cytokines like IL-1β and Tnf-α (Milnerowicz et al., 2015, Angosto et al., 2018). These exposures upregulate the genes that encode the inflammatory signals (Alexandrov et al., 2018). Cytokine release is thought to recruit leukocyte that produce more inflammatory chemokines and cytokines that worsen the inflammation (Jangra et al., 2015; Khameneh et al., 2017). In addition, chronic granuloma associated with chronic metal exposure and systemic inflammation related to increasing the concentrations of IL-6 and Tnf-α, have in exposed animals (Haag et al., 2014, Pogue et al. 2017). There is a crosstalk between inflammation and oxidative stress in numerous chronic diseases (Bartoli et al., 2011). Inflammatory response is usually amplified by oxidative stress through the modulation of associated genes that are involved in inflammation in vivo (Cao et al., 2017; Tan et al., 2018). Interestingly, nuclear transcription factor Nfkb amplified by virtue of oxidative stress was responsible for the acceleration of inflammatory response evidenced in this study via the induction and upregulation of the inflammatory makers (Ramamoorthy et al., 2017; Aliomrani et al., 2016). The present study has shown activation of Nfkb following HMM exposure which was compensated by treatment with banana peel extract even in the low levels. Undeniably, the activation of Nfkb pathway has been linked to liver fibrosis and hepatocyte damage (de Souza Basso et al 2021). So, the regulation of this pathway by banana peel extract is an indication of possible mechanism of action of the extract (Piazza et al., 2019).

From a functional standpoint, Nrf2 negatively modulates oxidative-stress mediated Nfkb activation through the Hmox-1 pathway (Bellezza et al., 2012). Nrf2 is bound to Keap1 protein in the cytosol under normal physiological situations but in oxidative stress, there is oxidation of sulfhydryl groups on Keap1 leading to conformational changes, release of Nrf2 and nuclear translocation of Nrf2 which binds with ARE genes like Hmox-1 and musculoaponeurotic fibrosarcoma (Maf) proteins (Bellezza et al., 2012). Hmox-1 is a vital enzyme involved in Nrf2-mediated Nfkb inhibition. Usually upregulation of Hmox-1 suppresses IL-1β- degradation, which inhibits Nfkb activity. Likewise, Hmox-1 inhibits the Tnf-α -dependent activation of Nfkb (Soares et al., 2004) whereas cellular upregulation of Nfkb results in decrease Hmox-1 thus confirming that Nfkb activation can act as an Nrf2 repressor (Sivandzade et al., 2019). It is known that Hmox-1 brings to bear its anti-inflammatory activities by the formation of carbon monoxide (CO), iron, and bilirubin. CO

is a known Nfkb inhibitor, which elicits reduced formation of pro-inflammatory responses. Taken together, the Nrf2/Hmox-1 pathway is directly involved in the inhibition of proinflammatory cytokines and activation of anti-inflammatory cytokines (Sandberg et al., 2014). Hence Nrf2 mediated increase in the expression of Hmox-1 is crucial for cross-talk between Nrf2 and Nfkb. Banana peel extract hampers this cross-talk in the hepatocytes of metal mixture treated rats.

The significant changes in various biochemical parameters following exposure to metals mixture were good indicators of hepatorenal toxicity and the interpretations were supported by corresponding changes: increase in liver and kidney weight and the observed histopathological alterations. There is evidence that chronic heavy metal administration leads to metal accumulation in the in liver and kidney and, in turn, lead to alterations in the liver function (Newairy et al., 2009, Nikolov et al., 2010).

The histological analysis revealed that HMM triggered hydropic degeneration of the hepatocytes over a sixty-day period in the toxicity control when compared to controls. These distortions are consistent with numerous investigations regarding metal toxicity in diverse animal models (Saha et al., 2016, Lim., 2020) and corroborate the pathogenic role of heavy metals induced inflammation and oxidative stress in the hepatocyte. The liver is usually the first organ that toxicants target because it is predominantly linked with biotransformation and detoxification activities (Saha et al, 2016, Lim., 2020). Thus, the possible reason for the observed lesions in the liver. The lymphocytes infiltration and sinusoid expansions as well as increased vacuolations of the hepatocytes observed in HMM treated group are clear signs of pathological responses following their exposure. Co-exposure of HMM with 200, 400 and 800 mg/kg BP showed lymphocytes infiltrations, nuclear pyknosis and expansion of the sinusoidal space, respectively. Overall, the present study confirmed that BP protect from the histopathological changes produced by HMM, which were consistent with the changes in oxidative stress and pro-inflammatory indicators. These results indicated that banana peel extract relieved metal mixture-induced hepatorenal cell apoptosis associated oxidative stress.

In spite of the immense nutritional benefit of banana peel, it contains also some antinutrients like glucosides, tannins, oxalate and phytate, which have untoward effects in humans (Arslan and Ozcan, 2010, Ozabor et al., 2020). High oxalate rich diets are feared to be risk factor in hyperoxaluria (Hulton, 2016) which may result in renal inflammation and acute renal failure

in cases of compromised kidney function (Getting et al., 2013). However, the oxalate content in banana peel is below the threshold value that can be absorbed in humans (40–50 mg/day) (Zaini et al., 2022). Dietary phytate induces lowering of blood glucose and lipids in addition to averting renal calcium crystallization (Schlemmer et al., 2009).

5. Conclusion

Chronic HMM (Pb, Al, Hg and Mn) exposure exacerbated oxidative dysfunction in the liver and kidney, which were demonstrated by hepatic and renal accumulation of Pb, Al, Hg and Mn, decrease in antioxidant parameters (CAT, SOD, GSH, GPx) and elevation of marker of lipid peroxidation – MDA. There was also upregulation of inflammatory markers and transcription factor markers that corroborated with severe histopathogical changes in the liver and kidney. However, in a dose dependent manner, banana peel extract annulled these effects. Banana peels decrease bioavailability of the metals in the tested mixture. Banana peel might have ameliorated hepatorenal injury and exerted anti-inflammatory and anti-apoptotic effects in heavy metal mixture mediated hepatotoxicity and nephrotoxicity by activation of Nrf2/Hmox-1 and inhibition of Nfkb pathway. These observations underscore the nutraceutical potentials of banana peel extract and the suggested beneficial role in the management of hepatorenal syndrome and other related metal-induced toxicity.

Funding

This work received no funding. No funding is available for open access publications from our institution or other sources.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We are grateful to World Bank Research Fund for Africa Centre of Excellence in Oilfield Chemicals Research (ACE-CEFOR), University of Port Harcourt for tuition waiver to BFE-

A. J.R. received postdoctoral fellowship from “Juan de la Cierva-incorporación” program of the Spanish “Ministerio de Ciencia, Innovación y Universidades” (IJC 2018-035126-I).

REFERENCES.

Abdel-Khalek, A.A., Hamed, A., Hasheesh, W.S.F. 2022. The Potential Use of Orange and Banana Peels to Minimize the Toxicological Effects of Silver Nanoparticles in Oreochromis Niloticus Bulletin of Environmental Contamination and Toxicology, 108 (5), pp. 985-994.

Ahmed, Y.F.; Eldebaky, H.A.A.; Mahmoud, K.G.M.; Nawito, M. 2012. Effects of lead exposure on DNA damage and apoptosis in reproductive and vital organs in female rabbits. Glob. Vet.; 9, 401–408.

Akamine Kiichiro, Tomoyuki Koyama and Kazunaga Yazawa. 2019. Banana Peel Extract Suppressed Prostate Gland Enlargement in Testosterone-Treated Mice, Bioscience, Biotechnology, and Biochemistry ;73(9): 1911-1914.

Al-Brakati, A.Y., Kassab, R.B., Lokman, M.S., Elmahallawy, E.K., Amin, H.K. and Abdel Moneim, A.E., 2019. Soursop fruit extract mitigates scopolamine-induced amnesia and oxidative stress via activating cholinergic and Nrf2/HO-1 pathways. *Metabolic Brain Disease*, *34*(3), pp.853-864.

Alexandrov, P. N., Pogue, A. I., and Lukiw, W. J. 2018. Synergism in aluminum and mercury neurotoxicity. *Integrative food, nutrition and metabolism*,;*5*(3).

Algandaby, M. M., El-Halawany, A. M., Abdallah, H. M., Alahdal, A. M., Nagy, A. A., Ashour, O. M., and Abdel-Naim, A. B. 2016. Gingerol protects against experimental liver fibrosis in rats via suppression of pro-inflammatory and profibrogenic mediators. *Naunyn-Schmiedeberg's archives of pharmacology*,;*389*(4): 419-428.

Alhusaini A, Faddaa L, Ali HM, Hassan I, El Orabi NF, Bassiouni Y. 2018. Amelioration of the protein expression of Cox2, NF κ B, and STAT-3 by some antioxidants in the liver of sodium fluoride-intoxicated rats. Dose-Response;16(3):1559325818800153

Aliomrani, M., Sepand, M.R., Mirzaei, H.R., Kazemi, A.R., Nekonam, S., Sabzevari, O. 2016. Effects of phloretin on oxidative and inflammatory reaction in rat model of cecal ligation and puncture induced sepsis. Daru.;24, 15.

Almeer RS, Alarifi S, Alkahtani S, Ibrahim SR, Ali D, Moneim A. 2018. The potential hepatoprotective effect of royal jelly against cadmium chloride-induced hepatotoxicity in mice is mediated by suppression of oxidative stress and upregulation of Nrf2 expression. Biomed Pharmacother;106:1490–1498.

Almenara, C.C.P., Broseghini-Filho, G.B., Vescovi, M.V.A., Angeli, J.K., Faria, T.O., Stefanon, I., Vassallo, D.V., Padilha, A.S. 2013 Chronic cadmium treatment promotes oxidative stress and endothelial damage in isolated rat aorta, PLoS One;8, 1–8.

Amadi, C.N., Offor, S.J., Frazzoli, C., Orisakwe, O.E. (2019). Natural antidotes and management of metal toxicity, Environ. Sci. Pollut. Res. - Int. 1–21.

Andjelkovic, M., Djordjevic, A.B., E. Antonijevic, B. Antonijevic, M. Stanic, J. Kotur-Stevuljevic, V. VSpasojevic-Kalimanovska, M. Jovanovic, N. Boricic, D. Wallace, Z. Bulat, (2019). Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney, Int. J. Environ. Res. Public Health 16 (274)1–22

Angosto, D., López-Muñoz, A., García-Alcazar, A., Meseguer, J., Sepulcre, M. P., and Mulero, V. (2018). Aluminum is a powerful adjuvant in teleost fish despite failing to induce interleukin-1β release. *Developmental & Comparative Immunology*, *85*, 18-24.

Anhwange, B.A., Ugye, T.J. and Nyiaatagher, T.D., 2009. Chemical composition of Musa sapientum (banana) peels. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(6), pp.437-442.

Anjum, S., Sundaram, S., Rai, G., 2014. Nutraceutical application and value addition of banana (Musa paradisica L. variety "Bhusawal Keli") peel: a review. Int. J. Pharm. Pharm. Sci. 6.

Ansari M, Aloliet R, Ganaie M, Khan T, Najeeb-ur-Rehman IF, Hamad AM (2019) Roflumilast, a phosphodiesterase 4 inhibitor, attenuates cadmium-induced renal toxicity via modulation of NF-κB activation and induction of NQO1 in rats. Hum Exp Toxicol 38(5):588–597

Anyanwu, B. O., Orish, C. N., Ezejiofor, A. N., Nwaogazie, I. F., Orisakwe, O. E. (2020). Neuroprotective effect of *Costus afer* on low dose heavy metal mixture (lead, cadmium and mercury) induced neurotoxicity via antioxidant, anti-inflammatory activities. Toxicology Reports 7 1032–1038.

Anyanwu, B.O., Ezejiofor, A.N., Igweze, Z.N. and Orisakwe, O.E., 2018. Heavy metal mixture exposure and effects in developing nations: an update. *Toxics*, *6*(4), p.65.

Arslan, D. and Özcan, M.M., 2010. Study the effect of sun, oven and microwave drying on quality of onion slices. *LWT-Food Science and Technology*, *43*(7), pp.1121-1127.

ATSDR (Agency for Toxic Substances and Disease Registry), 2008. Toxicological Profile for Aluminum. Atlanta: U.S. Department of Health and Human Services, Public Health Service.

Azarudeen, A.M. and Nithya, R., 2021. Pharmaceutical Aspects of Banana peel: A Review. Journal of Pharmaceutical Sciences and Research, 13(2), pp.112-117.

Badary, D.M., 2017. Folic acid protects against lead acetate-induced hepatotoxicity by decreasing NF-kB, IL-1b production and lipid peroxidation mediated cell injury. Pathophysiology 24, 39-44.

Bansal N, Roy KS. (1997). Histoenzymic effects of chlorpyrifos toxicity on rabbit genitalia. Indian J Anim Sci;67:127-7.

Barceloux, D.G., 1999. Copper. Journal of Toxicology Clinical Toxicology, 37 (2), 217–237.

Bartoli, M.L., Novelli, F., Costa, F., Malagrinò, L., Melosini, L., Bacci, E., Cianchetti, S., Dente, F.L., Di Franco, A., Vagaggini, B., Paggiaro, P.L., 2011. Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. Mediators Inflamm. 2011, 891752.

Bellezza, I.; Tucci, A.; Galli, F.; Grottelli, S.; Mierla, A.L.; Pilolli, F.; Minelli, A. 2012.Inhibition of NF-kappaB nuclear translocation via HO-1 activation underlies alpha-tocopheryl succinate toxicity. J. Nutr. Biochem., 23, 1583–1591.

Bethea JR. 2000. Spinal cord injury-induced inflammation: a dual-edged sword. Prog Brain Res; 128: 33-42.

Boison D, Adinortey CA, Babanyinah GK, Quasie O, Agbeko R, Wiabo-Asabil GK and Adinortey MB. 2019. Costus afer: a systematic review of evidence-based data in support of its medicinal relevance. Scientifica; 2019: 1-10.

Boots, A. W., Haenen, G. R., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. European Journal Pharmacology, 585(2–3), 325–337.

Buha, A.; Barali´c, K.; Djukic-Cosic, D.; Bulat, Z.; Tinkov, A.; Panieri, E.; Saso, L. (2021). The Role of Toxic Metals and Metalloids in Nrf2 Signaling. Antioxidants, 10, 630. https://doi.org/10.3390/ antiox10050630.

Canli, O., Nicolas, A.M., Gupta, J., Finkelmeier, F., Goncharova, O., Pesic, M., Neumann, T., Horst, D., Lower, M., Sahin, U., Greten, F.R., 2017. Myeloid cell derived reactive oxygen species induce epithelial mutagenesis. Canc. Cell 32, 869e883 e865

Cao, C., Luo, J., Li, X., Zhang, M., Zhang, H., Zhang, J., and Wang, K. (2018). Selenium-rich yeast protects against aluminum-induced renal inflammation and ionic disturbances. *Biological Trace Element Research*, *186*(2), 467-473.

Cao, L.P., Ding, W.D., Jia, R., Du, J.L., Wang, T., Zhang, C.Y., Gu, Z.Y., Yin, G.J., 2017. Anti-inflammatory and hepatoprotective effects of glycyrrhetinic acid on CCl4-induced damagein precision-cut liver slices from Jian carp (Cyprinus carpio var. jian) through inhibition of the nf-ƙB pathway. Fish Shellfish Immunol. 64, 234-242.

Casas, S.J. and Sordo, J. (2006). Lead Chemistry, Analytical Aspects, Environmental Impact and Health Effects; Elsevier: Amsterdam, The Netherlands, ISBN 9780444529459.

Cheng, J.-C., Dai, F., Zhou, B., Yang, L., & Liu, Z.-L. (2007). Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: Mechanism and structure–activity relationship. Food Chemistry, 104(1), 132–139.

Choudri, B.S., Charabi, Y., 2019. Pesticides and herbicides. Water Environ. Res. 91, 1342e1349.

Clairborne, A. Catalase activity, in: A.R. Greewald (Ed.), Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, 1995, pp. 237–242

Cory-Slechta, D.A., (2005). Studying toxicants as single chemicals: does this strategyadequately identify neurotoxic risk. Neurotoxicology 26 (4), 491–510.

de Freitas, A.S., Funck, V.R., dos Santos Rotta, M., Bohrer, D., Mörschbächer, V., Puntel, R.L., Nogueira, C.W., Farina, M., Aschner, M. and Rocha, J.B.T., 2009. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Research Bulletin*, *79*(1), pp.77-84.

Deng, Y., et al., 2015. Melatonin antagonizes mn-induced oxidative injury through the activation of keap1–Nrf2–ARE signaling pathway in the striatum of mice. Neurotox. Res. 27, 156–171.

de Souza Basso, B., Haute, G.V., Ortega-Ribera, M., Luft, C., Antunes, G.L., Bastos, M.S., Carlessi, L.P., Levorse, V.G., Cassel, E., Donadio, M.V.F. and Santarém, E.R., 2021. Methoxyeugenol deactivates hepatic stellate cells and attenuates liver fibrosis and inflammation through a PPAR-ɣ and NF-kB mechanism. Journal of Ethnopharmacology, 280, p.114433.

Dhir, V. and Dhand, P. (2010). Toxicological Approach in Chronic Exposure to Lead on Reproductive Functions in Female Rats (Rattus Norvegicus). Toxicology International Jan-Jun 2010 / Vol-17 / Issue-1. DOI: 10.4103/0971-6580.68340

Dong, J., Cong, Z., You, M., Fu, Y., Wang, Y., Wang, Y., Fu, H., Wei, L., Chen, J., 2019. Effects of perinatal di (2-ethylhexyl) phthalate exposure on thyroid function in rat offspring. Environ. Toxicol. Pharmacol. 67, 53-60.

Edenta, C.; James, D.B.; Owolabi, O.A.; Okoduwa, S.I.R. (2014). Hypolipidemic effects of aqueous extract of three Cultivars of Musa sapientum fruit peel on poloxamer-407 induced hyperlipidemic Wistar rats. Int. J. Pharm. Sci. Res., 5, 1046–1051.

Elkhadragy, M.F., Kassab, R.B., Metwally, D., Almeer, R.S., Abdel-Gaber, R., Al-Olayan, E.M., Essawy, E.A., Amin, H.K. and Abdel Moneim, A.E., 2018. Protective effects of Fragaria ananassa methanolic extract in a rat model of cadmium chloride-induced neurotoxicity. *Bioscience reports*, *38*(6), 1-14.

Elzagallaai, A.A., Sultan, E.A., Bend, J.R., Abuzgaia, A.M., Loubani, E., Rieder, M.J., 2020. Role of oxidative stress in hypersensitivity reactions to sulfonamides. J. Clin. Pharmacol. 60, 409-421.

Farombi, E.O., Tahnteng, J.G. Agboola, A.O., Nwankwo, J.O., Emerole, G.O. 2002. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a *Garcinia kola* seed extract, Food Chem. Toxicol. 38, 535-541.

Fasinu, P.S. and Orisakwe, O.E., 2013. Heavy metal pollution in sub-Saharan Africa and possible implications in cancer epidemiology. *Asian Pacific journal of cancer prevention*, *14*(6), pp.3393-3402.

Flora, G.; Gupta, D.; Tiwari, A. Toxicity of lead: A review with recent updates. Interdiscip. Toxicol. 2012, 5, 47–58.

Flora, S.J.S.; Agrawal, S. 2017. Arsenic, Cadmium, and Lead; Academic Press: Cambridge, MA, USA, ; ISBN 9780128042397.

Franco, J.L., Posser, T., Missau, F., Pizzolatti, M.G., Santos, A.R., Souza, D.O., Aschner, M., Rocha, J.B., Dafre, A.L. and Farina, M., 2010. Structure–activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. *Environmental Toxicology and Pharmacology*, *30*(3), pp.272-278.

Frazzoli, C., Ruggieri, F., Battistini, B., Orisakwe, O.E., Igbo, J.K. and Bocca, B., 2022. E-WASTE threatens health: The scientific solution adopts the one health strategy. *Environmental Research*, *212*, p.113227.

Gao D, Xu Z, Zhang X, Wang H, Wang Y, *et al*. (2013) Molecular cloning, immunohistochemical localization, characterization and expression analysis of caspase-9 from the purse red common carp (Cyprinus carpio) exposed to cadmium. Aquat Toxicol 142–143: 53–62.

Getting, J.E., Gregoire, J.R., Phul, A. and Kasten, M.J., 2013. Oxalate nephropathy due to 'juicing': case report and review. *The American journal of medicine*, *126*(9), pp.768-772.

Gifford FJ, Gifford RM, Eddleston M, Dhaun N. 2017. Endemic nephropathy around the world. Kidney Int Reports; 2:282–92.

Goodarzi, Z., Karami, E., Yousefi, S., Dehdashti, A., Bandegi, A.R., Ghanbari, A. (2020). Hepatoprotective effect of atorvastatin on Cadmium chloride induced hepatotoxicity in rats, Life Sci. 254, 117770.

Grant, C.N., Mojica, S.G., Sala, F.G., Hill, J.R., Levin, D.E., Speer, A.L., Barthel, E.R., Shimada, H., Zachos, N.C. and Grikscheit, T.C., 2015. Human and mouse tissue-engineered small intestine both demonstrate digestive and absorptive function. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *308*(8), pp.G664-G677.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. Tannenbaum, S.R. (1982) Analysis of nitrate, nitrite and [15N] nitrate in biological fluids, Anal. Biochem. 126 131–138.

Haag, C. K., Dacey, E., Hamilton, N., and White, K. P. (2019). Aluminum granuloma in a child secondary to DT aP-IPV vaccination: A case report. *Pediatric dermatology*, *36*(1), e17-e19.

Habtemariam, S., 2019. Modulation of reactive oxygen species in health and disease.Antioxidants 8.

Hallauer, J., Geng, X., Yang, H.C., Shen, J., Tsai, K.J., Liu, Z., 2016. The effect of chronic arsenic exposure in zebrafish. Zebrafish 13, 405e412.

Hasanein, P., Ghafari-Vahed, M., Khodadadi, I., 2017. Effects of isoquinoline alkaloid berberine on lipid peroxidation, antioxidant defense system, and liver damage induced by lead acetate in rats. Redox Rep. 22, 42-50.

Homma, T., Fujii, J. (2015). Application of glutathione as anti-oxidative and anti-aging drugs, Curr. Drug Metab. 16 560–571.

Hrycay, E.G., Bandiera, S.M., 2015. Involvement of cytochrome P450 in reactive oxygen species formation and cancer. Adv. Pharmacol. 74, 35e84.

Hulton, S.A., 2016. The primary hyperoxalurias: a practical approach to diagnosis and treatment. *International Journal of Surgery*, *36*, pp.649-654.

Igbokwe, I.O., Igwenagu, E., and Igbokwe, N.A., 2019. Aluminium toxicosis: a review of toxic actions and effects. Interdisciplinary Toxicology, 12 (2), 45–70.

Institoris, L., Kovac, D., Kecskemeti-Kovacs, I.., Lukacs, A., Szabo, A., Lengyel, Z., Papp, A., Nagymajtenyi, L., Desi, I., (2006). Immunotoxicological Investigation of subacute combind exposure with low doses of Pb, Hg and Cd in rats. *Acta. Biol. Hunh*., 57(4),pp.433-439.

IRIS (Integrated Risk Information System), 1999. Cincinnati, OH: U.S. Environmental Protection Agency.

Jangra, A., Kasbe, P., Pandey, S. N., Dwivedi, S., Gurjar, S. S., Kwatra, M., ... and Lahkar, M. (2015). Hesperidin and silibinin ameliorate aluminum-induced neurotoxicity: modulation of antioxidants and inflammatory cytokines level in mice hippocampus. *Biological trace element research*, *168*(2), 462-471.

Järup, L.; Åkesson, A. 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol., 238, 201–208.

Jayasumana C. 2019. Chronic Interstitial Nephritis in Agricultural Communities (CINAC) in Sri Lanka. Semin Nephrol;39(3):278–83.

Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R. (1974). Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite, Pharmacol. 11 151–169.

Jomova, K. and Valko, M. (2011). Advances in metal-induced oxidative stress and human disease, Toxicology 283 (2–3), 65–87.

Joseph, P. Mechanisms of cadmium carcinogenesis. Toxicol. Appl. Pharmacol. 2009, 238, 272–279.

Karri, V., Schuhmacher, M., Kumar, V. Heavy metals (Pb, Cd, As and MeHg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain (2016) Environmental Toxicology and Pharmacology, 48, pp. 203-213.

Ding, K., Wang, H., Xu, J., Li, T., Zhang, L., Ding, Y., Zhu, L., He, J. and Zhou, M., 2014. Melatonin stimulates antioxidant enzymes and reduces oxidative stress in experimental traumatic brain injury: the Nrf2–ARE signaling pathway as a potential mechanism. *Free Radical Biology and Medicine*, *73*, pp.1-11.

Khameneh, H.J., Ho, A.W., Laudisi, F., Derks, H., Kandasamy, M., Sivasankar, B., Teng, G.G. and Mortellaro, A., 2017. C5a regulates IL-1β production and leukocyte recruitment in a murine model of monosodium urate crystal-induced peritonitis. Frontiers in pharmacology, 8, p.10.

Khan R, Ali S, Mumtaz S, Andleeb S, Ulhaq M, Tahir HM, Khan MKA, Khan MA, Shakir HA (2019) Toxicological effects of toxic metals (cadmium and mercury) on blood and the thyroid gland and pharmacological intervention by vitamin C in rabbits. Environ Sci Pollut Res 26(16):16727–16741

Kim J, Seok YM, Jung KJ, Park KM. 2009. Reactive oxygen species/oxidative stress contributes to the progression of kidney fibrosis following transient ischemic injury in mice. Am J Physiol Renal Physiol;2009(297): F461–70.

Kim S, Cheon HS, Kim SY, Juhnn YS, Kim YY (2013) Cadmium induces neuronal cell death through reactive oxygen species activated by GADD153. BMC Cell Biol 14: 4.

Krewski, D., Yokel, R.A., Nieboer, E., Borchelt, D., Cohen, J., Harry, J., Kacew, S., Lindsay, J., Mahfouz, A.M. and Rondeau, V., 2007. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. *Journal of Toxicology and Environmental Health, Part B*, *10*(S1), pp.1-269.

Li Y, Lim SC (2007) Cadmium-induced apoptosis of hepatocytes is not associated with death receptor-related caspase-dependent pathways in the rat. Environ Toxicol Pharmacol 24: 231–238.

Li, S., Tan, H.Y., Wang, N., Zhang, Z.J., Lao, L., Wong, C.W., Feng, Y. (2015). The role of oxidative stress and antioxidants in liver diseases, Int. J. Mol. Sci. 16 26087–26124.

Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR. 2017. Oxidative stress in hemodialysis patients: a review of the literature. Oxidative Med Cell Longev; 2017:3081856.

Lim, A.K., 2020. Abnormal liver function tests associated with severe rhabdomyolysis. *World journal of gastroenterology*, *26*(10), p.1020.

Lim, J.O., Jung, T.Y., Lee, S.J., Park, S.W., Kim, W.I., Park, S.H., Kim, J.H., Heo, J.D., Kim, Y.B., Shin, I.S. and Kim, J.C., 2022. Evaluation of 28-day repeated oral dose toxicity of aluminum chloride in rats. *Drug and Chemical Toxicology*, *45*(3), pp.1088-1097.

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry, 103(3), 1003–1008.

Lin, Y.C., Wang, F.F., 2008. Mechanisms underlying the pro-survival pathway of p53 in suppressing mitotic death induced by adriamycin. Cell. Signal. 20, 258e267.

Maccioni, R.B., Rojo, L.E., Fernandez, J.A. Kuljis, R.O. (2009). The role of neuroimmunomodulation in Alzheimer's disease, Ann. N. Y. Acad. Sci. 1153 (1),240–246.

Maduabuchi, J.M.U., Nzegwu, C.N., Adigba, E.O., Oragwu, C.I., Agbo, F.N., Agbata, C.A., Ani, G.C. and Orisakwe, O.E., 2008. Iron, manganese and nickel exposure from beverages in Nigeria: a public health concern?. *Journal of Health Science*, *54*(3), pp.335-338.

Mahieu, S., del Carmen Contini, M., González, M. and Millen, N., 2009. Melatonin reduces oxidative damage induced by aluminium in rat kidney. *Toxicology Letters*, *190*(1), pp.9-15.

Matovi´c, V., Buha, A., D. Duki´c-´C osi´c, Z. Bulat, 2015. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys, Food Chem. Toxicol. 78 130–140

Matović, V., Buha, A., Bulat, Z. and Đukić-Ćosić, D., 2011. Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. *Arhiv za higijenu rada i toksikologiju*, *62*(1), pp.65-75.

Methods for Oxygen Radical Research. CRC Press, Boca Raton, 1995, pp. 237–242.

Milnerowicz, H., Ściskalska, M., and Dul, M. 2015. Pro-inflammatory effects of metals in persons and animals exposed to tobacco smoke. *Journal of Trace Elements in Medicine and Biology, 29,* 1-10.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 3170–3175.

Moghaddam AH, Nabavi SF, Nabavi SM, Loizzo MR, Roohbakhsh A, Setzer WN 2015 Ameliorative effects of curcumin against sodium fluoride-induced hepatotoxicity. Progress Nutr 17(4):324–330.

Mohapatra, D., Mishra, S. and Sutar, N., 2010. Banana and its by-product utilisation: an overview., J. Sci. Ind. Res. (India). 69, 323–329.

Morris, D. Khurasany, M., Nguyen, T., Kim, J., Guilford, F., Mehta, R., Gray, D., Saviola, B. Venketaraman, V. Glutathione and infection, Biochim. Biophys. Acta 1830 (2013) 3329–3349.

Newairy, A.S., et al., 2009. Propolis alleviates aluminum-induced lipid peroxidation and biochemical parameters in male rats. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 47 (6), 1093–1098.

Nikolov, I.G., Joki, N., Vicca, S., Patey, N., Auchère, D., Benchitrit, J., Flinois, J.P., Ziol, M., Beaune, P., Drüeke, T.B. and Lacour, B., 2010. Tissue accumulation of lanthanum as compared to aluminum in rats with chronic renal failure–possible harmful effects after long- term exposure. *Nephron Experimental Nephrology*, *115*(4), pp.e112-e121.

Nkpaa KW, Onyeso GI (2018) Rutin attenuates neurobehavioral deficits, oxidative stress, neuro-inflammation and apoptosis in fluoride treated rats. Neurosci Lett 682:92–99

Oboh Ganiyu, Adedayo Oluwaseun Ademiluyi , Sunday Idowu Oyeleye, Tosin Abiola Olasehinde & Aline Augusti Boligon (2017) Modulation of some markers of erectile dysfunction and malonaldehyde levels in isolated rat penile tissue with unripe and ripe plantain peels: identification of the constituents of the plants using HPLC, Pharmaceutical Biology, 55:1,1920-1926.

Ohkawa H. and Ohishi N. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry, 95(2). 351-358.

Okoye, E.A., Bocca, B., Ruggieri, F., Ezejiofor, A.N., Nwaogazie, I.L., Domingo, J.L., Rovira, J., Frazzoli, C. and Orisakwe, O.E., 2021. Metal pollution of soil, plants, feed and food in the Niger Delta, Nigeria: Health risk assessment through meat and fish consumption. Environmental Research, 198, p.111273.

Onk, D., Onk, O.A., Erol, H.S., Özkaraca, M., Çomaklı, S., Ayazoğlu, T.A., Kuyrukluyıldız, U. and Ünver, S., 2018. Effect of melatonin on antioxidant capacity, ınflammation and apoptotic cell death in lung tissue of diabetic rats. Acta Cirurgica Brasileira, 33, pp.375-385.

Orisakwe, O.E., Frazzoli, C., Ilo, C.E. and Oritsemuelebi, B., 2019. Public health burden of e-waste in Africa. Journal of Health and Pollution, 9(22). 2019 Jun 4;9(22):190610.

Ou, S., Kwok, K.-C., 2004. Ferulic acid: pharmaceutical functions, preparation and applications in foods. J. Sci. Food Agric. 84, 1261–1269.

Owumi, S.E. and Dim, U. J. (2019). Manganese suppresses oxidative stress, inflammation and caspase-3 activation in rats exposed to chlorpyrifos. Toxicology Reports 6 202–209

Oyibo, J.N., Wegwu, M.O., Uwakwe, A.A., Osuoha, J.O. (2018). Analysis of total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and risk assessment of heavy metals in some selected finfishes at Forcados Terminal, Delta State, Nigeria Environmental Nanotechnology, Monitoring and Management, *ELSEVIER*, 9, pp. 128- 135.

Ozabor, P.T., Ojokoh, A.O., Wahab, A.A. and Aramide, O.O., 2020. Effect of Fermentation on the Proximate and Antinutrient Composition of Banana Peels. *The International Journal of Biotechnology*, *9*(2), pp.105-117.

Paithankar, J.G., Saini, S., Dwivedi, S., Sharma, A. and Chowdhuri, D.K., 2021. Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. *Chemosphere*, *262*, p.128350.

Parameswaran, N., Patial, S. (2010).Tumor necrosis factor-α signaling in macrophages, Crit. Rev. Eukaryot. Gene Expr. 20 87–103.

Piazza S, Pacchetti B, Fumagalli M, Bonacina F, Dell'Agli M, Sangiovanni E (2019). Comparison of two ginkgo biloba L. extracts on oxidative stress and inflammation markers in human endothelial cells. Mediators Inflamm.

Pivtoraiko VN, Stone SL, Roth KA, Shack JJ. 2009. Oxidative stress and autophagy in the regulation of lysosome-dependent neuron death. Antioxid Redox Signal;11: 481–96.

Pogue, A. I., Jaber, V., Zhao, Y., and Lukiw, W. J. (2017). Systemic inflammation in C57BL/6J mice receiving dietary aluminum sulfate; up-regulation of the pro-inflammatory cytokines IL-6 and TNFα, C-reactive protein (CRP) and miRNA-146a in blood serum. *Journal of Alzheimer's disease & Parkinsonism*, *7*(6).

Policegoudra, R.S., Rehna, K., Rao, L.J., Aradhya, S.M. (2010). Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (Curcuma amada Roxb.) rhizome, J. Biosci. 35 (2) ,231–240.

Ramamoorthy, H., Abraham, P., Isaac, B., Selvakumar, D., 2017. Role for NF-κB inflammatory signalling pathway in tenofovir disoproxil fumarate (TDF) inducedrenal damage in rats. Food Chem Toxicol. 99, 103-118.

Roberts, I.I. and Orisakwe, O.E., 2011. Evaluation of potential dietary toxicity of heavy metals in some common Nigerian beverages: A look at antimony, tin and mercury. *QScience Connect*, *2011*(1), p.2.

Rotruck, J. T. Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (1973) 588– 590

Saha, S., Sadhukhan, P., Sinha, K., Agarwal, N. and Sil, P.C., 2016. Mangiferin attenuates oxidative stress induced renal cell damage through activation of PI3K induced Akt and Nrf-2 mediated signaling pathways. *Biochemistry and biophysics reports*, *5*, pp.313-327.

Salim, S., Chugh, G., Asghar, M. (2012). Inflammation in anxiety, in: Advances in Protein Chemistry and Structural Biology, 88, Academic Press, 2012, pp. 1–25.

Sánchez-Valle, V., Chávez-Tapia, N.C., Uribe, M., Méndez-Sánchez, N. Role of oxidative stress and molecular changes in liver fibrosis: a review, Curr. Med. Chem. 19 (2012) 4850–4860.

Sandberg, M.; Patil, J.; D'Angelo, B.; Weber, S.G.; Mallard, C. 2014**.** NRF2-regulation in brain health and disease: Implication of cerebral inflammation. Neuropharmacology, 79, 298–306.

Sannadia, S., Kadeyalaa, P. K., Gottipolub, R.R. (2013). Reversal effect of monoisoamyl dimercaptosuccinic acid (MiADMSA)for arsenic and lead induced perturbations in apoptosis and antioxidant enzymes in developing rat brain. Int. J. Devl Neuroscience 31, 586–597

Sarpong-Kumankomah, S., Gibson, M.A., Gailer, J. (2018). Organ damage by toxic metals is critically determined by the bloodstream, Coord. Chem. Rev. 374, 376–386.

Satarug, S.; Garrett, S.H.; Sens, M.A.; Sens, D.A. (2010). Cadmium, environmental exposure, and health outcomes. Environ. Health Perspect. 2010, 118, 182–190.

Schlemmer, U., Frølich, W., Prieto, R.M. and Grases, F., 2009. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular nutrition & food research*, *53*(S2), pp.S330-S375.

Seif, M.M., Madboli, A.N., Marrez, D.A., Aboulthana, W. (2019). Hepato-renal protective effects of egyptian purslane extract against experimental cadmium toxicity in rats with special emphasis on the functional and histopathological changes, Toxicol. Rep. 6 625–631.

Shahbandeh M. 2021. Production of bananas worldwide by region 2020. Fruit: banana production worldwide by region 2020 | Statista accessed 11th April 2022.

Singh, N., Kumar, A., Gupta, V.K., Sharma, B., 2018. Biochemical and molecular bases of lead-induced toxicity in mammalian systems and possible mitigations. Chem. Res. Toxicol. 31, 1009-1021

Sivandzade, F.; Prasad, S.; Bhalerao, A.; Cucullo, L. 2019. NRF2 and NF-KB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. Redox Biol., 21, 101059.

Soares, C.M.; Baptista, A.M.; Pereira, M.M.; Teixeira, M. 2004. Investigation of protonatable residues in Rhodothermus marinus caa3 haem-copper oxygen reductase: Comparison with Paracoccus denitrificans aa3 haem-copper oxygen reductase. J. Biol. Inorg. Chem. , 9, 124–134.

Su, H., Zhou, L., Samuel, S., Fiati, K., Hongbo, S., Yafei, W., Xin, S., Yuanliang, G., Tabatha, B., Joni, A., Baobo, Z., Min, D., Jinshun, Z. I.D and Xialu, L. (2017). Joint Toxicity of Different Heavy Metal Mixtures after a Short-Term Oral Repeated-Administration in Rats. *Int. J. Environ. Res. Public Health*, 14, 1164.

Tan, X., Liu, B.Y., Lu, J.J., Li, S.Y., Baiyun, R.Q., Lv, Y.Y., Lu, Q., Zhang, Z.G., 2018. Dietary luteolin protects against HgCl2-induced renal injury via activation of Nrf2-mediated signaling in rat. J Inorg Biochem. 179, 24-31.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. Exper. Suppl. (Basel) 101, 133e164.

Tejada-Tovar, C., Gonzalez-Delgado, A.D., Villabona-Ortiz, A. Characterization of residual biomasses and its application for the removal of lead ions from aqueous solution (2019) Applied Sciences (Switzerland), 9 (21), art. no. 4486.

Vu, H.T., Scarlett, C.J., Vuong, Q.V., 2017. Optimization of ultrasound-assisted extraction conditions for recovery of phenolic compounds and antioxidant capacity from banana (Musa Cavendish) peel. J. Food Process. Preserv. 41 (5), e13148 n/a–n/a.

Vu, H.T., Scarlett, C.J., Vuong, Q.V., 2018. Phenolic compounds within banana peel and their potential uses: a review. J. Funct. Foods 40, 238–248.

Waisberg, M.; Joseph, P.; Hale, B.; Beyersmann, D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003, 192, 95–117.

Wang, G.; Fowler, B.A. (2008). Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. Toxicol. Appl. Pharmacol., 233, 92–99.

Wang, Y., Su, H., Gu, Y., Song, X., Zhao, J., 2017c. Carcinogenicity of chromium and chemoprevention: a brief update. OncoTargets Ther. 10, 4065-4079.

Weaver VM, Fadrowski JJ, Jaar BG. 2015. Global dimensions of chronic kidney disease of unknown etiology (CKDu): a modern era environmental and occupational nephropathy? BMC Nephrol; 16:145.

Wei, X., Wei, H., Yang, D., Li, D., Yang, X., He, M., Lin, E. and Wu, B., 2018. Effect of aluminum exposure on glucose metabolism and its mechanism in rats. *Biological trace element research*, *186*(2), pp.450-456.

Willhite, C.C., Karyakina, N.A., Yokel, R.A., Yenugadhati, N., Wisniewski, T.M., Arnold, I.M., Momoli, F. and Krewski, D., 2014. Systematic review of potential health risks posed by pharmaceutical, occupational and consumer exposures to metallic and nanoscale aluminum, aluminum oxides, aluminum hydroxide and its soluble salts. *Critical reviews in toxicology*, *44*(sup4), pp.1-80.

Wohlt, D., Schwarz, E., Schieber, A., & Bader-Mittermaier, S. (2021). Effects of Extraction Conditions on Banana Peel Polyphenol Oxidase Activity and Insights into Inactivation Kinetics Using Thermal and Cold Plasma Treatment. *Foods (Basel, Switzerland)*, *10*(5), 1022. https://doi.org/10.3390/foods10051022

World Health Organization (WHO), (2007). Health risks of heavy metals from long range trans boundary air polution, Jt. WHO l Conv. Task Force Heal. Asp. Air Pollut 2–144.

World Health Organization (WHO), Ten Chemicals of Major Public Health Concern, World Health Organization, 2010, pp. 1–4.

Yang, X., Han, Y., Mu, Y., Yang, P., Gu, W., Zhang, M., 2020. Multigenerational effects of cadmium on the lifespan and fertility of Drosophila melanogaster. Chemosphere 245, 125533.

Yuan, C. Y., Lee, Y. J. & Hsu, G. S. W. (2012). Aluminum overload increases oxidative stress in four functional brain areas of neonatal rats. *J Biomed Sci* **19,** 51

Zaini, H.M., Roslan, J., Saallah, S., Munsu, E., Sulaiman, N.S. and Pindi, W., 2022. Banana peels as a bioactive ingredient and its potential application in the food industry. *Journal of Functional Foods*, *92*, p.105054.

Zhang, Y., Liu, Q., Yin, H., Li, S., 2020. Cadmium exposure induces pyroptosis of lymphocytes in carp pronephros and spleens by activating NLRP3. Ecotoxicol. Environ. Saf. 202, 110903.

Zhao, L.L., Ru, Y.F., Liu, M., Tang, J.N., Zheng, J.F., Wu, B., Gu, Y.H., Shi, H.J., 2017. Reproductive effects of cadmium on sperm function and early embryonic development in vitro. PloS One 12, e0186727.

Zhuang, S., Demirs, J.T., Kochevar, I.E. p38 mitogen-activated protein kinase mediates bid cleavage, mitochondrial dysfunction, and caspase-3 activation during apoptosis induced by singlet oxygen but not by hydrogen peroxide, J. Biol. Chem. 275 (2000) 25939–25948.

Zhuang, Y., Ma, Q., Guo, Y. and Sun, L., 2017. Protective effects of rambutan (Nephelium lappaceum) peel phenolics on H_2O_2-induced oxidative damages in HepG2 cells and d-galactose-induced aging mice. *Food and Chemical Toxicology*, *108*, pp.554-562.

Table 1: Effect of banana peel extract on the body weight, absolute and relative weight of liver and kidney, feed and fluid intake in female albino rats exposed to heavy metal mixture.

GROUPS	**Absolute wt liver (g)**	**Absolute wt kidney(g)**	**BODY WEIGHT (g)**	**Relative wt (%) liver**	**Relative(%) wt kidney**
Control	5.5±0.00[a]	1.3±0.00 [a]	I = 125.82±16.43 F = 200.00±14.14 %wt gain=62.91	2.75 [a]	0.65
Heavy Metal Mixture (HMM)	4.8±0.28[b]	1.4±0.28 [a]	I = 116.86±8.05 F = 180.50±0.71 %wt gain=64.92	2.66 [a]	0.78
HMM+ Low Dose Banana Peel Extract (BPE)	4.44± 0.62 [b]	1.2± 0.14 [a]	I = 103.57±11.50 F= 168.50±38.89 %wt gain=61.47	2.64 [a]	0.71
HMM + Medium Dose Banana Peel Extract (BPE)	4.68±0.11 [b]	1.45±0.35 [a]	I = 87.57±4.79 F =179.50±28.99 %wt gain=48.79	2.61 [a]	0.81
HMM + High Dose Banana Peel Extract (BPE)	4.15±0.21 [b]	1.03±0.18 [b]	I = 94.86±6.67 F = 147.50±19.09 %wt gain=64.31	2.81 [b]	0.7

Values are presented as Mean ±SD values
with different superscripts are significantly different from each other at $p< 0.05$), while values with the same superscripts are not significantly different.

Table 2: Person's correlation between biomarkers of oxidative stress, pro-inflammatory markers, apoptotic marker and transcription factors in the liver

Variables	GSH	GPX	CAT	SOD	MDA	Cas 3	Hmox	IL6	Nrf2	Nfkb	Tnfα	NO
GSH	**1.00**											
GPX	**0.88**$^{\#}$	**1.00**										
CAT	**0.72***	**0.76***	**1.00**									
SOD	**0.92**$^{\#}$	**0.82**$^{+}$	0.59	**1.00**								
MDA	**-0.66***	-0.51	-0.48	**-0.77**$^{+}$	**1.00**							
Cas-3	**-0.88**$^{\#}$	**-0.71***	-0.57	**-0.82**$^{+}$	**0.76**	**1.00**						
Hmox	**-0.65***	-0.44	-0.46	**-0.73***	0.57	0.62	**1.00**					
IL-6	**-0.73***	-0.47	-0.50	**-0.79**$^{+}$	**0.74***	**0.74***	**0.96**$^{\#}$	**1.00**				
NFR2	**-0.90**$^{\#}$	**-0.82**$^{+}$	**-0.64***	**-0.97**$^{\#}$	**0.74***	**0.79**$^{+}$	**0.82**$^{+}$	**0.85**$^{+}$	**1.00**			
NFKB	**-0.67***	-0.32	-0.51	-0.53	0.56	**0.79**$^{+}$	**0.69***	**0.80**$^{+}$	0.55	**1.00**		
TNF-α	**-0.76***	-0.47	-0.59	**-0.72***	**0.69***	**0.81**$^{+}$	**0.89**$^{\#}$	**0.95**$^{\#}$	**0.77**$^{+}$	**0.93**$^{\#}$	**1.00**	
NO	-0.55	-0.22	-0.37	-0.59	**0.66***	**0.65***	**0.91**$^{\#}$	**0.95**$^{\#}$	**0.66***	**0.85**$^{+}$	**0.94**$^{\#}$	**1.00**

Values in bold are different from 0 with a significance level p<0.05 (*), ii) p<0.01 (+) and iii) p<0.001 (#).

Table 3: Pearson R correlation between biomarkers of oxidative stress, pro-inflammatory markers, apoptotic marker and transcription factors in the kidney

Variables	GSH	GPX	CAT	SOD	MDA	Cas 3	Hmox	IL6	Nrf2	Nfkb	Tnfα	NO
GSH	**1.00**											
GPX	**0.76***	**1.00**										
CAT	0.44	0.51	**1.00**									
SOD	0.43	**0.75***	**0.71***	**1.00**								
MDA	-0.46	-0.58	**-0.84**$^{+}$	**-0.79**$^{+}$	**1.00**							
Cas-3	-0.59	**-0.69***	**-0.67***	**-0.80**$^{+}$	0.50	**1.00**						
Hmox	**-0.78**$^{+}$	**-0.85**$^{+}$	**-0.72***	**-0.80**$^{+}$	**0.64***	**0.94**$^{\#}$	**1.00**					
IL-6	-0.57	**-0.68***	**-0.75***	**-0.87**$^{\#}$	**0.71***	**0.90**$^{\#}$	**0.90**$^{\#}$	**1.00**				
Nrf2	**-0.65***	**-0.82**$^{+}$	-0.60	**-0.73***	0.47	**0.86**$^{+}$	**0.86**$^{+}$	**0.67***	**1.00**			
Nfkb	-0.63	**-0.73***	**-0.80**$^{+}$	**-0.87**$^{+}$	**0.75***	**0.90**$^{\#}$	**0.93**$^{\#}$	**0.99**$^{\#}$	**0.73***	**1.00**		
Tnf-α	-0.57	**-0.68***	**-0.83**$^{+}$	**-0.84**$^{+}$	**0.94**$^{\#}$	0.61	**0.75***	**0.85+**	0.51	**0.87**$^{\#}$	**1.00**	
NO	**-0.68***	**-0.82**$^{+}$	**-0.79**$^{+}$	**-0.89**$^{\#}$	**0.68***	**0.95**$^{\#}$	**0.97**$^{\#}$	**0.92**$^{\#}$	**0.90**$^{\#}$	**0.95**$^{\#}$	**0.79**$^{+}$	**1.00**

Values in bold are different from 0 with a significance level p<0.05 (*), ii) p<0.01 (+) and iii) p<0.001 (#).

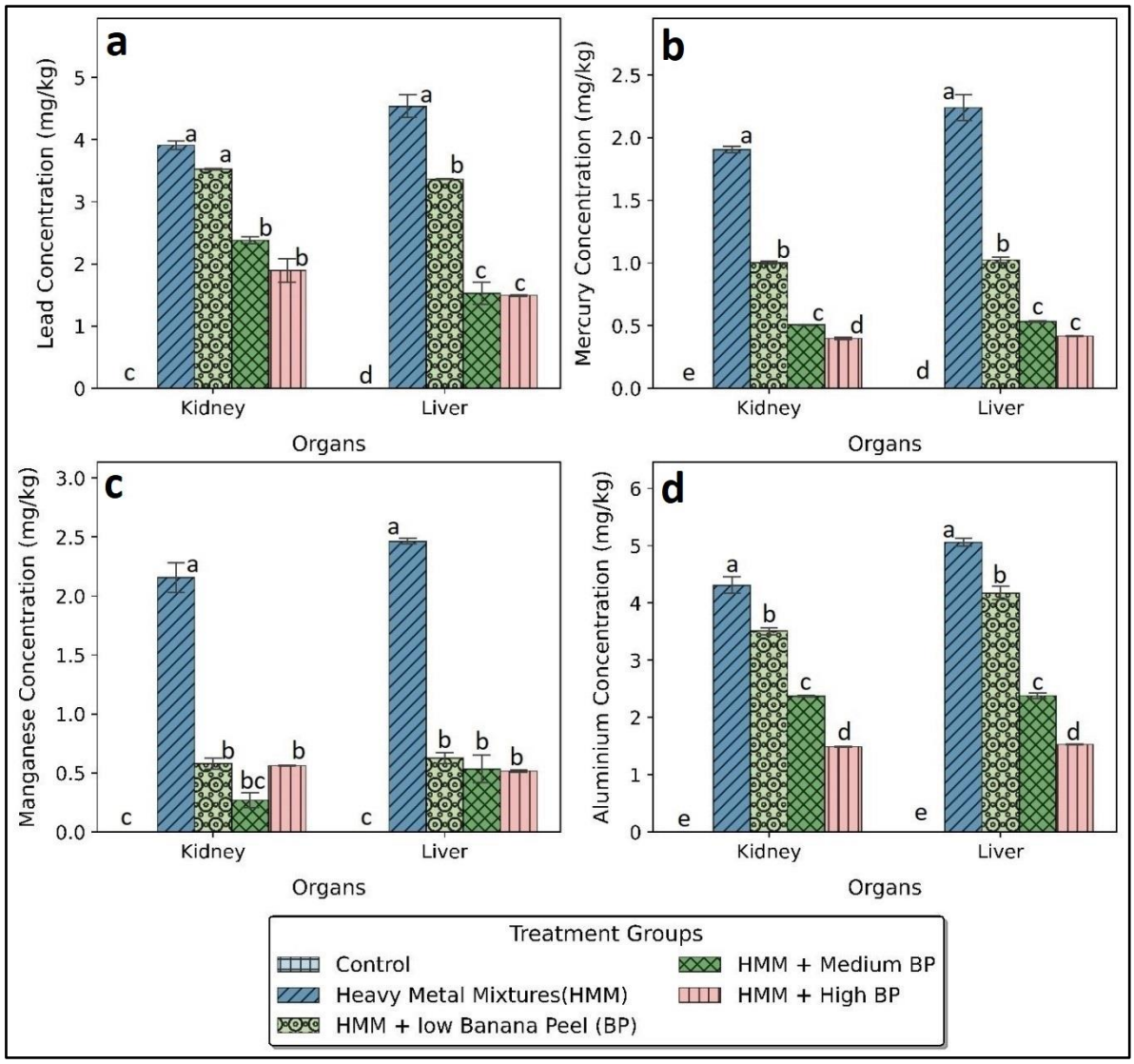

Figure 1: Effect of banana peel extract on metal levels in liver and kidney of rats exposed to heavy metal mixture (MM) and banana peel extract (BP). Different superscripts (a, b, c. d e) indicated significant ($p<0.05$) differences between groups.

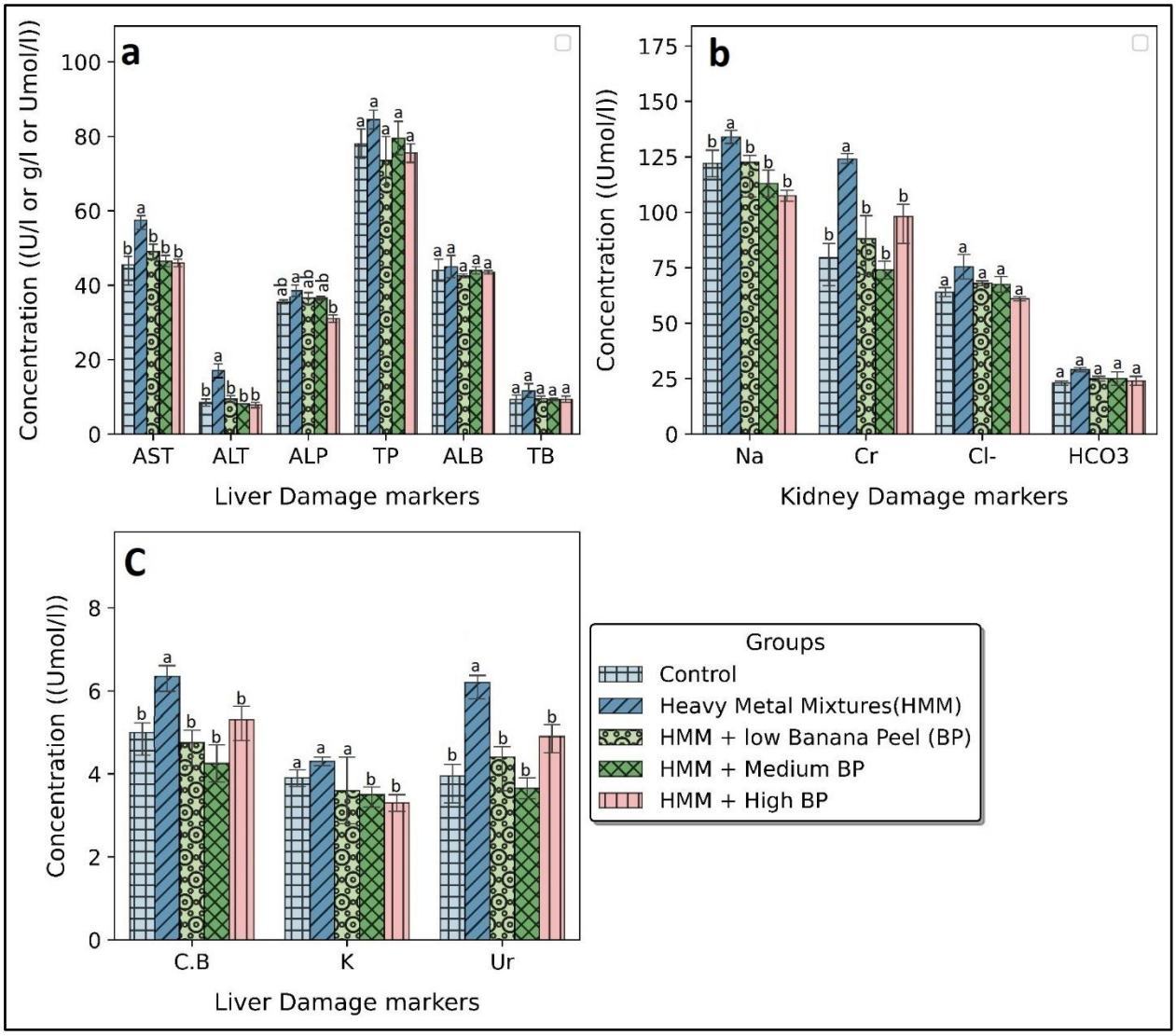

Figure 2a-c. Effect of banana peel extract on liver function markers and electrolytes/renal function markers of rats exposed to metal mixture. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

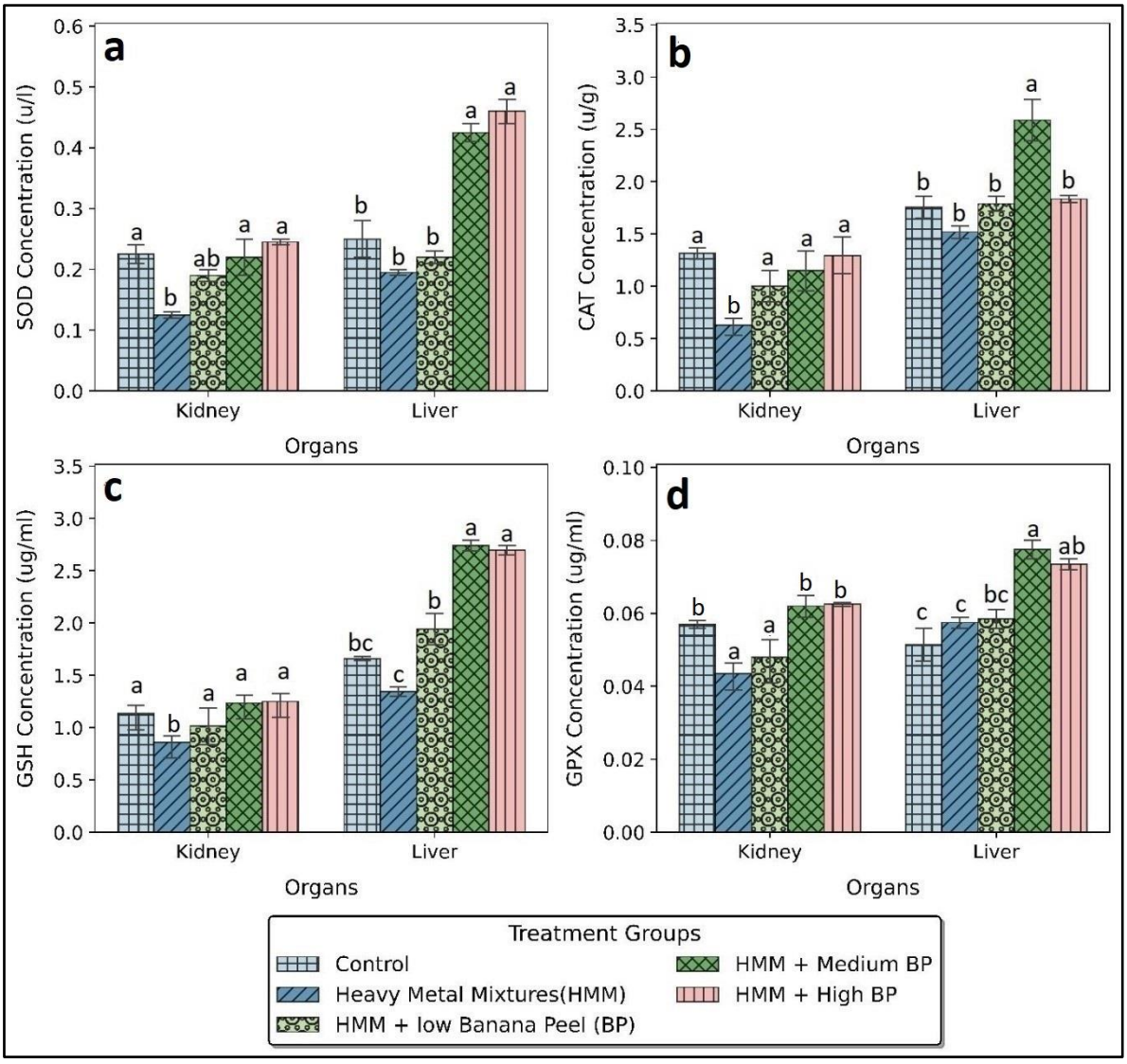

Figure 3: Antioxidants profile in liver and kidney of rats treated or not with Banana peel extract and exposed to metal mixture. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

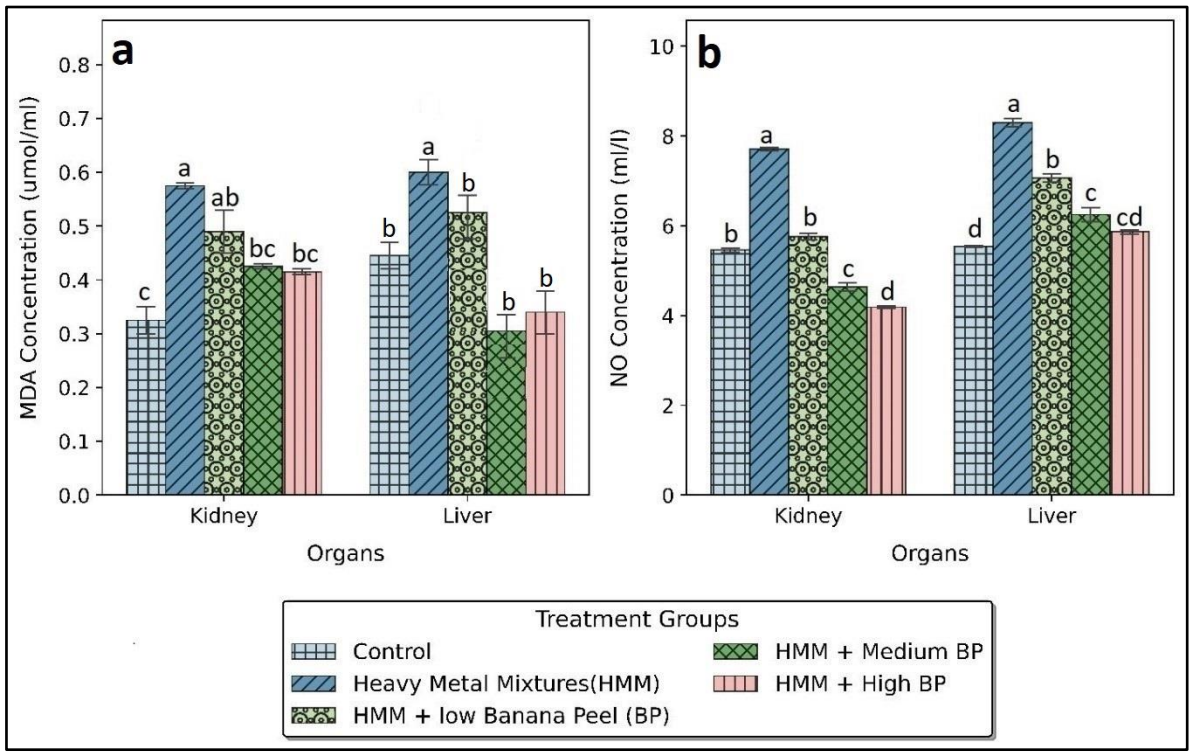

Figure 4: Lipid peroxidation and oxidative stress profile in liver and kidney of rats treated with Banana peel extract after exposure to metal mix exposure. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

.

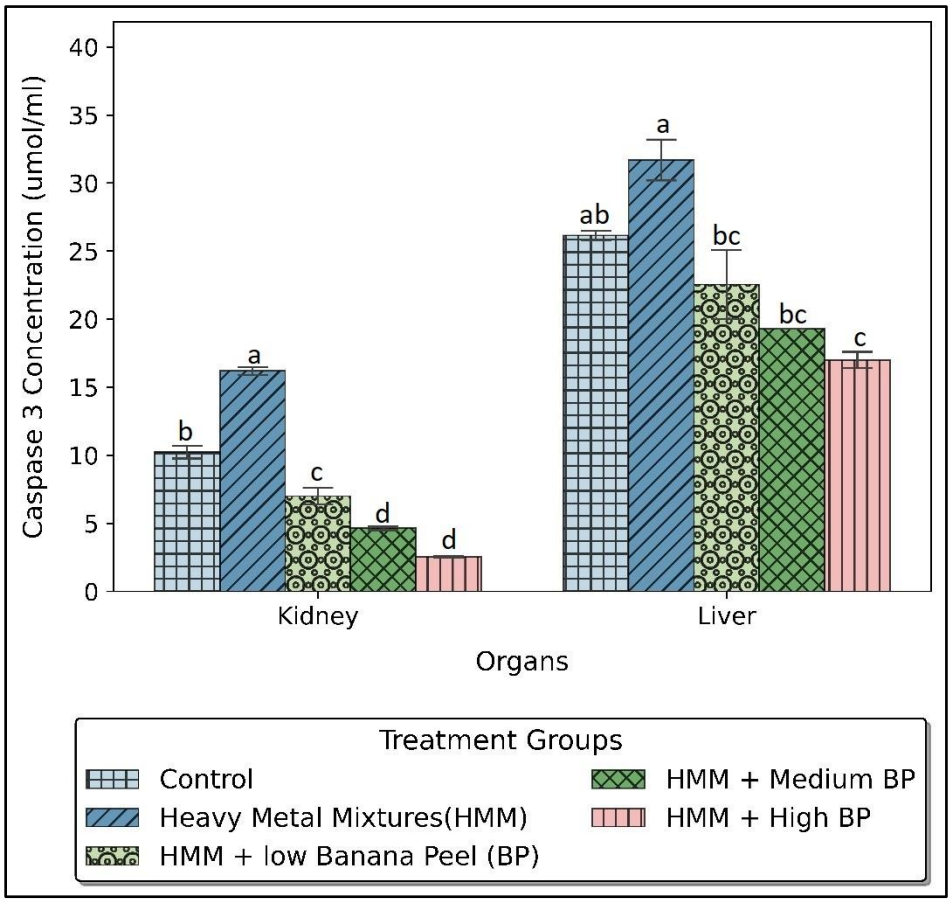

Figure 5: Effect of banana peel extract treatment on Caspase 3 activity in the Liver and kidney of rats exposed to heavy metal mixture. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

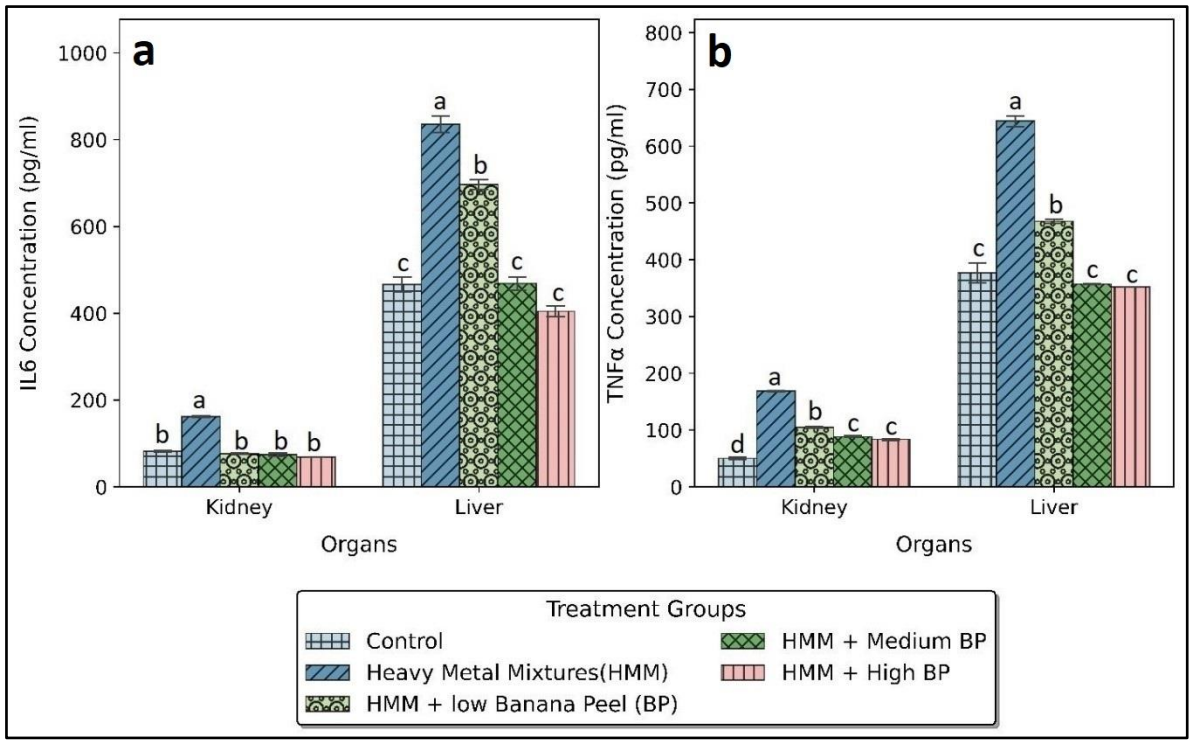

Figure 6: Effect of banana peel extract on pro-Inflammatory markers (IL-6 and Tnf-α in the liver and kidney of rats exposed to heavy metal mixture. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

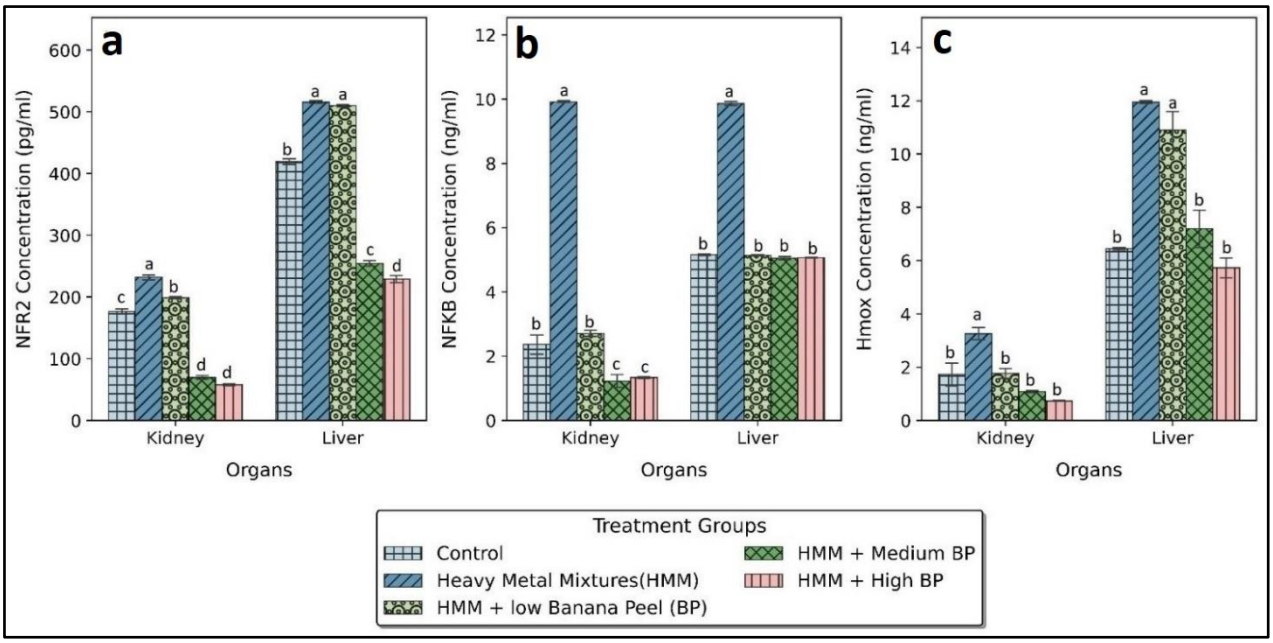

Figure 7: Effect of banana peel extract on transcription factors (Nfkb and Nfr2) and Hmox-1 in the liver and kidney of rats exposed to heavy metal mixture. Different superscripts (a, b, c) indicated significant ($p<0.05$) differences between groups.

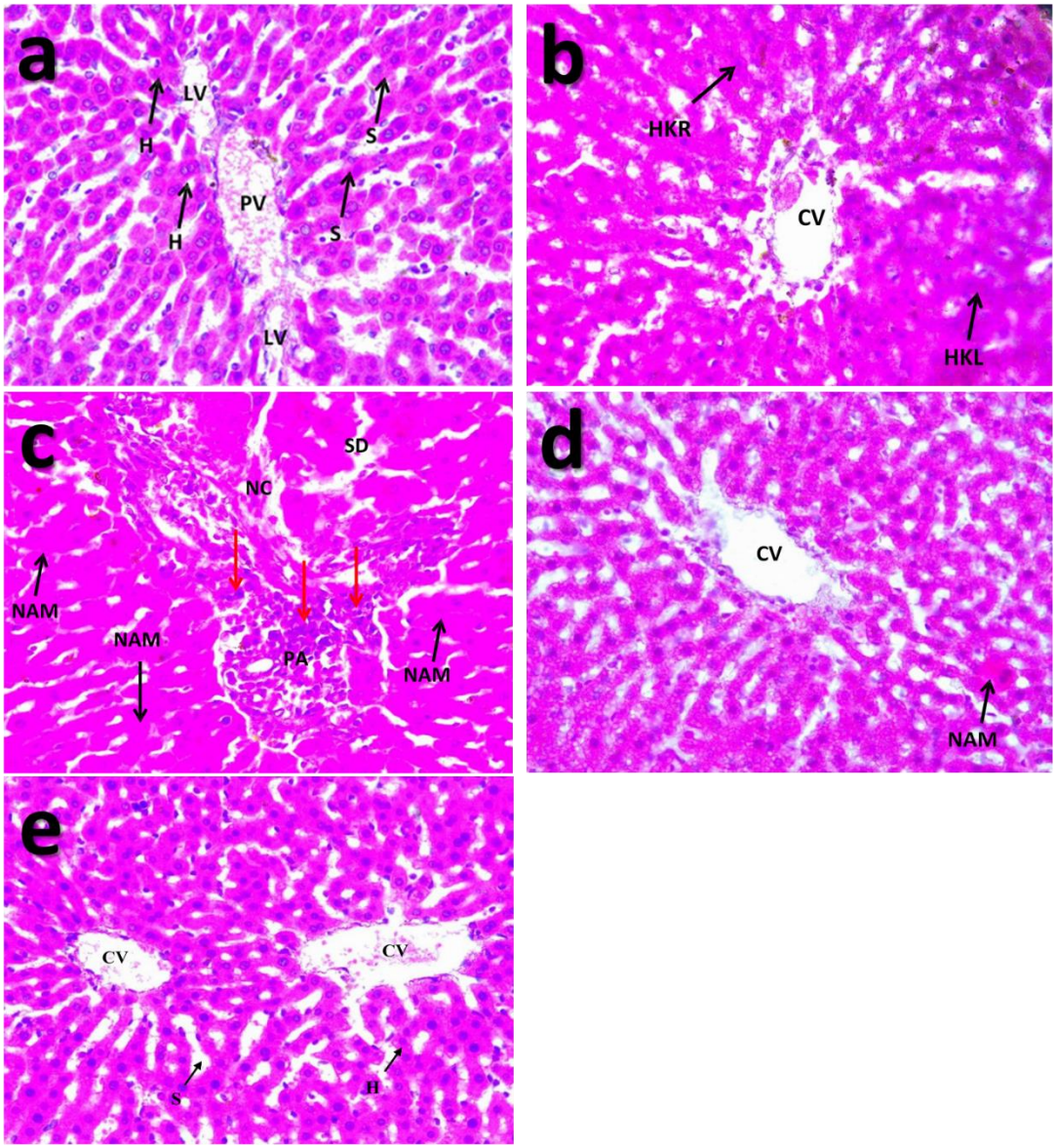

Figure 8: Photomicrograph section of liver tissue of rats: a) control group; b) metal mixture c), d), and e) metal mixture with banana peel extract at 200, 400 and 800 mg·kg^{-1}. Portal Vein (PV), Lymphatic Vessels (LV), Hepatocytes (H) , sinusoids(S), Central Vein (CV), Hepatocyte Necrosis (HN), multifocal areas of Hepatocyte Karyorrhexis (HKR) and Hepatocyte Karyolysis (HKL), Necrotic acidophil mass (NAM), portal area (PA), Sinusoid dilation (SD).

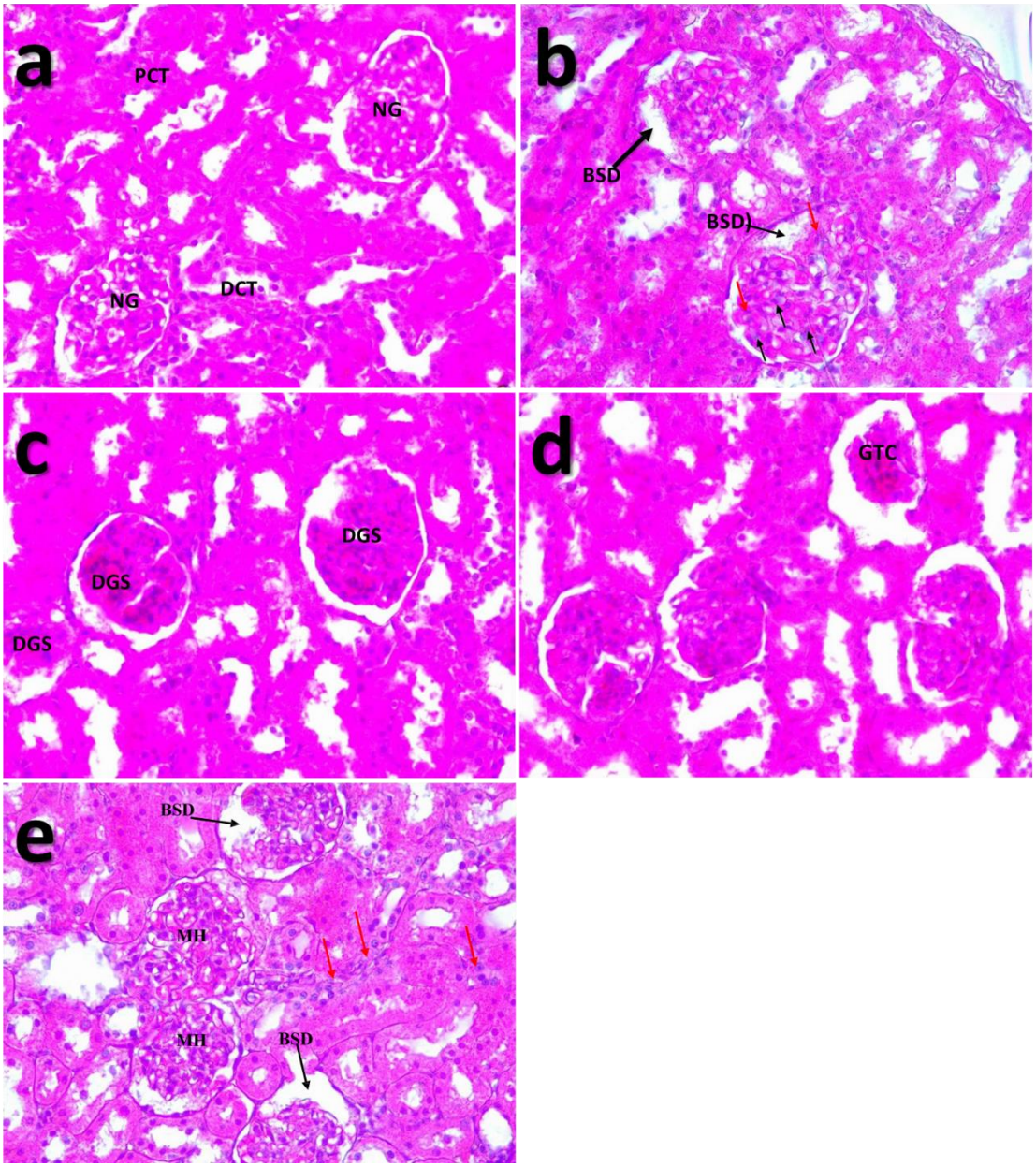

Figure 9: Photomicrograph section of kidney tissue of rats: a) control group; b) metal mixture c), d), and e) metal mixture with banana peel extract at 200, 400 and 800 mg·kg^{-1}. Normal Glomerulus (NG), Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT), Bowman Space Dilation (BSD), Diffuse Glomerular Sclerosis (DGS), Glomerula tuft collapse (GTC), mesangial hypercellularity (MH)