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Histopathologic effect of lead and cadmium and the mitigating effect of standard metal chelator and spice mixture

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Abstract

Lead and cadmium are toxic to the liver and kidney. Standard metal chelators have deleterious effects on the body. Hence, natural spices like ginger and garlic have been studied as alternative mitigants. This study uniquely evaluates the possible synergistic mitigating effect of the mixture of both spices (SM) in comparison with that of calcium sodium ethylenediaminetetraacetic acid (CaNaEDTA), a standard metal chelator (MC). A total of 10 groups of 6 rats each were used and they were fed with finisher mash and water *ad libitum*. Group 1 was given only feed and water, Additionally, Group 2 was given 50 mg/kg lead chloride ($PbCl_2$), group 3 was given 15 mg/kg cadmium chloride (CdCl), while group 4 received 15 mg/kg $CdCl_2$ plus 50 mg/kg $PbCl_2$. Group 5, 6 and 7 received the same treatment as group 2,3 and 4 plus 15 mg/kg CaNaEDTA; while Group 8, 9 and 10 received the same treatment as group 2,3 and 4 respectively, plus 300 mg/kg of SM. Treatments were given at 72-hour intervals for 6 weeks through oral gavage. The rats were sacrificed, and their harvested organs were analyzed histologically. Lead and cadmium induced severe inflammatory changes, necrosis, fatty degeneration, and pyknosis in the liver and kidney. Co-administration of the metals and MC resulted in moderate fatty changes, moderate degeneration, and mild inflammation in both organs. Likewise, co-administration of the metal with SM produced moderate degeneration and moderate fatty changes with mild inflammation in the kidney. The study shows that the ameliorating effect of the SM on lead and cadmium-induced renal toxicity is comparable to that of the MC. However, SM did not produce any appreciable ameliorative effect in the liver. Therefore, these readily available spices can be used for prophylactic and therapeutic purposes against heavy metal-induced renal toxicity.

Key words: Lead, Cadmium, Histopathologic, Liver and Kidney

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INTRODUCTION

Lead and cadmium are extremely toxic heavy metals that negatively impact almost every organ and system of the body when inhaled or swallowed (Mitra *et al*., 2022, Tchounwou *et al.*, 2012). Lead causes several histopathological changes in the liver and kidney such as fatty or hydropic degeneration, haemosiderosis, large foci of extramedullary erythropoiesis in the liver as well as inflammatory changes such as infilteration of inflammatory cells (Nguyen *et al*., 2023), with high infiltration of lymphocytes and plasma cells, hyperplasia of Kupffer cells, nuclear pyknosis of hepatocytes, cytoplasmic inclusions and cellular necrosis in the liver (Jarrar and Taib, 2012). Offor *et al.* (2017) observed degeneration and necrosis of the renal parenchymal cells with high levels of inflammatory cell infiltration and vacuolar degeneration of the renal cortex in rats exposed to lead poisoning. Lead has been shown to cause degeneration of the bowman's capsule, pyknotic glomeruli, severe haemohrage and infilteration of inflammatory cells in the interstitial tissues as well as acute necrosis in the epithelial cells linings of the kidney tubules (Amal, 2023). Cadmium was also shown to cause some damage in the liver tissues such as pyknotic nuclei, cell membrane breakdown and in the renal system such as swollen and pyknotic glomeruli (Gattea *et al,* 2021). It has also been demonstrated that co-administration of the two metals produces more severe lesions in the liver and kidney, such as, vesicular degeneration, necrosis, nuclei concentration etc. in the liver. In the kidney, they cause severe inflammatory cell infiltration in the glomeruli and tubules, swelling of the glomerulus, obstruction of the nephric tubules, renal tubular epithelial cell degeneration, necrosis etc. (Yuan *et al.,* 2014). Treatment of lead and cadmium poisoning has mostly relied on the use of chelating agents which help to remove the metal from circulation, and on the use of antioxidants that counteract the toxic effects of the compounds (Wani *et al.,* 2015). Some of the known chelating agents include dimercaprol or British Anti-Lewisite (BAL), calcium disodium ethylenediaminetetraacetic acid ($CaNa_2$ EDTA), D-penicillamine and succimer (Henretig, 2001). Other compounds that act by mitigating the toxic effects of the compounds are Taurine, N-acetylcysteine, Thiamine, Alpha-lipoic acid, Methionine, and homocysteine (Sajitha *et al.,* 2010; Wani *et al.,* 2015). However, the use of these synthetic chelators has been associated with some unpleasant side effects such as gastrointestinal disturbances, rash, alteration of some liver enzymes serum levels, loss of appetite, hypertension, tachycardia, hypocalcemia etc. (Venkatesh and Shambhavi, 2013).

Recently, interests have been drawn to the use of alternative medicine, such as the use of herbs, or natural products in the management of cadmium and lead poisoning. Some natural products that have demonstrated usefulness in lead and cadmium toxicity include ginger, garlic, cabbage, onions etc. (Eteng *et al.,* 2012; Mohammed *et al.,* 2013, Gabr *et al.,* 2017). The phytochemical constituents of garlic are allicin, ajoene, flavonoids, essential oils, sulfides,S-allyl-cysteine, O-Butylisourea, formic acid, phenol, cytidine (Yunus *et al*., 2021) while that of ginger are flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins (Kela *et al*, 2023). *Coriandrum sativum* (Commonly known as Coriander) and *Curcuma longa* have been shown to have positive effects on the treatment of lead and cadmium toxicity (Mustafa, 2021; Park *et al.,* 2021). According to the World Health Organization, it is estimated that 240 million people are overexposed and 99 % of those with high blood levels above 20 µg/dl are in the developing world, and about 1 million people die annually due to lead poisoning (WHO, 2022). Lead and cadmium are two important heavy metals prevalent in Ebonyi State, South–Eastern Nigeria (Obasi *et. al.,* 2019) and which have been studied as a major threat to the health of citizens in this part of the country (Elom *et al.,* 2017). Hence the need to explore natural and readily available mitigants for the management of heavy metal toxicity in contaminated areas. Since there is paucity of studies that evaluated

the mixture of ginger and garlic as mitigants, and the need to compare the effect of alternative natural spices with that of a standard metal chelator and to assess possible synergistic effect of combining garlic and ginger, the aim of this study was to compare the ameliorative effect of a spice mixture containing garlic and ginger with that of $CaNa_2EDTA$ on the histopathological changes induced by lead and cadmium poisoning in the liver and kidney of rats.

MATERIALS AND METHODS

Materials: Analytical grades lead chloride ($PbCl_2$), cadmium chloride $CdCl_2$ and EDTA were obtained from Riedel-De HaenAGSeelze, Hannover, Germany. Other chemicals used were formalin, normal saline, diethyl ether, formaldehyde, Absolute Alcohol, Xylene, Paraffin wax, Egg Albumin, Hematoxylin, and Eosi (Dantegahospital reagent and equipment, Enugu). Equipment: Microscope Olympus (Japan), hot air oven (Search Tech China), water bath (Search Tech China), Microtome (Shadon England), Hot plate (Search Tech China)

Animals

Sixty [60] one month old male rats of 38 – 97g of weight were bred at Pet Field Resources LTD, Nsukka Enugu state, were purchased and kept in clean metal cages at Ebonyi State University animal house Presco, fed commercial chick mash *ad libitum* and acclimatised for one week before the commencement of research. All the rats received human care in accordance with the National Institute of health guidelines for the care and use of laboratory animals (NRC, 1985).

Preparation of extracts of spice mixture (sm) using ginger and garlic.

Fresh garlic cloves (500g) and ginger (500g) were purchased from Orie-Ugba market in Umuahia, Abia state Nigeria. They were thoroughly washed with clean water, and the bark of the ginger and garlic carefully peeled off. The cleaned garlic and ginger were carefully blended into a thick paste; the thick paste of spice mixture (SM) was passed through a muslin cloth. The filtrate was allowed to settle for about 8-10 hrs before decanting off the clear water. The slurry was further poured into a cheese bag for the removal of water, and further sun dried to obtain a dry powdery spice mixture. The dried SM powder was collected and stored in a dry polythene bag at room temperature for further use.

Experimental design

Sixty (60) male rats of one month old were randomly divided into ten (10) groups of six rats per group. Group 1 was given feed and water only, group 2 was given 50 mg/kg lead chloride ($PbCl_2$), group 3 was given 15 mg/kg cadmium chloride ($CdCl_2$) (El Tarras*et al*, 2016), group 4 was given 15 mg/kg $CdCl_2$ and 50 mg/kg $PbCl_2$. Group 5 received 50mg/kg of $PbCl_2$ and 15mg/kg of $CdCl_2$ plus 15 mg/kg calcium sodium ethylenediaminetetraacetic acid (CaNaEDTA) as a standard metal chelator (MC) (Chandran and Cataldo, 2010), group 6 received 50mg/kg of $PbCl_2$ and 15mg/kg of EDTA while group 7 was given 15mg/kg of $CdCl_2$ and 15mg/kg of EDTA. Group 8 received 50mg/kg of lead and 300 mg/kg of Spice Mixture (Ugwuja *et al.,* 2019), group 9 received 15mg/kg of $CdCl_2$ and spice mixture while group 10 received 50mg/kg of $PbCl_2$ and 15mg/kg of $CdCl_2$ plus spice mixture. All administration were through oral intubation/gavage at 72 h intervals for 42 days (6 weeks). The weights of the animals were taken weekly for proper administration of the right dosages of chemicals and spice mixtures.

Collection of samples

At the end of 42 days, the animals were sacrificed under light ether anesthesia. The liver and kidney were excised and preserved in 10% formalin for histological analyses.

Histological analysis

The liver and kidney fixed in 10% buffered formalin were processed for paraffin sectioning by dehydration in 50%, 70%, 90% alcohol and grade 1, grade 2 and grade 3 absolute alcohol for 45 minutes each. The tissue was fixed in 10% normal saline in a container with light-fitting lids for 3 days to prevent autolysis, improve staining quality and aid the optical differentiation of cells (Okorie *et al.,* 2020). The tissue was then dehydrated to remove water that was not miscible with xylene and wax using different grades of alcohol ranging from 50% to 90% absolute alcohol for 30 minutes each. The

dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it in 3 changes of xylene for 30 minutes each. The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temp of 55^{O}C by passing it through three changes of molten paraffin in a hot air oven for 30 minutes. The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify. The Paraffin block of tissue was attached to a wooding block with the aid of a hot spatula held in between the wood block and paraffin wax, the spatula melted the wax which solidified when the spatula was removed. Finally, the block of tissues was sectioned using a rotary microtome, it was trimmed to obtain the cutting surface of the tissue at 15 microns and was sectioned at 5micron and dried on a hot plate for staining.

The sectioned tissues were dewaxed in xylene for 30 minutes. Afterwards, the xylene was removed by rinsing in absolute alcohol 90, 70 and 50% alcohol for two seconds each, and then washed in 2 changes of water. The tissues were stained in haematoxylin for 20mins, washed in water, differentiated in 1% acid, blued in tap water and then washed in water. The tissues were counter-stained in Eosin for 5 minutes, washed in water, dried and cleared in xylene. Finally, the stained tissues were mounted in dibutyl phthalate polystyrene xylene and dried for micrograph and interpretation

RESULTS

Figure 1 depicts photomicrograph of sections of kidney (X400)(H/E) of control group 1 (1R1 & 1R2) showing normal renal architecture with glomeruli (G), bowman space (BS), renal tubules (RT) and active tubular cell (TC). Photomicrograph of group 2 (2R1 & 2R2) sections of the kidney (x400) (H/E) exposed to lead only shows severe degeneration of the renal tissue, severe intra-renal inflammation (IRI), and severe fatty change (FC). The overall features are consistent with acute nephritis. Photomicrograph of group 3 (3R1 & 3R2) sections of the kidney (x400) (H/E) exposed to only cadmium shows severe degeneration on the renal tissue with severe intra renal inflammation (IRI) with severe tubular atrophy (TA) severe and fatty change (FC) the overall feature is consistency with chronic hepatitis. Photomicrograph of group 4 (4R1 & 4R2) section of the kidney (x400) (H/E) exposed to lead and cadmium shows severe degeneration on the renal tissue with acute persistent infiltration of the inflammatory cell (IRI) replacing the renal tubules, pyknotic glomeruli and severe fatty change (FC) the overall features are consistent with acute tubulointerstitial nephritis. Photomicrograph of group 5 (5R1 & 5R2) sections of the kidney (x400) (H/E) which received lead, cadmium, and Metal Chelator (MC) shows only moderate degeneration with active tubular cell (TC) and moderate fatty changes (FC). Photomicrograph of group 6 (6R1 & 6R2) sections of the kidney (x400) (H/E) which received lead and MC shows moderate degeneration mild fatty changes (FC) and mild infilteration of inflammatory cell (IIC) around the glomeruli.

Figure 2 shows a photomicrograph of group 7 (7R1& 7R2) sections of the kidney (x400) (H/E) that received cadmium and metal chelator shows moderate degeneration of the renal tissue and mild infiltration of the inflammatory cell (IIC) and mild area of hemorrhage (H) with active glomeruli (G). Photomicrograph of group 8(8R1 & 8R2) sections of the kidney (x400) (H/E) which received lead and spice mixtures (SM) shows moderate degeneration with moderate fatty changes (FC) and active glomeruli (G). Photomicrograph of group 9 (9R1 & 9R2)sections of the kidney (x400) (H/E) which received cadmium and SM shows moderate sege with mild pyknotic glomeruli (PG) and mild infiltration of the inflammatory cell (IIC). Photomicrograph of group 10 (10R1 & 10R2) sections of kidney (x400) (H/E) which received lead, cadmium and SM shows mild degeneration with moderate extravasated red blood cell (EVRBC) and focal area of necrosis (FAN).

In Figure 3, a photomicrograph of sections of the liver (x400) (H/E) of the control group (1r1 & 1r2), showing normal hepatic architecture with portal triad (PT) central vein (CV) and hepatocyte (H) was observed.

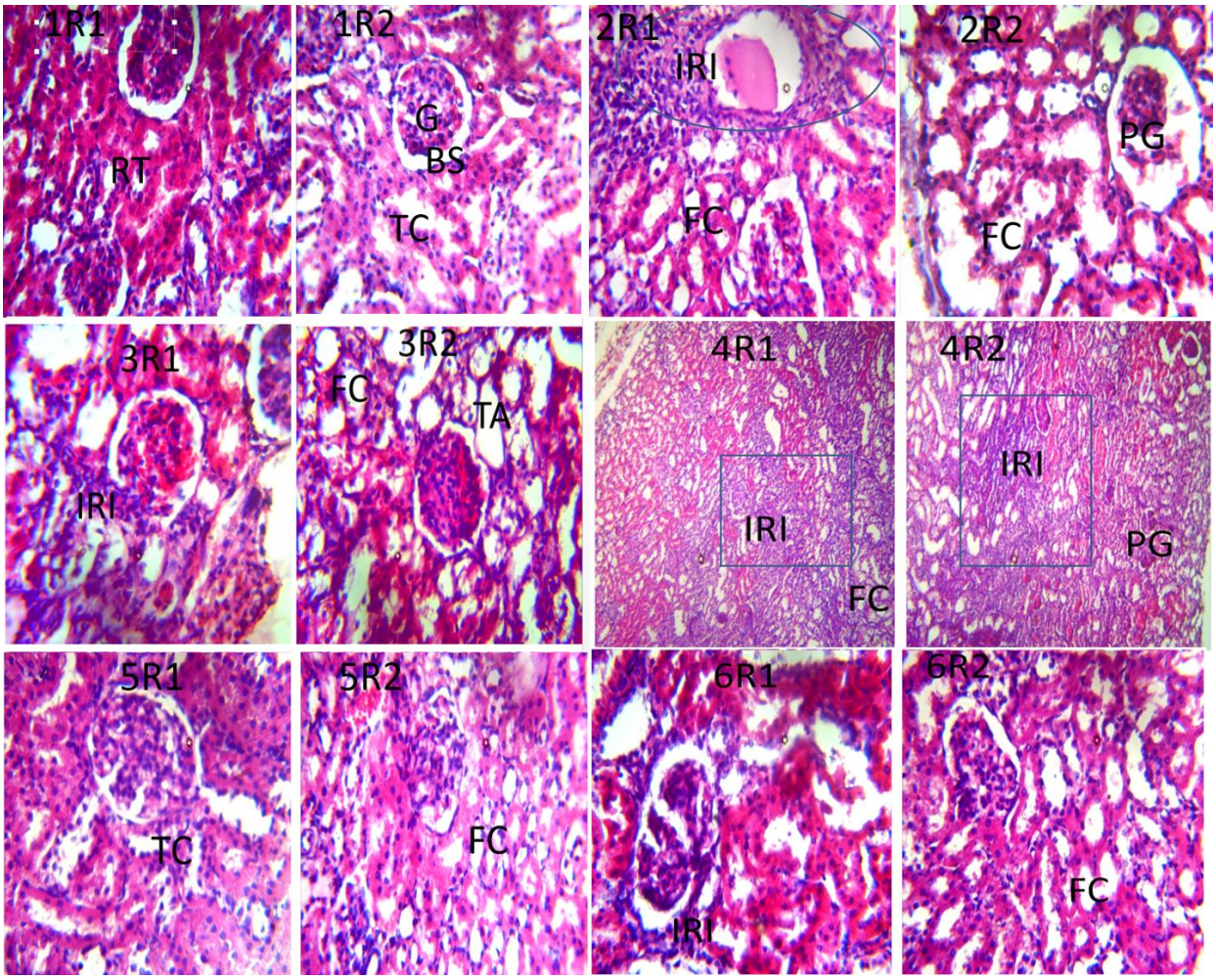

Figure 1: Photomicrograph of kidney sections A

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Photomicrograph of group 2 (2R1 & 2R2) sections of the liver (x400) (H/E) exposed to lead only showing severe effect on the hepatic tissues with a focal area of necrosis (N) and severe intrahepatic inflammation (IHI) the overall feature is consistent with chronic hepatitis. Photomicrograph of group 3(3R1 & 3R2) sections of the liver (x400) (H/E) exposed to cadmium showing the severe effect on the hepatic tissues with severe intrahepatic inflammation (IHI) and pyknotic (P) hepatocyte in some area the overall feature is consistent with chronic Inflammation. Photomicrograph of group 4 (4R1 & 4R2) sections of the liver (x400) (H/E) exposed to both lead and cadmium shows the severe effect on the hepatic tissues with severe intrahepatic inflammation (IHI) and focal area of intrahepatic hemorrhage (IHH) the overall feature is consistent with chronic hepatitis. Photomicrograph of group 5(5R1 & 5R2) sections of liver (x400) (H/E) exposed to lead, cadmium, and metal chelator (MC)shows moderate healing with moderate to mild intrahepatic aggregate of inflammatory cell (AIC), clumping (C) and pyknotic(P) hepatocyte. Photomicrograph of group 6 (6R1 & 6R2) sections of the liver (x400) (H/E) that was given lead and MC shows moderate healing with mild intrahepatic inflammation (IHI) and congestion of the portal vein (CPV)

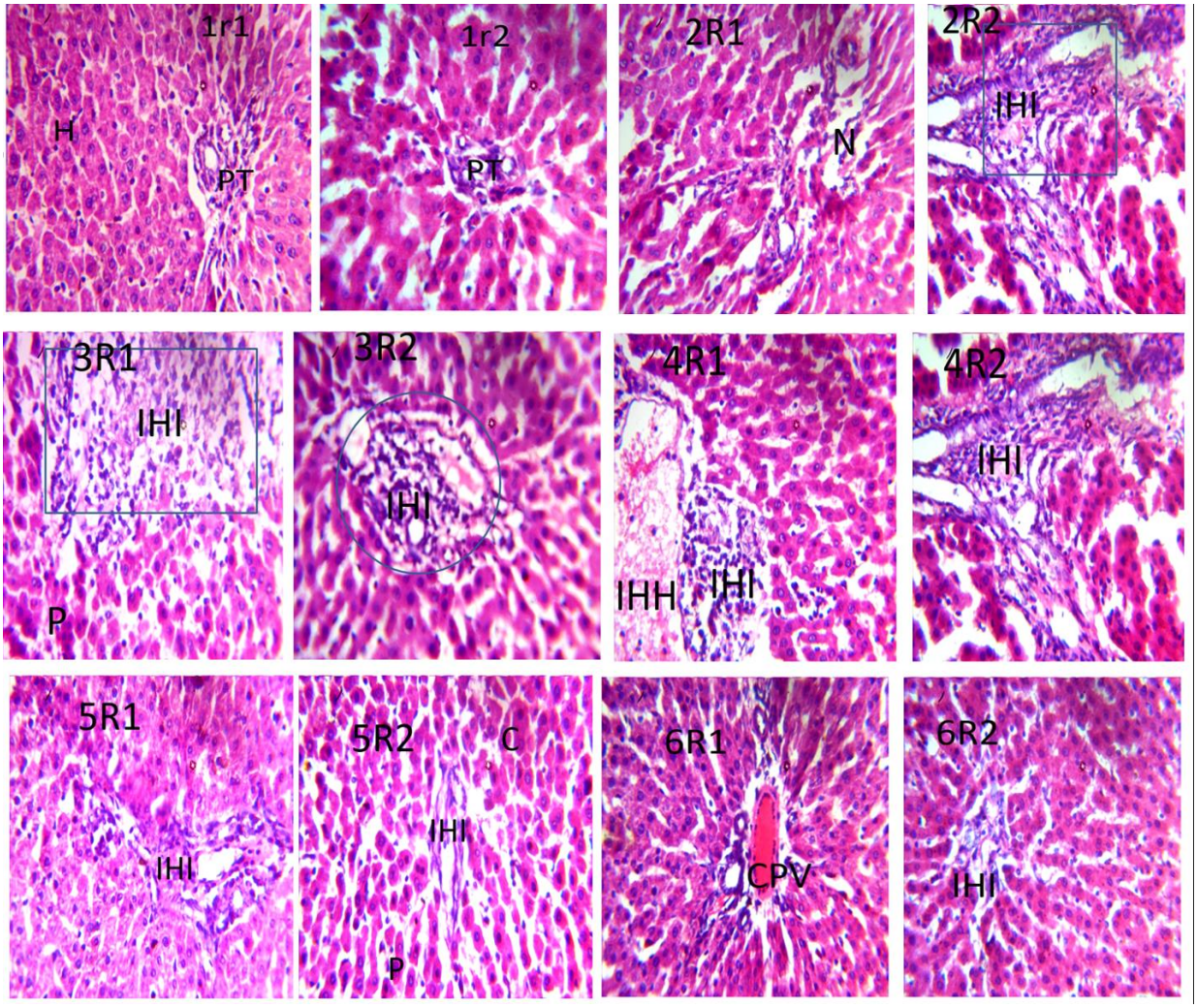

Figure 2: Photomicrograph of kidney sections B

Figure 4 depicts a photomicrograph of group 7 (7R1 &7R2) sections of liver (x400) (H/E) that was given cadmium and metal chelator showing moderate healing of the hepatic tissues with moderate intra hepatic inflammation (IHI). Photomicrograph of group 8 (8R1 & 8R2) sections of liver (x400) (H/E) given lead and spice mixture (SM) shows mild to moderate effect on the hepatic tissues with moderate intra hepatic inflammation (IHI). Photomicrograph of group 9(9R1 & 9R2) sections of liver (x400) (H/E) given cadmium and spice mixture shows severe effect on the hepatic tissues with severe intra hepatic inflammation (IHI). The overall features are consistent with chronic inflammation. Photomicrograph of group 10 (10R1 & 10R2) sections of liver (x400) (H/E) that received lead, cadmium and spice mixtures show severe effect on the hepatic tissues with severe intra hepatic inflammation (IHI) and fatty change (FC).

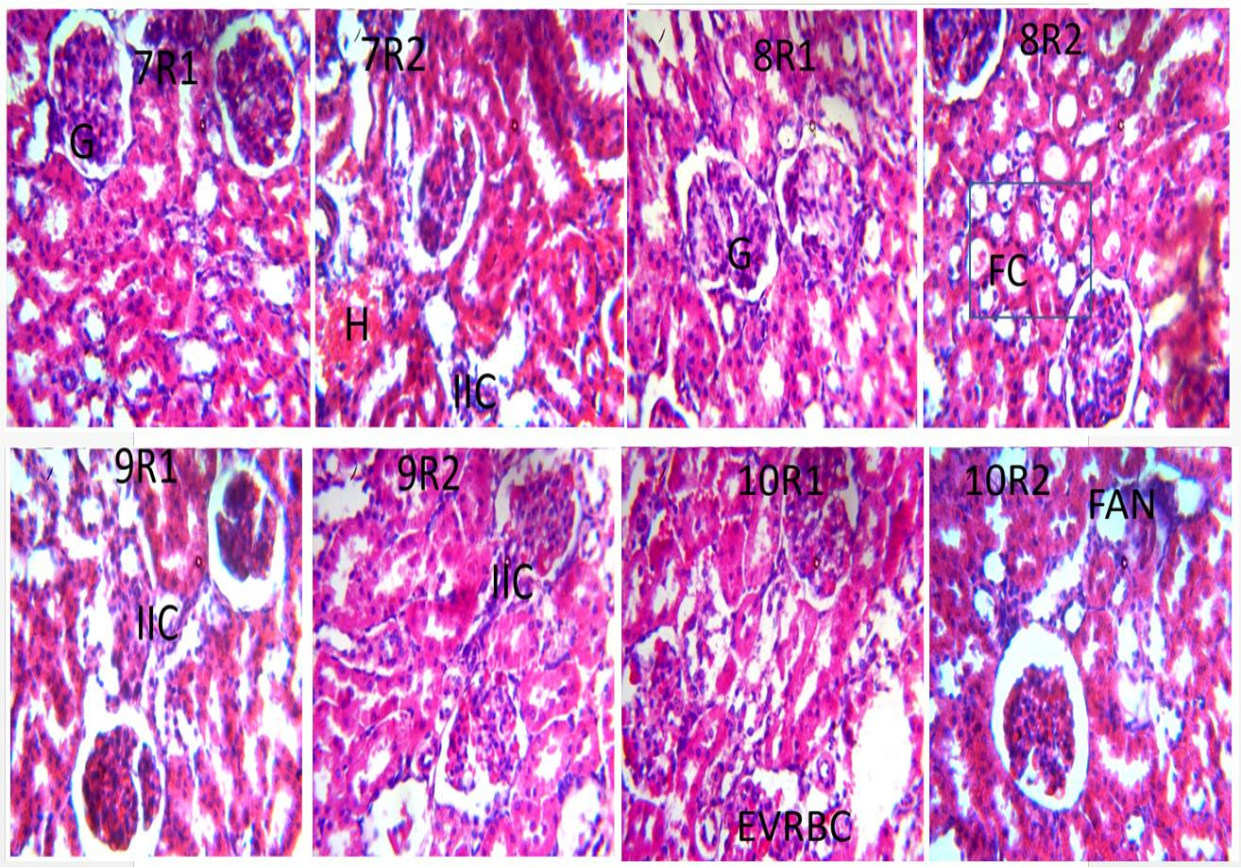

Figure 3: Photomicrograph of liver sections A

DISCUSSION

Lead (Pb) and cadmium (Cd) have been ranked second and seventh place on the priority list of dangerous substances (Andjelkovic *et al.,* 2019). It has been suggested that Pb and Cd can cause toxic effects either through oxidative stress, binding to ligands of important molecules such as oxygen, sulphur and nitrogen, inhibition of apoptosis, alteration of DNA structure, prevention of repair of DNA and aberrant gene expression (Matovic *et al.,* 2015). Both metals have been shown to cause toxic damage to different organs of the body such as the liver, kidney, pancreas, and organs of the endocrine, immune, reproductive and cardiovascular systems. Treatment of Pb and Cd poisoning has mostly relied on the use of chelators which have been shown to have several adverse effects on the liver, kidney, gastrointestinal tracts etc. Other limitations with their usage are being expensive and difficulty in administration. We hereby assessed the efficacy of a mixture of ginger and garlic in the treatment of Pb and Cd poisoning. The photomicrograph of the kidney and liver of the control group in this experiment showed normal renal and hepatic architecture; the kidney showed the glomeruli surrounded by the Bowman's space and the renal tubules with active tubular cells. The liver section showed the portal triad with the central vein and hepatocytes. However, the kidneys of the rats challenged with lead and cadmium showed severe degeneration of renal tissue, intrarenal infiltration of inflammatory cells which could be seen displacing the renal tubules as well as fatty degeneration.

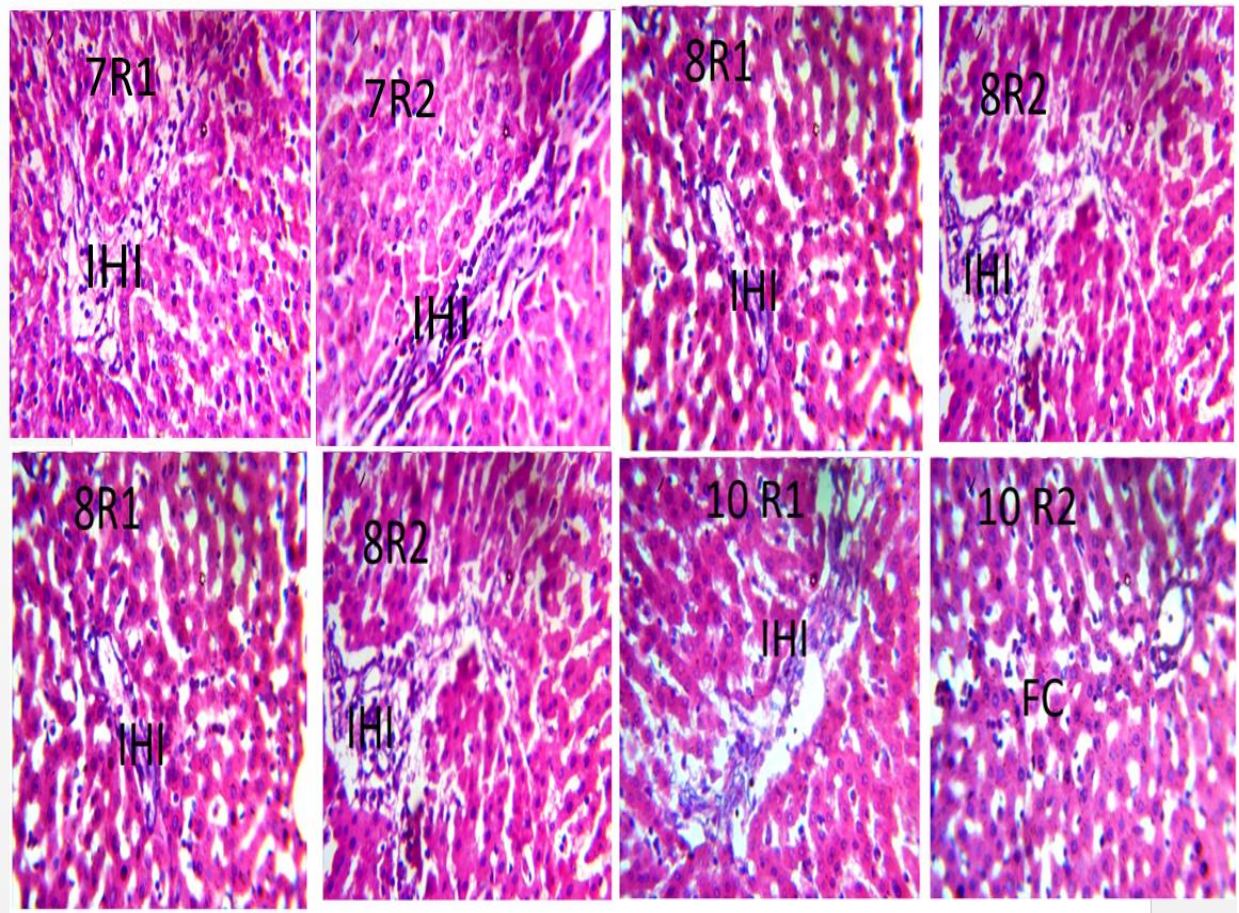

Figure 4: Photomicrograph of liver sections B

The group challenged with both toxins also showed pyknosis of the glomeruli. In the liver, severe pathologic changes involving focal areas of necrosis and severe intrahepatic inflammation were also seen in the groups administered with the two heavy metal toxins. Cadmium toxicity in addition was associated with pyknosis of the hepatocytes while its co-administration with lead caused intrahepatic haemorrhages. The occurrence of degenerative changes in renal tissues and massive infiltration of inflammatory cells following exposure to lead and cadmium poisoning have been reported by other studies (Bashir and Noory, 2012; Offor *et al.,* 2017, Nguyen et al., 2023, Amal, 2023). Reddy *et al.* (2014) reported architectural changes in the glomerulus and tubular epithelial cells; including necrosis, infiltration of inflammatory cells and fatty degeneration in between the glomeruli and intratubular region following lead poisoning. The toxic effects of lead in various organs of the body have been attributed to its binding to a wide range of proteins and its interference with the functioning of other essential cations such as calcium, zinc and iron (Halmo and Nappe, 2023). Lead-induced toxicity has much been associated with the oxidative stress and inflammatory responses it elicits; it has been shown to increase lipid peroxidation in both the kidney and liver and also impair the activities of antioxidants. It stimulates the production of proinflammatory cytokines, including IL-1β, TNF-

α and IL-6 as well as increases total and differential WBC counts (Matović *et al.,* 2015). Cadmium on the other hand, has been associated with disorders in the metabolism of zinc and copper in internal organs as well as oxidative stress on the endoplasmic reticulum which causes apoptosis or necrosis (Borowska *et al.,* 2017).

From the photomicrograph, the rats treated with the spice mixture (SM) and those treated with the metal chelator (MC) showed moderate degeneration of the renal tissues, reduced severity of the fatty changes occurring in the kidney and severity of the inflammatory cells infiltration induced by the metal toxins. In both rats co-administered cadmium with SM and MC, the glomeruli were seen to remain active. This shows that SM is comparable to MC in ameliorating nephrotoxicity induced by lead and cadmium. In the liver, it could be seen that the MC was able to reduce the severity of the toxicity induced by both lead and cadmium; there was *moderate* healing with moderate to mild intrahepatic inflammatory cell infiltration, clumping and pyknosis. However, the SM was only able to show a significant ameliorative effect on the lead-induced toxicity but did not have a significant positive effect on the cadmium only and cadmium + lead induced toxicity. $CaNa_2EDTA$ is a standard chelator commonly used for the treatment of lead and cadmium poisoning, it functions by forming bonds with the metal ions which then facilitates its excretion from the body (Kim *et al.,* 2015). However, its usage has been limited by multiple side effects such as acute tubular necrosis in the kidney, zinc deficiency, bone marrow depression, hypotension etc. (George and Brady, 2023). It has also been demonstrated that intravenous administration of $CaNa_2EDTA$ mobilizes lead and calcium from the trabecular bone while increasing its concentration in the soft tissues (Crinnion, 2011). Congestion of the portal vein in the liver of the MC + lead-treated group which was not observed in the lead-only group may be an adverse effect induced by the MC.

The use of herbs in the treatment of heavy metal poisoning, provides a good alternative to synthetic chelators. Though its usage has mostly been applied prophylactically, it has shown significant effectiveness in ameliorating the toxic effects of cadmium and lead poisoning. It also has the advantage of being less expensive and causing minimal side effects. Ginger and garlic serve as food spices globally. Several studies have demonstrated their potential in conferring protection against lead and cadmium induced toxicity in the liver, kidney, neurons and blood of rats as well as in preventing mitochondrial damage and apoptosis in tissue cultures (Lawal and Ellis, 2011; Waseem *et al.,* 2014; Zhai *et al.,* 2015).The ability of the SM to reduce the severity of the histopathological changes in the kidney induced by lead in this study is in line with the report of Gabr *et al.* (2017) who showed that rats treated with Cd along with ginger had regeneration, restoration of glomeruli, renal tubules and cells and the report of Reddy *et al.* (2014) who also demonstrated that rats co-administered lead and ginger had milder architectural changes in the kidneys than those exposed to lead alone. Their studies demonstrated that co-administration of ginger extract with lead significantly increased the activities of glutathione peroxidase, glutathione – 5 – transferase and catalase in the kidney. Okutu *et al.* (2019) also demonstrated the nephroprotective activity of the mixture of ginger and garlic using serum biochemical parameters, their study showed that the combination of the two spices produced a greater effect in ameliorating the toxicity induced by lead in the kidney than when applied individually. The ability of these spices to protect the organs from the toxic effects of these metals has been attributed to their antioxidant potentials, their chelation activity and their ability to prevent absorption of Cd and Pb in the intestine using its sulphur-containing amino acids.

Our study showed that the SM could not ameliorate the toxic effect of cadmium in the liver of the treated rats. This is contrary to the report of Odewumi *et al.* (2018) who demonstrated that diallyl disulfide, which is a major compound from garlic was able to ameliorate the toxic effects of Cd in liver cells via cytokines modulation. It is also contrary to the findings of Cobb-Abdullah *et al.* (2019) who reported that the garlic compound was able to alleviate the toxic effect of Cd on the transcriptome of liver cells. However, in the studies of Egwurugwu *et al.* (2019), it was observed that the ameliorative effect of ginger on the hepatotoxic effect of Cd was more significant when applied therapeutically than prophylactically as further cadmium intake was avoided for an extra five weeks with continued

ginger administration. Hence, our studies may have recorded better results on the ameliorative effect of the SM on the Cd-induced liver pathologies if the treatment was applied prophylactically.

In conclusion, this study found that the ameliorative effect of the mixture of ginger and garlic on lead and cadmium-induced histologic toxicity in the kidney, as well as in the liver of rats, is comparable to that of the standard metal chelator. The mixture of ginger and garlic can therefore, be used as an alternative to synthetic chelators in the prevention and management of lead and cadmium toxicity.

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Author contribution

ODG was involved in conceptualization and design of the work, acquisition, analysis, and interpretation of data, writing the original draft and critical review of the draft. UEI was involved in conceptualization and design of the work, acquisition, analysis, and interpretation of data for the work, and critical review of the draft. OU was involved in interpretation of data for the work, writing the manuscript, and critical review for important intellectual content. OAO was involved in analysis, interpretation of data and critical review of the original draft while OOV was involved in acquisition, analysis, and interpretation of data, writing the and review of the original draft of the manuscript.

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