ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

Biochemical and histopathological studies of hepatic effect of mixture of cadmium, chromium and lead in albino rats

KENNEDY OSOH[1], ALOYSIUS C. ENE[1], CHINWE S. ALISI[1], LINUS A. NWAOGU[1], CHRISTIAN O. NWEKE[2]

[1]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria
[2]Department of Microbiology, Federal University of Technology, Owerri, Nigeria

DOI: https://doi.org/10.5281/zenodo.7961629

Published Date: 23-May-2023

Abstract: **The joint effect of cadmium, chromium and lead on the liver of albino rats exposed to them simultaneously was investigated in this study. Seventy (70) male albino rats (Wistar rats) were used in the study. Specified doses of 5, 10, and 20mg/kg body weight respectively, of these metals were administered by gavage thrice weekly to 60 albino rats and 10 albino rats were used as control. There were four treatment groups Cd, Cr, Pb and Cd+Cr+Pb (i.e. Cd alone, Cr alone, Pb alone and Cd, Cr, Pb combined) per dose with five animals per treatment group. Body weights (BW) of the rats were measured weekly before treatment. The treatments were for 90 days, and salt solutions of the metals (i.e. $CdSO_4$, $K_2Cr_2O_7$, and $Pb(NO_3)_2$) were used while the control received only distilled water. The animals were sacrificed after 90 days and blood samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total bilirubin. Histopathological evaluation of liver was also done. Results of combined treatment showed hormetic response with regard to ALT, AST and total bilirubin as they were elevated in the low dose but decreased with high dose. This phenomenon was also observed in Cr individual treatment. The results also showed that LDH activity in the combined treatment group increased with increasing dose but was not significantly different from that of the most hazardous metal in the individual treatments. Histopathological evaluation showed tissue injury in liver in the 20mg/kg combined and individual treatment groups only. Conclusively, the results suggested that there was no significant health risk posed to the liver by simultaneous exposure to the metals beyond the risk already posed by the most hazardous individual metal for the endpoint of interest. Interactions where they occurred were predominantly less-than-additive.**

Keywords: **Liver, cadmium, chromium, lead, combined, treatment, hormetic, histopathological, interaction.**

1. INTRODUCTION

Some potential environmental hazards involve exposure to only a single compound but most instances of environmental pollution involve simultaneous or sequential exposures to a mixture of compounds that may induce similar or dissimilar effects over exposure periods ranging from short-term to lifetime [1]. The main objective in the risk assessment of chemicals in mixtures is to establish or predict how the toxicological effects of the mixture might turn out, often in comparison with exposure to individual compounds. One of the main points to consider is whether chemicals in a mixture interact and produce an increased (enhanced) or decreased (diminished) overall response compared to the expected sum of the effects if each chemical acts independently of each other [2].

Cadmium, chromium and lead are known environmental pollutants. They are present in the environment, food or water as a result of natural and anthropogenic activities. Industrialization and urbanization are main reasons for their presence and they are persistent in the environment being non biodegradable. Humans and other animals are exposed to them mainly through food, water, consumer products, and occupational exposures.

Cadmium produces toxic effects even at low doses due to accumulation in the body with a long biological half life [3]. Increased exposure to cadmium has been reported to cause abnormal liver function indicators, pathological degeneration, rat liver cell necrosis, and proliferation of collagen fibres [4]. Cadmium has also been reported to be a silent killer of the hepatic system through the induction of oxidative stress [5].

Lead exposure has been reported to be toxic to most organs including the liver, kidney and hematopoietic tissues. More than 33% of accumulated lead in the human body is found in the liver, followed by the kidney [6]. Induction of oxidative stress has been suggested as one of the mechanism of action of lead toxicity in the liver. It has been reported that oxidative stress through reactive oxygen species (ROS) can result in damaged function and histopathological damage to the liver, including peroxidation of lipid membranes [7].

The liver has been reported as the main target organ of the body after exposure to hexavalent chromium. It has been stated that hexavalent chromium can cause lipid peroxidation, DNA damage, and mitochondrial dysfunction in the liver [8].

Cadmium, chromium, and lead mixture have been chosen as the subject for this interaction study because it is a very frequently occurring ternary mixture at hazardous waste sites. This mixture was found in soil at 219 sites out of the 1,608 sites for which Agency for Toxic Substances and Disease Registry (ATSDR) has produced a Public Health Assessment [9], [10].

2. MATERIALS AND METHODS

2.1 Collection, preservation and treatmentof animals

Seventy (70) male albino rats (Wistar rats) aged between 10 – 12 weeks were used in the study. They were randomly selected into fourteen cages made from wood and wire mesh with five animals per cage. The test substances for treatment were lead nitrate ($Pb(NO_3)_2$) for lead, cadmium sulphate ($CdSO_4$) for cadmium, and potassium dichromate ($K_2Cr_2O_7$) for hexavalent chromium. The treatments were for 90 days, and salt solutions of the metals (i.e. $CdSO_4$, $K_2Cr_2O_7$, and $Pb(NO_3)_2$) were used while the control received only distilled water. Body weights (BW) of the rats were measured weekly before treatment. Specified doses of 5, 10, and 20mg/kg body weight respectively, of these pollutants/metals were administered by gavage thrice weekly to 60 albino rats and 10 albino rats were used as control. There were four treatment groups Cd, Cr, Pb and Cd+Cr+Pb (i.e. Cd alone, Cr alone, Pb alone and Cd, Cr, Pb combined) per dose with five animals per treatment group. The animals were sacrificed after 90 days and blood samples were analyzed for some biomarkers of liver function namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and lactate dehydrogenase (LDH). Histopathological evaluation of liver was also done.

2.2 Assay of serum alanine aminotransferase (ALT) activity

This test was done using the method described by Reitman and Frankel [11].

Test Principle:

α - oxoglutarate + L – alanine $\xrightarrow{\text{ALT}}$ L – glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 – dinitrophemyl hydrazine.

2.3 Assay of serum aspartate aminotransferase (AST) activity

This test was done using the method described by Reitman and Frankel [11].

Test Principle:

α - oxoglutarate + L – aspartate $\xrightarrow{\text{AST}}$ L–glutamate+ oxaloacetate

AST is measured by monitoring the concentration of oxloacetate hydrazone formed with 2, 4 – dinitrophenyl hydrazine.

2.4 Assay of serum alkaline phosphatase (ALP) activity

This test was done using the method described by Roy [12].

Test Principle:

Alkaline phosphatase acts upon AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

2.5 Determination of serum total bilirubin concentration

This test was done using the colorimetric method described by Jendrassik and Grof [13].

Test Principle:

Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

2.6 Assay of serum lactate dehydrogenase (LDH) activity

This assay was done applying the method described by Henry, Chiamori, Golub, and Berkman [14].

Test Principle:

Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate with simultaneous oxidation of reduced NADH to NAD^+. The rate of decrease in absorbance due to formation of NAD^+ is measured at 340nm and is proportional to the LDH activity in the sample.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{lactate} + NAD^+$$

(NAD = nicotinamide adenine dinucleotide)

2.7 Histopathological evaluation of liver

Histological evaluation of liver was done using the method described by Okoro [15] with minor modifications.

Pieces of liver tissue were cut out with surgical blade and placed in tissue cassette. They were placed in four (4) increasing grades/concentrations of isopropyl alcohol (IPA) i.e. 70%, 80%, 90%, 100% for one (1) hour each and subsequently two (2) changes of xylene for one (1) hour each. They were solidified with molten paraffin wax. Thin sections were cut and floated in water bath and picked with clean slide. Tissue sections were stained using Haematoxylin and Eosin and covered with cover glass. The sections were viewed and interpreted using Leica DM 750 Binocular microscope with photomicrographic facilities and then photomicrographed.

2.8 Statistical analysis

Results of the study were presented as mean ± standard deviation and in charts. They were analyzed using Stats Tester software and one way analysis of variance (ANOVA). Multiple t-test (with Bonferroni correction) was used to compare means at $p<0.05$.

3. RESULTS

Figure 1 showed that in the three treatment doses, there was significant increase ($p<0.05$) in mean serum ALT activity in Cd, Cr, Pb individual and combined treatment groups compared with control. Figure 1 also showed that in the Cr and combined treatment groups, a hormetic effect was observed with regard to serum ALT activity as treatment with the low dose (5mg/kg) produced a stimulating effect which was reduced at the high dose (20mg/kg).

Figure 2 showed that in 5mg/kg and 10mg/kg treatment doses, there was significant increase ($p<0.05$) in mean serum AST activity in Cd, Cr, Pb individual and combined treatment groups compared with control but there was no significant difference ($p>0.05$) in mean serum AST activity in Cr individual and combined treatment groups compared with control in 20mg/kg treatment dose. Figure 2 also showed that in the Cd, Cr, Pb and combined treatment groups, a hormetic effect was observed with regard to serum AST activity as treatment with the low dose (5mg/kg) produced a stimulating effect which was reduced at the high dose (20mg/kg).

Figure 4 showed that in the combined treatment group, a hormetic effect was observed with regard to serum total bilirubin concentration as treatment with the low dose (5mg/kg) produced a stimulating effect which was reduced at the high dose (20mg/kg). Treatment with Pb caused a dose dependent increase in mean serum total bilirubin concentration as the dose increased.

Figure5 showed that in the three treatment doses, there was significant increase ($p<0.05$) in mean serum LDH activity in Cd, Cr, Pb individual and combined treatment groups compared with control but there was no significant difference ($p>0.05$)

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

in mean serum LDH activity in Cd, Cr, Pb individual treatment groups compared with the combined treatment group. Treatment with Cd, Cr, Pb individually and combined caused a dose dependent increase in mean serum LDH activity as the dose increased.

Results of histological sections of the livers in the control and various treatment groups are presented in Plates 1-13.

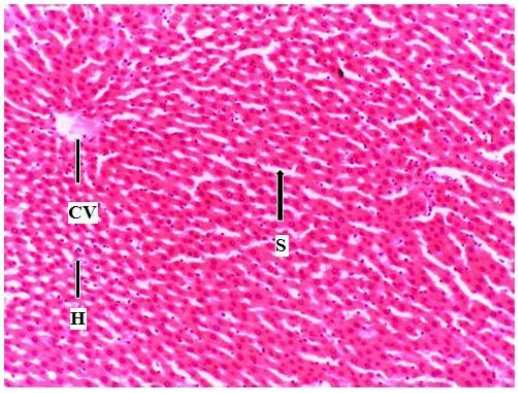

Plate 1: Photomicrograph of section of liver of albino rat in the Control group for 90 days showing normal tissue architecture. Within the interstitial tissue (stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes (H), Sinusoids (S), capillary sinusoids (CS), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

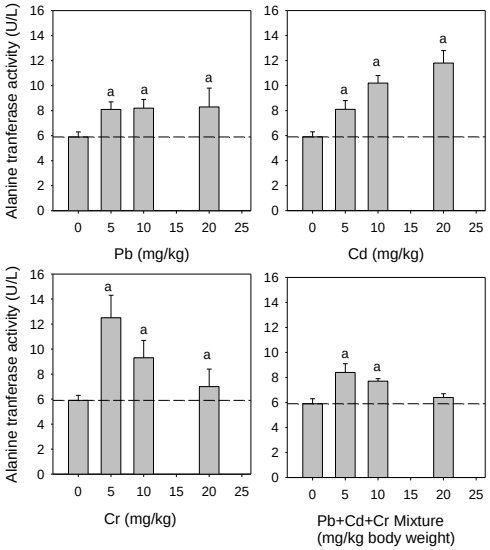

Figure 1: Dose-effect relationship for serum alanine aminotransferase (ALT) activity in albino rats treated with Pb, Cd and Cr individually and as a mixture

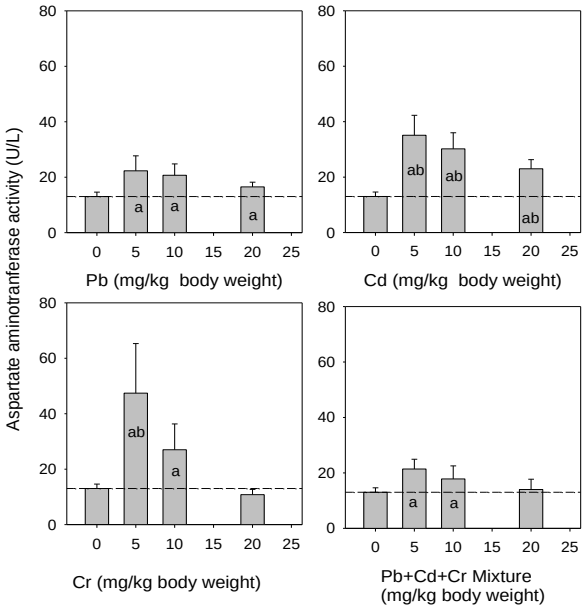

Figure 2: Dose-effect relationship for serum aspartate aminotransferase (AST) activity in albino rats treated with Pb, Cd and Cr individually and as a mixture

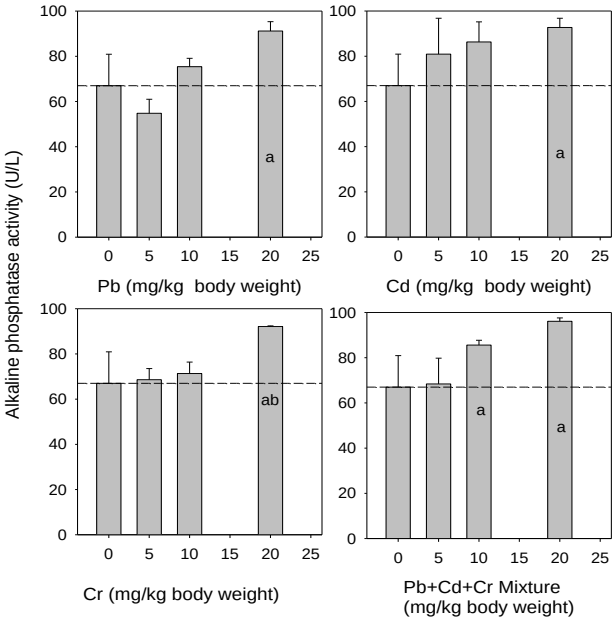

Figure 3: Dose-effect relationship for serum alkaline phosphatase (ALP) activity in albino rats treated with Pb, Cd and Cr individually and as a mixture

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

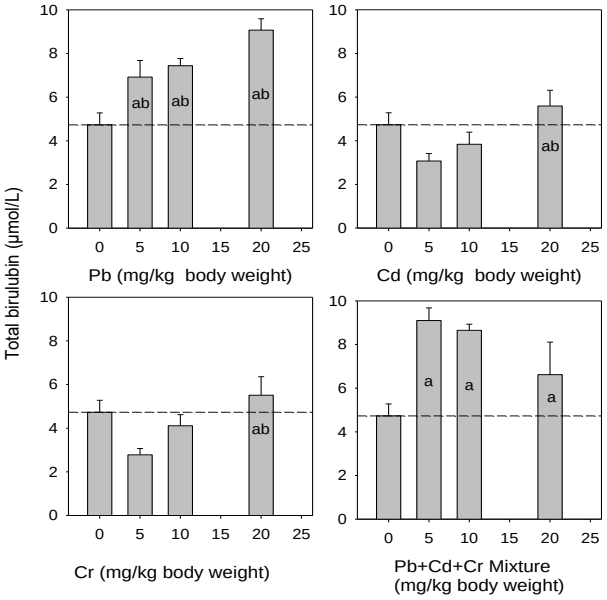

Figure 4: Dose-effect relationship for serum total bilirubin concentration in albino rats treated with Pb, Cd and Cr individually and as a mixture

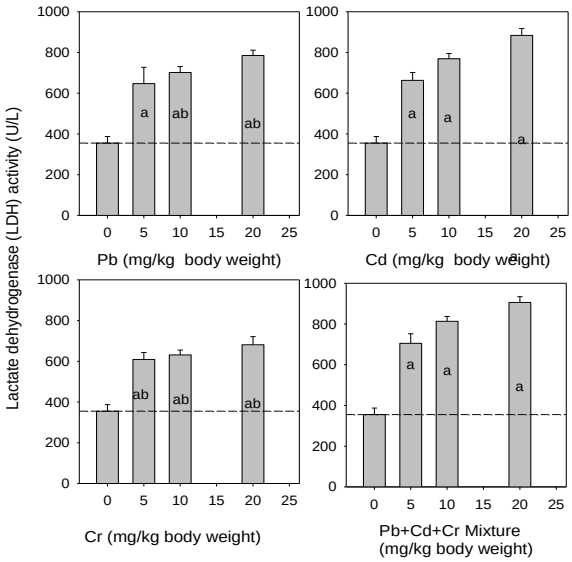

Figure 5: Dose-effect relationship for serum lactate dehydrogenase (LDH) activity in albino rats treated with Pb, Cd and Cr individually and as a mixture

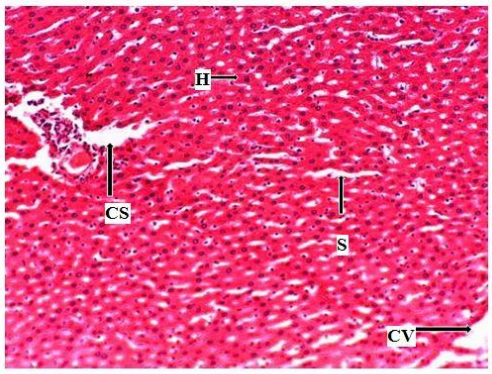

Plate 2: Photomicrograph of section of liver of albino rat administered 5mg/kg body weight of Cd for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes (H), Sinusoids (S), capillary sinusoids (CS), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

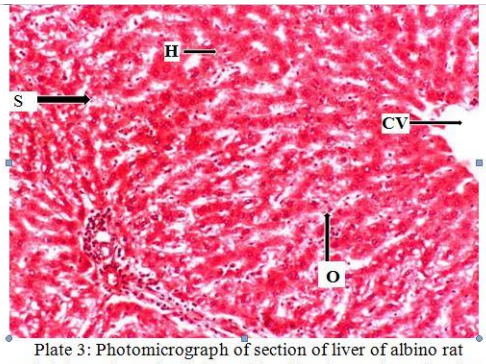

Plate 3: Photomicrograph of section of liver of albino rat administered 5mg/kg body weight of Cr for 90 days showing proliferation of stromal tissue (interstitial tissue) and hepatocytes (H) with few hepatocytes appearing swollen/enlarged,. Within the interstitial tissue (stroma) are seen Central vein (CV). The laminae (plate) of hepatocytes are not easily identifiable. The interstitial tissue is oedematous (O) and the sinusoids (S) are enlarged. In some areas there are foci of necrosis (x400), Stain: H and E.

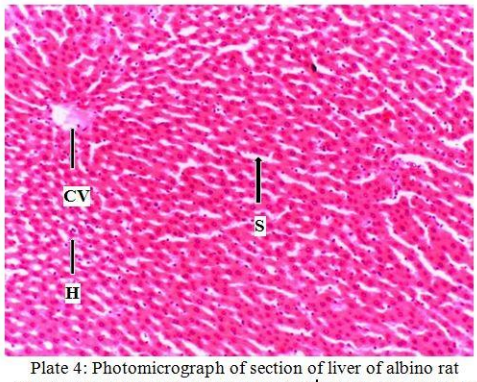

Plate 4: Photomicrograph of section of liver of albino rat administered 5mg/kg body weight of Pb for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes(H), Sinusoids(S), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

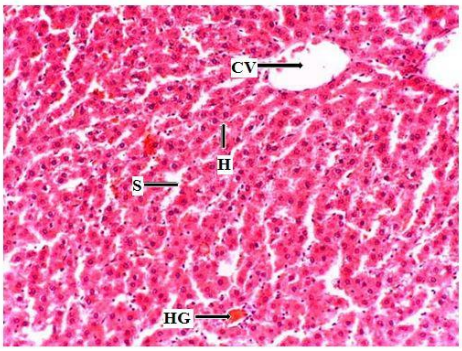

Plate 5: Photomicrograph of section of liver of albino rat administered 5mg/kg body weight Cd, Cr, Pb mixture for 90 days showing normal tissue architecture. Within the interstitial tissue (stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes (H), Sinusoids (S), Hepatocytes and Portal triad that appear normal. HG stands for hemorrhage (x400), Stain: H and E.

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

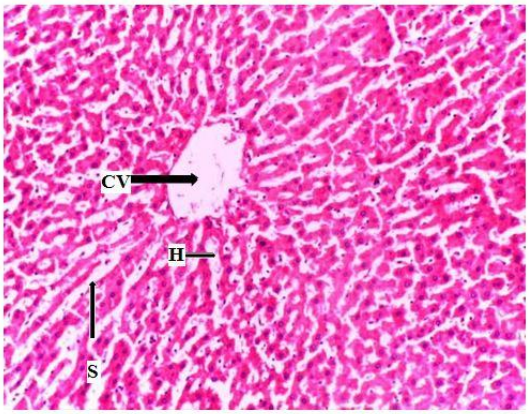

Plate 6: Photomicrograph of section of liver of albino rat administered 10mg/kg body weight of Cd for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes (H), Sinusoids (S), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

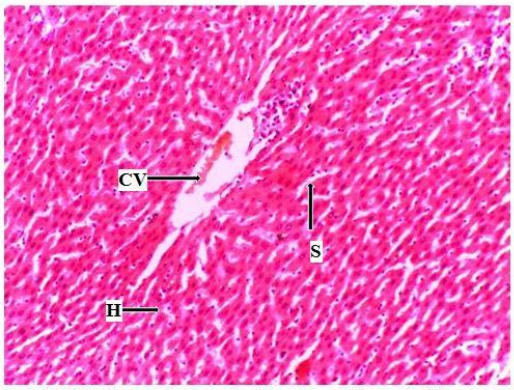

Plate 7: Photomicrograph of section of liver of albino rat administered 10mg/kg body weight of Cr for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV),Laminae(plate) of hepatocytes(H), Sinusoids(S), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

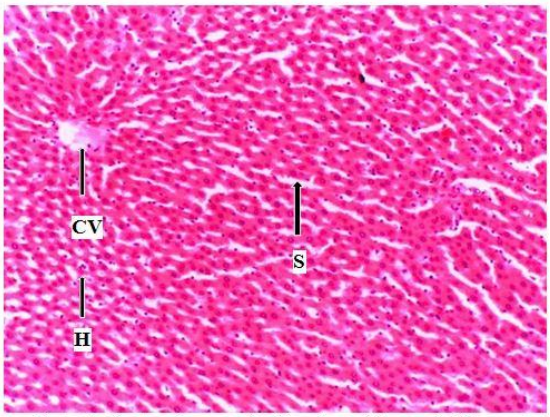

Plate 8: Photomicrograph of section of liver of albino rat administered 10mg/kg body weight of Pb for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV), Laminae (plate) of hepatocytes (H), Sinusoids (S), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E

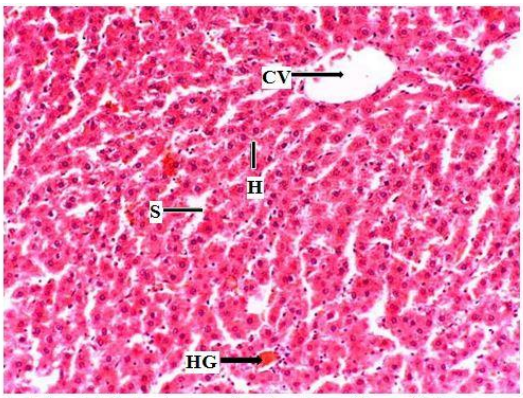

Plate 9: Photomicrograph of section of liver of albino rat administered 10mg/kg body weight Cd, Cr, Pb mixture for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV),Laminae(plate) of hepatocytes(H), Sinusoids(S), Hepatocytes and Portal triad that appear normal. HG stands for hemorrhage (x400), Stain: H and E.

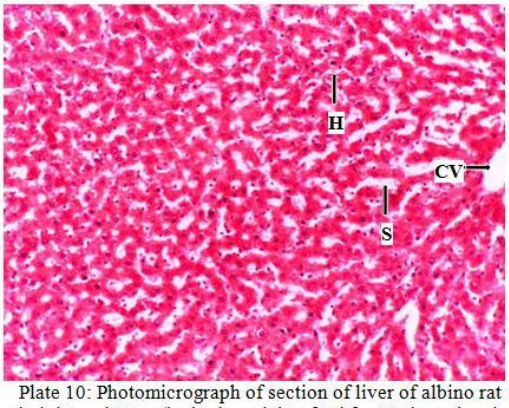

Plate 10: Photomicrograph of section of liver of albino rat administered 20mg/kg body weight of Cd for 90 days showing proliferation of stromal tissue (interstitial tissue) and hepatocytes (H) with few hepatocytes appearing swollen/enlarged. Within the interstitial tissue (stroma) are seen Central vein (CV). The laminae (plate) of hepatocytes are not easily identifiable. The interstitial tissue is oedematous (O) and the sinusoids (S) are enlarged. In some areas there are foci of necrosis (x400), Stain: H and E.

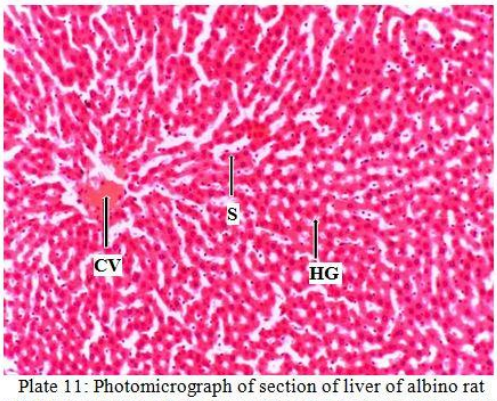

Plate 11: Photomicrograph of section of liver of albino rat administered 20mg/kg body weight of Cr for 90 days showing proliferation of stromal tissue (interstitial tissue) and hepatocytes (H) with few hepatocytes appearing swollen/enlarged. Within the interstitial tissue (stroma) are seen Central vein (CV). The laminae (plate) of hepatocytes are not easily identifiable. The sinusoids (S) are enlarged. In some areas there are foci of necrosis. HG stands for hemorrhage (x400), Stain: H and E.

ISSN 2350-1049

International Journal of Recent Research in Interdisciplinary Sciences (IJRRIS)
Vol. 10, Issue 2, pp: (24-31), Month: April - June 2023, Available at: www.paperpublications.org

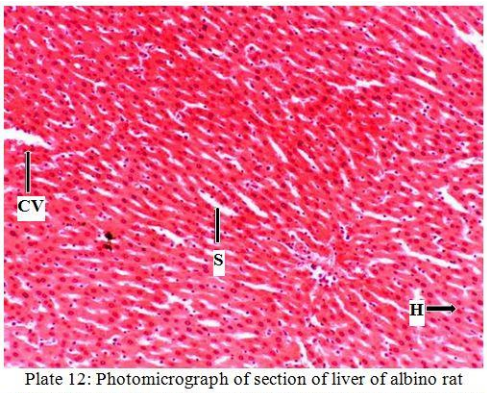

Plate 12: Photomicrograph of section of liver of albino rat administered 20mg/kg body weight of Pb for 90 days showing normal tissue architecture. Within the interstitial tissue(stroma) are seen Central vein (CV), Laminae(plate) of hepatocytes(H), Sinusoids(S), Hepatocytes and Portal triad that appear normal (x400), Stain: H and E.

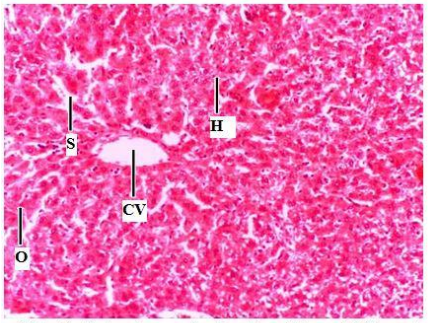

Plate 13: Photomicrograph of section of liver of albino rat administered 20mg/kg body weight Cd, Cr, Pb mixture for 90 days showing proliferation of stromal tissue (interstitial tissue) and hepatocytes (H) with few hepatocytes appearing swollen/enlarged. Within the interstitial tissue (stroma) are seen Central vein (CV). The laminae (plate) of hepatocytes are not easily identifiable. The interstitial tissue is oedematous (O) and the sinusoids (S) are enlarged. In some areas there are foci of necrosis (x400), Stain: H and E.

4. DISCUSSION

The results obtained from this study suggest hepatotoxicity by the metals (Cd, Cr, Pb) both singly and in combination (as a mixture). This was manifested by the effects on the levels of biomarkers of hepatotoxicity such as serum ALT, AST, ALP, LDH activities and serum total bilirubin concentration. Simultaneous exposure to these three toxicants (Cd, Cr, Pb) was expected to produce increased adverse hepatic effect but this was not the observation. Instead, the observed hepatotoxic effect of the mixture was either same as one of the individual metals or less than that of the individual metals. This suggests an interaction (less than additive) in which the mode of action of one metal is affected by the other metal(s). The decrease in adverse effect observed in the metal mixture (combined) treatment with increasing dose with respect to serum ALT, AST, total bilirubin in the three treatment doses is hormetic. It is proposed that the mixture at low dose caused a stimulating effect which may have shifted the redox status of the liver cell and activated the cell defense and repair mechanism through the Nrf2 transcription factor leading to decrease in adverse effect observed at high dose of the metal mixture (combined) treatment [16]. This was also observed for Cr individual treatment group with respect to ALT and AST suggesting that the Cr component of the mixture may be responsible for the shift in the redox status of the cell as Cr mechanism for toxicity had been said to include production of reactive oxygen species following its reduction from hexavalent Cr to trivalent Cr [17]**.** This is similar to the observation of Fan *et al.* [18] which reported that single and combined Cd and Pb treatment induced hormesis in soil microbial populations and that the mixture hormetic effects were related to the effect of single Cd or Pb.

Results of histopathological evaluation indicated no tissue injury in the liver in both combined and individual treatments of the metals (Cd, Cr, Pb) for 5 & 10mg/kg treatment doses as tissue architecture appeared normal while tissue injury was observed in the liver in both combined and individual treatments of the metals for 20mg/kg treatment dose. The 20mg/kg treatment group showed proliferation of stromal tissue (interstitial tissue) and hepatocytes with few hepatocytes that appeared swollen/enlarged. The laminae (plate) of hepatocytes were not easily identifiable. The interstitial tissue was oedematous and the sinusoids were enlarged. In some areas there were foci of necrosis. This result suggests injury in the liver resulting from increased dose of the treatment metals in the combined and individual treatment.

5. CONCLUSION

Simultaneous exposure to these three hepatic toxicants was expected to produce increased adverse hepatic effect but this was not the observation. Instead, the observed hepatic effect of the mixture was either same as one of the individual metals or less than that of the individual metals. Conclusively, this study has shown that simultaneous to these metals (Cd. Cr and Pb) posed no greater health risk to the liver of albino rats than the exposure to the individual metals as the observed adverse effects in the mixture were similar to those of individual metals, making it safe to assess/estimate health risk due to the mixture from the risk of the individual metals. Hormesis should be considered in their risk assessment.

ACKNOWLEDGEMENT

Late Mr. Uchechukwu I. Arukwe is acknowledged for giving his best in assistance to make this work practically possible in the laboratory. Late Prof. O.A. Ojiako, Late Prof. A.I. Ukoha and emeritus Prof. (Mrs.) N.C. Agha are acknowledged for their assistance in the commencement of this study.

REFERENCES

[1] Hernandez, A.F., Buha, A., Constantin, C., Wallace, D.R., Sarigiannis, D., Neagu, M., … Tsatsakis, A. (2019). Critical assessment and integration of separate lines of evidence for risk assessment of chemical mixtures. *Archives of Toxicology, 93*, 2741-2757.

[2] Vincenti, S.R. and Filippini, T. (2021). Public health and public law issues for the toxicological risk assessment of chemical mixtures. *Public Health Toxicology, 1*(2), 6.

[3] Rehman, N.U., Ansari, M.N., Ganaie, M.A., Madkhali, H.A., Saeedan, A.S., Imam, F., and Hamad, A.M. (2020). Cadmium-induced hepatotoxicity and oxidative stress in rats: Protection by Roflumilast via NF-kB and HO-1 pathway. *International Journal of Pharmacology, 16*(2):154-163

[4] Zhang, H., Yan, J., Xie, Y, Chang, X., Li, J., Ren, C., … Li, X. (2022). Dual role of cadmium in rat liver: Inducing liver injury and inhibiting the progression of early liver cancer. *Toxicology Letters, 1*, 355:81. Doi:10.1016/ j.toxlet.2021.11.004

[5] Banerjee, A., Nandi, P., Bhattacharya, C., Kabir, Z., Mukherjee, S., and Maji, B.K. (2019). Cadmium acts as a silent killer of liver by inducing oxidative stress and hepatocellular injury and a possible amelioration by vitamin B_{12} and folic acid in rat model. *Progress in Health Science, 9:*1

[6] Ilesanmi, O.B., Adeogun, E.F., Odewale, T. T., and Chikere, B. (2022). Lead exposure-induced changes in hematology and biomarkers of hepatic injury: protective role of Trevo™ supplement. *Environmental Analysis Health and Toxicology, 37*(2):e2022007

[7] Agency for Toxic Substances and Disease Registry (ATSDR). (2020). *Toxicological Profile for Lead*. Retrieved from https://www.atsdr.cdc.gov/ToxProfiles/tp13.pdf

[8] Yang, Q., Han, B., Li, S., Wang, X., Wu, P., Liu, Y., …. Zhang, Z. (2022). The link between deacetylation and hepatoxicity induced by exposure to hexavalent chromium. *Journal of Advanced Research, 35:*129-140. Doi:10.1016/ jare.2021.04.002

[9] Agency for Toxic Substances and Disease Registry (ATSDR). (2004). *Interaction Profile for Arsenic, Cadmium, Chromium, and Lead.* Retrieved from https://www.atsdr.cdc.gov/interactionprofiles/ip-metals1/ip04-c5.pdf

[10] Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R., and Sadeghi, M. (2021). Toxic mechanism of five heavy metals: Mercury, lead, chromium, cadmium and arsenic. *Frontiers in Pharmacology*. https://doi.org/10.3389/ fphar2021.643972

[11] Reitman, S., and Frankel, S. (1957**)**. A colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *American Journal of Clinical Pathology, 28*(1), 56-63.

[12] Roy, A.V. (1970). Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. *Clinical Chemistry, 16*, 431-436.

[13] Jendrassik**,** L., and Grof, P. (1938). Simplified photometric methods for the determination of bilirubin. *Biochemical Journal, 297*, 81-89.

[14] Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S. (1960). Revised spectrophotometric methods for the determination of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. *American Journal of Clinical Pathology, 34*, 381-396.

[15] Okoro, I. (2002). *Manual of practical of histology* (2nded.). Owerri, Nigeria: Peace.

[16] Calabrese, E.J., and Kozumbo, W.J. (2021). The hormetic dose-response mechanism: Nrf2 activation. *Pharmacological Research,* 167, 105526. https://doi.org/10.1016/j.phrs.2021.105526.

[17] Singh, V., Singh, N., Verma, M., Kamal, R., Tiwari, R., Chivate, M.S., … Mishra, V. (2022). Hexavalent-chromium-induced oxidative stress and the protective role of antioxidants against cellular toxicity. *Antioxidants, 11*(12), 2375. https://doi.org/10.3390/antiox11122375

[18] Fan, D., Sun, J., Liu, C., Wang, S., Han, J., Agathokleous, E., and Zhu, Y. (2021). Measurement and modeling of hormesis in soil bacteria and fungi under single and combined treatments of Cd and Pb. *Science of the Total Environment, 783*, 147494