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Does Simultaneous Exposure to Cadmium, Chromium and Lead Enhance or Diminish Renal Injury in Albino Rats?

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Abstract: **This study was aimed at investigating the joint effect of cadmium, chromium and lead on the kidney of albino rats exposed to them simultaneously. 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 urea, creatinine, potassium (K), chloride (Cl^-), and inorganic phosphorus. Histopathological evaluation of kidney was also done. Results of combined treatment showed hormetic response with regard to urea 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 K and inorganic phosphorus levels 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 kidney in the 20mg/kg combined and individual treatment groups only. Conclusively, the results suggested that there was no significant health risk posed to the kidney 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: **Combined, hazardous, histopathological, hormetic, interaction, kidney, treatment.**

1. INTRODUCTION

Humans and animals are exposed to complex and variable combinations of chemical compounds. In a situation of multiple chemical exposures, the single chemicals may act independently as in a single exposure, or a number of the chemicals may interact to modulate the effects of the total multiple exposure. Significant questions exist when comparing single-to-mixture-chemical toxicity concerning additivity, synergism, potentiation, or antagonism [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.

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Cadmium is considered a cumulative toxicant [3]. The kidney is target organ in cadmium toxicity as it has been observed that cadmium causes damage even at low concentrations and the damage can progress to chronic kidney disease [4]. There appears to be a critical concentration of cadmium in the renal cortex that, once exceeded, is associated with tubular dysfunction [5].

Lead has been shown to affect virtually every organ and system in both humans and animals. The most sensitive effects of lead appear to be neurological (particularly in children), hematological, renal and cardiovascular [6]. Acute lead nephrotoxicity consists of proximal tubular dysfunction and can be reversed by treatment with chelating agents. Chronic lead nephrotoxicity consists of interstitial fibrosis and progressive nephron loss, azotaemia and renal failure [7].

Hexavalent chromium is acutely toxic, with most reports of human toxicity occurring as a result of accidental or intentional ingestion. Renal effects included accumulation of lipids and inhibition of membrane enzymes in rats given chromium (VI) at 13.5 mg/kg/day by gavage, and proteinuria in rats given chromium (VI) at 98 mg/kg/day from drinking water [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 kidney function namely urea, creatinine, potassium (K), chloride (Cl^-), and inorganic phosphorus. Histopathological evaluation of kidney was also done.

2.2 Determination of serum urea concentration

This test was done using the method described by Fawcett and Scott [11]. The test principle is that urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{phenol} \longrightarrow \text{indophenols (blue compound)}$$

2.3 Determination of serum creatinine concentration

This test was done applying the method described by Bartels and Bohmer [12] using Jaffe's reaction. The test principle is that creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

2.4 Determinaton of serum potassium concentration

This test was done using the method described by Engelbrecht and McCoy [13]. The test principle is that the amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2 – 7mEq/l.

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2.5 Determination of serum chloride concentration

This test was done applying the method described by Iwasaki, Utsumi, and Ozawa [14].

The test principle is that chloride ions form a soluble, non-ionized compound with mercuric ions and will displace thiocyanate ion from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480mm. The intensity of the colour produced is directly proportional to the chloride concentration.

$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$

$2SCN^- + Fe^{3+} \longrightarrow 4Fe(SCN)_3$ red complex

2.6 Determination of serum phosphorus (inorganic) concentration

This test was done applying the method described by Amador and Urban [15].

The test principle is that inorganic phosphorus reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex which is measured at 340nm.

2.7 Histopathological evaluation of kidney

Histological evaluation of kidney was done using the method described by Okoro [16] with minor modifications.

Pieces of kidney 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 urea concentration in Cd, Cr, Pb individual and combined treatment groups compared with control. Figure 1 also showed that combined treatment with the three metals did not produce significant increase in serum urea concentration compared with the individual metal treatments for the three treatment doses instead it caused a decrease or same effect as the individual metal treatments.

Figure 2 showed that in the three treatment doses, there was no significant difference ($p>0.05$) in mean serum creatinine concentration in Cd, Cr, Pb individual and combined treatment groups compared with control. Also, there was no significant difference ($p<0.05$) in mean serum creatinine concentration in Cd, Cr, Pb individual treatment groups compared with the combined treatment group.

Figure 3 showed that in the three treatment doses, there was significant increase ($p<0.05$) in mean serum potassium concentration in Cd, Cr, Pb individual and combined treatment groups compared with control. Figure 3 also showed that combined treatment did not produce any significant increase in serum potassium concentration compared with the individual treatments instead a decrease as observed in 5mg/kg treatment group.

Figure5 showed that in the three treatment doses, there was significant increase ($p<0.05$) in mean serum inorganic phosphorus concentration in Cd, Cr, Pb individual and combined treatment groups compared with control but there was no significant difference ($p>0.05$) in mean serum inorganic phosphorus concentration 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 inorganic phosphorus concentration as the dose increased.

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

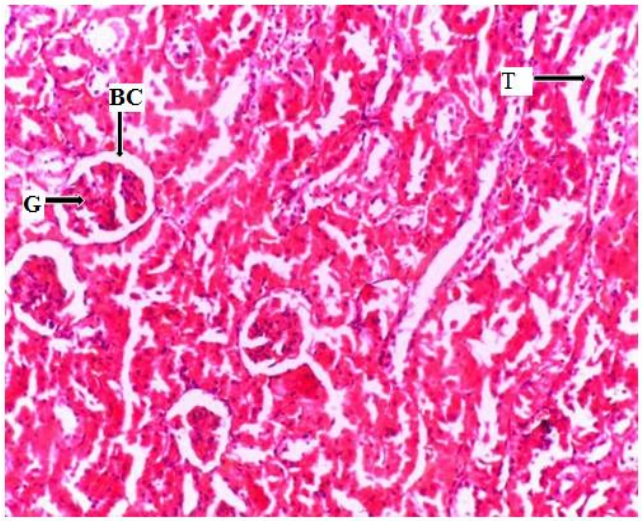

Plate 1: Photomicrograph of section of kidney of albino rat in the Control group for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal (x400), Stain: H and E

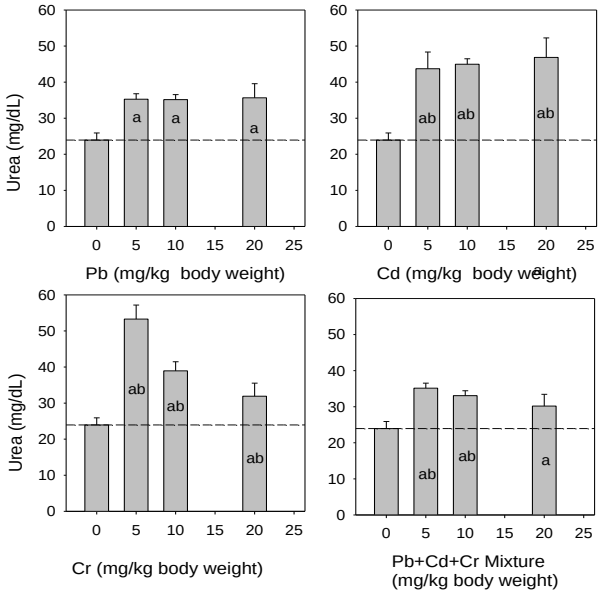

Figure1: Dose-effect relationship for serum urea concentration in albino rats treated with Pb, Cd and Cr individually and as a mixture

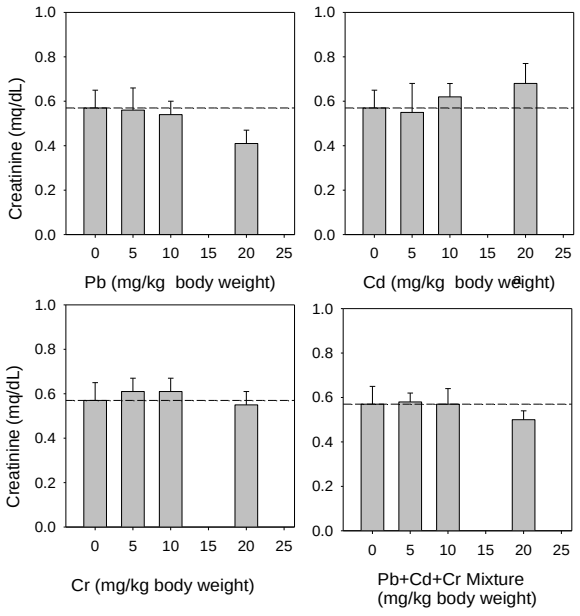

Figure 2: Dose-effect relationship for creatinine concentration in albino rats treatedwithPb, Cd and Cr individually and as a mixture

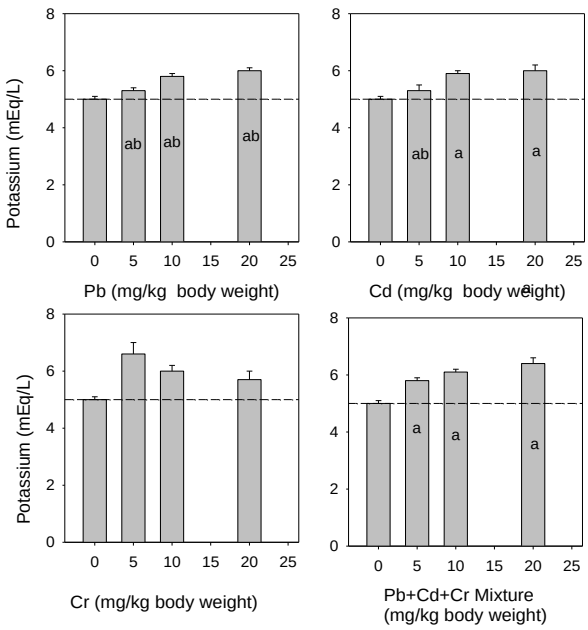

Figure 3: Dose-effect relationship for serum potassium concentration in albino ratstreated with Pb, Cd and Cr individually and as a mixture

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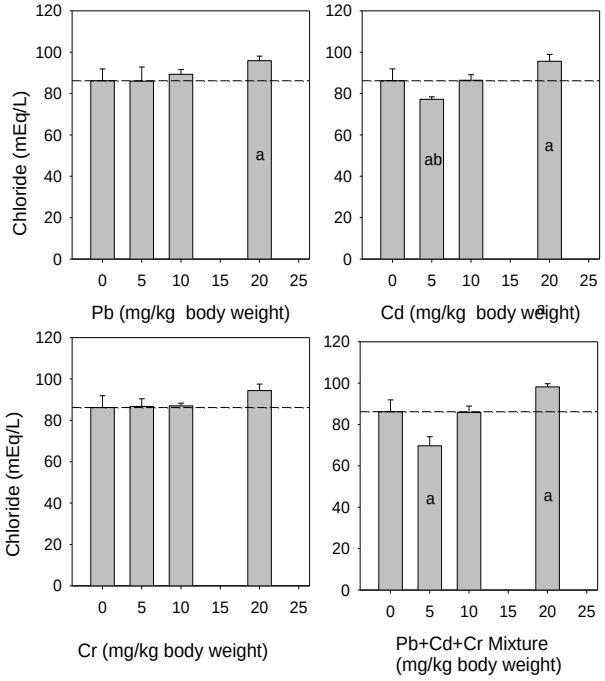

Figure 4: Dose-effect relationship for serum chloride concentration in albino ratsreated with Pb, Cd and Cr individually and as a mixture

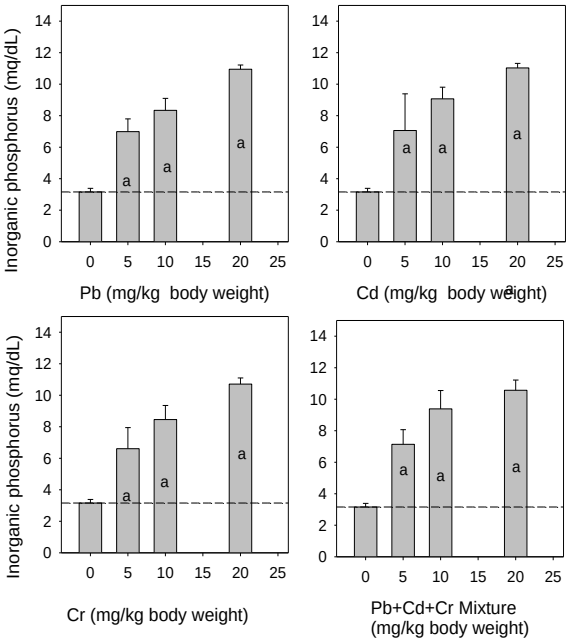

Figure 5: Dose-effect relationship for phosphorus concentration in albino rats treatedwith Pb, Cd and Cr individually and as a mixture

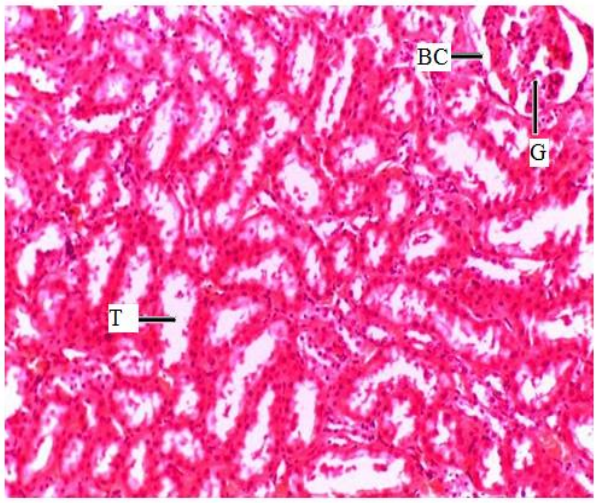

Plate 2: Photomicrograph of section of kidney of albino rat administered 5mg/kg body weight of Cd for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal (x400), Stain: H and E

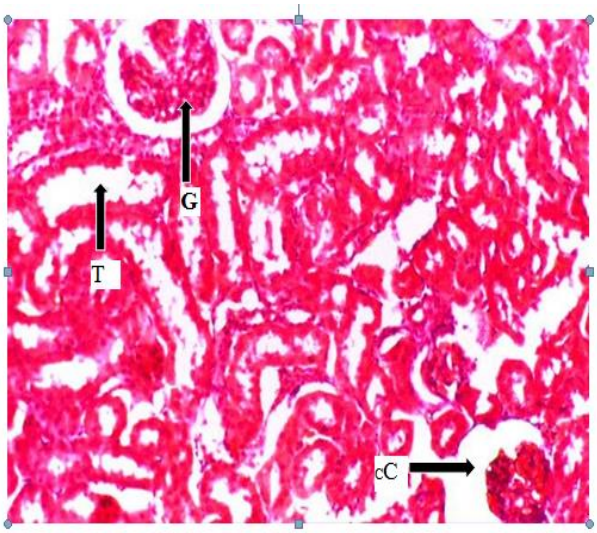

Plate 3: Photomicrograph of section of kidney of albino rat administered 5mg/kg body weight of Cr for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal (x400), Stain: H and E

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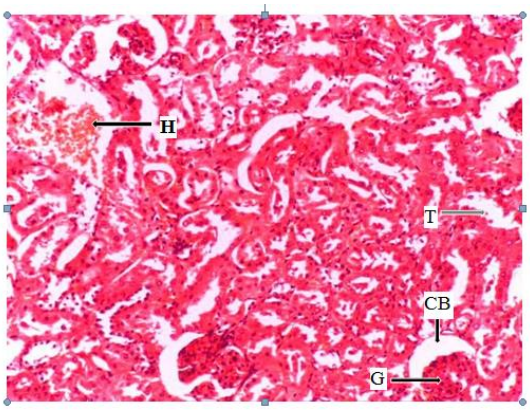

Plate 4: Photomicrograph of section of kidney of albino rat administered 5mg/kg body weight of Pb for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. H stands for hemorrhage (x400), Stain: H and E

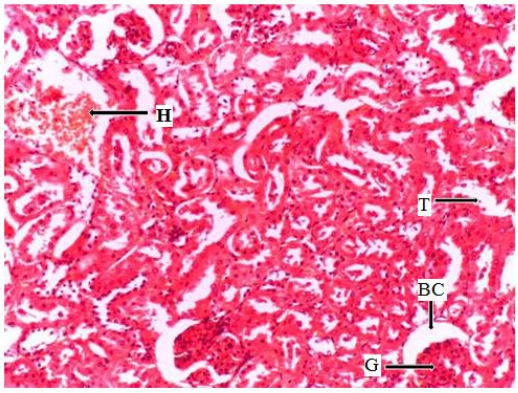

Plate 5: Photomicrograph of section of kidney of albino rat administered 5mg/kg body weight Cd, Cr, Pb mixture for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. H stands for hemorrhage (x400), Stain: H and E

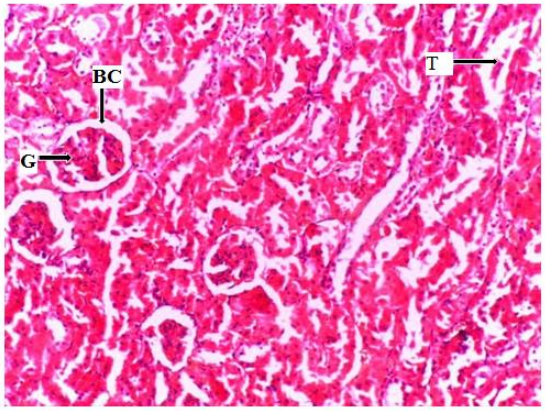

Plate 6: Photomicrograph of section of kidney of albino rat administered 10mg/kg body weight of Cd for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. (x400), Stain: H and E

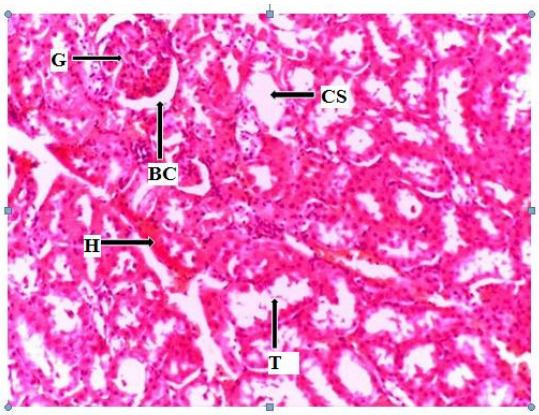

Plate 7: Photomicrograph of section of kidney of albino rat administered 10mg/kg body weight of Cr for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. H stands for hemorrhage. Also there is cystically dilated space (CS) filled with eosinophilic materials (x400), Stain: H and E

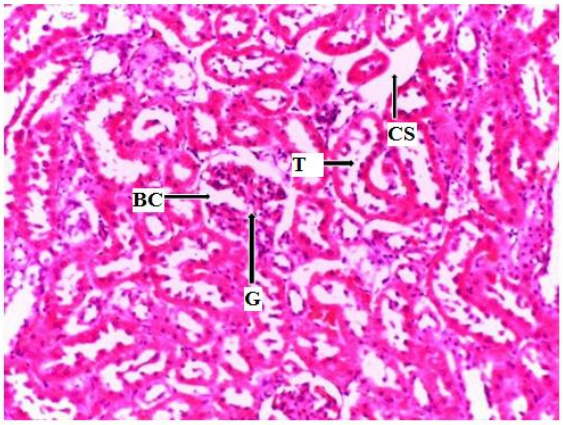

Plate 8: Photomicrograph of section of kidney of albino rat administered 10mg/kg body weight of Pb for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. Also there is cystically dilated space (CS) filled with eosinophilic materials (x400), Stain: H and E

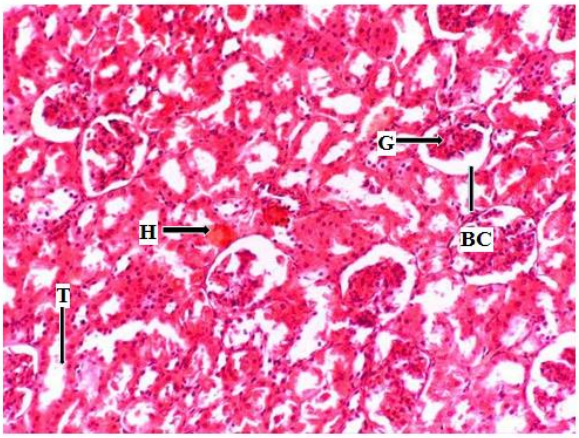

Plate 9: Photomicrograph of section of kidney of albino rat administered 10mg/kg body weight Cd, Cr, Pb mixture for 90 days showing normal tissue architectural disposition. The glomeruli (G), Bowman's capsule (BC) (glomerular capsule), Glomerular tuft, Endothelial cells and Tubules (T) all appear normal. H stands for hemorrhage (x400), Stain: H and E

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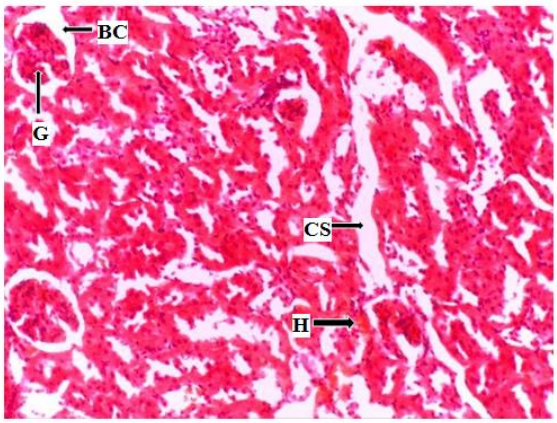

Plate 10: Photomicrograph of section of kidney of albino rat administered 20mg/kg body weight of Cd for 90 days showing some slightly and obviously shrunken glomeruli (atrophy) (G) with increased bowman's space (BC). H stands for hemorrhage. The endothelial cells appear normal. Also there is cystically dilated space (CS) filled with eosinophilic materials (x400), Stain: H and E

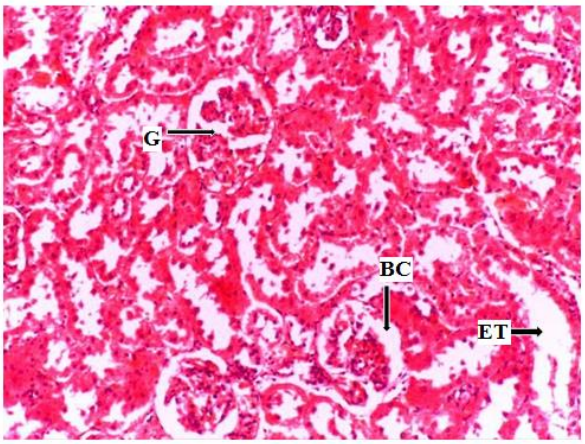

Plate 11: Photomicrograph of section of kidney of albino rat administered 20mg/kg body weight of Cr for 90 days showing some slightly and obviously shrunken glomeruli (atrophy) (G) with increased bowman's space (BC). ET is enlarged tubule. The endothelial cells appear normal (x400), Stain: H and E

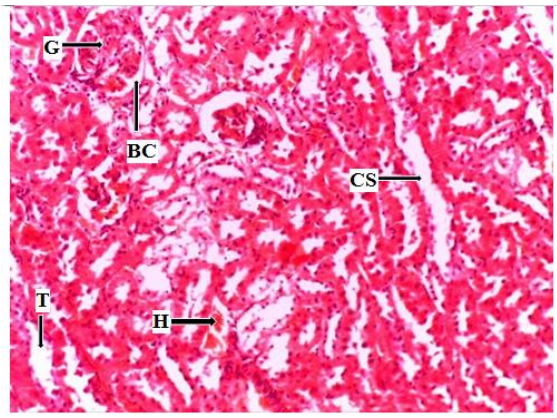

Plate 12: Photomicrograph of section of kidney of albino rat administered 20mg/kg body weight of Pb for 90 days showing some slightly and obviously shrunken glomeruli (atrophy) (G) with increased bowman's space (BC). The interstitial tissue (stromal tissue) is fibrous with attempt at compressing some tubules (T) and in some cases loss of identifiable tubules. H stands for hemorrhage. The endothelial cells appear normal. Also there is cystically dilated space (CS) filled with eosinophilic materials (x400), Stain: H and E

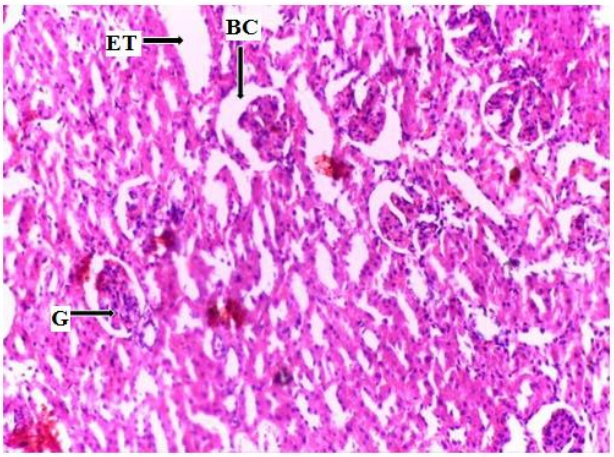

Plate 13: Photomicrograph of section of kidney of albino rat administered 20mg/kg body weight Cd, Cr, Pb mixture for 90 days showing some slightly and obviously shrunken glomeruli (atrophy) (G) with increased bowman's space (BC). ET is enlarged tubule. The endothelial cells appear normal (x400), Stain: H and E

4. DISCUSSION

Serum urea and creatinine concentration, are common tests used to evaluate renal function but creatinine is a late marker of kidney injury [17]. Increased serum urea due to renal failure causes results from disease or toxicity of the renal parenchyma [18]. From the results of the study, treatments with the metals individually and combined caused an increase in serum urea concentration relative to control suggesting renal injury due to the treatments. But the combined treatment did not cause an increased renal injury compared to the individual metal treatments suggesting a less than additive interaction amongst the metals. The less-than-additive interaction observed in the mixture treatment with regard to serum urea for the three treatment doses may be due to the Cr component of the mixture as the effect due to the mixture also decreased with increasing mixture dose just as observed in Cr. This dose response phenomenon is known as hormesis. Hormesis refers to adaptive responses of biological systems to moderate environmental or self-imposed challenges through which the system improves its functionality and/or tolerance to more severe challenges [19]. It is proposed that the Cr component of the mixture at low dose may have caused a stimulating effect by shifting the redox status of the cell [20] which may have activated the cell defense and repair mechanism through the Nrf2 transcription factor [21] leading to decrease in adverse effect observed at high dose of the metal mixture (combined) treatment.This is responsible for the decreased adverse effect observed as the mixture dose increased contrary to the increased adverse effect observed in the individual treatments (Cd and Pb).

Another clinical chemistry finding sometimes observed when renal function is significantly impaired is increased serum inorganic phosphorus concentration. Increased inorganic phosphorus is primarily due to reduced filtration and is observed in chronic kidney disease [18], [22]**.** From the result of the study, this was observed in both the combined and individual metal treatments and increased with increasing treatment dose. But the renal injury observed in combined treatment with regard to inorganic phosphorus was not higher but rather same as observed with individual metal treatments.

Results of histopathological evaluation indicated no tissue injury in the kidney 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 kidney in both combined and individual treatments of the metals for 20mg/kg treatment dose. This is contrary to the observation of Yasmin&Hussain (2021) [23] who treated albino rats with 10mg/kg Cr (VI) orally daily for 42 days. They observed obliteration of the Bowman's space due to mesangial proliferation in glomeruli. The difference may be because we treated the rats thrice weekly as against their daily dosing and as such the bioavailability of Cr in the kidney may have been altered by earlier clearance during the intervals of treatment thereby altering its adverse effect on the kidney

5. CONCLUSION

Simultaneous exposure to these three renal toxicants was expected to produce increased adverse renal effect but this was not the observation. Instead, the observed renal 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 kidney 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

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