

Toxicological Outcome of Combined Exposure to Potassium Dichromate and Levonorgestrel in the Kidney of Female Rats

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Abstract: Co-exposure to chromate (VI) compound and oral contraceptives is common in our environment especially among women working in chromate-related industries. Exposure to either chromate (VI) or oral contraceptives is linked with the etiology of several diseases including cancers and renal injury. However, there is paucity of information on the toxic effect of combined co-exposure to both compounds. The present study examines the toxicity of combined exposure to potassium dichromate (PDC) and an oral contraceptive, levonorgestrel in the kidney of female rats. Control animals were fed distilled water, while experimental rats were injected 12 mg/kg body weight of PDC once a week for six weeks and oral daily exposure to 15µg/kg body weight of levonorgestrel either alone or in combination. Absolute and relative kidney weight, renal function, oxidative stress and pathological lesion were assessed in plasma and kidney of control and experimental rats. The PDC and levonorgestrel significantly ($p < 0.05$) increased plasma urea creatinine and malondialdehyde levels in treated-rats, while renal superoxide dismutase and glutathione-S-transferase activities were reduced by both compounds. Moreover, histopathological lesions including necrotizing nephritis was observed in the kidney of PDC-treated rats, while tubular epithelial degeneration and necrosis was observed in levonorgestrel-treated rats. Combined exposure to both compounds aggravated the increase in urea, creatinine and renal damage. Additionally, the antioxidant enzymes were further repressed in the co-treatment group. The study suggests that combined exposure to potassium dichromate and levonorgestrel worsened nephrotoxicity in rats by increasing oxidative stress.

Keywords: Potassium dichromate, levonogestrel, chromate (VI), oral contraceptive, kidney, free radicals oxidative stress, nephrotoxicity.

INTRODUCTION

Hormonal contraceptives are among the most commonly used medications. Over 100 million women worldwide use contraceptives to prevent unwanted pregnancies. One of the most effective and widely used contraceptives is levonorgestrel. It is often used as an emergency contraceptives that is taken orally as 0.75 mg pill at most 72 hours after unprotected sex and administration of the same dose after 12 hours of taking the first dose. It is also slowly released into body over a long period of time in the form of levonorgestrel-intrauterine device. It creates an unfavorable environment for sperm penetration by suppressing luteinizing hormone secretion and constricting the lining of the uterus [1]. Although efficient in preventing unwanted pregnancies, the drug is associated with many side effects including menstruation irregularities, weight gain, nausea, breast tenderness, headache, abdominal pain, irritability, loss of libido and fatigue [2]. A long list of unusual adverse reactions and events

including cancer was recently documented [3]. Experimental studies have demonstrated that levonogestrel administration is associated with nephrotoxicity [4,5].

Many women using contraceptives are inadvertently co-exposed to numerous occupationally and environmental toxicants including chromate (VI) that may influence toxicity outcomes. Chromium (VI) is an environmental and occupational toxicant that is used in numerous industrial processes including: chrome pigment production, chrome plating, leather tanning, metal finishes and wood preservation [6]. Workers in these industries are occupationally exposed to Cr (VI) compounds mainly by inhalation and ingestion. Additionally, Cr (VI) is discharged into the environment by chromate related industries. Consequently, the public is exposed to Cr (VI) compounds in soil, food, air and water [7-8], especially in developing countries, where regulation on effluent disposal are not strictly enforced. The Cr (VI) exposure affects many tissues including the kidney, principally because it is the main organ for its elimination. Chromate (VI) that bio-accumulate in the kidney are converted to active

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metabolites and free radicals. These metabolites and radicals can cause lipid peroxidation and inactivation of cellular antioxidant enzymes, resulting in renal damage and impairment of renal function [9-10]. In addition, Cr (VI) impairs kidney health and function in humans and animals [11-13].

Co-exposure to chromate (VI) compound and oral contraceptives is common in our environment especially among women working in chromate related industries including textile, paint and dye industries. Low level co-exposure is also possible in contaminated water. However, there is paucity of information on combined effect of co-exposure to both substances. In the present study, we report the toxic effect of combined exposure to potassium dichromate (PDC) and levonorgestrel in the kidney of female albino rats.

MATERIALS AND METHODS

Chemical Reagents

Potassium dichromate (PDC) from Sigma Chemical Co., St. Louis, MO, USA was dissolved in deionised water and administered at a dose of 12 mg/kg body weight. Urea and creatinine kits were obtained from Cobas Diagnostic kits (Mannheim, Germany). All other chemical reagents used were of analytical grade. Levonorgestrel was in the form of a commercial product, Postinor-2 (Gedeon Richter PLC, Budapest, Hungary), containing 0.75 mg of the compound per tablet obtained from the Family Planning Clinic of the Ogun State Hospital, Ota, Ogun State, Nigeria. The drug was dissolved in deionised water.

Experimental Animals

Twenty four female albino rats with an average age of 12 weeks obtained from the Animal House of the Department of Physiology, University of Ibadan, Ibadan were used for the experiment. The animals were housed, six per cage with wood shaven beddings that were constantly changed every two days in polypropylene cages under standard environmental conditions and were kept in the Animal House, Department of Chemical and Food Sciences, Bells University of Technology, Ota, Ogun State. They were fed standard chow and water *ad libitum*. The animals were allowed to acclimatize for two weeks before the commencement of the experiment. All animal handling and procedures were conducted in consonance with the National Institutes of Health procedures for the care and use of laboratory animals [14].

Experimental Protocol

The experimental animals were divided randomly into four groups of six animals each. Animals in group 1 (control) were given distilled water and normal diet throughout the experiment. Animals in group 2 were injected intraperitoneally with 12 mg/kg body weight PDC once a week for six weeks. Group 3 rats were intubated orally with 15 µg/kg body weight, while Group 4 animals were co-exposed to 12 mg/kg body weight PDC and 15 µg/kg /kg body weight levonorgestrel. The dose of PDC in this study has been consistently reported in our laboratory to have toxic effects in rats [15,16], while that of levonorgestrel is similar to a medium dose that was recently reported to mimic contraception related changes in women and useful in evaluating its impacts on non-reproductive tissues [17].

After the period of treatment, the final weights of the animals were recorded. The rats were anesthetized through injection of ketamine and xylazine (i.p) and blood was collected from each rat by ocular puncture into pre-labelled heparinized tubes. The rats were sacrificed by cervical dislocation, dissected and the paired kidney were harvested. The paired kidneys were washed in cold saline, blotted on a filter paper and weighed. The left kidney was used to evaluate the levels of the oxidative stress markers, while the right kidney was stored in formalin for histopathological analysis.

Assessment of Renal Health and Function Markers

The blood samples in heparinized tubes were centrifuged at 3000 x g for 10 minutes to separate plasma. Diagnostic kits obtained from Cobas Diagnostic (Mannheim, Germany) was used to estimate plasma urea and creatinine levels in an automated chemistry analyzer (Roche/Hitachi C311 – Mannheim, Germany).

Assessment of Renal Oxidative Stress Parameters

Superoxide dismutase (SOD), catalase (CAT) glutathione-S-transferase (GST) and malondialdehyde (MDA) in the kidney were determined according to the methods of Sun and Zigma [18], Aebi [19], Habig *et al.* [20] and Estabauer and Cheeseman [21] respectively.

Histopathological Examination of Kidney

Histopathological changes in the kidney were examined as previously reported by Akinwumi *et al.*,

[22]. Briefly, the kidney stored in formalin was washed in phosphate buffer pH 7.4 at 4 °C for 12 hours and dehydrated in graded ethanol. Subsequently, the tissue was embedded in paraffin, cut into 5 µm sections, stained with haematoxylin-eosin dye and scored using an Olympus BX 41 photomicroscope.

Statistical Analysis

Data were expressed as mean \pm SE. They were analyzed with one way analysis of variance (ANOVA) and Duncan Multiple Range Test using the 23rd version of IBM SPSS (IBM, SPSS Inc., NY, USA). The level of statistical significance was put at $p < 0.05$.

RESULT

The effect of administration of PDC and levonorgestrel either alone or in combination on the kidney and relative kidney weight is presented in Table 1. Rats intoxicated with PDC presented with a significant ($p < 0.05$) increase in kidney and relative kidney weight when compared to the control group. The increase in kidney and relative kidney weights were further markedly ($p < 0.01$) enhanced in rats co-exposed to levonorgestrel and PDC as compared to control rats. The kidney and relative kidney weight in the group treated with levonorgestrel only were similar to control value.

Table 1: Effects of Potassium Dichromate and Levonorgestrel on Absolute and Relative Kidney Weight in Test and Control Animals

GROUP	Kidney Weight (g)	Relative Kidney Weight (%)
1	0.72 \pm 0.06	0.53 \pm 0.05
2	0.90 \pm 0.03*	0.71 \pm 0.052*
3	0.71 \pm 0.04	0.56 \pm 0.05
4	0.91 \pm 0.01*	0.69 \pm 0.02*

*Significantly different from control at $p < 0.05$.

The effect of administration of PDC and levonorgestrel on plasma concentration of urea and creatinine is presented in Table 2. Following treatment, creatinine and urea concentrations were significantly ($p < 0.05$) increased when compared with the control. Both values were further significantly ($p < 0.01$) raised by co-exposure to PDC and levonorgestrel also as compared to the control.

The effect of administration of PDC and/ or levonorgestrel on renal superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and malondialdehyde (MDA) activities is presented in Table

Table 2: Effects of Potassium Dichromate and Levonorgestrel on Kidney Function Markers in Test and Control Groups

GROUP	Creatinine (mg/dl)	Urea (mg/dl)
1	0.23 \pm 0.04	15.21 \pm 2.6 9
2	0.49 \pm 0.03*	46.92 \pm 3.92*
3	0.43 \pm 0.06*	45.99 \pm 4.31*
4	0.73 \pm 0.05**	80.27 \pm 10.14**

*Significantly different from control at $p < 0.05$.

**Significantly different from control at $p < 0.01$.

3. Administration of PDC or levonorgestrel to the rats resulted in significant ($p < 0.05$) 60.41% and 39.59% decreases in renal SOD activity respectively when compared to the control. Moreover, co-exposure to PDC and levonorgestrel further declined renal SOD activity to 67.73% as compared to the control. However, renal CAT in the treated groups were not significantly different from the control. Renal GST activity was also significantly ($p < 0.05$) decreased in the groups treated with PDC or levonorgestrel when compared to the control. Co-administration of PDC and levonorgestrel exacerbated renal GST activity as compared to the control. In contrast, MDA level was significant ($p < 0.05$) increased by PDC or levonorgestrel when compared to the control. Concomitant administration of PDC and levonorgestrel resulted in a slight and insignificant increase in renal MDA when compared to either PDC or levonorgestrel-treated groups.

The effect of PDC and levonorgestrel on the kidney histopathology of test and control rats is presented in Figure 1A-E. Unlike the kidney of control that showed normal architecture, kidney of PDC-treated rats showed necrotizing nephritis. Tubular epithelial degeneration and necrosis was found in levonorgestrel-treated rats. The kidney of rats exposed to combination of PDC and levonorgestrel showed tubular epithelial degeneration and necrosis, ectasia of glomerular space and tubules.

DISCUSSION

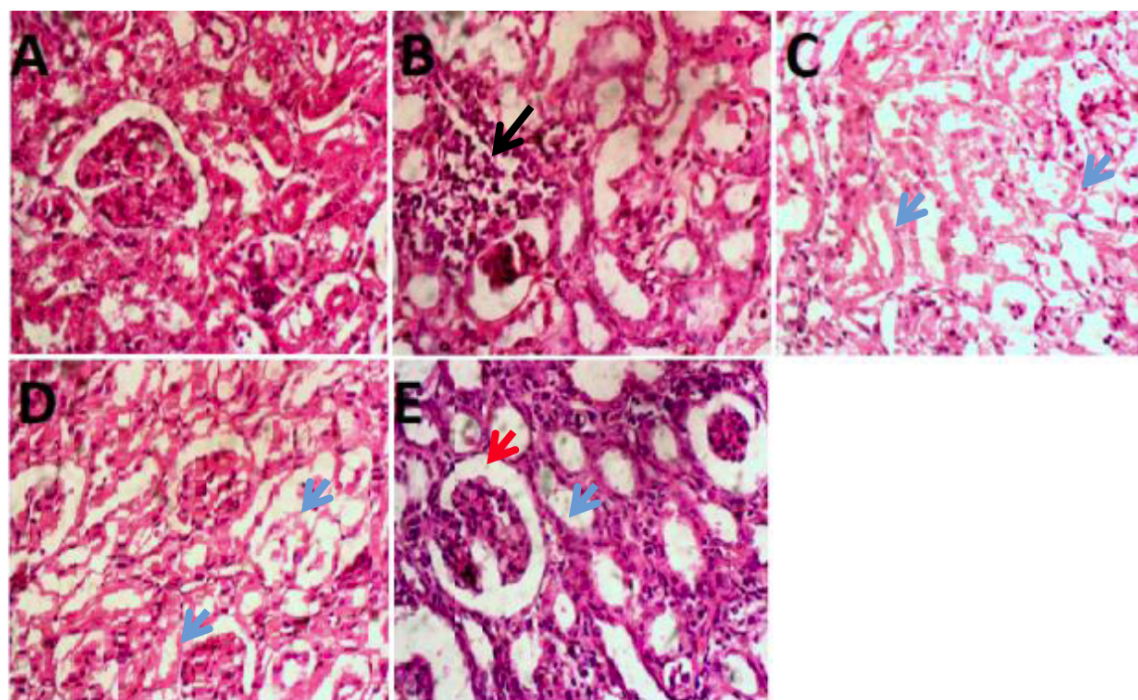
Contraceptives are used by millions of women all over the world, but there is limited information on their toxicity. In addition, many women on contraceptives are inadvertently co-exposed to numerous occupationally and environmental toxicants including chromate (VI) that may influence toxicity outcomes. However, studies on the toxicological consequences of combined exposure to chromate (VI) and contraceptives are

Table 3: Effects of Potassium Dichromate and Levonorgestrel on Kidney Antioxidant Enzymes and Lipid Peroxidation in Test and Control Animals

GROUP	KSOD	KCAT	KGST	KMDA
1	4.37 ± 0.76	0.18 ± 0.02	172.58 ± 9.98	1.62 ± 0.23
2	1.73 ± 0.52*	0.12 ± 0.00	102.00 ± 9.88**	3.03 ± 0.09*
3	2.64 ± 0.54*	0.17 ± 0.01	136.41 ± 13.26*	2.96 ± 0.19*
4	1.41 ± 0.11*	0.16 ± 0.01	98.14 ± 11.57**	3.14 ± 0.12*

*Significantly different from control at $p < 0.05$.**Significantly different from control at $p < 0.01$.

Keys:

KSOD = Kidney superoxide dismutase activity ($\mu\text{mol/min/g}$ tissue weight).KCAT = Kidney catalase activity (mmol/min/g tissue weight).KGST = Kidney glutathione S transferase activity ($\mu\text{mol/min/g}$ tissue weight).KMDA = Kidney malondialdehyde level (nmoles/g tissue weight).**Figure 1:** The effect of potassium dichromate and levonorgestrel on the kidney histopathology of test and control animals. (A) Control rat with normal kidney structure; (B) potassium dichromate treated rat with necrotizing nephritis (black arrow); (C) levonorgestrel treated rat with tubular epithelial degeneration and necrosis (blue arrows); (D & E) Rats exposed to a combination of PDC and levonorgestrel with tubular epithelial degeneration and necrosis (blue arrows), ectasia of glomerular space and tubules (red arrow).

scarce. In the present investigation, the toxic effect of combined exposure to PDC and levonorgestrel was evaluated in female Sprague Dawley rats. Our data suggest that repeated exposure to each of the two compounds is nephrotoxic. The nephrotoxicity could be worsened by co-exposure to both compounds.

The observed increase in kidney and relative kidney weight in the groups treated with PDC alone and in combination with levonorgestrel is suggestive of inflammation and nephrotoxicity. The increase in absolute and relative kidney weight in the PDC group in this study is in line with previous studies [23].

Nephrotoxicity of individual administration of PDC and levonorgestrel was clearly demonstrated by raised plasma urea and creatinine level in the treated groups. Elevated blood creatinine is associated with reduction in glomerular filtration rate, while raised plasma urea is related to enhanced protein catabolism and amino acid deamination in addition to challenges in the ability of nephrons to eliminate nitrogenous and other metabolic wastes. Elevation of urea and creatinine in both PDC and levonorgestrel in this study is consistent with the works of Bashandy *et al.* [23] and Ekhatior *et al.* [4], who found increased serum urea and creatinine levels in rodents exposed to PDC and levonorgestrel-

containing oral pills respectively. Moreover, enhanced urea and creatinine observed in co-treatment group suggests that nephrotoxicity is further compounded by simultaneous exposure to the compounds

The kidney plays prominent role in the metabolism and elimination of both PDC and levonorgestrel [4, 11]. Active metabolites and radical species generated during metabolism can inhibit antioxidant enzymes. Therefore, the inhibition of renal SOD and GST in the current investigation could have resulted from extensive utilization of the enzymes in counteracting free radicals and enhancing detoxification in the kidney. Decline in renal SOD activity would subject renal cells to attack by O^{2-} and propagate free radical-mediated reaction. Similarly, reduction in GST activity could limit neutralization of free radicals and conjugation of bio-transformed toxic metabolites with reduced glutathione for efficient elimination from the body. A resultant effect of sustenance of free radical-mediated reactions is oxidation of cellular macromolecules including membrane lipids. Lipid peroxidation was manifested in this study by elevation of MDA in rats exposed to either PDC and / or levonorgestrel. The reduction in antioxidant enzymes together with lipid peroxidation, also known as oxidative stress have recently been documented for each of the compounds [11-12,24]. Additionally, co-exposure to PDC and levonorgestrel exacerbated oxidative stress and nephrotoxicity as evident by further decline in kidney SOD and GST in this study. Oxidative stress has deleterious toxicological consequences and could be responsible for the on-set of metabolic dysregulation and development of several diseases including nephrotoxicity and cancers that have been linked to PDC and levonorgestrel exposure.

In fact, oxidative stress could have led to the renal damage observed in this study. Lesion observed in the kidney of the group treated with PDC are similar to our earlier observation [25] and that of others [10-11]. Moreover, levonorgestrel impairs energy metabolism and microsomal electron transportation in the proximal tubules and other parts of the nephron [4], which may also explain the tubular epithelial degeneration and necrosis observed in the levonorgestrel-treated group in this study. The extent of renal damage was aggravated by co-administration of levonorgestrel and PDC as manifested by ectasia of glomerular space and tubules in addition to tubular epithelial degeneration and necrosis seen in the co-exposure group. Thus confirming that co-exposure to PDC and levonorgestrel could worsen nephrotoxic outcome.

CONCLUSION

Data obtained in this study suggests that combined exposure to potassium dichromate and levonorgestrel worsened nephrotoxic outcome in rats by aggravating oxidative stress.

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