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ORIGINAL ARTICLE

Comparative evaluation of vehicle, dose, and duration-related biochemical and cardiotoxic effects of chromium (VI) and doxorubicin in adult male Wistar rats: Implications for environmental exposure

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ABSTRACT

BACKGROUND:

Doxorubicin (Dox) cardiotoxicity has been established while the bioaccumulation of chemicals such as chromium, manganese, and lead have been observed by researchers in several studies, which impair the normal functioning of body organs.

OBJECTIVE:

This study compared vehicle, dose, and duration-related oxidative, cardiotoxic, inflammatory, and histologic responses of chromium (VI) compound (Cr(VI) or Cr^{6+}) and Dox intoxication in rats' heart by standard protocols.

METHODS:

The rats were respectively intoxicated with Cr(VI) and Dox in 3 different phases. In the first phase, sixty rats were assigned to six groups of ten each. Group 1 served as the control, while groups 2, 3, and 4 were treated with oral doses of 10, 20, and 30 mg/kg body weight (b.wt) of potassium dichromate (K2Cr2O7) solution, while groups 5 and 6 received intraperitoneal administration of 15 and 20 mg/kg b.wt Dox for two days, respectively, before the sacrifice. The procedure was repeated in the second and third phases, but for 60 days. Oxidative, cardiotoxic, inflammatory and histologic indices were determined in the rats' heart.

RESULTS:

The results indicated that exposure to either Dox or Cr(VI) caused a significant (P<0.05) dose, vehicle and duration-dependent decrease in superoxide dismutase, glutathione peroxidase, catalase activities, and nitric oxide levels while the levels of cardiac croponin levels, creatinine-kinase, C-reactive protein, aspartate transaminase (AST), lactate dehydrogenase and malondialdehyde increased when compared to the control. Heart histopathology of Dox- and Cr(VI)-treated rats showed dose, vehicle and duration-dependent cardiac tissue oedema, hyaline necrosis and displacement of adjacent myocytes compared to control.

CONCLUSION:

Overall, Cr(VI) compound indicated cardiotoxicity, oxidative stress, inflammatory and histopathologic responses relative to Dox induction in the rats' heart.

KEYWORDS:

Cardiotoxicity; Cardiac tissue oedema; Chromium(VI); Doxorubicin; Exposure; Nitric oxide; Oxidative stress

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INTRODUCTION

Several studies deduced the indiscriminate release of chromium (VI) compounds (Cr (VI)) during industrial activities like photo production, chrome plating, paint making, welding, electroplating, and leather tanning as the major ways of Cr-pollution in the ecosystem[1-4]. Chromium (VI) toxicity has been implicated in various organ toxicities like respiratory, gastrointestinal, hepatic, renal, neurological, and reproductive systems. It alters general development, causes increased reactive oxygen species (ROS) production, which limits the activities of antioxidant enzymes, protein oxidation, and DNA damage[1,3,5-6]. The health effects of Cr, like other trace elements and chemicals of concern, may vary with the route of exposure, possibly, through inhalation, dermal contact, and ingestion of contaminated water and food crops grown in Cr-polluted soils[7,8]. Chromium (Cr) has varying oxidation states and solubility[9]. However, the Cr (VI) forms are more hazardous to health because they make up very strong oxidising agents that get absorbed easily across the membranes through non-specific anion carriers[10].

Cardiotoxicity results in myocardial and electrophysiology dysfunctions, which affect the heart's ability to pump blood efficiently, resulting in various co-morbidities and deaths[5,11]. Doxorubicin (Dox), an established cardiotoxic agent, is a broad-spectrum antitumor drug used to treat various cancers but could lead to cardiac failure and cardiomyopathies, even after treatment cessation[12,13]. The early damage occurs 1 to 3 days after drug administration, often resulting in apoptosis, necrosis and oxidative stress, which results in irreversible cardiotoxicity[12]. The molecular mechanisms of Dox-induced cardiotoxicity are unclear. Although, the suggestive possible mechanisms of action of Dox include Dox-mediated attack on the mitochondria, increase in ROS, iron ferroptosis, disruption of calcium homeostasis, topoisomerase dysregulation, and elevation in cardiac enzymes, such as Aspartase transaminase (AST), Lactose Dehydrogenase (LDH), and Creatine Kinase-MB (CK–MB), and Troponin[13,14]. Like Dox, Cr(VI)-induced cardiotoxicity can occur through the induction of hypertrophic signalling, apoptosis, necrosis, and disrupting redox-dependent vascular wall signalling processes resulting in cardiac remodelling[1,14,15-16].

Previously, high levels of Cr(VI) were detected in soils and food crops grown around industrial areas in the Southeast of Nigeria[1,3]. This could indicate high level of Cr(VI) contamination and possible health risks.
The dearth of information on the cardiotoxic effect of Cr(VI) compounds as well as the growing concerns of possible cardio-related ailments observed among the residents and industrial workers in the areas[1,2,8,17] warranted this present study. Therefore, this study aimed to evaluate and compare the vehicle, dose, and duration-based oxidative, cardiotoxic, inflammatory and histologic responses of administration of Cr(VI) (from potassium dichromate) and Dox in the heart of adult male Wistar rats.

METHODS

Ethical approval and consent to participate

All investigations involving the experimental animals adhered strictly to the guidelines for the Committee on Care and Use of Laboratory Animals, US Department of Health and Human Services, Public Health Service, Institute of Laboratory Animal Resources, USA. The experiment protocols were performed after the ethical approval was granted by the College of Natural Sciences, Research Ethics Committee (CTREC/002/21), Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Animal Husbandry

A total of one hundred and eighty adult male Wistar albino rats (age: 12weeks and body weight: 120-140 g) procured from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for this study. The animals were housed in well-ventilated plastic cages at the Animal holding facility of the department of Biochemistry, College of Natural Sciences, Michael Okpara University of

Agriculture, Umudike, Abia State, Nigeria and subjected to a photoperiod of 12h light 12h dark cycle. They were allowed access to rat chow and drinking water ad libitum for 14 days. All investigations involving the experimental animals were strictly followed regarding the guidelines for the care and use of animals as defined by the National Institute of Health Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, USA[18]. The experiment protocols were performed after the ethical approval was granted by the College of Natural Sciences, Research Ethics Committee (CREC/002/21), Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Experimental Design

Sixty (60) rats in three groups were used for each phase of treatment. The treatment phases included an acute exposure phase and two chronic exposure phases.

In the 1st phase (acute phase exposure), group 1; control rats received an equal volume of normal drinking water orally for 21 consecutive days while groups 2, 3 and 4 rats were given oral (using gavage) treatment of 10, 20, and 30 mg/kg K2Cr2O7 compound (Cr(VI)) respectively for 21 consecutive days. Two days before sacrifice, animals in group 5 and 6 received 15 and 20 mg/kg (i.p) of doxorubicin (Dox) respectively.

The protocol in the 2nd phase (chronic phase exposure) was as in the 1st phase but lasted for sixty (60) consecutive days.

In the 3rd phase (chronic food supplementation phase), group 1, control rats were fed with a standard pellet diet and received an equal volume of distilled water. Group 2 rats received 0.01% of K2Cr2O7 [Cr(VI)] compound in feed i.e 10mg/kg of K2Cr2O7 [Cr(VI)] compound per kg body weight in 100 g of Rat feed for 60 days. Group 3 rats received 0.02% of K2Cr2O7 [Cr(VI)] compound in feed i.e 20 mg/kg of K2Cr2O7 [Cr(VI)] compound per kg body weight in 100g of Rat feed for 60 days. Group 4 rats received 0.03% of K2Cr2O7 [Cr(VI)] compound in feed i.e 30mg/kg of K2Cr2O7 [Cr(VI)] compound per kg body weight in 100g of Rat feed for 60 days. Group 5 and 6 rats respectively received intraperitoneal administration of 15 mg/kg and 20 mg/kg body weight of doxorubicin (2 days before sacrifice). The doses of K2Cr2O7 used in this study were based on the pilot study and as previously published[19,20] while doses of, and methods used for Dox administration were based on earlier published reports[15,16].

Preparation of heart samples

All analyses, including biochemical and histopathology assessment of heart tissue carried out in the first phase, were repeated in the second and third phases. On the 22nd and 61st day, i.e., after overnight fasting. Ketamine injection was administered intraperitoneally to anaesthetize the animals, followed by prompt excision of the respective rats' heart tissues. Part of the heart was rinsed in normal saline. One gram was weighed, sliced, and ground with laboratory mortar and pestle in a 5 mL phosphate buffer solution. The resulting solution was centrifuged at 2200rpm for 25 minutes in a cold centrifuge at 4 °C. The supernatant (homogenate) was collected, labelled, and preserved in the freezer until analysis. The heart homogenates were used to assess Creatine Kinase MB (CK-MB), C-reactive protein (CRP), Aspartate transaminase (AST), Lactate Dehydrogenase (LDH), and Cardiac troponin I (CTnI). Also, part of the heart was excised and fixed in 10% buffered formalin for a histopathology examination.

Body Weight and Relative Organ Weight

The body weight of each rat in the groups was weighed at the beginning of the study and on the day of sacrifice. The excised organs (heart) were weighed during the sacrifice to calculate the relative organ weight (using the formula below) and observed for gross lesions.

$$\text{Relative organ weight} = \frac{\text{Organ weight (g) of animal on sacrifice day (g)}}{\text{Body weight (g) of animal on sacricice day}} \; X \; 100$$

Biochemical Assay

Superoxide dismutase (SOD) activity was assayed by the method of[21,22]. Glutathione peroxidase (GPX) assay was carried out according to the method of[22]. The activity of Catalase (CAT) was assayed by the method of[23]. Lipid peroxidation product, malondialdehyde (MDA) was assayed according to the method described by[24]. LDH assay was carried out according to the method used by[25]. CK-MB assay was carried out according to the method of[26]. AST assay was according to the method of[27]. Nitric Oxide (NO) assay was also carried out according to the method described by[28]. Spectrophotometric reading was assessed using D3900 UV-VIS Spectrophotometer (Hach Technologies Loveland USA.)

Immuno Assays

The cardiac troponin I test (CTnI) was carried out according to the method of Melanson et al[29] to measure cTnI levels in heart homogenate. C-reactive proteins (CRP) were analyzed using ELISA kits, following the manufacturer's instructions.

Preparation of slides for histopathological evaluation

The hearts of the rats were isolated immediately after sacrifice, washed with cold phosphate- buffered saline (pH = 7.4) and fixed in 10% buffered formalin. Tissues were embedded in paraffin, and 5m serial sections of the heart were taken. These sections were stained with Hematoxylin and Eosin (H&E). Changes in the histopathology were observed under a light microscope (Olympus BX10, Tokyo, Japan), and images were taken using a digital camera. The individual performing the evaluation was blinded to the treatment given to the respective groups. Tissue sections of the heart were collected for histopathological studies. Prior to the commencement of tissue preparation, the heart samples were fixed in formalin (10% buffered) after washing with cold phosphate- buffered saline (7.4). The tissues were prepared by trimming and dehydrating in 70%, 80%, 90%, and absolute alcohol, clearing in 3 grades of xylene, and solidifying the tissues in molten wax. On solidifying, the tissue-containing wax blocks were cut into 5µm thick sections with a rotary microtome, floated in a water bath, and incubated at 60°C for 30 minutes. The 5µm thick sectioned tissues were cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80%, and 70%). The sections were then stained with haematoxylin for 15 minutes. Blueing was done with ammonium chloride, and differentiation was done with hydrochloric acid alcohol (1% / 70%) before counterstaining with eosin. Permanent mounts were made on degreased glass slides using a mountant, DPX. The prepared slides were examined with a Motic™ compound light microscope using x4, x10, and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x400 magnifications.

Statistical Analysis

The least significant difference (LSD) was used to compare differences in each sample within treatments. Data were reported as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was also used to determine significant differences between groups, considering a level of significance of less than 0.05 ($p<0.05$) using SPSS Statistics v27.

RESULTS

Effect of K2Cr207 and Doxorubicin treatments on heart and body weight

The absolute and relative heart weights of rats treated orally (by administration with gavage) with K2Cr2O7 for 21 days are presented in Table 1. The experimental rats' absolute and relative heart weights indicated no significant difference compared to the Control ($p < 0.05$). However, a slight increase in the absolute and relative heart weight of groups treated with K2Cr2O7 for 60 days was observed compared to the Dox and normal (control) groups for all treatment routes. The rats also showed increased final body weights across groups, as seen in table 1. However, we observed a statistically marked increase in body weight of the control groups compared to those that received K2Cr2O7 and Dox.

Table 1. Effect of K2Cr2O7 and Doxorubicin on the body weight, absolute and relative heart weights of rats (g)

		Heart Weight		Body Weight	
		Absolute	Relative	Final	Initial
Control	Acute exposure	0.52±0.03	0.36±0.04	145.69±1.42	131.4±1.51
	Chronic Exposure	0.57±0.03	0.33±0.06	168.73±1.48	126.4±1.75
	Chronic exposure in food.	0.54±01	0.34±0.02	161.73±0.03	133.40±0.03
10 mg/kg Cr	Acute exposure	0.51±0.05	0.36±0.02	140.80±1.52	127.25±1.32
	Chronic Exposure	0.58±0.05	0.37±0.02	156.80±2.66	138.25±1.84
	Chronic exposure in food	0.60±0.01	0.38±0.01	156.73±0.02	132.28±0.02
20 mg/kg Cr	Acute exposure	0.53±0.10	0.37±0.02	141.75±3.50	121.75±2.91
	Chronic Exposure	0.55±0.10	0.35±0.02	155.75±3.56	137.75±3.58
	Chronic exposure in food	0.47±0.03	0.29±0.01	161.43±0.02	132.55±0.01
30 mg/kg Cr	Acute exposure	0.54±0.12	0.38±0.01	141.23±2.51	130.75±0.80
	Chronic Exposure	0.59±0.12	0.38±0.01	153.23±3.97	135.75±0.85
	Chronic exposure in food	0.59±0.01	0.37±0.12	160.25±0.03	136.20±0.02
15 mg/kg Dox	Acute exposure	0.53±0.06	0.37±0.02	144.50±2.00	122.40±1.14
	Chronic Exposure	0.58±0.06	0.38±0.01	159.50±2.73	126.40±1.24
	Chronic exposure in food	0.47±0.02	0.30±0.13	156.43±0.02	135.70±0.02
20 mg/kg Dox	Acute exposure	0.55±0.07	0.38±0.01	143.5±2.17	120.10±1.20
	Chronic Exposure	0.57±0.07	0.35±0.01	163.5±2.15	124.00±1.6813
	Chronic exposure in food	0.47±0.01	0.30±0.01	156.15±0.03	2.83±0.01

Each value represents the mean ± SEM; Values are significantly different at $p<0.05$

Different doses of K2Cr2O7 and Doxorubicin impaired Antioxidant enzymes following acute and chronic exposure to Rats in the Treatment Phases (Phase 1, 2 and 3)

Results presented in Figure 1 (A, B and C) show that rats exposed to K2Cr2O7 and Dox respectively elicited a significant (*p0.05*) decrease in glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) activities in the treatment phases relative to the control (Group 1).

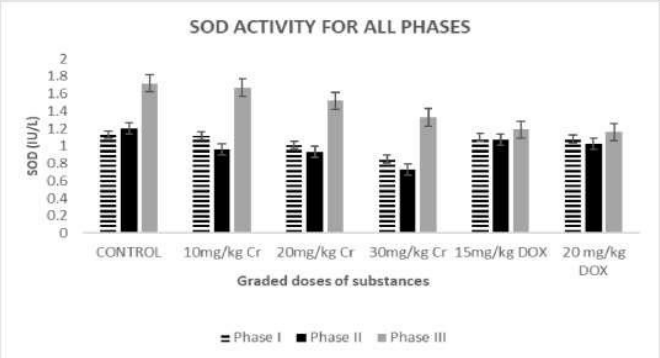

Figure 1A. Different doses of K2Cr2O7 and Dox impaired SOD activity in the heart tissue of exposed rats in all treatment phases. Values are presented as Mean ± Standard error of mean.

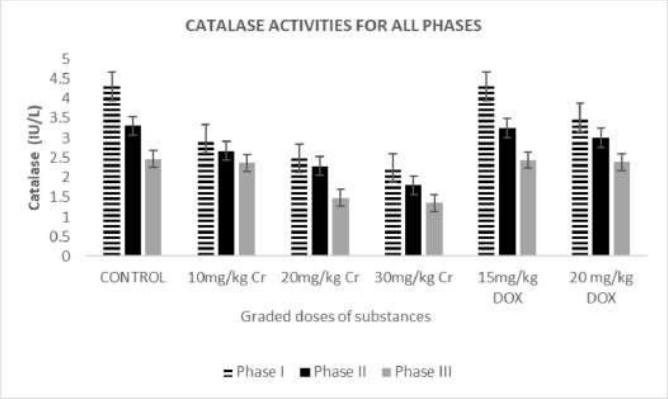

Figure 1B. Different doses of K2Cr2O7 and Dox impaired Catalase activity in heart tissue of exposed rats for all three treatment phases. Values are presented as Mean ± Standard error of mean.

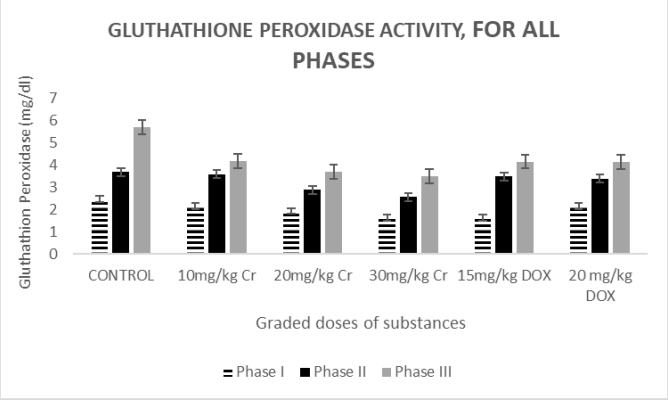

Figure 1C. Different doses of K2Cr2O7 and Dox impaired Glutathione peroxidase activity in heart tissue of exposed rats for all three treatment phases. Values are presented as Mean ± Standard error of mean.

In phase 1,The SOD activities decreased by 0.89%,10.62%, 24.78% K2Cr2O7 treated Rats, CAT activities also decreased by 30.86%,42.23% and 48.03% in K2Cr2O7 treated rats, likewise, GPx activities decreased by 12.96%, 21.96% and 34.01% in rats treated with 10mg/kg, 20mg and 30 mg/kg K2Cr2O7 respectively, relative to the normal control animals. Similarly, animals treated with 15 mg/kg and 20 mg/kg Dox in groups 5 and 6 showed decreased GPx, CAT, and SOD activities. The percentage decreases in the enzyme activities for groups 5 and 6 relative to the control group were 2.65% and 4.24%(SOD), 0.23% and 18.33% (CAT), and 34.4% and 12.55% (GPx).

In the Second phase, the SOD activities decreased by 20%, 22.5%, 39.17 % in K2Cr2O7 treated rats, CAT activities also decreased by 19.34%, 30.82% and 45.62% in K2Cr2O7 treated rats. Likewise, GPx activities decreased by 2.70%, 21.95% and 30.62% in Rats treated with 10mg/kg, 20mg and 30mg/kg K2Cr2O7 respectively relative to the Normal control animals. Similarly, animals that received 15 mg/kg and 20 mg/kg Dox in Groups 5 and 6 showed decreased GPx, CAT, and SOD activities by 10.38% and 14.17%, 1.81%, and 9.37%, 5.42%, and 8.40% respectively compared to the enzyme activities recorded for the normal control (Group 1).
In the third phase, the SOD activities decreased by 2.90%, 11.63%, and 22.67% K2Cr2O7 in treated Rats. CAT activities also decreased by 4.04%, 40%, and 45% in K2Cr2O7 treated rats. Likewise, GPx activities decreased by 26.92%, 35.49% and 38.11% in Rats treated with 10 mg/kg, 20 mg/kg and 30 mg/kg K2Cr2O7 respectively relative to the normal control animals.

Similarly, animals treated with 15 mg/kg and 20 mg/kg Doxorubicin (Dox) in Groups 5 and 6 decreased the enzyme activities by 30.81% and 32.56% (SOD), 1.21% and 3.24%(CAT), and 27.27% and 27.47% (GPx) compared to the normal control (Group 1).

Treating rats with different doses of K2Cr2O7 and Dox induced Oxidative Stress in all the treatment phases (First, Second and Third Phases)

The results presented in Figure 2 show the level of malondialdehyde (MDA) investigated in the hearts of the Control, K2Cr2O7, and Dox treated Rats. The Rats that received both K2Cr2O7 and Dox showed a significant increase in MDA levels following the First, Second and Third treatment Phases when compared to the normal control, the percentage increase in MDA levels for the Rats treated with 10 mg/kg, 20 mg/kg, and 30mg/kg K2Cr2O7 was 16.67%,

respectively, 63.33%, 90.00%, respectively, in the first phase.

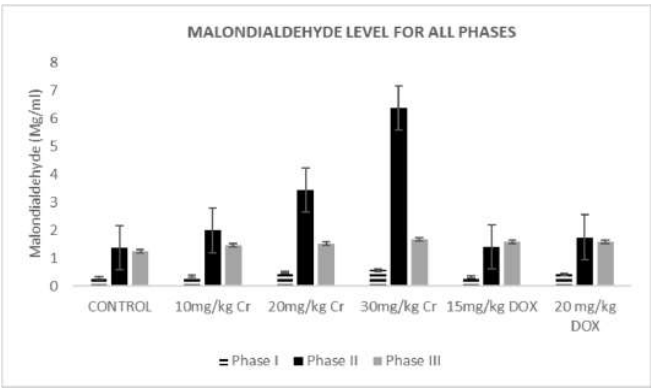

Figure 2. MDA levels in control male Wistar rats and those treated with K2Cr2O7 and Dox. Values are presented as Mean ± Standard error of mean.

In the second phase, the MDA levels increased significantly by 46%, 151.47%, and 368.38% compared to the levels observed in the control. Similarly, the percentage increases observed compared to control for the third treatment phases were 18.55%, 23.39% and 33.87% for 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7 respectively.

In the Dox groups (5 and 6), the rats treated showed similar spikes ($p<0.05$) in the levels of MDA compared to the levels observed in the control. The percentage increases were 3.33% and 40% (First Phase), 2.21% and 27.94%(Second phase), and 26.61% and 28.23% (Third Phase) for rats treated with 15 mg/kg and 20 mg/kg Dox treated groups respectively.

Rats treated with different doses of K2Cr2O7 and Dox showed increased Cardiac markers (AST, LDH, and CK-MB) activities in all treatment phase

Results presented in Figure 3 (A, B and C) show that rats exposed to K2Cr2O7 and Dox respectively elicited a significant (*p<0.05*) increase in aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatine kinase MB (CK-MB) activities in the treatment phases relative to the control (Group 1).

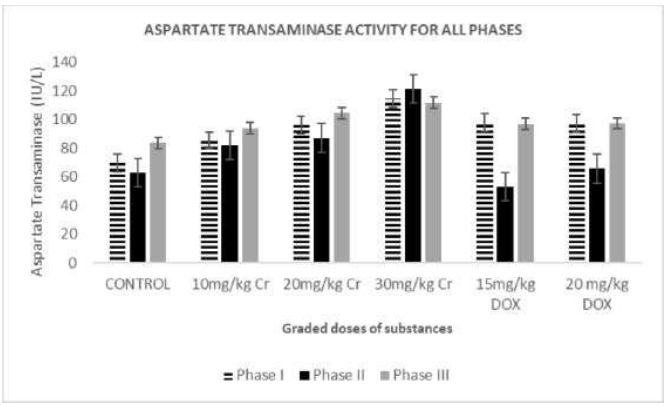

Figure 3A. Rats treated with different doses of K2Cr2O7 and Dox showed increased activity of Aspartate transaminase (AST). Values are presented as Mean ± Standard error of mean.

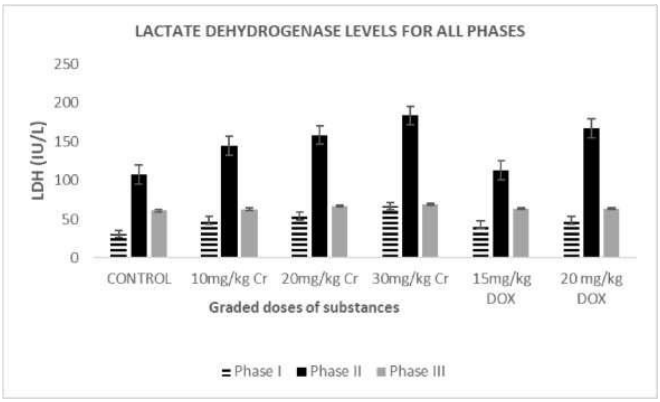

Figure 3B. Rats treated with different doses of K2Cr2O7 and Dox showed increased activity of Lactate Dehydrogenase (LDH). Values are presented as Mean ± Standard error of mean.

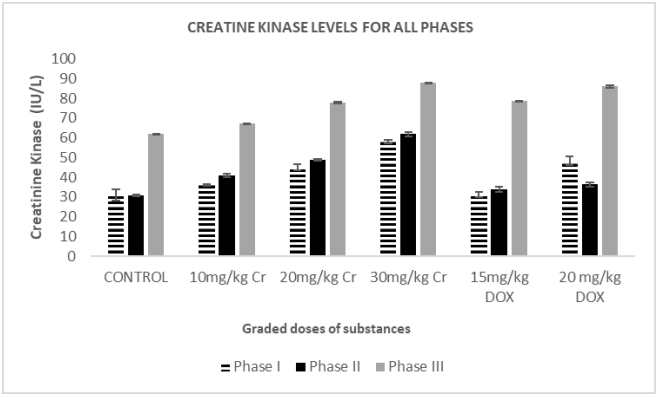

Figure 3C. Rats treated with different doses of K2Cr2O7 and Dox showed increased activity of creatinine kinase (CK-MB). Values are presented as Mean ± Standard error of mean.

In the second phase, the AST results indicated 30.76%, 38.73%, and 93.56% increases in enzyme activities. LDH activities also increased by 34.37%, 47.17%, and 70.25%. Also, CK-MB activities followed the increasing trend by 33.15%, 58.80%, and 101.12% for rats treated with 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7, respectively, when compared to the Normal group. Likewise, the rats treated with 15 mg/kg and 20 mg/kg Doxorubicin (Dox) in groups 5 and 6, respectively, indicated increases in AST, LDH, and CK-MB activities. The percentage increases in activities for groups 5 and 6 compared to the control group were 5.28% and 4.56% (AST), 38.33% and 56.43% (LDH), and 9.71% and 18.13% (CK-MB).

In the third phase, the AST results indicated 12.35%, 25.20%, and 33.89% increases in enzyme activities. LDH activities also increased by 3.56%, 10.32%, and 13.48%. Also, CK-MB activities followed the increasing trend by 8.55%, 25.48%, and 101.12% for rats treated with 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7, respectively, when compared to the normal group.

Similarly, the rats treated with 15mg/kg and 20mg/kg Doxorubicin (Dox) in groups 5 and 6, respectively, showed elevated levels of AST, LDH, and CK-MB activities. The percentage increases in activities for groups 5 and 6 compared to the control group were 5.28% and 4.56% (AST), 38.33% and 56.43% (LDH), and 9.71% and 18.13%(CK-MB).

Rats treated with different doses of K2Cr2O7 and Dox increased Cardiac Troponin I (CTnI) levels in all the treatment phases(First, Second and Third Phases)

The result in Figure 4 shows the levels of CTnI investigated in the hearts of the Control, K2Cr2O7, and Doxtreated rats with a significant increase in CTnI levels in all the treatment Phases. The percentage increase in CTnI levels for the rats treated with 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7 compared to the normal control was 29.18%, 35.01%, 38.91% for the first phases.

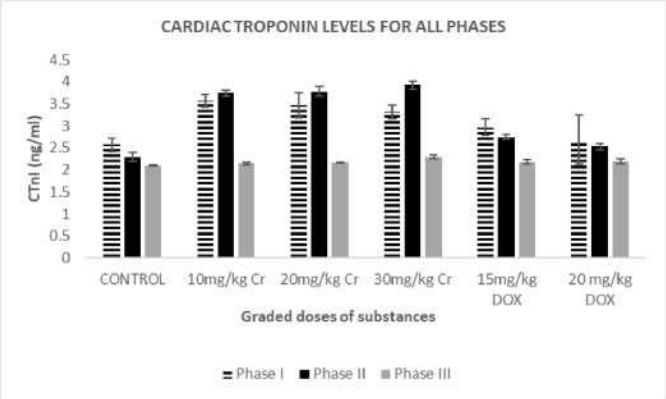

Figure 4. Rats treated with different doses of K2Cr2O7 and Doxorubicin showed increased Cardiac Troponin I (CTnI) levels in all the treatment phases (First, Second and Third Phases). Values are presented as Mean ± Standard error of mean.

In the second phase, the CTnI levels increased significantly by 63.76%, 65.07%, and 71.62% compared to the levels observed in the control. Also, the percentage increases observed compared to control for the third treatment phase were 2.28%, 3.33% and 9.52% for 10mg/kg, 20mg/kg, and 30mg/kg K2Cr2O7, respectively.

Rats treated with different doses of K2Cr2O7 and Dox indicated increased C-Reactive Proteins (CRP) in all the treatment phases (First, Second and Third Phases)

The result presented in Figure 5 show spikes in C-reactive protein (CRP) levels compared to control, K2Cr2O7, and Dox-treated rats. The percentage increase in CRP levels for the rats treated with 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7 compared to the normal control were 35.62%, 45.21%, 82.19% for the first phases. In the second phase, the CRP levels increased significantly by 113%, 229%, and 300% compared to the observed control values.

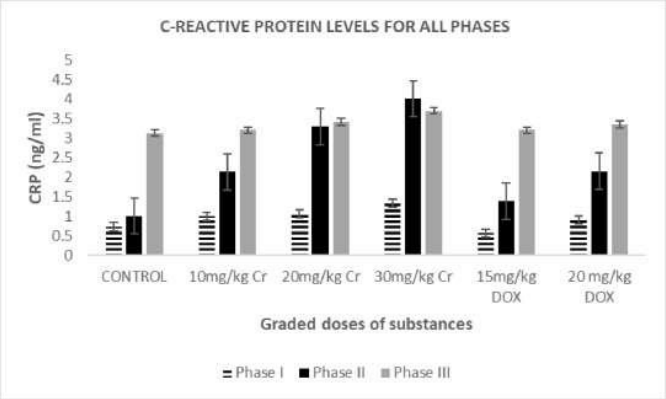

Figure 5. Results for rats treated with different doses of K2Cr2O7 and Doxorubicin indicated increased C- reactive Proteins (CRP) in all the treatment phases. Values are presented as Mean ± Standard error of mean.

Also, the percentage increases observed compared to Control for the third treatment phases were 2.56%, 9.29% and 18.27% for 10mg/kg, 20mg/kg, and 30mg/kg K2Cr2O7 respectively. In the Dox groups (5 and 6), the Rats treated indicated similar spike increases ($p<0.05$) in the levels of CRP compared to the levels observed in the control. The percentage increases were 23.28% and 23.29% (First Phase), 38% and 114% (Second phase), and 2.56% and 7.05% (Third phase) for Rats treated with 15mg/kg and 20mg/kg Dox treated groups respectively.

Different doses of K2Cr2O7 and Dox inhibited Nitric Oxide levels in the heart of treated Rats in all phases (First, Second and Third Phases)

The result presented in Figure 6 shows decreases in the levels of nitric oxide (NO) availability in the hearts of K2Cr2O7 and Dox-treated rats compared to the control. The percentage decrease in NO levels for the rats treated with 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7 compared to the normal control were 4.65%, 18.61%, 27.91% for the first phase. Conversely, in the second phase, NO indicated an increase of 31.7%, 41.7%, and 76.8% compared to the control.

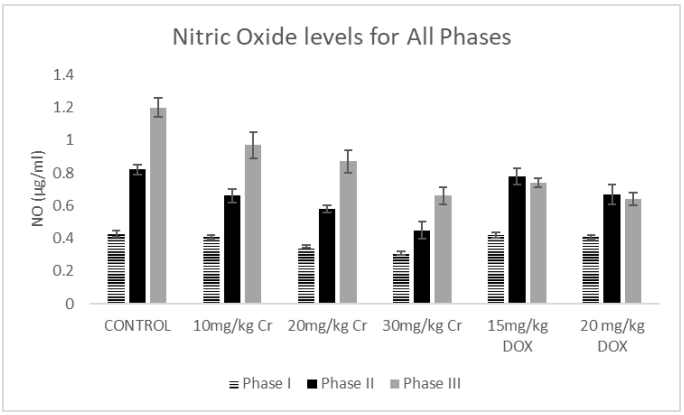

Figure 6. Different doses of K2Cr2O7 and Doxorubicin inhibited Nitric Oxide (NO) levels in the heart of treated Rats in all phases. Values are presented as Mean ± Standard error of mean.

Also, percentage increases were observed compared to control for the third treatment phases were as follows: 385%, 885% and 1200% for 10 mg/kg, 20 mg/kg, and 30 mg/kg K2Cr2O7, respectively. The NO levels in Dox groups (5 and 6) in the first phase indicated a slight decrease compared to control levels. In contrast, a significant increase ($p<0.05$) was observed in the levels of NO in the second and third phases compared to the Control. The percentage decreases were 2.31% and 4.65% (first phase), while the percentage increase was 19.5% and 1.22% (second phase), and 750% and 1115% (third phase) for rats treated with 15 mg/kg and 20 mg/kg Dox treated groups respectively.

The effect of administering K2Cr2O7 and Doxorubicin intraperitoneal injection on the myocardial histoarchitecture of animals treated in the first phase, Second and Third Phase

Plate 1 shows the effect of K2Cr2O7 treatment in phase 1, revealing control rats (a) as having the normal histoarchitecture of the heart, as the myocardium showed a rich network of vasculature, capillaries, nucleus, and pericytes. In contrast, the groups treated with K2Cr2O7 (b, c and d) indicated areas of mild tissue oedema due to treatments exposure. The Dox groups (e and f) showed a higher degree of cardiac damage as there was myotocytolysis and hyaline necrosis in addition to

pulmonary oedema, the affected cells' loss of striation, fragmentation of the myocytes, intense eosinophilic staining of the sarcoplasm (myocytes cytoplasm) with nuclear pyknosis and karyorrhexis. In group 6, oedema seemed to be more severe and widespread.

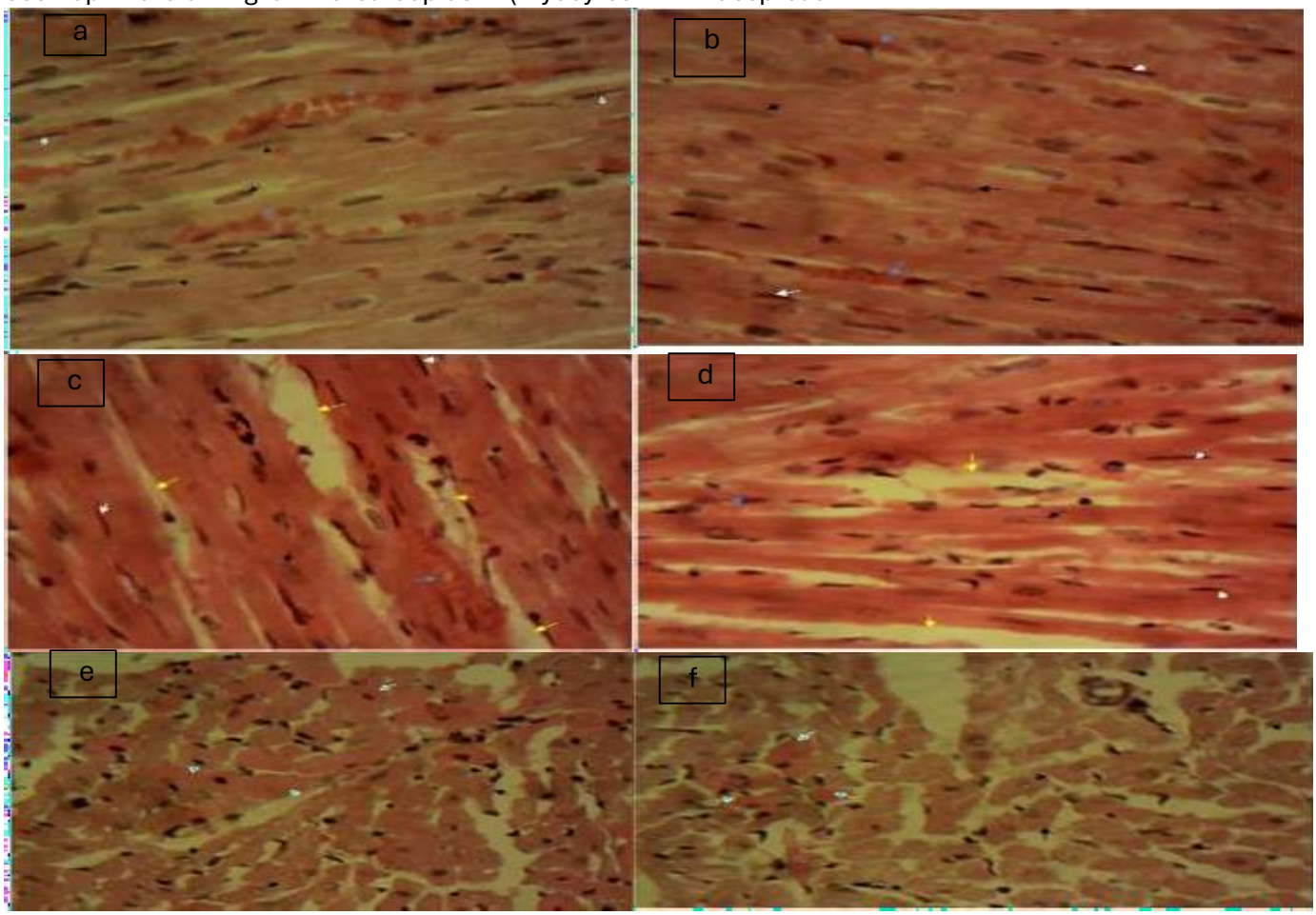

Plate 1: Effects of K2Cr2O7 and Doxorubicin cardiotoxicity on histopathological alteration in the heart of treated rats after 21 days (H & E X400, representative photomicrographs). (a) Control rats received an equal volume of normal drinking water orally for 21 consecutive days, (b) Rats were orally treated with 10 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 21 consecutive days, (c) Rats were orally treated with 20 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 21 consecutive days, (d) Rats were orally treated with 30 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 21 consecutive days, (e)15 mg/kg body weight doxorubicin (2 days before sacrifice) administered intraperitoneally,(f) 20 mg/kg body weight doxorubicin (2 days before sacrifice) administered intraperitoneally.

Similarly, Plates 2 and 3 revealed the histopathology examination of K2Cr2O7 and Dox treated Rats in phase 2 and 3 showing Control groups (g and m, respectively) expressed a normal heart structure while (h, I, j) and (n, o, p) indicated areas of myocyte displaced, indicating pulmonary oedema; Dox groups (k and l) and (q and r) similarly showed areas of pulmonary oedema, hyaline necrosis and myocytolysis for the second and third study, respectively.

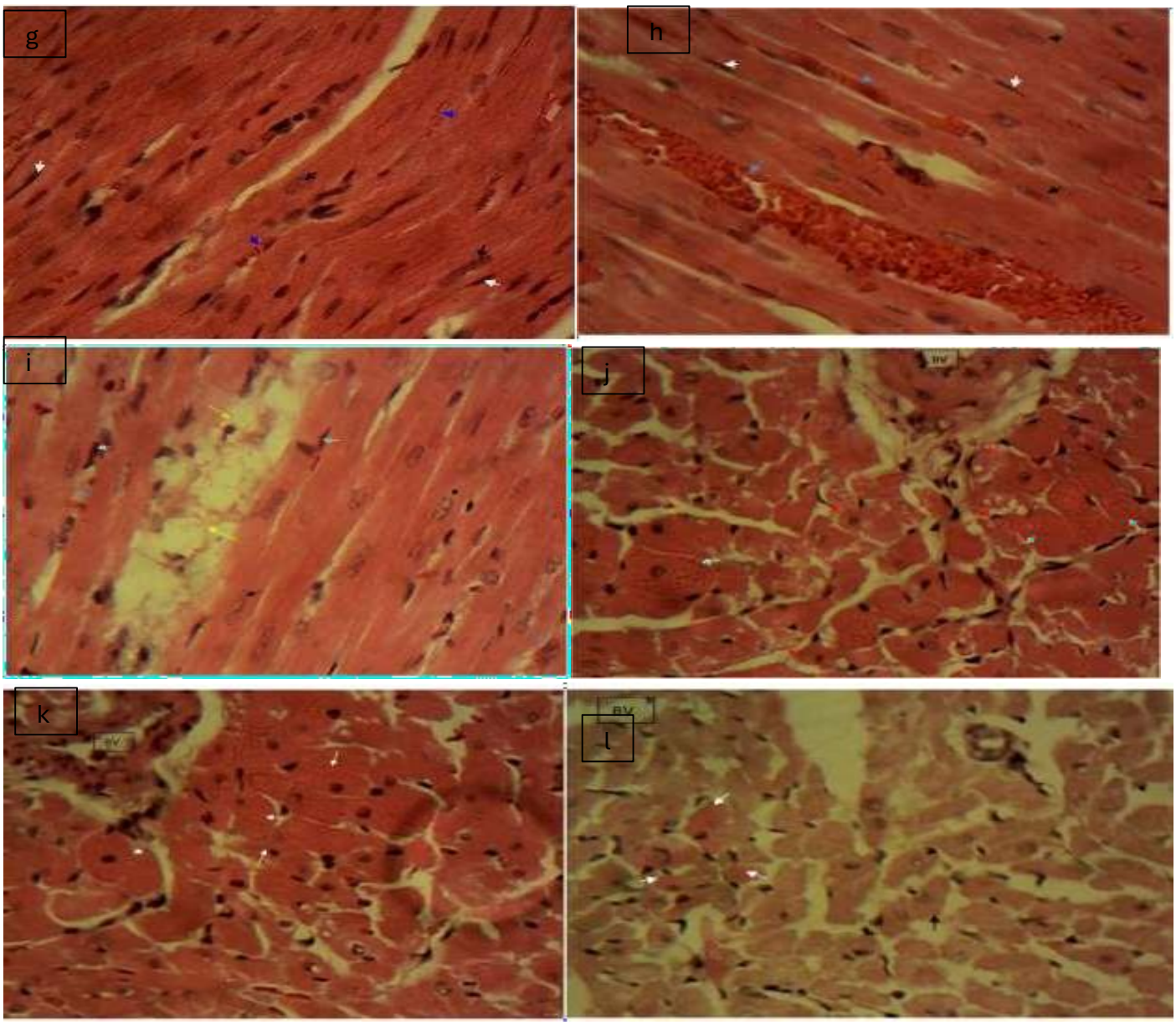

Plate 2: Effects of K2Cr2O7 and Doxorubicin in phase 2 on histopathological alteration in the heart of male Wistar Rats for 60 days (H & E X400, representative photomicrographs). (g) Control rats received an equal volume of normal drinking water orally for 60 consecutive days, (h) Rats were orally treated with 10 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 60 consecutive days, (i) Rats were orally treated with 20 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 60 consecutive days, (j) Rats were orally treated with 30 mg/kg body weight of K2Cr2O7 [Cr(VI)] compound for 60 consecutive days, (k)15 mg/kg body weight doxorubicin (2 days before sacrifice) administered intraperitoneally,(l)20 mg/kg body weight doxorubicin (2 days before sacrifice) administered intraperitoneally.

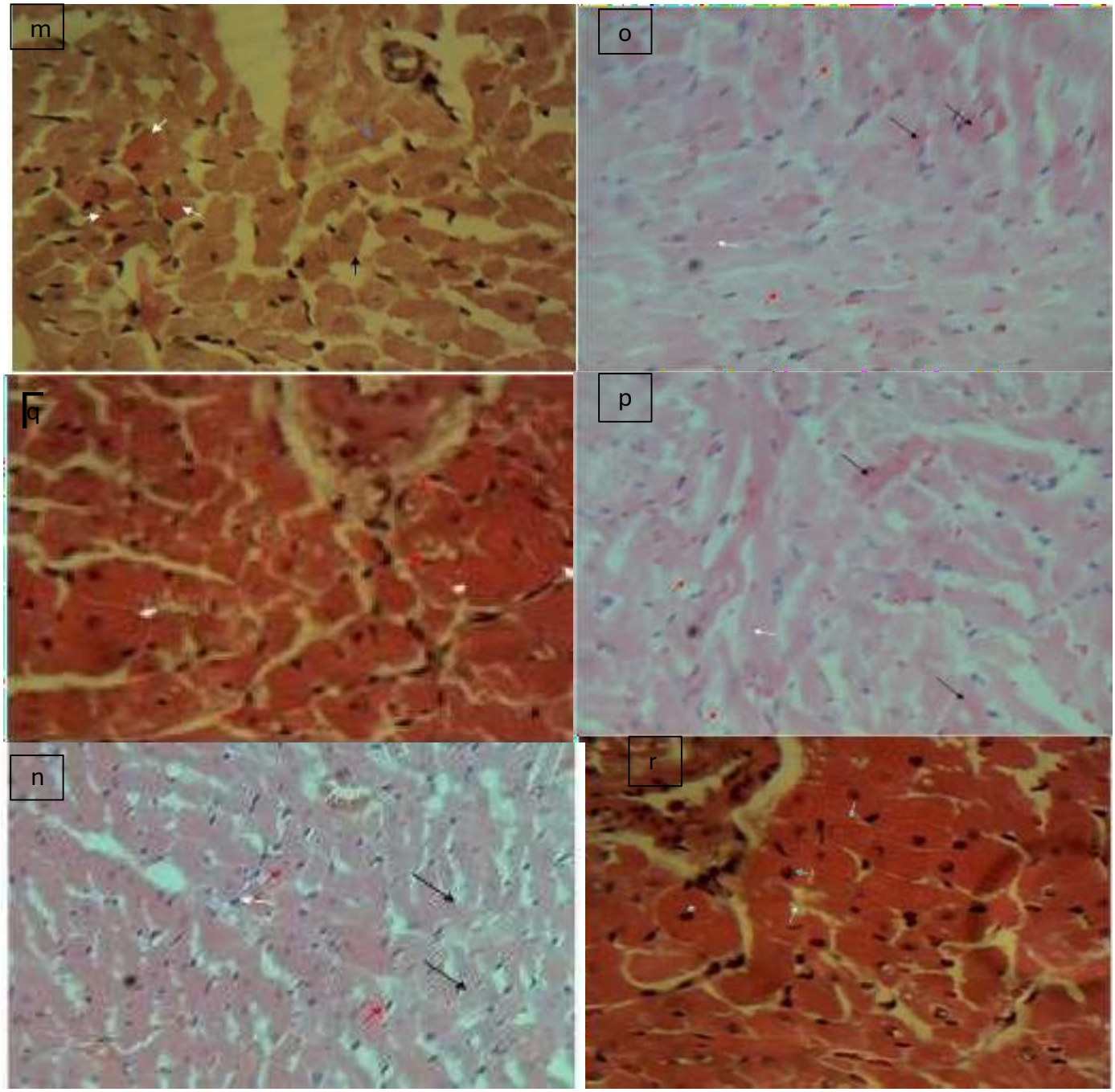

Plate 3: Effect of K2Cr2O7 and Doxorubicin in phase 3 on histopathological alteration in the hearts of Male Wistar Rats (H & E X400 representative photomicrographs). (m) Control rats were fed with a standard pellet diet and received an equal volume of distilled water, (n) 0.01% of K2Cr2O7 [Cr(VI)] compound in feed, i.e. 10mg/kg of K2Cr2O7 [Cr(VI)] compound per Kg Bodyweight in 100g of Rat feed for 60 days, (o) 0.02% of K2Cr2O7 [Cr(VI)] compound in feed, i.e. 20mg/kg of K2Cr2O7 [Cr(VI)] compound per Kg Bodyweight in 100g of Rat feed for 60 days, (p) 0.03% of K2Cr2O7 [Cr(VI)] compound in feed, i.e. 30mg/kg of K2Cr2O7 [Cr(VI)] compound per Kg Bodyweight in 100g of Rat feed for 60 days, (q) 15 mg/kg body weight Dox (2 days before sacrifice) administered intraperitoneally, (r) 20 mg/kg body weight doxorubicin (2 days before sacrifice) administered intraperitoneally.

DISCUSSION

It is interesting to note in this study that chromium(VI) (Cr^{6+}) exposure through the administration of potassium dichromate (VI) (K2Cr2O7) as well as doxorubicin (Dox) in all the treatment phases induced oxidative damage to the heart, as evident in the decrease in antioxidant activities (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx)) and increase in malondialdehyde (MDA) levels. These treatments when compared to the control groups elevated the levels of cardiac troponin I (CTnI), C-reactive protein (CRP) and nitric oxide (NO) in all phases except the first phase that witnessed a decrease in the level of NO.

Thus, this study demonstrated that Cr (VI) compared well with Dox in cardiotoxicity induction with accompanying oxidative stress, inflammatory and histo-hepatic responses in the rats. The dose and duration of treatments for the biochemical and myocardial indices are not dependent on body weight alone, hence may need pharmacokinetic modifications for conversion between animals and humans. Also, the mechanism that resulted in pulmonary oedema regarding the myocardial indices observed in this study is unclear warranting further studies for clarification and elucidation.

Many research data indicate that environmental exposure to toxic metals is implicated in the severe pathogenesis of chronic diseases[3,7]. In this present study, there was no mortality during the treatment phases. The percentage decrease in antioxidant enzymes observed in rats in the second phase of treatment by oral gavage administration was significantly higher than that of the third phase by diet supplementation. Our findings agree with Foster et al[31], whose results propose that bolus gavage administration of manganese could be associated with higher brain manganese levels and, consequently, a higher risk of neurotoxicity. Researchers have also elucidated the elevation of cardiac markers such as AST, LDH, CK–MB used as indicators of myocardial tissue damage. These includes ischemia and infarction[14,32,33] . This present study indicated the cardiotoxicity of K2Cr2O7 and Dox exposure as evidenced in the increased LDH, AST, and CK-MB activities in the phase 1 compared to the control rats. The increased levels observed may be associated with severe Cardiotoxic symptoms such as chest pain, hypertension, decreased heart function**,** myocarditis, cardiomyopathy, shortness of breath, arrhythmias, palpitations, vascular disease, acute coronary syndrome, oedema, and vasculitis[4,34,35]. Similar results were also observed in our findings in both the second treatment phase and the third treatment phase. Furthermore, the increase in NO levels in the DOx treated groups as seen in this study, especially in the second and third phases, indicates an upregulation of nitric oxide synthase activities as observed by other researchers leading to an increase in the concentration of nitric oxide, thereby enhancing the production of the highly toxic peroxynitrite[36]. K2Cr2O7 intoxication may have been responsible for the rats' observed relative heart weight reduction. This finding agrees with[37], where severe toxicity to brush border enzymes was observed by K2Cr2O7 intoxication. Likewise, Wuri[38] have also buttressed K2Cr2O7 toxicity in kidneys and oocytes of exposed rats suggesting that oxidative damage may be responsible for the reduced relative organ weight. Also, the trend of oxidant production was observed to be on the increase as the concentrations of both K2Cr2O7 and doxorubicin treatments increased, resulting in a decrease in the antioxidant enzyme activities (GPx, CAT, and SOD) due to the overwhelming burden of the toxic agents[39,40]. The percentage decrease in the enzyme activities was higher than the decrease observed in the first treatment phase. This may result from the longer duration of the animal exposure to K2Cr2O7, compromising the antioxidant defense system and resulting in cardiodamages[40,41]. The percentage increase in CTnI progressed with increased duration of the K2Cr2O7 exposure, i.e., 21 days and 60 days, respectively, compared to the control rats. The Dox groups similarly showed an increase in CTnI, indicating that both K2Cr2O7 and Dox may be implicated in a dose and duration-dependent cardiotoxicity. Cardiac troponin I changes in concentration levels are useful in diagnosing heart failure and other related cardiac diseases[42].

Clinical evidence has shown that patients who are often readmitted due to heart failure are those with persistent CTnI increases[42]. However, the percentage increases in CRP levels, were significantly elevated in the second and third phases, possibly due to the longer treatment duration compared to the first phase. CRP are pro-inflammatory markers produced by the liver and used clinically to diagnose heart failure. The discrepancy

observed in NO levels in all treatment phases compared to the normal Control may be due to an overexpression of iNOS, whose induction occurs primarily in conjunction with infection and inflammation as part of the defense response.

The weak antioxidant defenses in the heart make it a porous ROS target compared to other organs[39-41]. Hence, SOD, CAT, and GPx activities are impaired, resulting in cellular lipids, proteins, and DNA damage and the onset of many chronic diseases. Therefore, this present study suggests that the higher cardiotoxicity effect observed in the oral gavage- K2Cr2O7-treated rats compared to the supplemented diet- K2Cr2O7 treated rats may be responsible for the higher decreased antioxidant activities. These observations have raised concerns regarding the fitness of oral treatment administration using gavage against diet supplementation due to the endocrine disruptor effects observed by the gavage method[38,43]. Intestinal hypoperfusion may have occurred due to the translocation of orally-administered K2Cr2O7 from the stomach to the bloodstream as evidenced from previous researches, resulting in vascular inflammation and subsequent increased levels of CRP observed in this study[42-44] . Inducible nitric oxide synthase (iNOS) expression is minimal under normal conditions; however, it is upregulated in response to pro-inflammatory cytokines and thus generates much more NO than eNOS does. Hence, High NO levels may have occurred in response to inflammation due to K2Cr2O7 exposure for 60 days, while the decrease in NO levels in the groups treated with K2Cr2O7 and Dox may have resulted from the inhibition of eNOS expression due to treatment exposure for 21 days. This signals cardiotoxicity, owing to the development of systemic and pulmonary hypertension, as seen in the histopathology photomicrographs presented in plates 1, 2 and 3 for the 3 different phases of this research, this further buttress the effect chromium(VI) play in the pathophysiology of heart-related ailments[36,45]. Thus, continuous exposure to this toxic metal may be detrimental to immune, cardiovascular and other essential organs of the human body due to its bioaccumulation.

CONCLUSION

This study revealed that exposure to chromium(VI) (Cr(VI)) via potassium dichromate ($K_2Cr_2O_7$) and doxorubicin (Dox) caused significant oxidative stress, cardiotoxicity, inflammatory responses, and histological damage in rats. These effects were dose, vehicle, and duration-dependent, with Cr(VI) showing comparable cardiotoxicity to Dox. The observed reduction in antioxidant enzyme activities (SOD, CAT, GPx) and the increase in biomarkers such as cardiac troponin I, CRP, and NO underscore the detrimental impact on heart function. Oral gavage administration of Cr(VI) resulted in greater oxidative damage and cardiotoxicity compared to dietary supplementation, indicating that the route of exposure influences severity. To mitigate these harmful effects, it is crucial to enhance environmental and occupational safety by implementing stringent monitoring and regulation of Cr(VI) emissions and ensuring protective measures for workers. Additionally, reducing exposure to contaminants and including antioxidant-rich foods in the diet can help combat oxidative stress. Further research is needed to investigate the

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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AUTHORS' CONTRIBUTIONS

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REFERENCES

1. García-Niño WR, Zatarain-Barrón ZL, Hernández-Pando R, Vega-García CC, Tapia E, Pedraza- Chaverri J. Oxidative stress markers and histological analysis in diverse organs from rats treated with a hepatotoxic dose of Cr(VI): Effect of Curcumin. Biol Trace Elem Res. 2015 Sep 27; 167(1):130–145.
2. Onyedikachi UB, Belonwu DC, Wegwu MO. Human health risk assessment of heavy metals in soils and commonly consumed food crops from quarry sites located at Isiagwu, Ebonyi State. Ovidius Univ Ann Chem. 2018; 29(1):8–24.
3. Onyedikachi UB, Belonwu CD, Wegwu MO. Health risk assessment of chromium, manganese and arsenic through the consumption of food from industrial areas in South Eastern States of Nigeria. Annu Res Rev Biol. 2019; 31(6):1–20.
4. Hashem MM, Abo-EL-Sooud K, Abd-Elhakim YM, Badr YA hamid, El-Metwally AE, Bahy-ElDien A. The long-term oral exposure to titanium dioxide impaired immune functions and triggered cytotoxic and genotoxic impacts in rats. J Trace Elem Med Biol. 2020; 60(7):126473.
5. Awasthi Y, Ratn A, Prasad R, Kumar M, Trivedi SP. An *invivo* analysis of Cr^{6+} inducedbiochemical, genotoxicological and transcriptional profiling of genes related to oxidative stress, DNA damage and apoptosis in liver of fish, *Channa punctatus* (Bloch, 1793). Aquat Toxicol. 2018; 200:158–167.
6. Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology. 2002; 180(1):5-22.
7. Onyedikachi UB, Achi NK, Oriaku CE, Onyeabo C. Health risk assessment of heavy metal contamination of some indigenous vegetables grown in oil spilled agricultural area in Ogoniland, Rivers state, Nigeria. Niger Agric J. 2021; 52(1):138-151.
8. Das PK, Das BP, Dash P. Chromite mining pollution, environmental impact, toxicity and phytoremediation: A Review. Environ Chem Lett. 2021; 19(5):1369-1381.
9. Homa D, Haile E, Washe AP. Determination of spatial chromium contamination of the environment around industrial zones. Int J Anal Chem. 2016; 2016(1):7214932.
10. Pokhrel GR, Pokhre G. Effect of chromium on Human-health: A Review. BMC J Sci Res. 2022; 5(1):27–35.
11. Wu BB, Leung KT, Poon EN. Mitochodrial-targetd therapy for doxorubicin-induced cardiotoxicity. Int J Mol Sci. 2022; 23(3):1912-1931.
12. Hardaway BW. Adriamycin-associated cardiomyopathy: Where are we now updates in pathophysiology, dose recommendations, prognosis, and outcomes. Curr Opin Cardiol. 2019; 34(3):289-295.
13. Morelli MB, Bongiovanni C, Da Pra S, Miano C, Sacchi F, Lauriola M, et al. Cardiotoxicity of anticancer drugs: Molecular mechanisms and strategies for cardioprotection. Front Cardiovasc Med. 2022; 9:847012.
14. Fernandez-Chas M, Curtis MJ, Niederer SA. Mechanism of doxorubicin cardiotoxicity evaluated by integrating multiple molecular effects into a biophysical model. Br J Pharmacol. 2018; 175(5):763–781.
15. Occupational Safety and Health Administration (OSHA), Departmnt of Labour. Occupational exposure to hexavalent chromium. Fed Regist. 2006; 71(39):10099-10385.
16. Nur-E-Alam M, Mia MAS, Ahmad F, Rahman MM. An overview of chromium removal techniques from tannery effluent. Appl Water Sci. 2020; 10(8):205-217.
17. National Research Council (NRC). Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (U.S.). Guide for the care and use of laboratory animals. Washington, DC: National Academies Press; 2011.
18. Navya K, Phani Kumar G, Chandrasekhar Y, Kr A. Evaluation of potassium dichromate (K2Cr2O7)-induced liver oxidative stress and ameliorative effect of *Picrorhiza kurroa* extract in Wistar albino rats. Biol Trace Elem Res. 2018; 184(1):154–164.
19. Ounassa A. Effects of potassium dichromate on haematological parameters in female and male Wistar albino rats. Ass Univ Bull Environ Res. 2009; 12(2):87-98.

20. Sandamali JAN, Hewawasam RP, Jayatilaka KAPW, Mudduwa LKB. Dose dependent cardiac effects of doxorubicin in Wistar rats: A biochemical and histopathological analysis. Int J Pharm Sci Res. 2019; 10(6):2700-2710.
21. Karabulut D, Ozturk E, Kaymak E, Akin AT, Yakan B. Thymoquinone attenuates doxorubicin cardiotoxicity in rats. J Biochem Mol Toxicol. 2021; 35(1):e22818.
22. Arthur JR, Boyne R. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 1985 Apr 22; 36(16):1569-1575.
23. Sinha AK. Calorimetric assay of catalase. Anal Biochem. 1972; 47(2):389-394.
24. Wallin B, Rosengren B, Shertzer H, Camejo G. Lipoprotein oxidation and measurement of TBARSformation in single microtiter plate: its use for evaluation of antioxidants. Anal Biochem. 1993; 208(1):10–15.
25. Jung K, Nickel E, Pergande M. A microalbuminuria assay using bromphenol blue. Clinica Chimica Acta. 1990; 187(2):163-172.
26. Foreback CC, Chu JW. Creatinine kinase isoenzymes: Electrophoretic and quantitative measurements. Crit Rev Clin Lab Sci. 1981; 15(3):187-230.
27. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28(1):56-63.
28. Andrabi SM, Sharma NS, Karan A, Shahriar SMS, Cordon B, Ma B, et al. Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications. Adv Sci. 2023;10(30):e2303259.
29. Melanson SEF, Tanasijevic MJ, Jarolim P. Cardiac troponin assays: A view from the clinicalchemistry laboratory. Circulation. 2007; 116(18):e501-504.
30. Anderson HC, Mccarty M. Determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever. Am J Med. 1950; 8(4):445-455.
31. Foster ML, Bartnikas TB, Johnson LC, Herrera C, Pettiglio MA, Keene AM, et al. Pharmacokinetic evaluation of the equivalency of gavage, dietary, and drinking water exposure to manganese in F344 rats. Toxicol Sci. 2015; 145(2):244-251
32. Soudani N, Troudi A, Bouaziz H, Ben Amara I, Boudawara T, Zeghal N. Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. Ecotoxicol Environ Saf. 2011; 74(3):513–520.
33. Lakhani HV, Pillai SS, Zehra M, Dao B, Tirona MT, Thompson E, et al. Detecting early onset of anthracyclines-induced cardiotoxicity using a novel panel of biomarkers in West-Virginian population with breast cancer. Sci Rep. 2021; 11(1):7954.
34. Boriskin P, Gulenko O, Devyatkin A, Karimova R, Leonov V, Pavlova O. Correlation of the distribution of antioxidant enzyme concentrations in blood serum and heart tissue in rats. BIO WebConf. 2020; 17(2020):00234.
35. Nandi A, Yan LJ, Jana CK, Das N. Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxid Med Cell Longev. 2019; 2019:9613090.
36. Sharifi-Rad M, Anil-Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020; 11:694-708.
37. Fatima S, Arivarasu NA, Banday AA, Yusufi AN, Mahmood R. Effect of potassium dichromate on renal brush border membrane enzymes and phosphate transport in rats. Hum Exp Toxicol. 2005;24(12):631-638.
38. Wuri L, Arosh JA, Wu JZ, Banu SK. Exposure to hexavalent chromium causes infertility by disrupting cytoskeletal machinery and mitochondrial function of the metaphase II oocytes in superovulated rats. Toxicol Rep. 2022; 9(5):219–229.
39. Gandhi H, Patel VB, Mistry N, Patni N, Nandania J, Balaraman R. Doxorubicin mediated cardiotoxicity in rats: Protective role of felodipine on cardiac indices. Environ Toxicol Pharmacol. 2013; 36(3):787–795.
40. Yang F, Teves SS, Kemp CJ, Henikoff S. Doxorubicin, DNA torsion, and chromatin dynamics. Biochim Biophys Acta. 2014; 1845(1):84-89.
41. Ganatra S, Parikh R, Neilan TG. Cardiotoxicity of

Immune Therapy. Cardiol Clin. 2019; 37(4):385-397.
42. Magnussen C, Blankenberg S. Biomarkers for heart failure: small molecules with high clinical relevance. J Intern Med. 2018; 283(6):530-543.
43. Anand IS, Latini R, Florea VG, Kuskowski MA, Rector T, Masson S, et al. C-reactive protein in heart failure: Prognostic value and the effect of Valsartan. Circulation. 2005; 112(10):1428–1434.
44. Vulesevic B, Lavoie SS, Neagoe PE, Dumas E, Räkel A, White M, et al. CRP Induces NETosis in heart failure patients with or without diabetes. Immunohorizons. 2019; 3(8):378–388.
45. Wu Y, Ding Y, Ramprasath T, Zou MH. Oxidative stress, GTPCH1, and endothelial nitric oxide synthase uncoupling in hypertension. Antioxid Redox Signal. 2021; 34(4):750-764.