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Complete List of Authors:	Akinrinde, Akinleye; University of Ibadan, Department of Veterinary Physiology, Biochemistry and Pharmacology; University of Fort Hare, Department of Biochemistry and Microbiology Omobowale, Olutayo; University of Ibadan, Department of Veterinary Medicine Oyagbemi, Ademola; University of Ibadan, Department of Veterinary Physiology, Biochemistry and Pharmacology Asenuga, Ebunoluwa; University of Benin, Department of Veterinary Biochemistry Ajibade, Temitayo; University of Ibadan, Department of Veterinary Physiology, Biochemistry and Pharmacology
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Protective effects of Kolaviron and Gallic acid against cobalt chloride induced cardio-renal dysfunction via suppression of oxidative stress and activation of ERK signaling pathway

Akinrinde Akinleye Stephen^{1*}, Omobowale Olutayo², Oyagbemi Ademola¹, Asenuga Ebunoluwa³ and Ajibade Temitayo¹

¹ Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

² Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

³ Department of Veterinary Biochemistry, Faculty of Veterinary Medicine, University of Benin, Nigeria

***Correspondence:**

Dr. Akinrinde Akinleye Stephen, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria.

E-mail: 201514802@ufh.ac.za; leyesteve2000@yahoo.com

Abstract

Cobalt (Co) toxicity is a potential public health problem due to recent renewed use of Co in orthopedic implants, dietary supplements and blood doping in athletes and horses. We investigated the protective roles of Kolaviron, KV (a bi-flavonoid of *Garcinia kola*) and Gallic acid (GA) on cobalt chloride (CoCl_2) - induced cardio-renal damage in rats. CoCl_2 caused significant increases ($p < 0.05$) in serum creatine kinase-myocardial band (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST), xanthine oxidase (XO), urea, creatinine, malondialdehyde, H_2O_2 , nitric oxide, as well as C-reactive protein expression, along with significant ($p < 0.05$) reduction in cardiac and renal expression of extracellular signal regulated kinase (ERK) and the activities of superoxide dismutase, catalase and glutathione S-transferase. KV and GA prevented the toxic effects of CoCl_2 by stimulating ERK expression and reversing Co-induced biochemical changes. Administration of CoCl_2 alone did not significantly alter ECG patterns in the rats, although co-treatment with KV (200 mg/kg) produced QT-segment prolongation and also appeared to potentiate Co-hypotension. Histopathology of the heart and kidneys of rats treated with KV and GA confirmed the biochemical data. Kolaviron and Gallic acid thus protected against cardiac and renal damage in cobalt-intoxication via antioxidant and/or cell survival mechanisms, possibly involving ERK activation.

Keywords: Cobalt, Heart, kidneys, Kolaviron, Gallic acid, oxidative stress, ERK, ECG.

1.0 Introduction

Cobalt has been used historically in the treatment of pernicious anemia, due to its ability to stimulate the production of red blood cells and hemoglobin (Barceloux 1999). It was also employed in the 1960s as a foam stabilizer in beers (Alexander 1972). The use of this metal in these situations had been accompanied by toxic effects namely thyroid dysfunction in children and cardiomyopathies in some heavy beer drinkers (Paustenbach et al. 2013). The introduction of more efficacious drugs in the treatment of anemia and the occurrence of adverse effects led to the termination of cobalt use in these situations decades ago.

Recent concerns over cobalt toxicity have emerged from its potential use as a blood doping agent in humans and animals (Jelkmann and Lundby 2011; Lippi et al. 2006; Mobasheri and Proudman 2015), as well as its use in surgical patients with Co-containing hip implants (Hasegawa et al. 2012; Macnair et al. 2012). Furthermore, intense mining activities in certain areas have led to environmental contamination with potential widespread exposures to cobalt (Banza et al. 2009; Cheyins et al. 2014). The consumption of cobalt-containing dietary supplements is also believed to be on the rise in different parts of the world (Tvermoes et al. 2013).

Formation of reactive oxygen species, leading to oxidative stress and lipid peroxidation has been shown to be one of the major pathogenetic mechanisms for cobalt-induced tissue injuries (Ahmed and Siddiqui 2007; Catelas et al. 2005; Franco et al. 2009; Kubrak et al. 2011). Oxidative processes result in post-translational modifications of a variety of proteins including kinases (e.g. extracellular signal-regulated kinase, ERK; p38); proteases (e.g. caspases) and transcription factors (e.g. NF- κ B, AP-1, p53, Nrf 2) (Franco et al. 2009; Son et al. 2011). These proteins regulate diverse biochemical and cellular functions, ranging from cell survival to cell death (Haagenson and Wu 2010).

Antioxidants may act directly by scavenging reactive oxygen and nitrogen species (ROS/RNS) via direct consumption or chemical modification. Alternatively, antioxidants may act indirectly by controlling gene expression during oxidative stress by up-regulating phase II detoxifying and antioxidant enzymes (Kim and Jang 2014). Stimulation of these enzymes usually occurs by the activation of nuclear transcription factor-erythroid 2-related factor 2 (Nrf-2) via its release from Kelch-like ECH-associated protein 1 (Keap 1)-Nrf-2 complex (Park et al., 2004). In addition, Keap 1-independent activation of Nrf-2 can occur by its phosphorylation by several signal pathways involving ERK, phosphatidyl inositol 3-kinase (PI3K/Akt) and protein kinase C (PKC) (Baird and Dinkova-Kostova 2011; Itoh et al. 2004)

Flavonoids have found valuable therapeutic and prophylactic applications due to their excellent free radical scavenging activities (Havsteen 2002) and their ability to induce the expression of detoxifying and antioxidant enzymes (Hormann et al. 2011; Mann et al. 2009). Kolaviron (KV) is a de-fatted extract from bitter kola (*Garcinia kola*) seeds, widely reported to contain *Garcinia* biflavonoid 1 (GB1), *Garcinia* biflavonoid 2 (GB2) and kolaflavanone (Figure 1a). Its antioxidant and anti-inflammatory actions have been employed to protect against many pathological conditions (Adedara and Farombi 2012; Olaleye et al. 2000). Its cardio protective (Adaramoye and Lawal 2015) and nephro-protective (Adedara et al. 2015) actions have been reported. Gallic acid (Figure 1b) is an endogenous plant phenol that is found in many fruits, teas and wine (Ma et al. 2003; Singh et al. 2004). It has been widely reported for its strong antioxidant (Kroes et al. 1992), anti-inflammatory (Kim et al. 2002) and cardio-protective properties (Priscilla and Prince 2009).

In the present study, we attempted to evaluate the cardio- and nephro-protective effects of Kolaviron and Gallic acid on cardiac and renal damage induced by cobalt chloride administration

in rats. We attempted to demonstrate the possible mechanisms of the therapeutic efficacies of these compounds by studying the biochemical markers of injury to cardiac and renal tissues, antioxidant defense system, electrocardiographic and histopathological changes, as well as immunohistochemical staining patterns of a key signaling protein, ERK.

2.0. Materials and methods

2.1. Chemicals

Cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) was obtained from Tianjin Kermel[®] Chemical Reagent Co, China. Gallic acid, Glutathione, 1, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide, xylene orange, potassium hydroxide and hydrogen peroxide were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

2.2. Extraction of Kolaviron

Seeds of *Garcinia kola* were obtained from a local vendor in Ibadan, Nigeria. They were identified at the Department of Botany herbarium, University of Ibadan, Nigeria. The seeds were peeled, sliced, and dried at room temperature (25-28°C). Kolaviron was extracted according to the method of Iwu et al. (1985).

2.3. Animal treatment and Experimental design

Male Wistar rats aged between 10 and 12 weeks (180-200g) were obtained from the Experimental animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. They were housed in plastic cages placed in a well-ventilated animal house and were given *ad libitum* access to rat chow (Ladokun Feeds Ltd, Ibadan, Nigeria) and subjected to natural photoperiod of

12-h light : dark cycle. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments (Public Health Service, PHS 1996).

The rats were divided randomly into five groups of 8 rats each. Group A received clean tap water only, while Group B were treated with cobalt chloride (CoCl_2 , 350 ppm) in drinking water for 14 days. Group C and D rats were orally treated by gavage with 100 and 200 mg/kg Kolaviron (KV1 and KV2), respectively, along with CoCl_2 treatment in drinking water. Group E rats were treated by oral gavage with 120 mg/kg Gallic acid and also with CoCl_2 in the same manner as groups C and D. The doses of cobalt chloride, Kolaviron and Gallic acid have been carefully chosen based on previous studies (Farombi et al. 2012; Garoui et al. 2011, 2012; Oyagbemi et al. 2015).

2.4. Electrocardiography

Standard lead II electrocardiogram was recorded in rats immobilized with xylazine/ketamine combination using a 6/7-lead ECG machine (EDAN VE-1010, Shanghai, China). The machine was calibrated at 20 mm/mV paper speed and 50mm/s paper speed. From the electrocardiogram, parameters such as heart rate, PR interval, QRS wave duration, R-wave amplitude and QT/QTc values were determined.

2.5. Blood pressure measurements

After all the treatments, indirect blood pressure parameters, including systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures were determined without anesthesia, by tail plethysmography using an electrosphygmomanometer (CODA, Kent Scientific, USA). The average of at least nine readings, taken in the quiescent state, following acclimatization, was recorded per animal.

2.6. Animal necropsy and tissue preparation

Blood was drawn from the retro-orbital plexus into plain vials prior to sacrifice. All rats were sacrificed by cervical dislocation, twenty four hours after the last treatment. The heart and kidneys were quickly removed, rinsed in 1.15% KCl and homogenized in potassium phosphate buffer (0.1 M, pH 7.4), and homogenates were centrifuged at 10,000 g for 20 minutes to obtain the post-mitochondrial supernatant which was maintained at -4°C used for the biochemical assays. The collected blood was allowed to clot and the samples were centrifuged at 3,000 g for 10 minutes. Serum was collected as the supernatant and was also used for some biochemical assays.

2.7. Determination of cardiac and renal oxidative stress and antioxidant markers

Protein concentration was determined using the Biuret method according to the method of Gornal et al. (1949). Hydrogen peroxide (H_2O_2) generation was determined spectrophotometrically at 560nm as described by Wolff (1994). Lipid peroxidation was evaluated by estimating malodialdehyde (MDA) using the method of Varshney and Kale (1990). MDA content was quantified with a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ and expressed as micromole per gram tissue. Superoxide dismutase (SOD) activity was determined

by measuring the inhibition of the auto-oxidation of epinephrine at pH 7.2 at 30°C as described by Misra and Fridovich (1972) with slight modifications from our laboratory Oyagbemi et al. (2015). Briefly, 100 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. 0.01 ml of each sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) followed by the addition of 0.3 ml of 0.3 mM epinephrine. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. 1 unit of SOD activity represents the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute. The concentration of GSH was determined at 412 nm using the method described by Jollow et al. (1974). Glutathione-S-transferase (GST) activity was estimated by the method of Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Catalase (CAT) activity was determined according to the method of Sinha (1972). Nitric oxide (NO) content (NO) was measured as described by Olaleye et al. (2007) by indirectly measuring the nitrite concentration. The activity of xanthine oxidase in serum was determined according to method of (Akaike et al. 1990).

2.8. Evaluation of markers of cardiac and renal injury

Activities of creatine kinase myocardial band (CK-MB), lactate dehydrogenase (LDH) and aspartate transaminase (AST) and the concentrations of urea and creatinine in serum were assayed in in serum using commercial kits purchased from Randox Laboratories Ltd (Crumlin, UK).

2.9. Immunohistochemistry of ERK and CRP

Immunohistochemistry of paraffin embedded heart and kidney tissues was performed after the tissues were fixed with 10% formalin based on the methods described by Todorich et al. (2011)

with slight modifications. Briefly, paraffin sections were melted at 60°C in the oven. Dewaxing of the samples in xylene was followed by passage through ethanol of decreasing concentration (100-80%). Peroxidase quenching with 1% H₂O₂/methanol was followed by antigen retrieval performed by microwave heating in 0.01 M citrate buffer (pH 6.0) to boil. All the sections were blocked in normal goat serum (10%, HistoMark[®], KPL, Gaithersburg MD, USA) and probed with anti-ERK and anti-CRP antibodies, as appropriate (Bioss, San Diego, CA, USA), 1:200 overnight at room temperature. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0µg/ml) secondary antibody and subsequently, streptavidin peroxidase (Horse Radish Peroxidase- streptavidin) according to manufacturer's protocol (HistoMark[®], KPL, Gaithersburg MD, USA).

The reaction product was enhanced with diaminobenzidine (DAB, Amresco[®], USA) for 2-3 minutes and counterstained with high definition hematoxylin (Enzo[®], NY - USA), with subsequent dehydration in ethanol. The slides were covered with coverslips and sealed with resinous solution. The immune-reactive positive expression of ERK- and CRP- intensive regions were viewed starting from low magnification on each slide then with 400 × magnifications using a photo microscope (Olympus) and a digital camera (Toupcam[®], ToupTek Photonics, Zhejiang, China).

2.10. Histopathology

Small pieces of cardiac and renal tissues were collected in 10% buffered formalin (pH 7.4) for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 µm in thickness were made and stained with haematoxylin and eosin for histopathological examination (Drury et al. 1976).

2.11. Statistical analyses

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups with Least Significant Difference (LSD) post-hoc analysis, followed by the Student's *t*-test using GraphPad Prism software (version 6.01). *P* values <0.05 were considered statistically significant.

3.0. Results

3.1. Effects of CoCl₂ exposure and treatments with Kolaviron and Gallic acid on blood pressure parameters

Cobalt chloride caused significant reduction ($p<0.05$) in systolic blood pressures in the rats, compared to control. The reductions observed in diastolic and mean arterial pressures were, however, not statistically significant. Rats treated CoCl₂ + Kolaviron exhibited further dose-dependent reduction in systolic, diastolic and mean arterial pressures when compared with those treated with CoCl₂ alone. There was also blood pressure reductions in the group exposed to CoCl₂ + Gallic acid, although these were not as much as that caused by the higher dose of Kolaviron (Table 1).

3.2. ECG waves and intervals

Electrocardiographic measurements for control and experimental rats are presented in Table 2. No statistically significant differences were observed in the heart rate, P wave, PR interval, QRS duration, QT, QT corrected (Bazett) and R amplitude between rats treated with CoCl₂ alone and the control rats. Similarly, rats co-treated with KV and GA did not show any significant alterations in most of the ECG parameters including heart rate, P wave, PR interval, QRS

duration and R amplitude. The only noticeable significant ($p<0.05$) alteration was displayed by rats co-treated with CoCl_2 and KV (200 mg/kg), which displayed prolonged QT and QTc intervals, compared to control as well as the rats given CoCl_2 alone.

3.3. Effect of KV and GA on markers of cardiac and renal injury

In CoCl_2 -exposed rats, we observed significant increases ($p<0.05$) in serum levels of all the markers of myocardial injury measured in this study: CK-MB, Lactate dehydrogenase (LDH), aspartate transaminase (AST) and xanthine oxidase (XO) (Figure 2). Kolaviron exhibited dose-dependent reductions ($p<0.05$) in all these parameters, when compared with the CoCl_2 group, while Gallic acid also showed marked reduction in the activities of these enzymes. There were also significant increases ($p<0.05$) in the markers of renal injury, urea and creatinine in the CoCl_2 group compared to the control (Figure 3). The increased levels of creatinine was significantly reversed ($p<0.05$) by both KV and GA, while significant ($p<0.05$) amelioration of serum urea concentration was only obtained with KV at 200 mg/kg.

3.4. Effect of KV and GA on markers of oxidative stress and antioxidant defense system

Exposure to CoCl_2 caused the induction of oxidative stress in cardiac tissues, indicated by significant increases in hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and nitric oxide (NO) contents, compared to control (Table 3). Kidney tissues also showed significant elevation in MDA level. KV and GA exhibited significant antioxidant activity towards H_2O_2 in both the heart and kidneys, causing significant reduction ($p<0.05$) in this reactive oxygen species, compared to the CoCl_2 group. KV produced significant reduction ($p<0.05$) of MDA levels in the kidneys, as well as significant reduction ($p<0.05$) of NO in the heart.

From Table 4, no significant alterations were observed in the concentration of reduced glutathione (GSH) in both the heart and kidneys of rats exposed to CoCl_2 , with or without KV or

GA. However, we observed significant reduction ($p<0.05$) in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) in the heart of CoCl_2 -treated rats, while the kidney also showed significant reductions ($p<0.05$) in CAT and GST. KV demonstrated significant ($p<0.05$) dose-dependent restoration of SOD, CAT and GST activities in the heart and kidneys, when compared to the CoCl_2 group, while GA only exhibited significant elevation of SOD activity.

3.5. Effect of KV and GA on myocardial and renal histoarchitecture

Histopathological examination of the cardiac and renal tissues confirmed the biochemical findings of cardiac and renal damage due to cobalt intoxication in the rats (Figures 5 and 6). Hearts from rats that received cobalt chloride alone showed disseminated haemorrhagic lesions with congestion of coronary blood vessels and mild infiltration of the myocardium and atrium by inflammatory cells. Kidney damage was indicated histologically by severe loss of normal morphology, loss of tubular and glomerular outlines with marked peri-tubular inflammatory cell infiltration and vascular congestion. However, treatment with KV, especially at the higher dose, and GA, prevented much of the damage as the histological appearance of the tissues tended towards that in the control rats.

3.6. Expression of ERK and CRP in cardiac and renal tissues

Immunohistochemically, the cardiac tissues in the control group displayed faint ERK staining [Figure 4(a)], whereas the kidneys exhibited a much greater staining intensity [Figure 4(b)]. Exposure to CoCl_2 alone exhibited very weak ERK immunoreactivity in both the heart and kidneys. By contrast, rats treated with either KV or GA, showed a strong staining intensity for ERK in both the heart and kidneys. This effect appeared to be much stronger with KV at the higher dose.

CRP immunoreactivity was monitored only in the heart tissues and this study [Figure 4(c)]. Rats in the CoCl_2 group exhibited a strong staining intensity for CRP, compared to all other groups. The higher dose of KV, as well as GA, both demonstrated much lower immunoreactivity for CRP, while control rats only had faint expression of this protein.

4.0. Discussion

The chemopreventive property of phenolic antioxidants has become very attractive in their use for protection against toxic and neoplastic effects of many xenobiotics, including heavy metals. Their ability to induce several detoxifying and antioxidant enzymes explains, in large part, the mechanisms underlying their protective actions. In this study, two natural phenolics, Kolaviron and Gallic acid have been investigated for their protective activities against cobalt toxicity in cardiac and renal tissues of Wistar rats. Cobalt, as a metal, is currently growing in importance due to recent modern applications in medical, industrial and domestic settings.

Cobalt-induced cardiac damage in the present study was indicated by significant elevation in serum CK-MB, AST and LDH activities in the group of rats treated with CoCl_2 alone. Serum CK-MB, AST and LADH are well known diagnostic markers of myocardial damage. Damage to cardiomyocytes by different toxicants causes an increase in membrane permeability or rupture of the cells themselves. As a result, these enzymes, normally found intracellularly, can enter the bloodstream, thereby increasing their activities in serum (Priscilla and Prince 2009). CK-MB is highly specific as a marker of myocardial damage in this regard (Patel et al. 2010). Treatment with KV and GA effectively prevented the elevation in the activities of these enzymes. The protective effect obtained for KV was dose-dependent for all the enzymes.

The kidney is the main site of metabolism and elimination of many metals and other toxicants (Matos et al., 2009). Toxicants which affect the kidneys can interfere with the incorporation of amino acids into proteins can increase the serum concentration of nitrogen-containing products of protein metabolism, including urea and creatinine, due to the failure of their elimination (Alessahin et al. 2005; Cuzzocrea et al. 2002). Our study revealed increased levels of urea and creatinine in the serum of CoCl₂-treated rats, an indication of possible renal damage. Similar results were obtained by Garoui et al. (2012), who utilized the same concentration of cobalt chloride as in the present study. With respect to these renal parameters, KV, at the higher dose, produced better amelioration of cobalt chloride-induced renal damage than GA.

We found evidence for the involvement of ROS as mediators of cardiac and renal damage in CoCl₂-treated rats. There were significant increases in hydrogen peroxide levels in heart and kidneys, as well as increased malondialdehyde and nitric oxide levels, especially in cardiac tissues. This pattern of elevation of these parameters clearly indicates the induction of oxidative stress and lipid peroxidation. Increased lipid peroxidation might be responsible for increased cell membrane permeability that led to the leakage of intracellular enzymes such as CK-MB, LDH and AST into the serum. Further evidence of free radical generation was indicated by the elevated serum activity of xanthine oxidase (XO). XO is an important source of free radicals and an important marker of myocardial damage (Raghuvanshi et al. 2007). Oxidation of sulhydryl residues in xanthine dehydrogenase can easily give rise to xanthine oxidase, both enzymes being inter-convertible forms of what is collectively known as xanthine oxido-reductase (XOR) (Harrison 2002; 2004).

In ischemic/hypoxic conditions, cellular ATP is degraded to hypoxanthine which is converted by xanthine oxidase to oxygen free radicals (Hearse et al. 1986), such as superoxide radicals. XO-

derived superoxide radicals can also react with nitric oxide forming the highly cytotoxic peroxynitrite (Trujillo et al. 1998), which causes tissue injury. In addition, cardiac damage was indicated by increased myocardial expression of C - reactive protein (CRP) in CoCl₂-treated rats. CRP is a prototype inflammatory marker and is used as a predictor of coronary heart disease (Calabro et al. 2012). KV and GA caused reversal of CoCl₂-induced increases in oxidative stress parameters and increased CRP expression in the present study, affirming the profound antioxidant and anti-inflammatory activities of these compounds.

In order to understand the involvement of key signaling pathways in the protection of cobalt-induced injury by KV and/or GA, we investigated the expression of the extracellular signal-regulated kinase (ERK). ERKs are one of four families of proteins called Mitogen-activated protein kinases (MAPKs) which play vital roles in signaling events involved in cell proliferation, differentiation, metabolism, survival and apoptosis. The other proteins in this class are c-Jun N-terminal kinases (JNK), p38 and ERK5 (or Big MAPK) (Rose et al. 2010). While ERKs are known to be stimulated mainly by growth factors (Ramos 2008), JNK and P38 are activated by stress factors such as UV-light, oxidant stress, infection and osmotic shock (Kyriakis and Avruch 2001), and are both collectively called Stress-activated MAPKs (SAPKs). With immunohistochemistry, both the heart and kidneys of CoCl₂-treated rats consistently exhibited lower expressions of ERK. However, KV and GA produced considerably higher expressions of this protein in both the heart and kidneys.

Stimulation of the ERK pathway by various stimuli has been demonstrated by various studies to produce cardioprotection (Hausenloy and Yellon 2007). Similarly, other studies have suggested that a down-regulation of ERK 1/2 is involved in Doxorubicin-induced cardiac damage (Su et al. 2006; Xiang et al. 2009). These studies concluded that administration of substances that increase

ERK 1/2 leads to prevention of Doxorubicin-induced cardiotoxicity. Based on these studies and the findings from our study, it is reasonable to infer that ERK activation has mechanistic implications for the protection offered by KV and/or GA.

The explanation for the mechanistic involvement of ERKs, as observed in the present study, may be linked to their ability to directly phosphorylate Nrf-2, causing the stimulation of the transcription factor. Downstream effectors of Nrf-2 include phase II detoxifying enzymes, such as GSTs, as well as antioxidant enzymes like SOD, CAT and GPx. We found significantly elevated activities of SOD, CAT and GST in the cardiac and renal tissues of rats treated with KV and GA, compared to those treated with CoCl₂ alone. This points to a likely up-regulation of these enzymes by mechanisms possibly related to the activation of ERK, as observed in this study.

The data on blood pressure indicated a reduction in blood pressure indices and especially the systolic blood pressure, in rats treated with cobalt chloride alone. This is in agreement with previous reports that infusion of Co²⁺ (55 mg/kg) lowered blood pressure in Wistar rats by decreasing total peripheral resistance, suggesting that cobalt acted by vasodilator effects on blood vessels (Dugin et al. 1991). With rats co-treated with Kolaviron or Gallic acid, we observed further decreases in all blood pressure indices measured, suggesting that these compounds exerted synergistic effects with cobalt chloride to cause hypotension in the rats. Previous reports have identified lowering effects of *Garcinia kola* extracts on systolic and mean arterial blood pressures (Naiho and Ugwu 2009). Like cobalt, the blood pressure-lowering effect of *Garcinia kola* extracts was attributed to its relaxant effects on vascular smooth muscles. Substances that cause vasorelaxation could act by stimulating cyclic AMP which, by an energy-requiring Ca²⁺-binding mechanism, may produce a decrease in sarcoplasmic Ca²⁺ and hence,

relaxation. Juxtaposing our findings in the present study with those of Naiho and Ugwu (2009), it may be reasonable to suggest that the hypotension-inducing factor in *Garcinia kola* may, indeed be Kolaviron. To support this assertion, previous experiments in rat superior mesenteric arteries have provided functional evidence that Kolaviron produce vaso-relaxant effects by blockade of influx of extracellular Ca^{2+} , inhibition of intracellular Ca^{2+} release and opening of K^+ channels (Adaramoye and Medeiros 2009).

Electrocardiography is considered one of the most important clinical tests for diagnosis of cardiac dysfunction (Patel et al. 2010). ECG assessments in this study showed that rats treated with CoCl_2 alone did not show any statistically significant differences in the patterns of most ECG parameters, when compared to control rats. However, co-treatment of CoCl_2 with KV at 200 mg/kg produced a noticeable alteration in the form of prolonged QT and QTc intervals, compared to control. The QT interval is the time from the start of the QRS complex to the end of the T wave. QT is a measure of the time required for the rapid inflow of Na^+ and Ca^{2+} , resulting in the depolarization of the myocardium and the outflow of K^+ , ultimately resulting in repolarization (Moskovitz et al. 2013). Since the rate of depolarization and repolarization depends on a patient's heart rate, QT is usually corrected to the QTc value. Prolonged QT/QTc interval indicates delayed repolarization of the heart, and usually identifies patients with increased risk of acute cardiovascular complications including ventricular dysrhythmias, seizure-like activities or sudden cardiac death (Witchel et al. 2000). Our finding from the present study would suggest that KV at high doses may predispose to cardiovascular risks and must, therefore, be used with caution.

Histopathological findings of rats treated with Kolaviron and gallic acid showed a near normal morphology of the heart and kidney tissues, compared to those of rats treated with CoCl_2 alone,

which showed disseminated hemorrhagic lesions in the myocardium and inflammatory cell infiltration and vascular congestion in the kidneys.

Overall, our study clearly shows the cardio-protective and nephro-protective effects of Kolaviron and Gallic acid against tissue damage induced by cobalt chloride. Our findings lend further credence to previous reports on the health benefits of these compounds on the heart and kidneys, as have been reported elsewhere (Adaramoye and Lawal 2015; Adedara et al. 2015; Priscilla and Prince 2009). Significant correlation in ERK expression and the activities of antioxidant and detoxifying enzymes in this study provided some mechanistic insight into the protective abilities of these compounds. However, the use of Kolaviron in treating conditions involving oxidative stress must be with caution to avoid synergistic drug-drug interactions that may potentiates its effects on lowering of blood pressure, as well as the risk of cardiovascular complications associated with the use of high doses.

Conflict of interest

The authors declare that they have no conflict of interest

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Legends to Figures

Figure 1: Structures of a) Kolaviron and b) Gallic acid

Figure 2: Effect of cobalt chloride with or without Kolaviron or Gallic acid on cardiac marker enzymes

Values are presented as mean±standard deviation ($n=10$).

^a represents significant difference at $p < 0.05$, when compared with control; ^b represents significant difference at $p < 0.05$, when compared with CoCl₂ group

Figure 3: Effect of cobalt chloride with or without Kolaviron or Gallic acid on serum urea and creatinine

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Figure 4: Immunohistochemical staining patterns of a.) ERK in cardiac tissues; b.) ERK in kidney tissues, and c.) CRP in cardiac tissues, of rats exposed to cobalt chloride with or without treatment with Kolaviron and Gallic acid.

A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). Intensity of staining is indicated with black arrows

Figure 5: Photomicrographs of heart sections of rats exposed to cobalt chloride with or without treatment with Kolaviron and Gallic acid.

A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). Hemorrhagic lesions in the myocardium are indicated with blue arrows.

Figure 6: Photomicrographs of kidney sections of rats exposed to cobalt chloride with or without treatment with Kolaviron and Gallic acid.

A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). Hemorrhagic lesions and congested vessels are indicated with blue arrows; black arrows show severe inflammatory cell infiltration.

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Table 1: Effects of Kolaviron and Gallic acid on blood pressure parameters rats exposed to Cobalt chloride

	A	B	C	D	E
Systolic blood pressure (mmHg)	154.56±5.04	136.10±1.89 ^a	129.25±0.55 ^{a,b}	93.95±17.81 ^{a,b}	117.29±4.53 ^{a,b}
Diastolic blood pressure (mmHg)	125.00±6.36	117.55±11.86	114.08±5.05	82.60±17.94 ^{a,b}	94.50±17.30 ^a
Mean arterial blood pressure (mmHg)	134.44±5.92	123.41±8.05	118.92±3.50	85.98±17.94 ^{a,b}	101.76±12.90 ^{a,b}

Values are presented as mean±standard deviation (*n*=10). A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). ^a represents significant difference at *p* < 0.05, when compared with control; ^b represents significant difference at *p* < 0.05, when compared with CoCl₂ group

Table 2: Effect of Kolaviron and Gallic acid on electrocardiographic parameters in rats exposed to cobalt chloride

	A	B	C	D	E
Heart rate (/min)	278.00±20.42	291.33±38.42	274.33±19.40	282.00±49.50	303.50±38.89
P wave duration (milliseconds)	21.67±1.53	20.00±7.81	23.67±3.06	17.50±4.95	21.50±12.02
PR interval (milliseconds)	41.00±1.00	40.33±4.62	47.67±7.51	38.00±4.24	36.50±9.19
QRS duration (milliseconds)	16.67±1.53	19.00±5.29	19.33±4.16	21.00±5.66	14.50±9.19
QT segment (milliseconds)	78.67±9.02	76.33±8.51	69.33±1.16	99.50±9.19 ^{a,b}	71.00±5.66
QT corrected Bazett (milliseconds)	168.33±16.44	167.00±10.44	147.67±5.03	214.00±1.41 ^{a,b}	159.50±23.34
R amplitude (milliseconds)	0.24±0.036	0.22±0.056	0.29±0.035	0.31±0.129	0.21±0.066

Values are presented as mean±standard deviation ($n=10$). A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). ^a represents significant difference at $p < 0.05$, when compared with control; ^b represents significant difference at $p < 0.05$, when compared with CoCl₂ group

Table 3: Effects of Kolaviron and Gallic acid on markers of oxidative stress in cardiac and renal tissues of rats exposed to Cobalt chloride

		A	B	C	D	E
Hydrogen peroxide (micromole/min/mg protein)	Heart	10.95±0.46	12.08±0.74 ^a	11.33±0.35 ^b	10.87±0.50 ^b	10.77±0.70 ^b
	Kidney	10.31±0.27	11.17±0.50 ^a	9.86±0.40 ^b	9.83±0.61 ^b	10.15±0.26 ^b
Malondialdehyde (micromole/g tissue)	Heart	0.63±0.04	0.91±0.17 ^a	0.90±0.11	0.89±0.04	0.90±0.10
	Kidney	1.40±0.47	1.64±0.08	1.10±0.19 ^b	1.02±0.04 ^b	1.16±0.30
Nitric oxide (micromole/litre)	Heart	0.14±0.03	0.19±0.01 ^a	0.19±0.05	0.16±0.02 ^b	0.14±0.03 ^b
	Kidney	0.75±0.10	0.76±0.13	0.69±0.16	0.65±0.09	0.67±0.13

Values are presented as mean±standard deviation (n=10). A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg)

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Table 4: Effects of Kolaviron and Gallic acid on antioxidant systems in cardiac and renal tissues of rats exposed to Cobalt chloride

		A	B	C	D	E
GSH (micromole/g tissue)	Heart	43.80±1.07	43.90±0.37	44.18±0.53	43.95±0.51	44.50±0.61
	Kidney	47.57±0.47	47.20±0.71	47.02±0.63	46.54±0.66	47.48±1.53
SOD (units/ mg protein)	Heart	0.19±0.07	0.018±0.007 ^a	0.21±0.07 ^b	0.24±0.03 ^b	0.25±0.06 ^b
	Kidney	0.085±0.022	0.083±0.026	0.184±0.047 ^b	0.265±0.034 ^b	0.264±0.066 ^b
CAT (micromole H ₂ O ₂ consumed/min/mg protein)	Heart	31.57±1.36	26.95±1.42 ^a	31.78±0.97 ^b	31.50±1.31 ^b	27.90±1.45
	Kidney	89.33±4.10	79.69±2.38 ^a	79.18±7.01	78.86±5.11	77.16±4.70
GST (mmole CDNB- GSH complex formed/min/mg protein)	Heart	0.021±0.004	0.011±0.006 ^a	0.021±0.006 ^b	0.023±0.009 ^b	0.018±0.003
	Kidney	0.014±0.005	0.0021±0.0009 ^a	0.0065±0.0014 ^b	0.0078±0.0045 ^b	0.0076±0.004

Values are presented as mean±standard deviation (n=10). A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg)

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82x45mm (300 x 300 DPI)

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87x61mm (300 x 300 DPI)

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81x70mm (300 x 300 DPI)

Figure 5: Photomicrographs of heart sections of rats exposed to cobalt chloride with or without treatment with Kolaviron and Gallic acid.
A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). Hemorrhagic lesions in the myocardium are indicated with blue arrows.

67x58mm (300 x 300 DPI)

Figure 6: Photomicrographs of kidney sections of rats exposed to cobalt chloride with or without treatment with Kolaviron and Gallic acid.
A = Control; B = CoCl₂ alone; C = CoCl₂ + KV (100 mg/kg); D = CoCl₂ + KV (200 mg/kg); E = CoCl₂ + Gallic acid (120 mg/kg). Hemorrhagic lesions and congested vessels are indicated with blue arrows; black arrows show severe inflammatory cell infiltration.

69x62mm (300 x 300 DPI)