Original Research Article

The bi-flavonoid complex kolaviron reduces cadmium-induced cytotoxicity and production of reactive oxygen species by suppressing inflammatory response

Abstract

The effect of kolaviron (a bi-flavonoid complex extracted from *Garcinia kola*) on cadmium (Cd)-induced cell death/ production of reactive oxygen species (ROS) in monocyte U937 cells and production of inflammatory markers/ antioxidant enzymes in U937-derived macrophages was investigated. In the first experiment, U937 cells were incubated with or without kolaviron for 24 h before exposed to Cd (10 µM). Cell viability and ROS production were later assessed via MTT reduction and DCHF assays respectively. In the other experiment, U937 cells were transformed to the macrophage form using phorbol 12-myristate 13-acetate and incubated with or without kolaviron for 24 h before exposed to Cd. Subsequently, production of TNF-α, IL-6 were analysed via cytokine ELISA and the expression of NF-κB, iNOS, SOD and catalase were assessed using RT-PCR. Results revealed that Cd caused significant cell death and production of ROS in U937 cells which were reduced by kolaviron in a dose-dependent manner ($p < 0.05$). Kolaviron also reduced Cd-mediated secretion of TNF-α and IL-6 in U937-derived macrophages which was concentration-dependent for the reduction of TNF-α ($p < 0.05$). The bi-flavonoid complex also reduced Cd-mediated expression of the transcription factors (NF-κB and iNOS) and the antioxidant enzymes (SOD and catalase) but the observation was only concentration-

dependent for the reduction of catalase ($p < 0.05$). These shows that kolaviron reduced Cd-mediated alterations in monocyte U937 cells and macrophages. Implications are discussed.

Keywords: Garcinia kola; kolaviron; cadmium; macrophages; cell viability

1. INTRODUCTION

Cadmium (Cd) is one of the most toxic environmental pollutants whose levels are raised due to anthropogenic activities [1-2]. Human activities that produce cadmium include use of automobiles, batteries, paints, etc. These activities seem to be indispensable to life as such human exposure to cadmium will be continuous [3]. Human beings are exposed to Cd via food consumption, smoking and probably contaminated drugs however toxicity depends on the amount ingested, entry rate, distribution and excretion [4-7]. Following uptake, cadmium is mainly retained in the kidney where it binds to albumin and cystein-rich protein metallothionein for excretion however the toxicant causes significant renal tubular damage [5]. Cadmium also causes liver damage, teratogenic effects, neurotoxic effects and cancer [8-10].

Plants are widely used in traditional medicine for the treatment of various ailments thus have attracted a lot of attention all over the world. *Garcinia kola* Heckel (Clusiaceae) is a medium sized plant valued in many part of Western and Central Africa whose seeds are consumed to treat cough, liver diseases, laryngitis, infections and inflammation [11-13]. Kolaviron is a bi-flavonoid complex isolated from *Garcinia kola* with immense antioxidant power. It has been reported that the complex has anti-nephrotoxic, anti-diabetic, anti-diabetogenic, anti-inflamatory and anti-microbial effects [11, 14-17]. This current work investigates the effect of kolaviron on cadmium-induced toxicity and production of reactive oxygen species (ROS) in the monocyte cell line U937. The effect of kolaviron on cadmium-mediated alterations of

some transcription factors and antioxidant enzymes in U937-derived macrophages was also reported.

2. MATERIALS AND METHOD

2.1 Materials

Cadmium chloride, *L*-glutamine, 3-(4,5-dimetylthiazol-2-yl),-2,5-diphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), 2′ ,7′ -dichlorohydrofluorescein diacetate (DCHF-DA) and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich (USA). Penicillin, streptomycin and all antibodies were purchased from Gibco (USA). All other chemicals were of analytical grade and commercially available.

2.2 Maintenance of cell culture

The monocyte cell line U937 obtained from the American Type Culture Collection (Rockville, MD, USA) was maintained in RPMI-1640 medium (Sigma-Aldrich, USA) as described [18]. Cells were seeded in flasks and grown in RPMI-1640 containing 10 % heat inactivated fetal calf serum, 2 mM *L*-glutamine, 100 U/mL streptomycin and penicillin (100 mg/mL). Cells were kept at a temperature of 37°C in an atmosphere of 5 % CO_2/95 % air and maintained at 5 x 10^4/mL.

2.3 Extraction of kolaviron

Kolaviron was extract from *Garcinia kola* seeds according to Iwu [19]. Briefly, 4 kg of the powdered seeds were soxhlet-extracted with light petroleum ether (bp 40–60°C) for 24 h. The defatted dried marc was repacked and extracted with acetone. The extract was concentrated and diluted twice its volume with water and re-extracted with ethylacetate (6×300 ml). The ethylacetate fraction was concentrated to give a golden yellow solid known as kolaviron.

2.4 Cell viability and ROS production

Two hundred microlitres of cells (at 5 x 10^4 cells/mL) were delivered into the wells of culture plates. In order to evaluate the effect of kolaviron on cell viability, cells were incubated with or without kolaviron (10 µg/mL or 25 µg/mL) for 24 h before exposure to 10 µM Cd (as cadmium chloride). For controls, cells were supplemented with equivalent volumes of RPMI-1640. One hour after addition of Cd, cell viability was assessed via the MTT reduction assay as reported [20]. Briefly, MTT was added to each culture to a final concentration of 0.5 mg/mL and incubated for 1 h at 37°C. MTT was aspirated and culture supplemented with DMSO. Absorbance was finally measured at 570 nm using a microplate reader.

The production of ROS was assessed based on the oxidation of 2′,7′-dichlorohydrofluorescein by intracellular peroxides as reported [21] with a slight modification. Twenty-four hours following the addition of kolaviron (10 µg/mL or 25 µg/mL), medium was removed and replaced with RPMI-1640 supplemented with 50 µM DCHF-DA and incubated for 30 min at 37°C. Cells were washed with 0.02 M phosphate buffered saline (pH 7.4) and incubated with Cd (10 µM) for 1 h. Fluorescence of cells was measured at excitation and emission wavelength at 485 nm and 530 nm respectively. Antioxidant activity was expressed as percent inhibition of intracellular ROS following Cd exposure.

2.5 Secretion of cytokines

Cells were transformed to the macrophage form using PMA as described [18]. Medium was removed and replaced with kolaviron (10 µg/mL or 25 µg/mL). Twenty-four hours later, Cd (10 µM) was added and the supernatant of each cell culture analyzed for the production of tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) 1 hr later via cytokine ELISA as reported [18].

2.6 RNA isolation and RT-PCR

Following the transformation and treatment of cells with kolaviron and Cd (as in 2.5 above), total RNA was extracted from cells (after removing supernatants) using TRIzol reagent (Invitrogen) and quantified by measuring absorbance at 260 nm. The cDNA was synthesized using a Revert Aid cDNA synthesis kit according to the manufacturer's proctocol. For RT-PCR, 1 µg of the resulting cDNA was used to amplify regions specific to nuclear factor kappa B (NF-κB), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD) and catalase (CAT) in an ABI Prism 7500 system (Applied Biosciences) with primer pairs listed in table 1. Real-Time PCR data was analyzed and presented as fold change in expression to the GAPDH housekeeping gene of same sample.

Table 1. Primers pairs for RT-PCR

mRNA	Primer sequence (5′-3′)
iNOS	FP: GTGCCACCTCCAGTCCAG
	RP:GCTGCCCCAGTTTTTGATCC
NF-κB	FP:GCCTTGCATCTAGCCACAGAG
	RP:GATGTCAGCACCAGCCTTCAG
SOD	FP:GACTGAAGGCCTGCATGGATTC
	RP: CACATCGGCCACACCATCTTTG
CAT	CTTCGACCCAAGCAACATGC
	GATAATTGGGTCCCAGGCGATG
GAPDH	FP:GTCGGAGTCAACGGATTTGGTC
	RP:CTTCCCGTTCTCAGCCTTGAC

2.7 Statistical analysis

Where appropriate, data were expressed as mean ± SEM of six replicates. Data analysis was done using ANOVA followed by Tukey's range test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Cytotoxicity and ROS production

The effect of kolaviron on Cd-induced cell death is shown in figure 1. Treatment of U937 monocytes with Cd resulted in significant cell death (16.73 ± 4.12 % viability) when compared to untreated controls ($p < 0.05$). However kolaviron reduced Cd-induced cell death which was concentration-dependent ($p < 0.05$). Following the DCHF-DA assay, the results further revealed that kolaviron reduced Cd-induced production of ROS in a concentration-dependent manner ($p < 0.05$) (Figure 2).

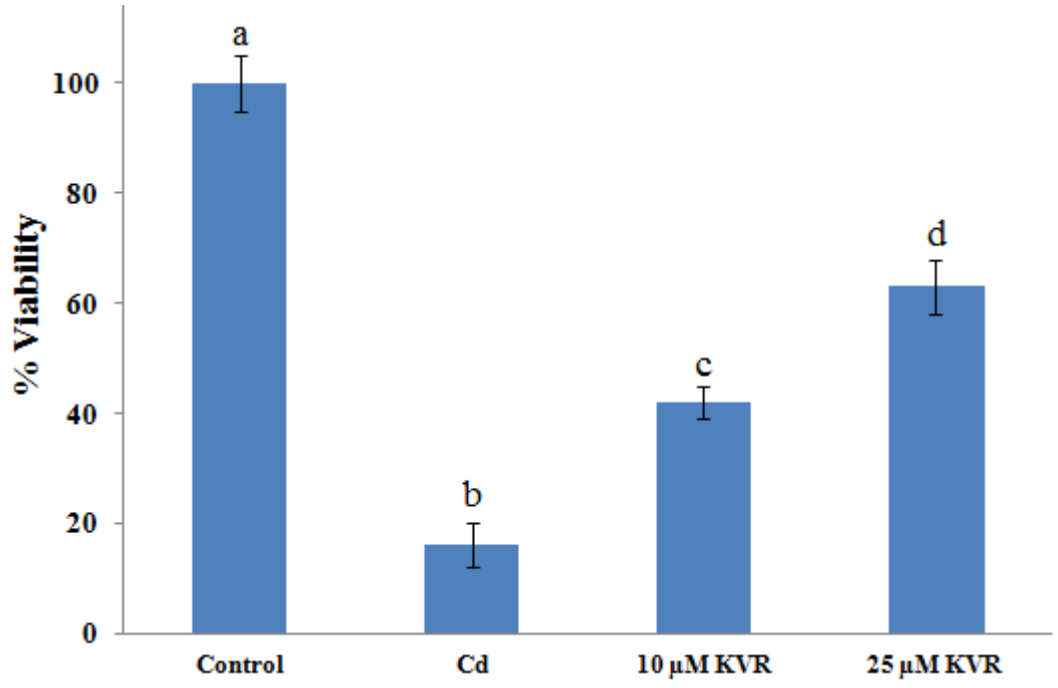

Figure 1. Effect of kolaviron on Cd-induced cell death in U937 monocytes assessed by MTT reduction assay. *Cd, cells treated with cadmium only. 10 µM KVR, cells treated with 10 µM kolaviron; 25 µM KVR, cells treated with 25 µM KVR before incubating with Cd.*

Each column represents mean ± SEM of six replicates. Values having different superscript letters differ significantly ($p < 0.05$).

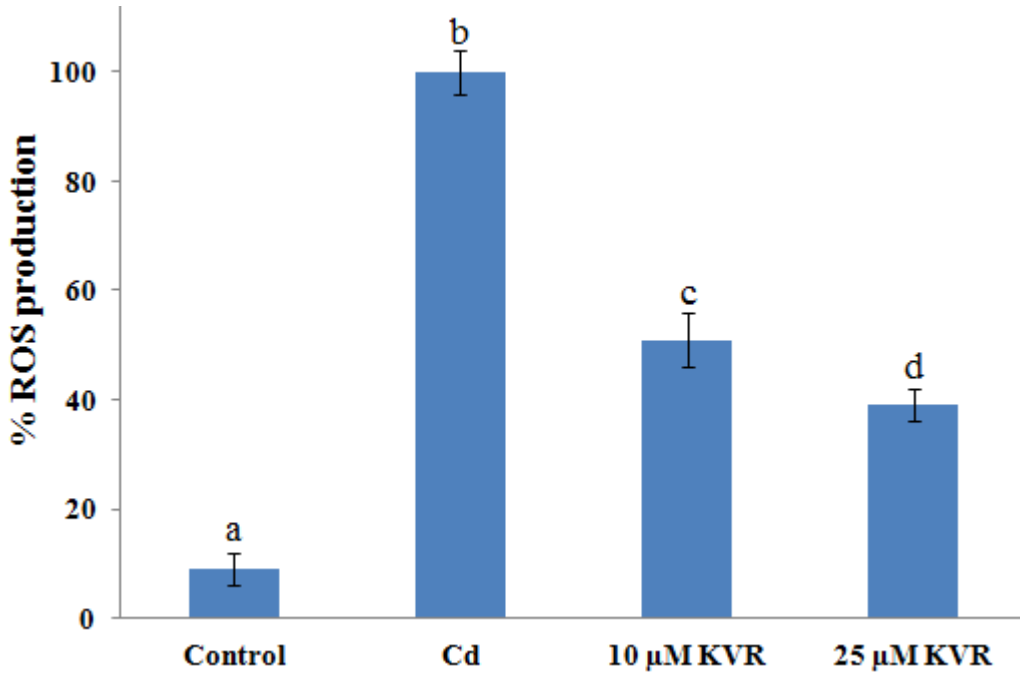

Figure 2. **Effect of kolaviron on Cd-induced production of ROS in U937 monocytes**. *Cd, cells treated with cadmium only. 10 µM KVR, cells treated with 10 µM kolaviron; 25 µM KVR, cells treated with 25 µM KVR before incubating with Cd. Each column represents mean ± SEM of six replicates. Values having different superscript letters differ significantly ($p < 0.05$).*

3.2 Effect of kolaviron on cytokine secretion

In order to investigate the effect of kolaviron on the production of the cytokines, the U937 monoytes were treatment with PMA (transforming them to the macrophage form) before exposed to kolaviron and Cd. The results revealed that kolaviron significantly reduced Cd-

mediated release of the cytokines i.e. TNF-α and IL-6 (Figures 3 and 4). While the reduction of TNF-α secretion was concentration-dependent ($p < 0.05$), the effect on IL-6 secretion was not ($p > 0.05$).

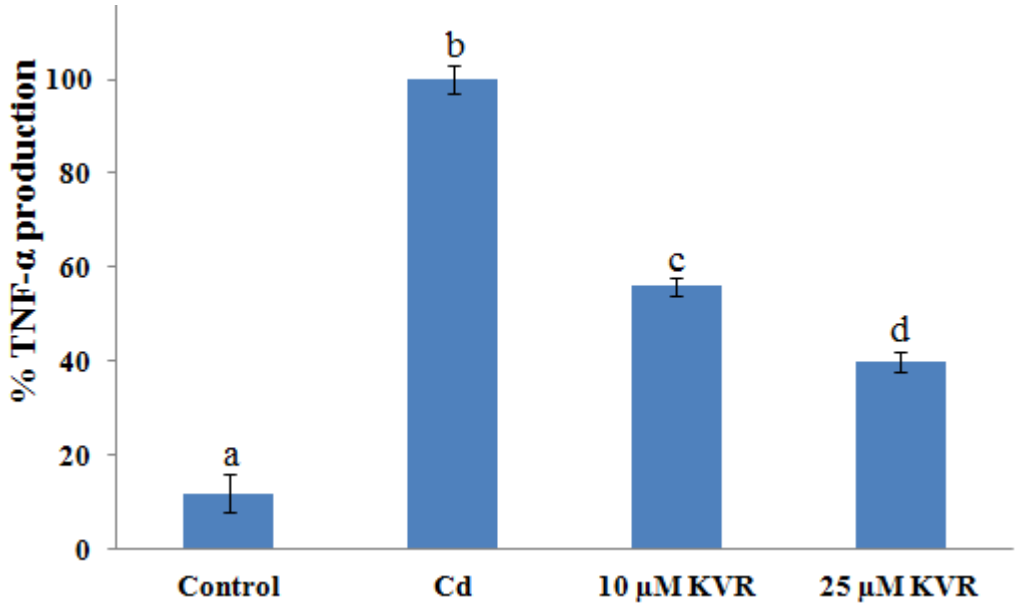

Figure 3. Effect of kolaviron on Cd-induced secretion of TNF-α in U937-derived macrophages. *Cd, cells treated with cadmium only. 10 µM KVR, cells treated with 10 µM kolaviron; 25 µM KVR, cells treated with 25 µM KVR before incubating with Cd. Each column represents mean ± SEM of six replicates. Values having different superscript letters differ significantly ($p < 0.05$).*

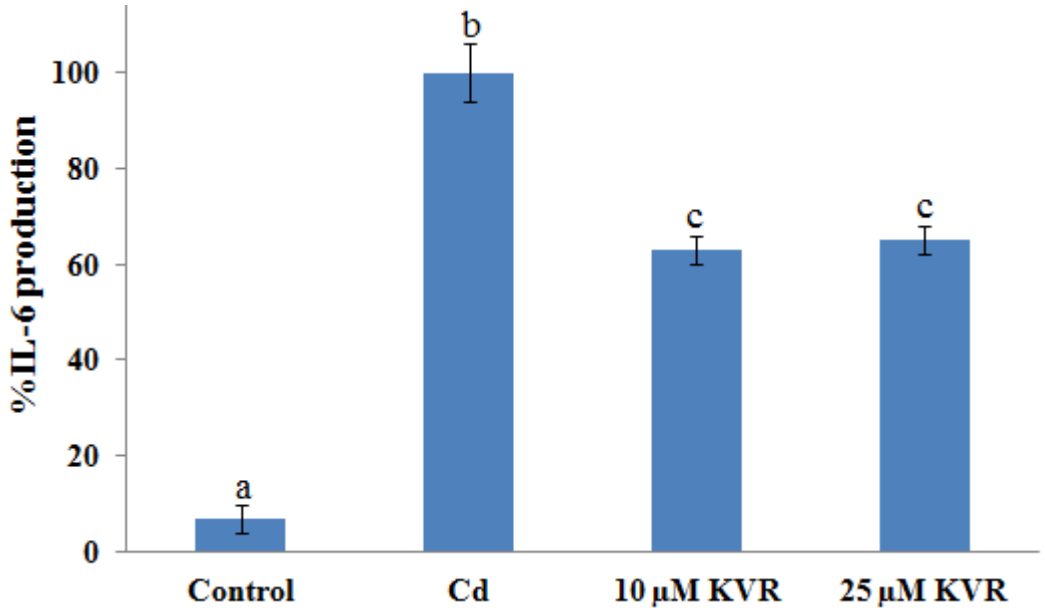

Figure 4. Effect of kolaviron on Cd-induced secretion of IL-6 in U937-derived macrophages. *Cd, cells treated with cadmium only. 10 µM KVR, cells treated with 10 µM kolaviron; 25 µM KVR, cells treated with 25 µM KVR before incubating with Cd. Each column represents mean ± SEM of six replicates. Values having different superscript letters differ significantly ($p < 0.05$).*

3.3 Expression of transcription factors and antioxidant enzymes

The effect of kolaviron on Cd-induced expression of the transcription factors (NF-κB, iNOS) and antioxidant enzymes (SOD and CAT) was assessed via RT-PCR (Figure 5). The findings reveal that Cd significantly up-regulated the expression of NF-κB, iNOS, SOD and CAT when compared to untreated cells ($p < 0.05$). Incubating the macrophages with kolaviron prior to the treatment with Cd reduced the expression levels closer to control levels. However, the effect of kolaviron was only concentration-dependent in the expression of CAT ($p < 0.05$).

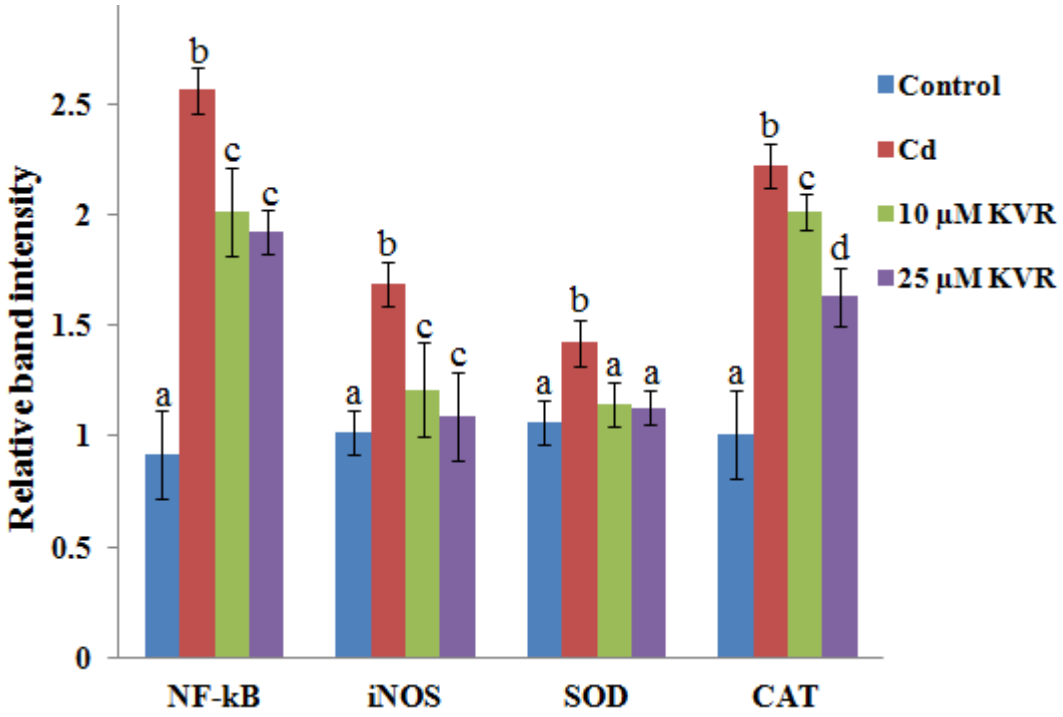

Figure 5. Expression of iNOS transcription factors and antioxidant enzymes in U937-derived macrophages. Values are % fold expressions over housekeeping gene GAPDH for RT-PCR of mRNA isolated from U937-derived macrophages. *Cd, cells treated with cadmium only; 10 µM KVR, cells treated with 10 µM kolaviron; 25 µM KVR, cells treated with 25 µM KVR before incubating with Cd. Each column represents mean ± SEM of six replicates. Values having different superscript letters differ significantly ($p < 0.05$). (Comparisons not made between different markers)*

4. DISCUSSION

Cadmium is considered a significant hazard because of its high toxicity at very low levels of exposure coupled with its prolonged half-life (over 20 years) [22]. The heavy metal interferes with several essential biological processes such as uptake of essential metals (e.g. calcium, zinc, Fe), oxidative phosphorylation and basal respiration and the consequences could be cell death [23-25]. This is in accord with the preliminary results of the toxic nature of cadmium. However, the results of the study also revealed that kolaviron reduced cadmium-induced cell death which was concentration-dependent (Figure 1). Flavonoids are cytoprotective because they chelate cadmium thereby reducing its cellular accumulation and enhancing the uptake of

essential elements [26]. Cd-induced cell death has been linked to the generation of ROS [27-28]. Cadmium is not a Fenton-like metal so its induction of ROS might be via an indirect mechanism as suggested [23]. The toxicant interferes with the uptake of essential metal ions which are key co-factors to important scavenger proteins such as superoxide dismutase, peroxidase, catalase therefore inactivates them [29]. The metal also stimulates the production of ROS by binding to complex III of the electron transport chain [30-31].

Several reports have it that flavonoids are cytoprotective due to their direct antioxidant potential [32-33]. However, flavonoids also possess indirect antioxidant activity as they could induce the up-regulation of glutathione, γ-glutamylcystein ligase, glutathione s-transferase and NAD(P)H:quinine oxidoreductase in different cell systems [34]. Another way flavonoids inhibit cell death is by suppressing oxidative stress [17].

Macrophages secrete cytokines (e.g TNF-α and IL-6) when activated and produce NF-κB and iNOS as part of the inflammatory response [35-36]. Thus Cd activates macrophages as part of the inflammatory response since it induced the secretion of pro-inflammatory cytokines TNF-α and IL-6 and expression of NF-κB, iNOS in the transformed U937 cells (otherwise known as U937-derived macrophages) (Figures 3 - 5). The excessive release of these factors has been implicated in many pathophysiological responses [37-38]. Nuclear factor kappa-B is a transcription factor that is up-regulated during inflammation and implicated in some disorders [39-40]. The activation of NF-κB is predisposing to cell death and linked with oxidative stress [41-43]. Thus the induction of NF-κB may accompany cadmium-induced oxidative damage. Though the monocyte form of the cell line could also release the cytokines, treatment with phorbol esters enhances their transformation to the macrophage form and enables them to maintain cell numbers [44].

Nitric oxide is an important ROS produced from arginine in a reaction catalyzed by inducible nitric oxide synthase (iNOS) thus upregulation of iNOS expression is key to oxidation. The expression of iNOS is dependent on the translocation of NF-κB into the nucleus and their co-expression has been linked to various tissue injuries. Excessive release of pro-inflammatory cytokines and other transcription factors by macrophages has been implicated in organ failure, septic shock, rheumatoid arthritis, asthma, cancer, chronic obstructive pulmonary diseases, viral infections, autoimmune diseases, hypotension and other systemic responses which could be traceable to the production of ROS [45-46]. Thus the inhibition of their expression is therapeutic [47-49].

The results from the present investigation revealed that kolaviron reduced Cd-mediated production of the cytokines TNF-α and IL-6 and expression of NF-κB, iNOS (Figures 3 – 5). Kolaviron has been shown to reduce inflammatory responses by inhibiting the secretion of various cytokines, nitric oxide and downregulation of transcription factors [50-53]. Flavonoids also suppress inflammatory processes by inhibiting NF-κB activation [54].

Since treatment of cells with cadmium resulted in the production of ROS, we thought this may also be due to the reduction in the levels of antioxidant enzymes thus the expression of superoxide dismutase (SOD) and catalase (CAT) in U937-derived macrophages was investigated. The results from this current study reveal that Cd (at 10 μM) increased the expression of both enzymes in the U937-derived macrophages (Figure 5). This seems to be at variance with some reports that Cd suppresses the activity of these enzymes via direct interaction [55-56]. In this experiment, the Cd-mediated elevation of the enzymes could be ascribed to their induction to compensate with the increase in ROS production which has been suggested [57]. The biflavonoid kolaviron reduced the cadmium-mediated production of

the antioxidant enzymes via an indirect mechanism which is attributable to the direct scavenging of cadmium. However, this could be further investigated.

5. CONCLUSION

The study reveals that kolaviron reduced Cd-induced cell death/ ROS production in the monocyte cell line U937 and activation of U937-derived macrophages. The production of the cytokines and transcription factors by macrophages is part of the inflammatory response which is implicated in some disorders. Thus the ability of kolaviron to reduce cell death may involve the suppression of the inflammatory response. Since some diseases are traceable to the oxidation by ROS and inflammation signals, kolaviron (and perhaps *Garcinia kola*) could be considered in their management. Even though further follow-up studies are still required, *Garcinia kola* is a valuable resource and should be exploited both nutraceutically and pharmacologically.

CONFLICT OF INTEREST

The authors declare that they have no conflict in interest.

REFERENCES

1. Satarug S, Vesey DA, Gobe GC. Health risk assessment of dietary cadmium intake: Do current guidelines indicate how much is safe? Environ Health Perspect. 2017;125:284-288.

2. García-Esquinas E, Pollan M, Tellez-Plaza M, Francesconi KA, Goessler W, Guallar E, Umans JG, Yeh J, Best LG, Navas-Acien A. Cadmium exposure and cancer mortality in a prospective cohort: the strong heart study. Environ Health Perspect. 2014;122:363-370.

3. Khan S, Khan MA, Rehman S. Lead and cadmium contamination of different roadside soils and plants in Peshawar City, Pakistan. Pedosphere 2011;21:351-357.

4. Röllin HB, Kootbodien T, Channa K, Odland JØ. Prenatal exposure to cadmium, placental permeability and birth outcomes in coastal populations of South Africa. PloS One. 2015;10: e0142455.

5. Fagerberg B, Borné Y, Barregard L, Sallsten G, Forsgard N, Hedblad B, Persson M, Engström G. Cadmium exposure is associated with soluble urokinase plasminogen activator receptor, a circulating marker of inflammation and future cardiovascular disease. Environ Res. 2017;152:185-191.

6. Pruvot C, Douay F, Hervé F, Waterlot C. Heavy metals in soil, crops and grass as a source of human exposure in the former mining areas. J Soils Sed. 2006; 6:215-220.

7. Ibraheem AS, Seleem AA, El-Sayed MF, Hamad BH. Single or combined cadmium and aluminum intoxication of mice liver and kidney with possible effect of zinc. J Basic Appl Zool. 2016; 77:91-101.

8. Afifi OK, Embaby AS. Histological study on the protective role of ascorbic acid on cadmium induced cerebral cortical neurotoxicity in adult male albino rats. J Micros Ultrastruc. 2016; 4:36-45.

9. Sanders A, Smeester L, Rojas D, DeBussycher T, Wu M, Wright F, Zhou YH, Laine J, Rager J, Swamy G, Ashley-Koch A. Cadmium exposure and the epigenome: Exposure-associated patterns of DNA methylation in leukocytes from mother-baby pairs. Epigenetics. 2014; 9:212-221.

10. Ezedom T, Asagba SO. Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats. Toxicol Reports. 2016; 3:708-715.

11. Tchimene MK, Anaga AO, Ugwoke CEC, Onoja OJ, Ezugwu CO, Okunji C, Iwu M M. Anti-diabetic profile of extract, kolaviron, biflavonoids and garcinoic acid from *Garcinia kola* seeds. Int J Curr Microbiol App Sci. 2016; 5:317-322.

12. Damian DC, Nweze EI, Onyeke CC. The in vivo anti-plasmodium activity of *Garcinia kola* Heckel. J Basic Pharmacol Toxicol. 2017; 1:27-31.

13. Oyagbemi AA, Bester D, Esterhuyse J, Farombi EO. Kolaviron, a biflavonoid of *Garcinia kola* seed mitigates ischemic/reperfusion injury by modulation of pro-survival and apoptotic signaling pathways. J Intercultural Ethnopharmacol. 2017; 6:42-49.

14. Oluwatosin A, Tolulope A, Ayokulehin K, Patricia O, Aderemi K, Catherine F, Olusegun A. Antimalarial potential of kolaviron, a biflavonoid from *Garcinia kola* seeds, against Plasmodium berghei infection in Swiss albino mice. Asian Pacific J Trop Med. 2014;7:97-104.

15. Oyagbemi, A, Omobowale T, Adedapo A, Oyekan A, Yakubu M. Antiproliferative effect of kolaviron, a biflavonoid complex from the seed of *Garcinia kola* on vascular smooth muscle cells (VSMs) and A549 cancer cell line. FASEB J. 2015; 29:945;17.

16. Akinmoladun AC, Akinrinola BL, Olaleye MT, Farombi EO. Kolaviron, a *Garcinia kola* biflavonoid complex, protects against ischemia/reperfusion injury: pertinent mechanistic insights from biochemical and physical evaluations in rat brain. Neurochem Res. 2015;40:777-787.

17. Ayepola OR, Cerf ME, Brooks NL, Oguntibeju OO. Kolaviron, a biflavonoid complex of *Garcinia kola* seeds modulates apoptosis by suppressing oxidative stress

and inflammation in diabetes-induced nephrotoxic rats. Phytomed. 2014;21:1785-1793.

18 Okoko T, Oruambo IF. Inhibitory activity of quercetin and its metabolite on lipopolysaccharide-induced activation of macrophage U937 cells. Food Chem Toxicol. 2009;47:809–812

19. Iwu MM. Antihepatoxic constituents of *Garcinia kola* seeds. Experientia 1985;41:699–700.

20. Zhao H, Liu W, Wang Y, Dai N, Gu J, Yuan Y, Liu X, Bian J, Liu ZP. Cadmium induces apoptosis in primary rat osteoblasts through caspase and mitogen-activated protein kinase pathways. J Vet Sci. 2015;16:297-306.

21. Koga T, Meydani M. Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. Am J Clin Nutr. 2001;73:941–948.

22. Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. Intern J Environ Health Res. 2014;24:378-399.

23. Nair AR, DeGheselle O, Smeets K, Van Kerkhove E, Cuypers A. Cadmium-induced pathologies: where is the oxidative balance lost (or not)?. Intern J Mol Sci. 2013; 14:6116-6143.

24. Ha TT, Burwell ST, Goodwin ML, Noeker JA, Heggland SJ. Pleiotropic roles of Ca^{2+}/calmodulin-dependent pathways in regulating cadmium-induced toxicity in human osteoblast-like cell lines. Toxicol Lett. 2016;260:18-27.

25. Erboga M, Kanter M, Aktas C, Donmez YB, Erboga ZF, Aktas E, Gurel A. Anti-apoptotic and anti-oxidant effects of caffeic acid phenethyl ester on cadmium-induced testicular toxicity in rats. Biol Trace Element Res. 2016;171:176-184.

26. Li Li X, Jiang X, Sun J, Zhu C, Li X, Tian L, Liu L, Bai W. Cytoprotective effects of dietary flavonoids against cadmium- induced toxicity. Ann New York Acad Sci. 2017: DOI; 10.1111/nyas.13344

27. Khojastehfar A, Aghaei M, Gharagozloo M, Panjehpour M. Cadmium induces reactive oxygen species-dependent apoptosis in MCF-7 human breast cancer cell line. Toxicol Mech Meth. 2015;25:48-55.

28. Selvaraj V, Armistead MY, Cohenford M, Murray E. Arsenic trioxide (As_2O_3) induces apoptosis and necrosis mediated cell death through mitochondrial membrane potential damage and elevated production of reactive oxygen species in PLHC-1 fish cell line. Chemosphere. 2013;90:1201-1209.

29. Szuster-Ciesielska A, Stachura A, Słotwińska M, Kamińska T, Śnieżko R, Paduch R, Abramczyk D, Filar J, Kandefer-Szerszeń M. The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. Toxicol. 2000;145:159-171.

30. Wang Y, Fang J, Leonard SS, Rao KM. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Rad Biol Med. 2004;36:1434-1443.

31. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Topics Med Chem. 2001;1:529-539.

32. Chow JM, Shen SC, Huan SK, Lin HY, Chen YC. Quercetin, but not rutin and quercitrin, prevention of H_2O_2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochem Pharmacol. 2005;69:1839-1851.

33. Chen TJ, Jeng JY, Lin CW, Wu CY, Chen YC. Quercetin inhibition of ROS-dependent and-independent apoptosis in rat glioma C6 cells. Toxicol. 2006;223:113-26.

34. Angeloni C, Spencer JP, Leonani E, Biagi PL, Hrelia S. Role of quercetin and its in vivo metabolites in protecting H9c2 cells against oxidative stress. Biochimie 2007;89:73–82

35. Duque GA, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers Immunol. 2014;5:491

36. Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, Martínez JA, García-Lafuente A. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. 2012;130:350-355.

37. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-β and TNF-α, by human macrophages. J Immunol. 1998;160:3513-3521.

38. Bai X, Feldman NE, Chmura K, Ovrutsky AR, Su WL, Griffin L, Pyeon D, McGibney MT, Strand MJ, Numata M, Murakami S. Inhibition of nuclear factor-kappa B activation decreases survival of *Mycobacterium tuberculosis* in human macrophages. PloS One. 2013;8(4):e61925.

39. Tak PP, Firestein GS. NF-κB: a key role in inflammatory diseases. J Clin Invest. 2001; 107:7-11.

40. Guo G, Cheng X, Fu R. Losartan Inhibits Nuclear Factor-κB activation induced by small, dense LDL cholesterol particles in human umbilical vein endothelial cells. Curr Therapeu Res. 2014;76:17-20.

41. Ghosh M, Manna P, Sil PC. Protective role of a coumarin-derived schiff base scaffold against tertiary butyl hydroperoxide (TBHP)-induced oxidative impairment and cell death via MAPKs, NF-κB and mitochondria-dependent pathways. Free Rad Res. 2011;45:620-637.

42. Gong P, Chen F, Liu X, Gong X, Wang J, Ma Y. Protective effect of caffeic acid phenethyl ester against cadmium-induced renal damage in mice. J Toxicol Sci. 2012;37:415-425.

43. Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ, Nawrot T. Cadmium stress: an oxidative challenge. Biometals. 2010;23:927-940.

44. Daigneault M, Preston JA, Marriott HM, Whyte MKB, Dockrell DH. The identification of markers of macrophage differentiation in PMA-Stimulated THP-1 Cells and monocyte-derived macrophages. PloS One 2010;5:e8668.

45. Lawrence T. The Nuclear factor NF-κB in inflammation. Cold Spring Harb Perspect Biol. (2009) 1:a001651.

46. Murray PJ, Allen JE, Biswas SK, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege J-L, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41:14-20.

47. Salamone F, Galvano F, Cappello F, Mangiameli A, Barbagallo I, Volti GL. Silibinin modulates lipid homeostasis and inhibits nuclear factor kappa B activation in experimental nonalcoholic steatohepatitis. Transl Res. 2012;159:477-486.

48. An HJ, Kim IT, Park HJ, Kim HM, Choi JH, Lee KT. Tormentic acid, a triterpenoid saponin, isolated from *Rosa rugosa*, inhibited LPS-induced iNOS, COX-2, and TNF-α expression through inactivation of the nuclear factor-κb pathway in RAW 264.7 macrophages. Intern Immunopharmacol. 2011;11:504-510.

49. Schuliga M. NF-kappaB signaling in chronic inflammatory airway disease. Biomolecules. 2015;5:1266-1283.

50. Abarikwu SO. Kolaviron, a natural flavonoid from the seeds of *Garcinia kola*, reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-κB, p38, Akt, pc-JUN and JNK. Biochim Biophy Acta. 2014; 1840:2373-2381.

51. Onasanwo SA, Velagapudi R, El-Bakoush A, Olajide OA. Inhibition of neuroinflammation in BV2 microglia by the biflavonoid kolaviron is dependent on the Nrf2/ARE antioxidant protective mechanism. Mol Cellular Biochem. 2016;414:23-36.

52. Farombi EO, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE. Kolaviron, a natural antioxidant and antiinflammatory phytochemical prevents dextran sulphate sodium- induced colitis in rats. Basic Clin Pharmacol Toxicol. 2013;113(1):49-55.

53. Ramyaa P, Padma VV. Quercetin modulates OTA-induced oxidative stress and redox signalling in HepG2 cells—up regulation of Nrf2 expression and down regulation of NF-κB and COX-2. Biochim Biophy Acta. 2014;1840:681-692.

54. Hou XL, Tong Q, Wang WQ, Shi CY, Xiong W, Chen J, Liu X, Fang JG. Suppression of inflammatory responses by dihydromyricetin, a flavonoid from

Ampelopsis grossedentata, via inhibiting the activation of NF-κB and MAPK signaling pathways. J Nat Prod. 2015;78:1689-1696.

55. Wang J, Zhang H, Zhang T, Zhang R, Liu R, Chen Y. Molecular mechanism on cadmium-induced activity changes of catalase and superoxide dismutase. Intern J Biol Macromol. 2015;77:59-67.

56. Das TP, Suman S, Damodaran C. Induction of reactive oxygen species generation inhibits epithelial–mesenchymal transition and promotes growth arrest in prostate cancer cells. Mol Carcinog. 2014;53:537-547.

57. Wang J, Zhu H, Liu X, Liu Z. N-acetylcysteine protects against cadmium-induced oxidative stress in rat hepatocytes. J Vet Sci. 2014;15:485-493.