Kolaviron protects against cisplatin-induced hepatic and renal oxidative damage in rats

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ABSTRACT
Kolaviron is an important bi-flavonoid complex isolated from Garcinia kola seeds which has been reported to possess significant bioactivities. Cisplatin is an antineoplastic drug, but tissue damage is a limiting factor to its chemotherapy. The effect of kolaviron on cisplatin-induced alterations of some biochemical parameters in rats was investigated. The research study included a control group (group I) that received neither cisplatin nor kolaviron while group II received only kolaviron (100 mg/kg/day) orally for the duration of the experiment. Rats in group III were given cisplatin (10 mg/kg/day) orally for three consecutive days intraperitoneally. In addition to the injection of cisplatin, rats in group IV were orally given kolaviron at 100 mg/kg/day for six consecutive days prior to the treatment with cisplatin, and for 3 days simultaneously with cisplatin. However, animals in group V were given kolaviron at 200 mg/kg/day for six consecutive days prior to the treatment with cisplatin, and for 3 days simultaneously with cisplatin. Thereafter, rats were anesthetized, dissected; serum and tissues were collected and analyzed. The intraperitoneal-administration of cisplatin (10 mg/kg) caused a significant increase in the serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen (Urea), and creatinine indicative of liver and kidney damage. Cisplatin also increased the levels of thiobarbituric acid reactive species, tumor necrosis factor-alpha, and interleukin-6, while significantly reducing the expression of superoxide dismutase (SOD) and catalase (CAT) in both liver and kidney. However, oral administration of kolaviron (100 and 200 mg/kg) for 6 days prior and 3 days co-treatment with cisplatin significantly reversed the cisplatin-induced alterations close to the control levels. Apart from the expression of SOD and CAT in the liver, the effect of kolaviron was dose-dependent (p < 0.05). Treating the rats with kolaviron (100 mg/kg) alone only significantly enhanced the expression of SOD and CAT in the liver over control (p < 0.05). The results reveal kolaviron as an effective chemoprotective agent against cisplatin-induced tissue damage in rats which can be exploited pharmacologically.

Introduction
Cisplatin (cis-diamminedichloroplatinum II) is an effective antineoplastic drug commonly used in the treatment of various solid tumors, however, dose-related nephrotoxicity is a limiting factor in its chemotherapy [1,2]. Though the mechanism of cisplatin-induced nephrotoxicity is poorly understood, it is believed to involve apoptosis and/or necrosis of renal parenchyma cells [3]. Systemic effects of cisplatin include renal proximal and distal tubuli damage which could lead to proteinuria, hypomagnesemia, hypokalemia, reduced glomerular filtration rate, increase serum creatinine, and elevated blood urea nitrogen (BUN) [1,4]. Other side effects of cisplatin chemotherapy include neurotoxicity, ototoxicity, emetogenicity, and hepatotoxicity [5–8]. Various plants and chemicals of natural origin have been shown to reduce cisplatin-induced tissue damage in experimental models [9–11].

One of the plants whose medicinal potential has been extensively studied is Garcinia kola Heckel (Clusiaceae). This is a largely cultivated evergreen tree whose seeds are highly valuable and consumed as an aphrodisiac and antidote for bronchitis.
Kolaviron protects against oxidative damage

diarrhea, laryngitis, liver diseases, cough, and so on [12–16]. A very potent compound isolated from *G. kola* is kolaviron (actually a bi-flavonoid complex), which is an effective chemoprotective agent in various models. The purpose of this study is to investigate the effect of kolaviron on cisplatin-induced alterations on some biochemical parameters of kidney and liver function in the rat. The liver and kidneys are central to metabolism, thus are frequent targets of toxicants. The liver is where the majority of substances are metabolized, thus have an impact on all the body’s systems [17]. The kidney also plays excretory, metabolic, and endocrine functions, thus the body is very sensitive to kidney insults [18].

**Materials and Methods**

**Extraction of kolaviron**

Kolaviron was isolated from *G. kola* seeds according to the method of Iwu et al. [19]. Briefly, powdered *G. kola* seeds (4 kg) were defatted with light petroleum ether for 24 hours using soxhlet. The defatted dried marc was repacked and further fractionated with acetone and the extract was diluted twice its volume with water and further extracted with ethyl acetate (6 × 300 ml). The ethylacetate fractions were concentrated to yield a golden yellow solid kolaviron.

**Animal treatment**

Male albino rats of the Wister strain (weighing 120–150 g) were obtained from the Nigerian Institute of Medical Research, Lagos. They were housed in plastic cages and maintained under 12-hour light/dark cycle at room temperature. The rats were acclimatized to laboratory conditions for 1 week before the experiment. All procedures were performed in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and approved by the Institutional Research Committee. The animals were allowed unlimited access to water and growers’ mash *ad libitum*.

Rats were split into five groups (five rats per group). The control group (group I) received neither cisplatin nor kolaviron while group II received only kolaviron (100 mg/kg/day) orally for the duration of the experiment. Rats in group III were given cisplatin (10 mg/kg/day) [20] for three consecutive days via the intraperitoneal route. In addition to the injection of cisplatin, rats in group IV were orally given kolaviron at 100 mg/kg/day for six consecutive days prior to the treatment with cisplatin, and for 3 days simultaneously with cisplatin. However, animals in group V were given kolaviron at 200 mg/kg/day for six consecutive days prior to the treatment with cisplatin, and for 3 days simultaneously with cisplatin. Thereafter, rats were anesthetized, dissected, and blood drawn via direct cardiac punctures. Livers and kidneys were also excised, washed in ice-cold physiological saline, and processed for further analysis.

**Determination of parameters in serum**

Blood was allowed to clot and centrifuged at 4°C for 10 minutes at 2880 × *g* to separate serum. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and BUN (urea) were determined using commercial diagnostic kits from Randox laboratories (UK).

**Determination of thiobarbituric acid reactive species and cytokines**

Liver and kidney homogenates were prepared with ice-cold phosphate buffered saline (0.05 M, pH 7.4) containing 1% protease inhibitor cocktail. Perinuclear fractions were later obtained via centrifugation. Each perinuclear fraction (1 ml) was mixed with 2 ml of reagent stock (0.375 % TBA, 50 % v/v concentrated HCl, 5% TCA) and boiled for 15 minutes, cooled and centrifuged for 5 minutes at 2880 × *g*. Absorbance of the supernatant was measured at 532 nm. TBARS was expressed as nanomoles of malondialdehyde per milligram tissue using the molar extinction coefficient of 1.56 × 10⁻⁵ M⁻¹ cm⁻¹. The levels of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-a) and interleukin-6 (IL-6) were determined in perinuclear fractions via cytokine capture Enzyme-linked immunosorbent assay (ELISA) as described [21].

**Assessment of antioxidant enzyme expression**

Total RNA was extracted from tissue (50 mg) by lysing using TRIzol reagent (Invitrogen) and quantified by measuring absorbance at 260 nm. Total RNA (1 µg) from each sample was subjected to cDNA synthesis using a Revert Aid cDNA synthesis kit according to the manufacturer’s protocol. The cDNA (1 µg) was used to amplify fragments corresponding to superoxide dismutase (SOD) and catalase (CAT) in an ABI Prism 7500 system (Applied Biosciences).
with primers listed in Table 1. Real-time Polymerase chain reaction (PCR) data were analyzed and presented as fold change in expression to the GAPDH house-keeping gene of the same sample.

**Statistical analysis**

Experimental data were presented as a mean ± standard error of mean (SEM) from five replicates. Data were subjected to analysis of variance followed by Tukey range test using the Minitab (version 18) Statistical package. Confidence exhibited at \( p < 0.05 \) was considered as significant.

**Results**

**Effect of kolaviron on markers of tissue damage in serum**

The effect of kolaviron on cisplatin-induced alterations on markers of tissue damage is shown in Figure 1. Cisplatin (group III) caused the elevation of ALT, AST, ALP, urea, and creatinine when compared with untreated controls (\( p < 0.05 \)). Administration of kolaviron prior to the treatment with cisplatin and for 3 days simultaneously with cisplatin reduced the levels of the markers closer to that of untreated controls. The effect of kolaviron on the markers analyzed in the serum was dose-dependent (\( p < 0.05 \)).

**Levels of TBARS, cytokines, and antioxidant enzyme expression in liver homogenate**

Perinuclear fractions were used for the assessment of TBARS, cytokines, and antioxidant enzyme expression, and the results are shown in Figure 2.

### Table 1. Primer sequences for RT-PCR.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer sequence (5’-3’)</th>
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| SOD  | FP: GACTGAAGGCCTGCATGGATTC  
RP: CACATCGGCAACAACATCTTG  |
| CAT  | FP: CTTCGACCCAAGCAACATGC  
RP: GATAATTGGGTCGCCAGCGATG  |
| GAPDH| FP: GTGGAGTCACCGGGATTTGGTC  
RP: CTTCCTTCTCAGGCTTGAC  |

![Figure 1](effect-of-kolaviron.jpg)

Figure 1. Effect of kolaviron on the levels of (A) ALT, (B) AST, (C) ALP, (D) urea, and (E) creatinine in serum. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; Group I, untreated controls; Group II, rats treated with 100 mg/kg kolaviron only; Group III, rats given 10 mg/kg cisplatin only; Group IV, rats treated with 100 mg/kg kolaviron and cisplatin (10 mg/kg); Group V, rats treated with 200 mg/kg kolaviron and cisplatin (10 mg/kg). Each bar represents mean ± SEM (\( n = 5 \)). Bars having different superscript letters differ significantly (\( p < 0.05 \)).
It revealed that cisplatin significantly elevated the levels of TBARS and cytokines (TNF-a and IL-6) while significantly reducing the expression of SOD and CAT when compared with untreated controls \((p < 0.05)\). But, kolaviron significantly reduced the levels of TBARS and cytokines (elevated by cisplatin) closer to control values. Kolaviron also enhanced the expression of SOD and CAT (reduced by cisplatin). While the effect of kolaviron was dose-dependent for alteration of TBARS and cytokine levels, the difference between treatment with 100 and 200 mg/kg kolaviron was not significant for the expression of SOD and CAT \((p > 0.05)\).

**Levels of TBARS, cytokines, and antioxidant enzyme expression in kidney homogenate**

The effect of kolaviron on cisplatin-induced alterations in the levels of TBARS, cytokines, and antioxidant enzyme expression in kidney homogenate is shown in Figure 3. Cisplatin significantly raised the levels of TBARS and cytokines (TNF-a and IL-6), while reducing the expression of the antioxidant enzymes (SOD and CAT) when compared with controls \((p < 0.05)\). Kolaviron reduced the levels of TBARS and cytokines (elevated by cisplatin) close to control values. Kolaviron also enhanced the expression of SOD and CAT. In all cases, the effect of kolaviron was dose-dependent \((p < 0.05)\).

**Discussion**

Though the mechanism of cisplatin is largely unknown, it is thought that its action is as a result of toxic metabolites which include free filterable platinum. Multiple cellular effects of the drug include but not limited to gene regulation, apoptosis, stimulating inflammation, fibrogenesis in renal tubular cells \([22,23]\). It is believed that cisplatin form conjugates with glutathione,
cysteinylglycine, or N-acetylcysteine. These conjugates have been reported to increase the toxicity of cisplatin in LLC-PK1 cells [23]. It has been argued that cisplatin-mediated cell death may be attributed to the generation of mitochondrial reactive oxygen species that influence multiple pathways including suppression of genes of proteins of the glycolytic and tricarboxylic acid pathways [24–26]. All these may account for the elevation of the markers of liver and kidney damage. The elevation of the serum levels of ALT, AST, ALP, creatinine, and BUN could also be ascribed to the compromised membrane integrity of the liver and kidney caused by the active conjugated metabolites of cisplatin. The alteration of these markers could be a secondary event following cisplatin-induced organ damage with the concomitant leakage from hepatocytes [27]. This is further evidenced by the elevation of TBARS in liver homogenate which clearly demonstrates the involvement of reactive oxygen species in cisplatin toxicity [28,29]. Cisplatin is thought to induce reactive oxygen species (ROS) production by binding to membrane lipids (which alters membrane fluidity) and by interacting with nuclear DNA, mitochondrial DNA and protein thiols [30]. Reactive oxygen species generated following cisplatin exposure include hydrogen peroxide, hydroxyl radicals, and superoxide radicals all of which are capable of attacking various cellular targets [27]. From the research study, cisplatin also reduces the expression of SOD and catalase in both liver and kidney. This reveals the antineoplastic drug disrupts antioxidant balance which could lead to further tissue damage. It is possible that the drug might interact with their DNA in order to suppress expression [31–33].

In most tissues, production of pro-inflammatory cytokines such as TNF-α and IL-6 is low but could be elevated in some pathological conditions.
such as liver diseases, cancer, and autoimmune diseases [34,35]. In the current work, cisplatin increased the levels of TNF-a and IL-6 in both liver and kidney which agrees with the findings by a recent research study [2]. Influx of bone marrow-derived inflammatory cells to the kidney follows cisplatin-induced nephrotoxicity [36]. The cytokines are thought to be the driving force of many disorders which often results in fibrosis and cirrhosis [37].

From the research study, kolaviron significantly reduced cisplatin-mediated alteration of the serum markers and the expression of SOD and CAT. The induction of antioxidant enzyme expression by kolaviron in tissues may involve the activation of the Nrf2 pathway especially in the liver [38]. It has been suggested that kolaviron exerts its protective effect by direct antioxidation and enhancing the level of detoxifying enzymes [39]. These may play a role in the detoxification of cisplatin-conjugates which are responsible for tissue damage. It has been reported that flavonoids induce phase II enzymes by up-regulating their expression which is thought to be sufficient for promoting antioxidant and protective activities [40]. The results also further support the ability of kolaviron to scavenge ROS following the exposure to cisplatin [14,15]. Giving the animals with kolaviron alone (i.e., group 2) enhanced the expression of SOD and CAT in liver but did not produce any significant response in other indices over untreated controls (i.e., group 1) \( p > 0.05 \) revealing the non-toxic nature of the bi-flavonoid complex.

‘Excessive release of cytokines has been implicated in many inflammatory diseases and the production of reactive oxygen species is key to their progression [41–44]. It has been reported that kolaviron suppresses the inflammatory response by improving tissue resistance to oxidative stress which could involve the up-regulation of antioxidant enzymes [45]. This activity could lead to the reduction in cytokines (such as TNF-a) as evidenced in the current work. In fact, the anti-inflammatory effect of flavonoids may be mediated by the down-regulation of TNF-a production probably by regulating gene expression [46,47].

Kolaviron is a direct scavenger of free radicals and other reactive conjugates (which are produced by cisplatin) [45,48]. It is believed that flavonoids (especially kolaviron) interrupt chain reactions that take place in the membrane and may involve chelation of redox active metals and other mechanisms [49].

**Conclusion**

Kolaviron is a potent bio-flavonoid complex and highly bioavailable because it survives first-pass metabolism [50]. This current work further provides the remarkable anti-inflammatory, tissue protective, and perhaps antioxidant potential of kolaviron. Apart from the expression of SOD and CAT in the liver, the effect of kolaviron on the cisplatin-induced alterations was dose-dependent. This could be exploited pharmacologically. Other experimental models to elucidate the biochemical potential of kolaviron are in progress.

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**References**


