Chemoprotection by Kolaviron of *Garcinia kola* in Benzene-induced leukemogenesis in Wistar rats

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**ABSTRACT**

Kolaviron (KV) is a biflavonoid phytoconstituent of defatted *Garcinia kola* seeds that possessed antiproliferative and pharmacological activities. Benzene is an industrial solvent that, however, constitutes occupation hazard, leading to hematological disturbance and leukemia. Therefore, the potency of kolaviron against benzene-induced hematological and myeloid toxicity leading to leukemia was investigated in a rat model. Preleukemic conditions were induced in Wistar rats by intravenous administration of benzene solution. Following induction, 200 mg/kg kolaviron was administered orally for seven days. Hematological parameters, percentage blast cell occurrence and blood cell morphology were compared between baseline control and leukemic rats with or without kolaviron treatment. Plasma activity of arylesterase of paraoxonase-1, total thiol and advanced oxidation protein products (AOPPs) along with clastogenicity and bone marrow architecture was assessed. Kolaviron restored altered hematology, reduced the occurrence of blasts and improved blood cell morphology. Kolaviron also decreased levels of AOPPs, increased total thiol, improved arylesterase activity and mitigated clastogenicity and dysplasia induced in leukemic rats. In conclusion, kolaviron protected against benzene-induced hematological and myeloid toxicities that are implicated in leukemia.

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**Introduction**

The global impact of hematopoietic malignancy and cancer at large on the health of people and economy of nation is becoming more worrisome [1]. Hematopoietic malignancies are diverse groups of disorders that include plasma cell tumors, lymphomas, myelodysplastic syndromes or myelodysplasia (MDS), mastocytosis and leukemias. The etiology of hematopoietic malignancies such as myelodysplastic syndromes and leukemia includes the exposure to environmental factors such as benzene, radiation and some chemotherapies [2]. Myelodysplasia is a hematopoietic disease that has its origin in the hematopoietic stem and progenitor cell compartment with variable degrees of cytopenias, morphological dysplasia and risk of progression to acute myeloid leukemia [3]. The symptoms of myelodysplasia include anemia, cytopenias, morphological dysplasia of precursor and mature bone marrow blood cells [4,5]. Myelodysplastic syndromes also called myelodysplasia are usually referred to as the premalignant condition as incurrence of additional genetic abnormalities may lead to the transformation of MDS into acute myeloid leukemia (AML). Leukemia is majorly a cancer of the white blood cells and bone marrow and present in most common form of cancer in children worldwide where it remains the second leading cause of death in children [6,7]. Currently among males, it is the 10th most commonly diagnosed cancer and 8th leading cause of male cancer mortality in 2020 [1].

Most cancer chemotherapy regimens and even the remission-induction therapy for the treatment of leukemia are accompanied by severe side effects besides their chemotherapeutic efficacy [8,9]. There is a need for modified treatment and intensive assessment of cytotoxic agents in the field of oncology [10] because many cytotoxic agents conferred severe side effects during the course of treatment [11,12]. Most commonly, some cancer chemotherapeutic agents are radiomimetic in nature especially alkylating agents affecting hematology, bone marrow cellularity and effective dysplasia formation in myeloid tissue, which may ultimately result in therapy-related myelodysplasia or acute myelogenous leukemia [13]. Therefore, leukemia burden has led to increased research in isolation and identification of more cytotoxic agents [14,15]. Considerably, herbal medicine that presents natural compounds of sufficient chemotherapeutic effect with little or no side effects may be investigated for cancer chemotherapy. One such natural compound is kolaviron, which is a biflavonoid isolate of the seed of *Garcinia kola* extract. It is a defatted fraction of *Garcinia kola* seed with valuable major constituents such as Garcinia biflavonoids GB1 and GB2 and kolaflavone [16,17]. It has organ protective capability [18], improved hematological indices and offered immunity boosting effects [19]. The safety profile of kolaviron, its antioxidant properties and antiproliferative capacity have been extensively studied in *vitro* and *in vivo* [20–21]. Moreover, it is known to offer protection against xenobiotic and chemical-induced oxidative stress-mediated toxicities in experimental murine models [22,23]. Therefore, the present work investigated the myeloprotective effect of kolaviron on benzene-induced bone marrow dysplasia in Wistar rats.

**Materials and methods**

**Extraction and isolation of kolaviron from Garcinia kola**

Kolaviron was extracted and isolated from the seeds of *Garcinia kola* following the procedure of Iwu et al. with slight modification [16]. The seeds of *Garcinia kola* were peeled then sliced, air-dried and ground into powder. The
powdered seeds were extracted with n-hexane to obtain a defatted marc, which was dried and subsequently extracted with methanol. The KV fraction, which gives a golden yellow solid, was obtained from methanol extract by the twin purification process of dilution using chloroform.

**Experimental animals**

Twenty four adult male Wistar strain rats of weight range 90–100 g were used for this study. The animals were obtained from the Department of Physiology University of Ibadan and acclimatized for 14 days in the animal house of the Department of Chemical Sciences, Ajayi Crowther University, Oyo. They were housed in plastic cages and fed with standard rat feed and clean tap water *ad libitum*. The designed work was conducted with the approval of the Faculty of Natural Sciences Ethical review of Ajayi Crowther University, Oyo with approval code: Fns/Erc/2,019,003 and the protocol conformed to the guidelines of the National Research Council for laboratory animal care and use [24].

**Animal treatments and groupings**

After 14 days of acclimatization, preleukemic conditions were induced in 90–100 g rats following the procedure of Akanni et al. [25] by intravenous injection of 0.2 ml of benzene solution (1:5:5 of benzene/2-propanol/distilled water v/v) every 2 days for 4 consecutive weeks. Following induction, 24 rats, which comprise 12 leukemic rats with traces of appearance of cell blasts in peripheral blood film (Figure 1) and 12 normal baseline control, were assigned into four experimental groups of 6 animals each as follows: Group CTRL, normal baseline rats; Group KV, normal baseline rats that received kolaviron (200 mg/kg) for 7 days, Group LKM, rats with benzene-induced preleukemic condition that received 0.2 ml intravenous injection of benzene:2-propanol:water mixture (1:5:5 v/v) for four consecutive weeks and Group LKM + KV, preleukemic rats that received kolaviron (200 mg/kg) for 7 days.

**Collection of blood and bone marrow**

After 24 hours of final treatment, blood samples were collected from each animal through retro-orbitals plexus into lithium heparinized tubes for biochemical assays and ethylene diaminetetraacetic acid (EDTA) bottles for hematological parameters such as hematocrit, total white blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin (Hb) and platelet counts estimation using the automated blood analyzer (SYSMEX KX21) and thereafter sacrificed. The femur bones were excised to obtain bone marrow for micronucleus assay and hematoxylin and eosin staining for histopathological examination.

**Assay for oxidative stress markers in the plasma**

Plasma AOPP was determined by the method described by Witko et al. [26] as modified by Zhang et al. [27]. Briefly, plasma (100 μl) was added to 400 μl of phosphate buffer saline (PBS) solution and 25 μl of 1.16 M potassium iodide was then added followed 2 min later by 50 μl of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 500 μl of PBS, 25 μl of 1.16 M potassium iodide and 50 μl of acetic acid. Plasma total thiol was measured spectrophotometrically using DTNB (2,2′-dinitro-5,5′-dithiodibenzonic acid) [28]. Arylesterase activity was determined using phenylacetate as the substrate following the procedure described by Erdem et al. [29].
Clastogenicity in preleukemic rats was evaluated in the bone marrow of the rats employing the micronucleus assay techniques as described by Heddle and Salmone [30], with modification by Heddle, et al. [31]. Briefly, bone marrow from femurs of rats was used for preparation of slides using the standard procedure by Matter and Schmid [32].

Statistical analysis

Data are presented as the mean ± standard deviation (SD) of six replicates. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan’s multiple comparison between control and treated rats in all groups using SigmaPlot® statistical package (Systat Software Inc., San Jose, CA, USA). P-values less than 0.05 (P < 0.05) were considered statistically significant.

Results

Effect of kolaviron on hematological parameters and blood morphology of benzene-induced leukemic rats

The effect of kolaviron on hematological parameters and blood morphology of benzene-induced leukemic rats is shown in Table 1. There were a decrease in the packed cell volume (PCV), erythrocyte counts and hemoglobin contents and a significant increase in WBC counts in leukemic rats when compared to the baseline control. Also, the peripheral blood film of the leukemic rats shows the presence of poikilocytosis, anisocytosis and appearance of blast. Upon treatment with kolaviron, the alteration in hematological parameters was normalized and the blast population was reduced when compared to untreated leukemia control.

Influence of kolaviron on bone marrow architecture of Benzene-induced leukemia in Wistar rats

The influence of kolaviron on bone marrow architecture of benzene-induced leukemia in Wistar rats is shown in Figure 2. Leukemic rats (LKM group) showed a hypercellularity in bone marrow tissue with severe dysplasia. However, in leukemic rats supplemented with kolaviron, the effect of benzene was alleviated, resulting in mild dysplasia observed in (KV + LKM) group.

Effect of kolaviron on the formation of Micronucleated polychromatic erythrocytes (mPCEs) in Benzene-induced Leukemia in Wistar rats

The influence of kolaviron on bone marrow formation of micronucleus on Benzene-induced leukemia in Wistar rats is shown in Figure 3. Leukemic rats showed a significant
increase in the frequency of micronucleus present in the bone marrow by 408.24% when compared to the control group. However, kolaviron supplementation in leukemic rats significantly alleviated the effect of benzene-induced genotoxicity by reducing the bone marrow micronucleus occurrence by 55.55% when compared with the leukemia control (LKM) group.

**Table 1. Effect of kolaviron on hematological parameters and blood morphology of benzene-induced leukemia in rats.**

|        | PCV % | HGB g/dl | RBC x 10¹²/L | PLT x 10⁹/L | WBC x 10⁹/L | % Blast | ANISD | POIK |
|--------|-------|----------|---------------|--------------|--------------|---------|-------|------|
| CTRL   | 45.33 | ± 0.52   | 15.00         | 7.60         | 649.67       | 9.17    | -     | -    |
| LKM    | 41.75 | ± 2.22   | 13.45         | ± 0.32       | ± 57.55      | ± 1.96  | 5     | ++   |
| KV     | 51.33 | ± 1.53   | 16.83         | ± 0.70       | ± 131.00     | ± 2.14  | -     | -    |
| KV + LKM | 44.67 | ± 5.51   | 14.83         | ± 0.25       | ± 32.36      | ± 0.85  | 2     | ++   |

PCV- Pack cell Volume, HGB- Hemoglobin, RBC- Red blood cell, PLT- Platelets, WBC- White blood cell, ANISD- Anisocytosis, POIK-Poikilocytosis
- = absent, + = mildly present and ++ = moderately present

**Effect of kolaviron on plasma oxidative stress status in Leukemic rats**

As presented in Figure 4, a significant decrease by 49.59% and 30.06% in plasma activity of arylesterase and total thiol content were observed, respectively, following leukemia induction with corresponding significantly elevated advanced oxidation protein products present in the blood plasma by 63.32% when...
compared to control. However, administration of kolaviron significantly ameliorated the decrease in plasma activity of arylesterase and total thiol content and increase in AOPPs (Figure 2).

Discussion
Routine hematological assessment is a diagnostic tool for monitoring the clinical status of blood-related conditions such as leukemia. The leukemogenic effect of benzene has been linked to hematological imbalance in human and animal models where reduced red blood cells, hemoglobin contents, packed cell volumes and platelets counts are common pictures [33,34].

Reductions in PCV, RBC count and Hb level were observed in rats induced with leukemic condition by benzene when compared to baseline rats. Abnormal occurrences of poikilocytosis and anisocytosis were also noted in leukemic rats relative to the baseline animals. The decrease in PCV, RBC count, Hb level and observable deformability in erythrocytes are reliable indications of anemia in animals [35]. A study has shown that hydroquinone impairs the maturation of granulocytes [36]. Moreover, exposure to hydroquinone was reported to induce neutrophilia, which may probably be due to intense mobilization of segmented cells from the bone marrow, leading to increased numbers of neutrophils at the peripheral compartment [37]. The increase in white blood cell counts was observed in leukemic rats in this study, which supported the earlier finding that related leukocytosis to the leukemic condition [25]. The occurrence of blasts in the peripheral blood film of leukemic rats in the present study is an indication of undifferentiated blood-forming cells in the blood mobilized from the marrow that had been associated with leukemia [38]. Kolaviron has been shown to cause an increase in red blood cell (RBC) counts and hemoglobin and hematocrit concentrations [39,40] due to its effectiveness as antioxidants in red cell survival and viability [41]. This is reflected in this present study where treatment with kolaviron offered a restoration of reduced Hb level, total WBC and RBC in benzene-exposed rats toward the normal level of control with concomitant reduction in the frequency of the blasts in the leukemic rat model when

**Figure 3.** Effect of kolaviron on formation of micronucleated polychromatic erythrocytes (mPCEs) in benzene-induced leukemia in wistar rats. Data are expressed as mean ± S.D for six rats in each group *significantly different from the control p < 0.05" significantly different from the LKM group
compared to leukemia control. This study also presented cross sectional examination of bone marrow severe dysplasia and hypercellularity, which were observed in the leukemic rats. Dysplasia occurrence in bone marrow is a morphological observation that has been linked to newly diagnosed acute myelogenous leukemia [42,43]. However, the supplementation of leukemic rats with kolaviron isolate reduced the dysplasia occurrences in the bone marrow tissue of the treated rats.

Myelodysplasia has been suggested to be a significant step in the generation of leukemia by benzene [36]. Benzene metabolite hydroquinone was earlier reported to promote proliferation and differentiation of the myeloblast into the myelocyte stage but inhibited the maturation of myelocyte into neutrophil [44]. The mutation of the clone of myelocytes without subsequent DNA repair may be further proliferated and promote the development of leukemia [45]. Reactive metabolites generated during benzene biotransformation can induce genotoxicity and cytotoxicity through diverse mechanisms [46–48]. The study had reported involvement of benzoquinones and other benzene reactive oxygen metabolites in the induction of oxidative DNA damage, lipid peroxidation and strand breaks in the DNA of bone marrow cells in benzene-induced toxicity [49–51]. The result of this study indicated the significant induction of clastogenicity in the marrow of the rats exposed to benzene as shown by generation of significantly high
occurrence of micronucleated polychromatic erythrocyte in the marrow of leukemic rats. However, administration of kolaviron significantly ameliorated the benzene-induced clasto-
genicity in the leukemic rats.

The induction of oxidative stress by benzene and its subsequent hematotoxicity had been reviewed [52]. The present work assesses the plasma redox status markers such as arylesterase activity, total sulhydryl and advanced oxidation protein products of leukemic rats to investigate a link between onco-hematological diseases and oxidative stress. Prolonged oxidative stress has been associated with the occurrence of tumors [53]. The result showed a decrease in the plasma total thiol content with a concomitant increase in advanced oxidation protein products in leukemic rats relative to normal control. Advanced oxidation protein products (AOPPs) are oxidized protein products of oxidative stress, which resulted from chlorinated oxidants on plasma proteins [26,54], and its elevated level has been reported in some pathological conditions such as chronic kidney disease, diabetes, uremia and rheumatoid arthritis [55–57]. The thiol group present on protein especially over albumin constitutes the major in vivo antioxidant and reducing group in the body fluid [58]. Therefore, antioxidant status is indicated by the level of total thiol where the low protein thiol content correlates with an increase in peroxides and advanced oxidation protein products [58,59]. The increase in AOPP and low protein content in this study suggests the induction of oxidative stress due to benzene reactive metabolites that consequently led to oxidant-mediated protein damage [60]. However, supplementation of kolaviron in this study significantly alleviated the increased AOPP level and restored the total thiol status when compared to the leukemia control group. Moreover, in this study, there was a significant reduction in the plasma activity of arylesterase in the leukemic rats when compared to control. Arylesterase of paraoxonase 1 (PON 1) is an esterase enzyme that possesses lipophilic antioxidant characteristics [61]. The susceptibility and occurrence of some cancers like prostate, breast and hematologic cancers have been linked to PON1 polymorphism [62,63]. However, the reduction in arylesterase activity was mitigated on treatment with kolaviron when compared with the leukemia control group. Therefore, kolaviron may reduce the tendency of the development of the leukemia through enhanced activity of arylesterase of paraoxonase 1.

Overall, kolaviron of *Garcinia kola* protected against hematological and myeloid assault in benzene-induced bone marrow dysplasia in Wistar rats through improvement on plasma antioxidant status.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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