

Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural biflavonoid of *Garcinia kola* seeds

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The chemopreventive effects of kolaviron, a natural anti-oxidant biflavonoid from the seeds of *Garcinia kola*, on aflatoxin B1 (AFB1)-induced genotoxicity and hepatic oxidative damage was investigated in rats. Kolaviron administered orally at a dose of 200 mg/kg once a day for the first 2 weeks and then 100 mg/kg twice a day for the last 4 weeks of AFB1 (2 mg/kg, single dose, intraperitoneal) treatment reduced the AFB1-increased activities of aspartate amino transferase (AST), alanine amino transferase (ALT) and gamma glutamyltransferase (γ -GT) by 62%, 56% and 72% respectively. Malondialdehyde (MDA) formation and lipid hydroperoxide (LHP) accumulation were observed in the livers of AFB1-treated rats. Kolaviron significantly reduced the AFB1-induced MDA and LHP formation. Vitamins C and E were protective in reducing the increase in the activities of AST, ALT and γ -GT as well as lipid peroxidation caused by AFB1 ($P < 0.01$). Administration of rats with kolaviron alone resulted in significant elevation in the activities of glutathione S-transferase, uridyl glucuronosyl transferase and NADH:quinone oxidoreductase by 2.45-, 1.62- and 1.38-folds respectively. In addition, kolaviron attenuated the AFB1-mediated decrease in the activities of these enzymes ($P < 0.01$). Pretreatment of rats with kolaviron, vitamins C and E alone did not exert genotoxicity assessed

by the formation of micronucleated polychromatic erythrocytes (MNPCEs) ($P > 0.05$). Co-treatment of rats intraperitoneally with kolaviron (500 mg/kg) 30 min before and 30 min after AFB1 (1 mg/kg) administration inhibited the induction of MNPCEs by AFB1 ($P < 0.001$) after 72 h. While vitamin C was effective in reducing AFB1-induced MNPCEs formation, vitamin E did not elicit any antigenotoxic response. These results indicate kolaviron as effective chemopreventive agent against AFB1-induced genotoxicity and hepatic oxidative stress. Thus kolaviron may qualify for clinical trial in combating the menace of aflatoxicosis in endemic areas of aflatoxin contamination of foods. *European Journal of Cancer Prevention* 14:207–214 © 2005 Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2005, 14:207–214

Keywords: Aflatoxin B1, antioxidants, chemoprevention, *Garcinia kola*, genotoxicity, hepatotoxicity, kolaviron, micronuclei

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Received 13 October 2003 Accepted 18 June 2004

Introduction

Aflatoxin B1 (AFB1), a potent hepatotoxic and hepatocarcinogenic agent, is produced by the mould *Aspergillus flavus*, which contaminates cereal grains and nuts in tropical regions of the world (Eaton and Gallagher, 1994). AFB1 and hepatitis B have been implicated in the aetiology of hepatocellular carcinoma, which has one of the poorest 5-year survival rates (Groopman *et al.*, 1992) and accounts for 15% of total cancer mortality (Premalatha and Sachdanandam, 2000).

It is generally believed that AFB1 is activated mainly by the cytochrome P450 group of enzymes to form the reactive intermediates AFB1-8,9-epoxide (AFBO) (Gallagher *et al.*, 1996). The subsequent covalent binding of this epoxide to nucleophilic centres in cellular macromolecules, forming *N*⁷-guanyl adducts with DNA, is considered to be critical in the carcinogenicity of AFB1 (Eaton and Gallagher, 1994). *N*⁷-Guanyl AFB1 has been detected in human populations with a high incidence of liver cancer (Autrup *et al.*, 1983).

It has been reported that AFB1 may disturb the integrity of cell membranes through stimulating phospholipid A₂ to initiate lipid peroxidation in cells (Amstad and Cerutti, 1983). Subsequently AFB1 was shown to induce formation of reactive oxygen species (ROS) (Shen *et al.*, 1996), lipid peroxidation (Shen *et al.*, 1994, 1995b) and formation of 8-hydroxydeoxyguanosine (8-OH dG) *in vivo* and *in vitro* (Shen *et al.*, 1995a). Considering the role of ROS in chemically induced carcinogenesis, the ability of AFB1 to induce oxidative damage to cells and DNA may, in addition to the formation of AFB1–DNA adducts, play an important role in AFB1 carcinogenicity (Yang *et al.*, 2000).

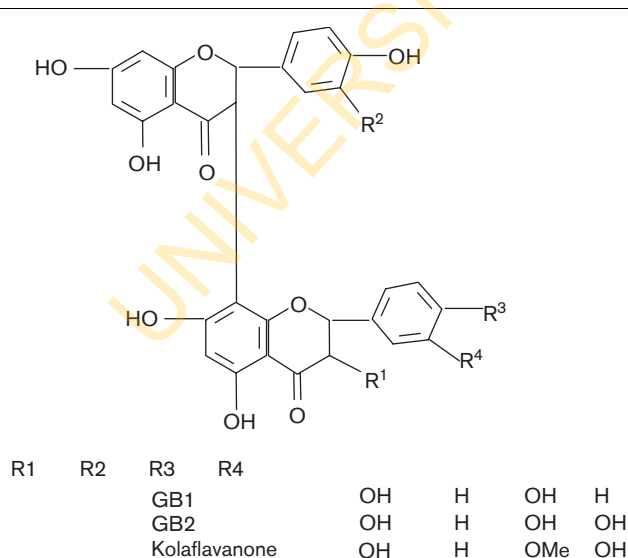
Chemopreventive and chemoprotective strategies designed to limit both exposure to and the adverse health effects from AFB1 are important public health goals in reducing the incidence of AFB1-induced neoplastic diseases. Since the complete elimination of exposure to AFB1-producing moulds is not possible, chemoprevention is an attractive strategy to protect individuals from the

risk of liver cancer caused by exposure to the mycotoxin (Kelly *et al.*, 2000).

Garcinia kola Heckel (Guttiferae) is a commonly cultivated tree in West and Central Africa and is highly valued for its edible nuts (Hutchinson and Dalziel, 1956). The seed commonly known, as 'bitter kola' is widely eaten and culturally acceptable in Nigeria. Extracts of the plant are employed in African herbal medicine for the treatment of ailments such as laryngitis, liver diseases (Iwu, 1982), cough and hoarseness of voice (Ayensu, 1978).

Kolaviron, a fraction of the deflated ethanol extract, containing *Garcinia* biflavonoid GB-1 (3'',4',4''',5,5'',7,7''-heptahydroxy-3,8'' biflavanone), GB-2 (3'',4',4''',5,5'',5''',7,7''-octahydroxy-3,8''-biflavanone) and kolaflavanone (3'',4',4''',5,5'',5''',7,7''-octahydroxy-4''-methoxy-3,8''-biflavanone) (Fig. 1) has been reported to significantly prevent hepatotoxicity mediated by galactosamine, amanita toxin (Iwu *et al.*, 1987), paracetamol (Akintonwa and Essien, 1990) and thioacetamide (Iwu *et al.*, 1990) in experimental animal models. Results of investigations in our laboratory have revealed the protective effects of kolaviron against hepatotoxicity and oxidative stress induced by 2-acetylaminofluorene and carbontetrachloride via antioxidant mechanisms and enhancement of xenobiotic-detoxifying enzymes (Farombi *et al.*, 2000; Farombi, 2000, 2003). We have also recently demonstrated the analgesic, anti-inflammatory and nephroprotective potentials of kolaviron (Olaleye *et al.*, 2000; Farombi *et al.*, 2002a,b). Although, our research group recently reported the inhibitory effects of kolaviron on AFB1-induced genotoxicity in human liver-derived HepG2 cells (Nwankwo *et al.*, 2000), protective effects on *in vivo* AFB1-induced carcinogenicity have not been demonstrated.

Fig. 1



Structure of kolaviron.

Accumulating epidemiological and compelling experimental evidence has revealed the influence of a number of naturally occurring and synthetic compounds on the incidence of AFB1-induced neoplasia (Primiano *et al.*, 1998; Premalatha and Sachdanandam, 2000). Using the rat as an experimental model, the phenolic antioxidants Butylated hydroxyanisole BHA and Ethoxyquin EQ and dithiolethione oltipraz have been reported to inhibit AFB1 hepatocarcinogenesis (Kelly *et al.*, 2000). At present, the antischistosomal drug oltipraz is being investigated as a chemopreventive agent against AFB1 hepatocarcinogenesis in humans (Wang *et al.*, 1999) but alternative therapies for this purpose are desirable. Moreover, since the increase in the use of synthetic chemicals in cancer therapy has led to many toxic effects, there is a worldwide trend to exploit naturally occurring plant sources which are therapeutically effective, culturally acceptable and economically within the reach of common people.

The present study was therefore designed to investigate the ability of kolaviron, a natural antioxidant and biflavonoid derived from the seeds of *Garcinia kola* to mitigate AFB1-induced genotoxicity and hepatotoxicity in an *in vivo* rat model.

Materials and methods

Chemicals

Aflatoxin B1, glucose 6-phosphate, *p*-nitroanilide, glycylglycine, UDP-glucuronic acid, NADP, NADH, NADPH, glutathione (GSH), 1-chloro-2,4-dinitrobenzene, α -tocopherol and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). Ascorbic acid and α -ketoglutaric acid were obtained from BDH Chemicals Ltd (Poole, Dorset, UK). All other reagents were of analytical grade and were obtained from BDH Chemicals.

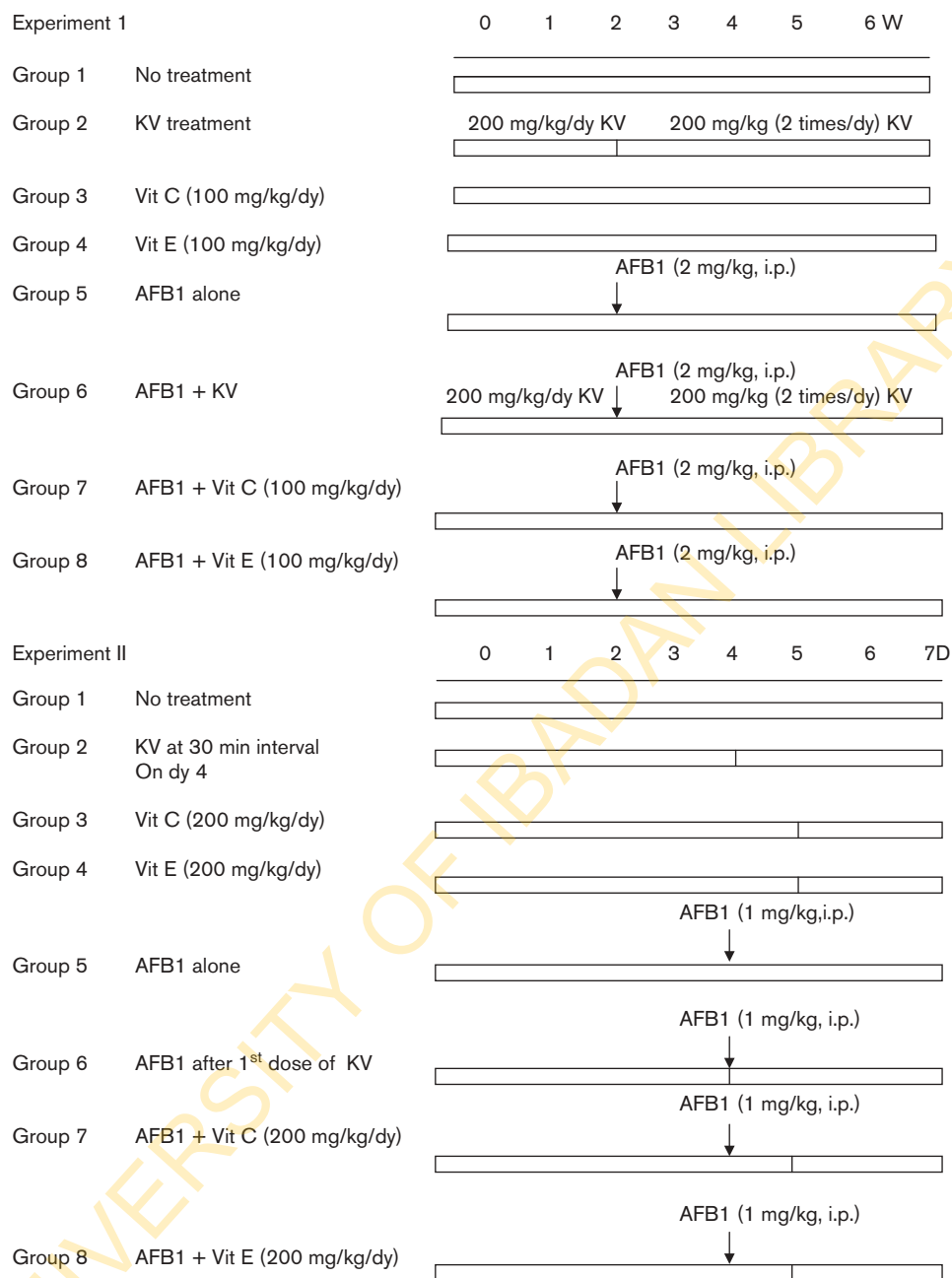
Extraction of kolaviron

Garcinia kola seeds purchased from a local market in Ibadan, Nigeria were certified at the herbarium in the Department of Botany, University of Ibadan, Nigeria. Five kilograms of the peeled seeds were sliced, pulverized with an electric blender and dried at 40°C in a Gallenkamp drying oven and then extracted according to the published procedure (Iwu, 1985). Briefly, the powdered seeds were extracted with light petroleum ether (bp 40–60°C) in a soxhlet 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 extracted with ethylacetate (6 × 300 ml). The concentrated ethylacetate yielded kolaviron as a golden yellow solid (Fig. 1).

Animals and treatment

The experimental protocols for the two experiments (hepatotoxicity and genotoxicity) we performed are shown in Figure 2.

Fig. 2



Experimental design for studying the effects of Kolaviron on AFB1-induced genotoxicity and hepatic oxidative damage. D=days; W=weeks; KV=Kolaviron; AFB1 (aflatoxin B1) administration.

Hepatotoxicity studies

Forty (40) male Wistar albino rats weighing (118–124 g) were randomly distributed into eight groups. The rats were used after 1 week of acclimatization. Group 1 served as control and was fed normal laboratory chow purchased from Ladokun Feeds, Ibadan, Nigeria. Rats in groups 2 and 6 were treated orally with 200 mg/kg kolaviron once a day for the first two weeks and then 100 mg/kg kolaviron twice a day for the last four weeks. Rats in groups 5, 6, 7 and 8 were given a single dose of AFB1 (2 mg/kg, i.p.) for

the last 4 weeks of the 6-week experimental period. Animals in groups 3 and 7 were treated orally with 100 mg/kg per day vitamin C, while rats in groups 4 and 8 were treated with 100 mg/kg per day vitamin E for 6 weeks. Animals were maintained on a 12 h light/dark cycle. Body weight and feed consumption were measured daily.

Preparation of microsomal and cytosolic fractions

The rats were sacrificed by cervical dislocation and livers were quickly removed, washed in 1.15% KCl, dried and

weighed. The liver was homogenized in 4 volumes of isotonic phosphate buffer, pH 7.4 and centrifuged at 9000 g for 20 min to obtain the postmitochondrial supernatant fraction. Microsomes were pelleted at 100 000 g for 90 min by subsequent centrifugation. The supernatant (cytosolic fraction) was immediately frozen on dry ice. Microsomes were resuspended in 0.25 mol/l sucrose solution. Aliquots of this suspension were stored at -80°C and thawed before use. All procedures were carried out at temperatures between 0 and 4°C .

Preparation of serum

Blood was collected by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10 min at 3000 g in an MSC bench centrifuge. The clear supernatant was used for the estimation of serum enzymes.

Protein determination

Protein content of serum and microsomal fractions were determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard.

Serum enzyme assay

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using a combination of the methods of Mohun and Cook (1957) and Reitman and Frankel (1957). γ -GT activity was assayed by following the *p*-nitroaniline released from γ -glutamyl-*p*-nitroanilide in the presence of glycyl glycine as glutamyl acceptor at 405 nm (Szasz, 1969).

Phase II drug metabolizing enzymes

Microsomal uridyl diphosphoglucuronosyltransferase (UDP-GT) activity was assayed by following the disappearance of *P*-nitrophenol upon glucuronidation as described by Gibson and Skett (1994).

Cytosolic glutathione *S*-transferase (GST) activity was determined by the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The reaction mixture (3 ml) contained 1.7 ml of 100 mmol/l phosphate buffer (pH 6.5), 0.1 ml of 30 mmol/l CDNB. After preincubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 ml diluted cytosol and the absorbance was followed for 5 min at 340 nm. Reaction mixture without the enzyme was used as blank. The specific activity of GST is expressed as nmoles of GSH-CDNB conjugate formed/min per mg protein using an extinction coefficient of 9.6 l/(mmol.cm).

NAD(P)H:quinone oxidoreductase (NQO) activity was measured as described by Singh *et al.* (2000) with NADH as the electron donor and 2,6-dichlorophenol-indophenol (DCPIP) as the electron acceptor at 600 nm. The activity was calculated using extinction coefficient of 21 l/(mmol.cm). One unit of enzyme activity is defined as amount of enzyme required to reduce 1 μ mole of DCPIP per min.

Determination of lipid peroxidation

Lipid peroxidation was evaluated as lipid hydroperoxide and malondialdehyde formation in rats. Lipid hydroperoxides (LHP) were determined using the thiocyanate method according to Cavallini *et al.* (1983). The TBA reacting substances (TBARS) assay was performed as described previously (Farombi *et al.*, 2000). Malondialdehyde (MDA) was quantitated by using $\Sigma = 1.56 \times 10^5$ l/(mol.cm) (Buege and Aust, 1978).

Genotoxicity studies

For the genotoxicity studies, a preliminary time-course and dose-response study was carried out. Rats were treated with a single i.p. administration of AFB1 at doses of 0.5, 1.0 and 1.5 mg/kg body weight. The animals were sacrificed at 0, 24, 48, 72 and 96 h and micronuclei assay was conducted. Control rats received an i.p. injection of saline alone.

In order to evaluate the chemopreventive potential of kolaviron and other antioxidants on AFB1 genotoxicity, 24 male rats were divided into eight groups of three rats per group. Group 1 served as control. Rats in groups 2 and 6 received double doses of kolaviron (500 mg/kg, i.p.) at 30-min interval. Animals in groups 3 and 7 were treated daily orally with vitamin C (200 mg/kg) for 5 days while rats in groups 4 and 8 were treated with vitamin E (200 mg/kg) orally for 5 days. Animals in groups 5, 6, 7 and 8 received AFB1 (1 mg/kg body weight, i.p.) after the first dose of kolaviron (group 6 only) and on the 4th day of vitamins C and E administration. Rats were sacrificed at 72 h after AFB1 treatment.

Bone marrow preparation and micronuclei assay

Bone marrow smears were prepared and micronucleus test carried out according to Asanami and Shimono (2000). Briefly, the femurs of each rat were removed and stripped clean of muscle. A syringe was then introduced into the marrow canal at the epiphyseal end and the marrow was flushed out through the hole at the iliac end with fetal bovine serum. The bone marrow was placed on a slide and mixed to obtain a homogeneous mixture and spread as a smear. After the slides were dried, they were fixed in absolute ethanol for 5 min and air dried to remove the solvent. The slides were stained in 5% Giemsa for 30 min and rinsed in phosphate buffer pH, 7.4 for 30 s and distilled water for 2 min and air dried. The slides were coded and screened to avoid bias and scored using a compound microscope with the aid of tally counter for the presence of micronucleated polychromatic erythrocytes.

Statistics

All variables were tested for normal distribution using the Kolmogorov-Smirnov test ($P > 0.05$) and for homogeneity of variance among groups using the Levene's test ($P > 0.05$). The groups were compared using one-way ANOVA. If significant differences were found ($P < 0.05$), the treatment groups were compared with the control

group using Dunnett's test. All the statistics were carried out in SAS (SAS System for windows, v8; SAS Institute Inc, Cary, NC, USA).

Results

Inhibition of AFB1-mediated hepatotoxicity by kolaviron

Figure 3 shows the effect of kolaviron, vitamin C and vitamin E on the activities of serum enzymes of rats treated with 2 mg/kg body weight (single dose) of AFB1. As expected, AFB1 significantly increased the activities of rat serum AST, ALT and γ -GT compared with controls. Kolaviron administered before, during and after AFB1 treatment reduced the AFB1-increased activities of AST, ALT and γ -GT by 62%, 56% and 72% respectively. Similarly vitamin C and vitamin E ameliorated the increase induced by AFB1 significantly.

Prevention of AFB1-induced oxidative stress by kolaviron

The results presented in Figure 4 indicate the effect of kolaviron, vitamins C and E on rat liver lipid peroxidation induced by AFB1. Kolaviron ($P < 0.001$) attenuated the increase in the formation of malondialdehyde and lipid hydroperoxide induced by AFB1. Likewise vitamin C and the radical chain breaking antioxidant vitamin E significantly reduced the increase in MDA and LHP induced by AFB1 (Fig. 4).

Induction of carcinogen detoxifying enzymes by kolaviron

Figure 5 shows the effects of kolaviron, vitamins C and E on the activities of drug detoxifying enzymes following treatment with AFB1. In rats treated with kolaviron alone, there were significant increases in the activities of GST, UDP-GT and NQO by 2.45-, 1.62- and 1.38-fold

respectively. Also kolaviron augmented the decrease mediated in the activities of these enzymes by AFB1. Although, vitamins C and E had no inductive effects on these enzymes but attenuated the AFB1 decrease in their activities ($P < 0.01$) (Fig. 5).

Time-course and dose-response genotoxic study

Treatment of rats with AFB1 at the tested doses of 0.5, 1.0 and 1.5 mg/kg induced significant formation of MNPCEs at 24, 48, 72 and 96 h (data not shown). The maximum frequency of MNPCEs (0.86%) was observed at a dose of 1.0 mg/kg 72 h after treatment. While the other doses of 0.5 mg/kg and 1.5 mg/kg induced micronuclei, the frequencies (0.71% and 0.75%, respectively) were lower than that at the 1.0 mg/kg dose (0.86%). Therefore, the dose of 1.0 mg/kg and sampling time of 72 h were used in the study.

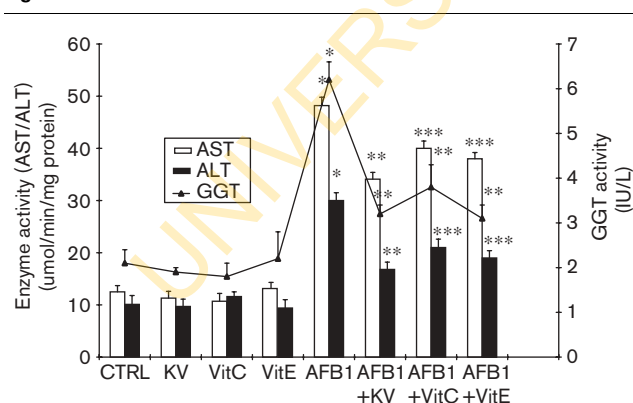
Antigenotoxic potential of kolaviron

The effects of kolaviron, vitamins C and E on AFB1-induced formation of MNPCEs are shown in Figure 6. Kolaviron, vitamins C and E treatment did not induce MNPCEs. Treatment of rats with kolaviron significantly inhibited the induction of MNPCEs by AFB1 ($P < 0.001$) after 72 h. While vitamin C significantly inhibited AFB1-induced MNPCE formation, vitamin E exerted no inhibitory effects.

Discussion

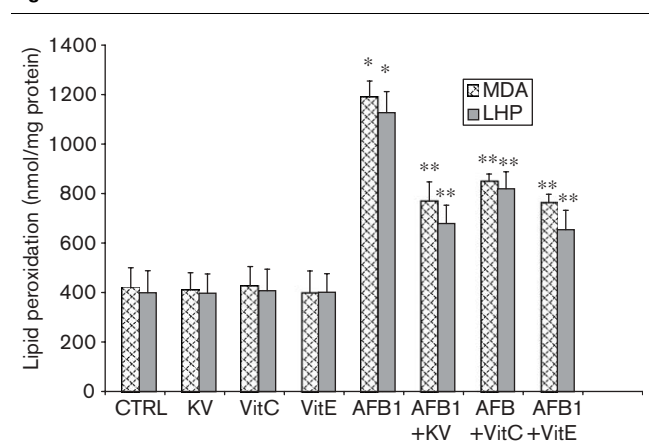
Chemoprevention is an attractive and valuable approach to improve public health in areas of the world where AFB1-producing *A. flavus* mould is prevalent. The risk of developing hepatic diseases from AFB1 exposure is highest in developing countries. The identification of naturally occurring modulators of AFB1 hepatocarcinogenesis that can be incorporated into the human diet at

Fig. 3



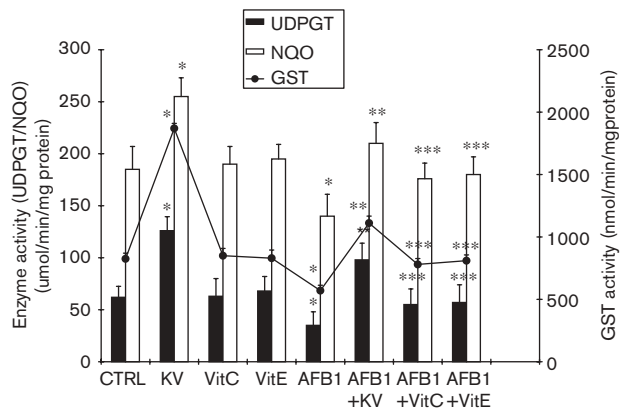
Effects of kolaviron, vitamins C and E on the activities of serum alanine aminotransferase (ALT), aspartate amino transferase (AST) and gamma glutamyltransferase (γ -GT) of rats treated with aflatoxin B1 (AFB1). * $P < 0.001$ significantly different from control; values are mean \pm SD for five rats in each group. ** $P < 0.01$ significantly different from AFB1 group; *** $P < 0.05$ significantly different from AFB1 group.

Fig. 4



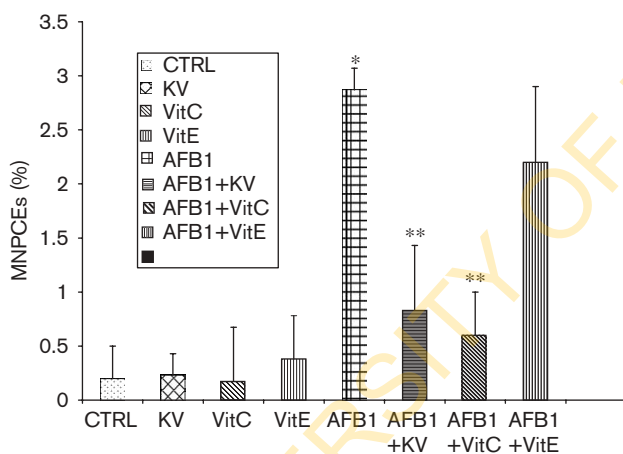
Effects of kolaviron, vitamins C and E on aflatoxin B1 (AFB1)-induced formation of rat liver malondialdehyde (MDA) and lipid hydroperoxide (LHP). Values are mean \pm SD for five rats in each group. * $P < 0.001$ significantly different from control; ** $P < 0.01$ significantly different from AFB1 group.

Fig. 5



Effects of kolaviron, vitamins C and E on the activities of uridyl glucuronosyltransferase (UDP-GT), NADH:quinone oxidoreductase (NQO) and glutathione S-transferase (GST) of rats treated with aflatoxin B1 (AFB1). Values are mean \pm SD for five rats in each group. * $P < 0.001$ significantly different from control; ** $P < 0.01$ significantly different from AFB1 group; *** $P < 0.05$ significantly different from AFB1 group.

Fig. 6



Effects of pretreatment with kolaviron, vitamins C and E on the frequency of occurrence of micronucleated polychromatic erythrocytes (MNPCEs) in rats treated with aflatoxin B1 (AFB1). Values are mean \pm SD for five rats in each group. * $P < 0.001$ significantly different from control; ** $P < 0.01$ significantly different from AFB1 group.

minimal cost would be relevant in such areas, where economic limitations mean that the majority of the populace are unable to purchase prophylactic drugs.

Coincidentally, the edible *Garcinia kola* nut from which kolaviron is obtained occupies a prominent position in the social customs of the people in Nigeria and other parts of West Africa. In spite of its very bitter taste, this nut is eaten as a refreshing pastime and offered to guests at homes and shared at social gatherings. Several experi-

mental studies have demonstrated the antioxidant and antihepatotoxic properties of kolaviron in various experimental models (Iwu *et al.*, 1990; Akintonwa and Essien, 1990; Farombi *et al.*, 2000, 200b; Farombi, 2000). Recently, our research group showed that kolaviron could protect against AFB1-induced genotoxicity in human liver-derived HepG2 cells (Nwankwo *et al.*, 2000). In the present study, we further demonstrate *in vivo* that kolaviron can inhibit AFB1-induced micronuclei induction, hepatotoxicity and oxidative stress in rats.

In this study, liver cell damage caused by AFB1 was measured by increase in serum ALT, AST and γ -GT activities. The apparent significant reduction in the AFB1-induced increase in the activities of these enzymes by kolaviron confirms its antihepatotoxic effects on similar environmental and hepatotoxic agents (Iwu *et al.*, 1990; Farombi *et al.*, 2000). Furthermore, the ability of kolaviron to attenuate AFB1-induced increase in γ -GT, an enzyme useful in early diagnosis of liver cancer, indicates that it may be relevant in the aetiology of AFB1 hepatocarcinogenesis.

Our study demonstrates a reduction in the activity of the phase 2 enzymes GST, UDP-GT and NQO following a single intraperitoneal dose of AFB1 to rats. Similar observations have been reported in AFB1-mediated tumorigenesis (Primiano *et al.*, 1995), lung tumour-bearing rats (Dogra *et al.*, 1985) and 41% reduction in GST activity was reported in hepatomas (Mouelhi *et al.*, 1987). Activation of AFB1 *in vivo* is believed to be mediated by the cytochrome P450 group of enzymes through epoxidation of the 8,9-double bond to form the highly reactive electrophile AFB1-8,9-epoxide. In most mammalian species studied, the primary pathways for AFB1 detoxification is through the GST-mediated conjugation of the epoxide metabolite with reduced glutathione to form mercapturic acid (Hayes *et al.*, 1991).

The decreased activity of GST in AFB1-treated rats may be due to excessive utilization of this enzyme in AFB1-GSH conjugation. UDP-GT is associated with phospholipids of the microsomal membrane (Erickson and Zakim, 1978) and NADH:quinone reductase is generally induced coordinately with other phase 2 detoxifying enzymes (Talalay, 1989) and hence the observed decrease in the activities of these enzymes may be due to ROS produced from AFB1 metabolism (Shen *et al.*, 1995b) leading to peroxidative damage to the microsomal lipid and inactivation of the enzyme activities. Previous investigations have also demonstrated the inhibition of conjugation enzymes in AFB1 (Premalatha and Sachdanandam, 2000), benzo(a)pyrene (Badary *et al.*, 1999) and carbon tetrachloride-treated animals (Farombi, 2000) and also in primary hepatic tumours (Mouelhi *et al.*, 1987).

One of the major mechanisms of chemoprevention is the induction of enzymes involved in the metabolism of carcinogens, particularly phase 2 enzymes (Kwak *et al.*, 2001). Mechanistic studies in rodent models for chemoprevention of AFB1-induced hepatocarcinogenesis suggest that induction of phase 2 enzymes is a sufficient condition for obtaining chemoprevention and this has been achieved by administering many naturally occurring and synthetic agents such as oltipraz, BHA, ethoxyquine and coumarin (Kelly *et al.*, 2000).

In the present study, kolaviron mediated an induction of UDP-GT, GST and NQO. This indicates the ability of kolaviron to induce drug-detoxifying enzymes and as such may play a role in the detoxification of AFB1 reactive metabolites. Induction of NQO also suggests that it may protect against the cytotoxic effects of quinones and their metabolites by inhibition of redox cycling (Prochaska and Talalay, 1992). In addition, kolaviron when administered together with AFB1 significantly reversed the toxic effect of AFB1 on the activities of these enzymes. Similarly, in a previous experiment, kolaviron mitigated the toxic onslaught of carbon tetrachloride on phase 2 drug-metabolizing enzymes (Farombi, 2000).

A more recently reported probable mechanism accounting for AFB1-induced hepatocarcinogenesis, apart from the activation of AFB1 by CYP450 enzymes to the highly reactive electrophilic AFB1-8,9-epoxide (Gallagher *et al.*, 1996), is the ability of AFB1 to induce ROS in cultured rat hepatocytes (Shen *et al.*, 1996), lipid peroxidation in rat liver (Shen *et al.*, 1994, 1995b) and the formation of 8-hydroxyguanosine in rat hepatic DNA (Shen *et al.*, 1995a).

The present study, in consonance with previous observation, demonstrates a significant increase in MDA formation and lipid hydroperoxides (markers of lipid peroxidation) in rats treated with AFB1. Lipid peroxidation is one of the cellular pathways involved in oxidative damage. ROS-mediated lipid peroxidation may also react with DNA to cause oxidative damage and this has been found to play an important role in chemical carcinogenesis (Shen *et al.*, 1994). Furthermore, studies have implicated lipid peroxidation in DNA damage since many of its products are capable of interacting with DNA to cause oxidative DNA damage (Agarwal and Draper, 1992). Lipid peroxidation may therefore possibly contribute to AFB1 genotoxicity. In this study, kolaviron, like vitamins C and E, was found to significantly inhibit AFB1-induced lipid peroxidation. The protective effect of kolaviron, via an antioxidant mechanism, against lipid peroxidation induced by some carcinogens in animal models has been reported (Farombi *et al.*, 2000, 2002b).

We also investigated the ability of kolaviron to prevent AFB1 genotoxicity using bone marrow micronucleus

induction as biomarker. This test is commonly used to assess the genotoxic potential of various chemicals and the antigenotoxic activity of many compounds both natural and synthetic. When rats were administered AFB1, micronucleus formation in mouse bone marrow cells was observed. In contrast, the frequency of occurrence of polychromatic micronucleated cells among bone marrow cells were reduced significantly when kolaviron was administered before and after AFB1 injection. The antigenotoxic effect of kolaviron may be due to the prevention of DNA damage induced by AFB1 via scavenging of active oxygen radicals produced by AFB1 metabolism. The present *in vivo* observation may be related to the previously reported *in vitro* inhibitory effects of kolaviron on AFB1 genotoxicity in human liver-derived HepG2 cells (Nwankwo *et al.*, 2000). As positive control, two classical antioxidant vitamins C and E were included in the assay. While vitamin C prevented AFB1 induction of micronucleus, vitamin E failed to elicit any protective effect. Our study confirms several reports of the antigenotoxic effects of vitamin C against AFB1 (Karekar *et al.*, 2000) and other mutagens in various models (Sai *et al.*, 1992; Anderson *et al.*, 1995). Vitamin E is reported to act differently in different model systems and in particular it is a lipid-soluble antioxidant which acts primarily against hydroxy and lipid peroxy radical-induced lipid peroxidation in cellular membranes (Terao *et al.*, 1994). Several studies have similarly demonstrated the inability of vitamin E to protect against micronucleus induction by chemical agents (Anderson *et al.*, 1995; Karekar *et al.*, 2000).

In conclusion, the present report demonstrates the ability of kolaviron to prevent AFB1 micronucleus induction and hepatic oxidative damage. It may therefore be implicated as a cancer chemopreventive agent which may possibly help to enhance the detoxification and scavenging reaction of the cell and thus may be an effective blocking agent against AFB1-induced carcinogenesis. Long-term carcinogenesis studies on the intervention of kolaviron on AFB1-DNA interaction and on the expression of specific AFB1 detoxification enzyme proteins such as aflatoxin aldehyde reductase and glutathione *S*-transferase A5 (GST A5) are necessitated by the present investigation.

Acknowledgements

We thank Mr Opeoluwa Odusan of Abbot Pharmaceuticals, Chicago, USA for the gift of aflatoxin B1. The Biotechnology Unit of the International Institute of Tropical Agriculture (IITA), Ibadan is thanked for making available the use of ultracentrifuge.

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