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Kolaviron protects against benzo[*a*]pyrene-induced functional alterations along the brain-pituitary-gonadal axis in male rats

Isaac A. Adedara[a,*], Olatunde Owoeye[b], Motunrayo A. Aiyegbusi[a], Joshua O. Dagunduro[a], Yetunde M. Daramola[a], Ebenezer O. Farombi[a]

[a] Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Exposure to benzo[*a*]pyrene (B[*a*]P) is well reported to be associated with neurological and reproductive dysfunctions. The present study investigated the influence of kolaviron, an isolated biflavonoid from the seed of *Garcinia kola*, on functional alterations along the brain-pituitary-gonadal axis in male rats exposed to B[*a*]P. Benzo[*a*]pyrene was orally administered at a dose of 10 mg/kg alone or orally co-administered with kolaviron at 100 and 200 mg/kg for 15 consecutive days. Administration of B[*a*]P significantly ($p < 0.05$) decreased plasma levels of pituitary hormones namely follicle-stimulating hormone (FSH) and prolactin but increased luteinizing hormone (LH) by 47%, 55% and 20.9%, respectively, when compared with the control. The significant decrease in gonadosomatic index (GSI) was accompanied by significant decrease in testosterone production and sperm functional parameters in the B[*a*]P-treated rats. Moreover, B[*a*]P-treated rats showed significant elevation in the circulatory concentrations of pro-inflammatory cytokines and oxidative stress indices in the brain, testes and sperm of B[*a*]P-treated rats. Light microscopy revealed severe necrosis of the Purkinje cells in the cerebellum, neuronal degeneration of the cerebral cortex, neuronal necrosis of the hippocampus and testicular atrophy in B[*a*]P-treated rats. Kolaviron co-treatment significantly ameliorated B[*a*]P mediated damages by suppressing pro-inflammatory mediators and enhancing the antioxidant status, neuroendocrine function, sperm characteristics and improving the architecture of the brain and testes in B[*a*]P-treated rats. The findings in the present investigation highlight that kolaviron may be developed to novel therapeutic agent against toxicity resulting from B[*a*]P exposure.

1. Introduction

Benzo[*a*]pyrene (B[*a*]P, Fig. 1A) is a widely distributed environmental contaminant belonging to a member of polycyclic aromatic hydrocarbon (PAH) family. The release of B[*a*]P into the environment could be due to industrial processes, products manufacturing, anthropogenic activities including incineration of municipal refuse, automobile emissions, cigarette smoke as well as culinary processes namely curing, frying and roasting (ATSDR, 1995; Hecht, 1999; Ramesh et al., 2004). Human exposure to B[*a*]P is principally through ingestion of contaminated food and water and also inhalation of particulates (Archibong et al., 2008). Exposure to B[*a*]P is hazardous to humans because it is associated with neurological abnormalities such as cognitive impairment, parasympathetic dysfunction, learning difficulties, and loss of short-term memory (Majchrzak et al., 1990; Kilburn and Warshaw, 1995; Qiu et al., 2011). Besides, B[*a*]P is a potent mutagenic, carcinogenic and pro-oxidative agent in both humans and experimental animals (Wijnhoven et al., 2000; Wester et al., 2012). B[*a*]P is bio-transformed by cytochrome P450 monooxygenases to produce reactive metabolites such as B[*a*]P-7,8-epoxide, BaP-7,8-dihydrodiol 9,10-epoxide and quinones which are well-known to damage the DNA and generate excessive reactive oxygen species (Flowers et al., 1997; Archibong et al., 2008). Moreover, B[*a*]P and/or its metabolites affect the brain tissues by crossing the blood–brain barrier to elicit detrimental effect directly on the central nervous system (Saunders et al., 2006). Indeed, B[*a*]P has been reported to induce behavioral deficits in rats and humans (Saunders et al., 2001; Niu et al., 2010; Chen et al., 2012).

* Corresponding author.
E-mail address: dedac2001@yahoo.co.uk (I.A. Adedara).

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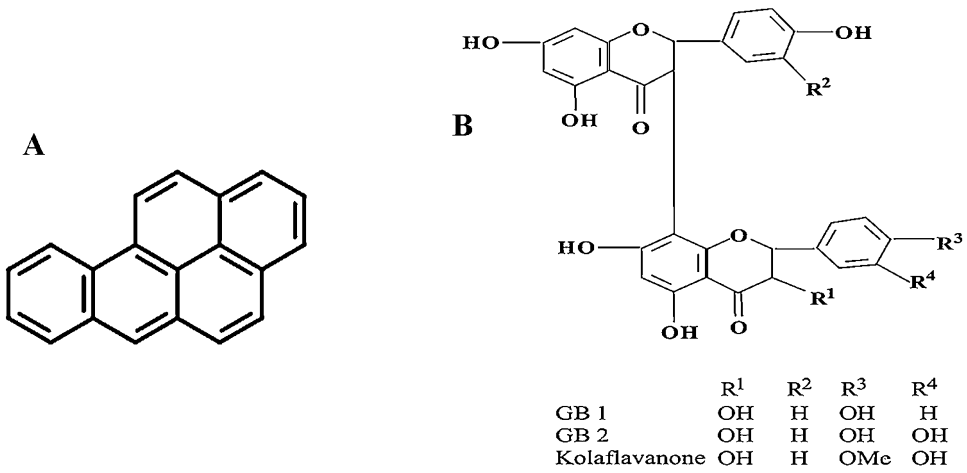

	R^1	R^2	R^3	R^4
GB 1	OH	H	OH	H
GB 2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Fig. 1. Chemical structures of tested compounds: (A) benzo[*a*]pyrene (B[*a*]P) and (B) kolaviron (KV).

Considering the brain-pituitary-gonadal (BPG) axis, it is clear that the brain and the gonad are susceptible targets during exposure to B[*a*]P. Male reproduction in vertebrates is intricately controlled by the B-P-G axis (Kime, 1998). Normally, the hypothalamus of the brain secretes gonadotropin-releasing hormone (GnRH) which stimulates the pituitary to produce two gonadotropins namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that in males control Leydig cell function. Testicular Leydig cells are predominantly responsible for the biosynthesis and secretion of testosterone, which supports sperm production and regulates the male phenotype (Hancock et al., 2009; Adedara et al., 2014). Previous investigations have implicated B[*a*]P in generation of increased reactive oxygen species which subsequently cause oxidative damage of macromolecules (Murawska-Cia"owicz et al., 2011; Curtis et al., 2011). Furthermore, B[*a*]P reportedly impaired testicular function of spermatogenesis and steroidogenesis in laboratory animals (Archibong et al., 2008; Chung et al., 2011; Liang et al., 2012). The fundamental role of oxidative stress in the B[*a*]P-mediated tissue damage can be use as significant biological target to prevent the noxious effect in exposed individuals.

Kolaviron (Fig. 1B), a biflavonoid compound isolated from the seed of *Garcinia kola* Heckel (Family Guttiferae), is well reported to have several pharmacological effects including anti-inflammatory, anti-oxidant, anti-hyperglycemic, and anti-genotoxic activities (Nwankwo et al., 2000; Olaleye et al., 2000; Terashima et al., 2002; Adaramoye and Adeyemi, 2006). The neuroprotective effect of kolaviron against vanadium, anticonvulsant phenytoin and sodium azide mediated toxicity in rats has been reported (Igado et al., 2012; Owoeye et al., 2014; Olajide et al., 2015). Moreover, the seed is traditionally called "male kola" because of its aphrodisiac and fertility enhancing activities (Okoko, 2009; Ralebona et al., 2012) which has been attributed to its vasodilator effects on the genitalia smooth muscles (Adegbehingbe et al., 2008). Kolaviron has been reported to protect against a variety of reproductive toxicants such as di-n-butyl phthalate, carbendazim, atrazine and ethylene glycol monoethyl ether *via* enhancement of antioxidant enzymes, inhibition of stress proteins and apoptosis, and steroidogenic dysfunction in rats (Farombi et al., 2007; Abarikwu et al., 2011; Adedara and Farombi, 2012; Adedara et al., 2013).

Compounds that would exhibit protection to both the brain and the testes would be beneficial to individuals who are exposed to B[*a*]P. Therefore, the objective of the present investigation was to evaluate the influence of kolaviron on functional alterations in brain-pituitary-gonadal (B-P-G) axis following exposure to B[*a*]P in male rats.

2. Materials and methods

2.1. Chemicals

Benzo[*a*]pyrene (B[*a*]P), glutathione, epinephrine, thiobarbituric acid, hydrogen peroxide, 5′,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and 1-chloro-2,4-dinitrobenzene (CDNB) were procured from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were purchased from the British Drug Houses (Poole, Dorset, UK).

2.2. Isolation of kolaviron

Kolaviron was isolated from the seeds of *Garcinia kola* according to published procedure (Iwu, 1985; Farombi et al., 2009). Briefly, the fresh *Garcinia kola* seeds were sliced, air dried and powdered. Extraction of the powdered seeds was carried out using light petroleum ether (bp 40–60 °C) in a soxhlet for 24 h. The defatted dried product was repacked and extracted with acetone. The extract was concentrated and diluted to twice its volume with distilled water and followed by subsequent extraction with ethylacetate (6× 300 mL). The concentrated ethylacetate yielded a golden yellow solid termed kolaviron which was identified by direct comparison of the 1H nuclear magnetic resonance (NMR), ^{13}C NMR and electron ionization (EI)-mass spectral result with previously published data (Iwu, 1985). The purity and identity of kolaviron was determined by subjection to Thin-Layer Chromatography using Silica gel GF 254-coated plates with a solvent consisting of a mixture of methanol and chloroform in a ratio 1:4 (v/v). Purity of isolated kolaviron was 96%.

2.3. Animal model

Forty male Wistar rats (8 weeks old; 158 ± 3 g) were obtained from the Department of Biochemistry, University of Ibadan, Ibadan. The animals were fed rat pellets, given drinking water *ad libitum*, and were subjected to natural photoperiod of 12 h light and 12 h darkness daily. Animal care and experimental protocols were executed according to the approved guidelines set by the University of Ibadan Ethical Committee, which is in agreement with the Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health.