Section 4

16

Pharmacology

A Naturally-occurring Antioxidant and Anti-inflammatory Bioflavonoid from the Seed of *Garcinia kola*

Nigeria

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Total cost: USD 50,000

Summary

Garcinia kola is a medium-sized tree found in moist forests, is widely distributed throughout west and central Africa, and is highly valued in for its edible nuts. The seed, commonly known as bitter kola, is a masticatory and an important kola substitute at home and at social ceremonies, playing a major role in African ethno-medicine. Traditionally, the seeds are used in the treatment of inflammatory disorders, laryngitis, cough, hoarseness of voice and liver diseases. For instance, extracts of the seeds led to remarkable improvement in liver function in patients with chronic hepatitis and cholangitis after treatment for 14 days at a Nigerian herbal home (Iwu, 1982). Our studies show that *G. kola* possesses immunomodulatory, antiviral, anti-inflammatory and antioxidant activities.

Laboratory experiments conducted using animals show that Kolaviron, a bioflavonoid extracted from *G. kola*, is beneficial to the body's system by helping to combat free radicals, chelate dangerous metals, boost the body's detoxification system, and reverse the noxious effects of inflammatory proteins and certain transcription factors that cause stress to the body's system. It also boosts sperm production, increases sperm motility and the sperms' antioxidant defence system and, as such, is useful in the male reproductive system, justifying its reputation in folklore as an aphrodisiac. Importantly, our recent data show that Kolaviron crosses the blood-brain barrier. It protected rats and human-derived brain cell cultures against drug-induced brain damage and as such has opened a new therapeutic window and application in neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. A Phase 1 clinical trial with *G. kola* showed it has relevance in the management of osteoarthritis of the knee and other inflammation-related disorders. Overall, this compound is a promising candidate drug in cancer chemoprevention and management of other diseases in which inflammation and free radicals have been implicated.

Background and Justification

The African continent is home to a rich diversity of plants used as herbs, health foods and for therapeutic purposes. This is largely due to the geographical spread spanning a land mass of approximately 2,166,340 km^2 of closed forest. Over 5,000 different substances from plant species have been recognized as occurring in these areas (Iwu, 1993), many of which have been found useful in traditional medicine for prophylaxis and the cure of diseases. This great biodiversity offers economic promise, particularly in the rapidly-emerging biotechnology industry. In spite of the heterogeneous nature of the continent and a deluge of information on the composition and biological activity of many plant

substances, little effort has been devoted to the development of chemotherapeutic and prophylactic agents from these plants. In the case of *Garcinia kola* it has been recognised as playing a great role in traditional medicine in the management of various ailments but no in-depth scientific studies have been carried out to prove and to justify its wide range of medicinal properties.

Recognising the many traditional values and medicinal potential of the *G. kola* plant, such as its antibacterial properties, cough-relieving effect and the relevance of its seed in the management of liver diseases, it became imperative to systematically and scientifically evaluate its therapeutic uses.

The identification of *G. kola* seed as a naturally-occurring modulator of liver disease qualifies it as candidate that can be incorporated in the human diet at minimal cost since the majority of the population in developing countries are not yet sufficiently economically-buoyant to be able to purchase prophylactic drugs. Coincidentally, the edible seed occupies a prominent position in the social customs of the people in West Africa and elsewhere on the continent. In spite of its very bitter taste, this nut is consumed as a refreshing habit and plays an important role in social ceremonies.

The first step by our scientific working group was the isolation and identification of a bioflavonoid compound, termed Kolaviron, which was shown to elicit significant hepatoprotective properties in animal models.

Description

The risk of developing liver diseases is highest in developing countries, due to combined exposure to aflatoxin B1 and the hepatitis B virus. This deadly duo has been implicated as a major risk factor in the development of liver cancer, which has been shown to present with the poorest 5-year survival rates and accounts for 15% of total cancer deaths. However, we have identified a commonly consumed, locally available and accessible seed in the region, *G. kola*, from which an active compound, Kolaviron, is derived. From studies using experimental rats, we reported the chemopreventive effects of Kolaviron against several liver damaging chemical compounds, namely carbon tetrachloride, 2-acetylamino fluorine (2-AAF), acetaminophen, galactosamine, phalloidin, potassium bromate (a nephrotoxic compound) and, most notably, aflatoxin B1. This latter compound is produced by the *fungus Aspergillus* flavus, known as a contaminant of maize, peanuts and other food products in the tropics (Farombi *et al*., 2005). Our recent study to unravel the molecular mechanisms of the hepatoprotective properties of Kolaviron also revealed its role in protection against dimethyl nitrosamine-mediated liver damage and the

downward regulation of certain transcription factors implicated in cancer (Farombi *et al*., 2009). These findings indicate that Kolaviron may protect against carcinogen- and drug-induced oxidative and membrane damage and as such may be relevant in the chemotherapy of liver and kidney diseases.

In implementing the project, we investigated the mechanisms by which Kolaviron protects against hepatotoxicity and possibly human liver cancer, which hitherto had not been investigated by any researcher. One major mechanism of hepatoprotection is the ability of a compound to elicit antioxidative activity. Therefore, in several assays involving reactive oxygen species (ROS), we evaluated the antioxidant properties of Kolaviron. Kolaviron exhibited significant reducing power, a dose-dependent inhibition of oxidation of linoleic acid, and inhibition of hydrogen peroxide activity together with significant scavenging of superoxides and of hydroxyl radicals. The ability of Kolaviron to scavenge hydroxyl radicals by inhibiting the oxidation of deoxyribose may directly relate to its inhibitory action of lipid peroxidation and thus account for its hepatoprotective properties in animal models. In human lymphocytes and rat liver cells, our data demonstrate that Kolaviron prevented DNA damage by eliciting a concentration-dependent decrease in hydrogen peroxide-induced DNA strand breaks. Furthermore, in rats treated with Kolaviron for 7 days, lipoprotein resistance to copper-induced oxidation was greatly improved. In addition, Kolaviron inhibited the Cu^{2+}-induced oxidation of rat serum lipoprotein in a concentration-dependent manner, exhibiting a chelating effect on Fe^{2+}, thus demonstrating that Kolaviron protected against the oxidation of lipoprotein by mechanisms involving metal chelation and antioxidant activity, and, as such, might be a good candidate for preventing atherosclerosis and heart-related diseases (Farombi *et al*., 2004).

Traditionally, *G. kola* seed plays important role as an aphrodisiac. We investigated this claim in a series of experiments in which rodents were exposed to Kolaviron as well as *G. kola* in the presence of environmental compounds known to cause testicular damage. Our data showed that Kolaviron treatment in rats ameliorated the toxicity posed to the testicular system by several environmental compounds that humans are occupationally and dietarily exposed to. In addition, in rats treated with *G. kola* at 0, 250, 500 and 1000 mg/kg for six consecutive weeks, there was an improvement in the spermatozoa characteristics and a boost in testicular antioxidant status. Furthermore, *G. kola* increased testosterone levels in these rats, reinforcing its reputation in enhancing reproductive capacity and thus justifying its folkloric use as aphrodisiac (Farombi *et al*., 2013).

The contribution of environmental contaminants to the aetiology of neurodegenerative diseases, such as Parkinson's disease, has been broadly recognized. Atrazine (ATR), a widely used agricultural pesticide, has been reported to work as a potential contributory risk

factor for Parkinson's disease and other neurological disorders in which dopamine levels are affected. At the same time, neuroprotection to delay or halt progressive degeneration of specified neurons is now proposed as a causal therapeutic strategy for Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (Abarikwu *et al*., 2011).

We investigated the effects of Kolaviron on ATR-induced cytotoxicity on human neuroblastoma cells in order to find a possible therapeutic intervention by application of a natural compound against degenerative diseases. Kolaviron elicited significant protection against ATR-mediated toxicity in these brain cells. Our data therefore demonstrate that Kolaviron's antioxidative property and its ability to protect against programmed cell death (Fig. 1) makes this natural molecule potentially protective against ATR-induced cytotoxicity. The results may open up a new clinical perspective in progressive neurodegenerative diseases such as Parkinson's disease.

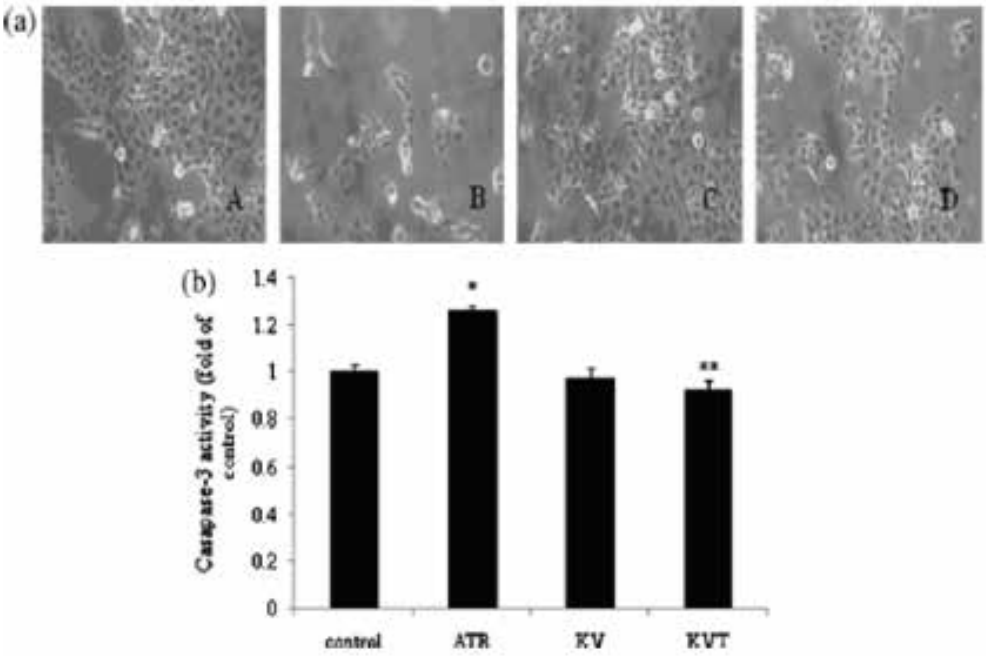

Figure 1: (a) Proliferation of SH-SY5Y cells treated with 0.3 mM atrazine (ATR) and/or Kolaviron (KV). A: control; B: 0.3 mM ATR; C: 60 IM KV; D: 0.3 mM ATR + 60 IM KV. (b) Caspase-3 enzymatic activity after 24 h exposure of SH-SY5Y cells to ATR in the presence or absence of KV. (Adapted from Abarikwu *et al*., 2011).

Results obtained so far, many of which have been published in peer-reviewed journals, indicate that this naturally occurring bioflavonoid, Kolaviron, from the seed of *G. kola*, has positive modulating and chemopreventive/chemoprotective effects on the liver, brain and reproductive system as well as on the noxious effects of environmental contaminants through various mechanisms including antioxidant effects, the induction of xenobiotic detoxifying enzymes, metal chelation, scavenging of ROS, attenuation of ROS-induced

DNA damage and programmed cell death, the down-regulation of certain transcription factors and stress-response proteins and up-regulation of phase 2 antioxidant genes.

These results, derived from a series of innovative experiments, justify the important role the seed of *G. kola* plays in African traditional medicine in the treatment of coughs, colds, hoarseness of voice, liver diseases, accentuating longevity and as an aphrodisiac.

Partnerships

In the course of implementing this project, we collaborated with some international scientists in the field of chemoprevention with plant-based medicinal compounds. They included:

- Professor Lars O. Dragsted, Institute of Food Safety and Nutrition, Soborg, Denmark;
- Professor Young Joon Surh, Seoul National University, South Korea; and
- Professor Beatrice Pool Zobel (late), Institute of Nutrition, University of Jena, Germany.

Impact

The project has had a significant impact on the scientific community locally and internationally and has built capacity in the field of traditional medicine research. Notably:

We have published about 40 articles in peer-reviewed international and regional journals on the beneficial role of *G. kola* and Kolaviron.

- We have made several presentations at both regional and international conferences on the success of this innovative research on *G. kola*.
- Several MSc and 10 PhD students have been trained and mentored, and many of them are actively practicing in the field.
- Several prizes, honours and distinctions have been won by reason of the series of innovative work on *G. kola*. For instance the first author has recently been admitted as a Fellow of the Nigerian Academy of Science (FAS), which represents the apogee of scientific achievement in Nigeria. Also some of the works and recognition in the field led to his winning the Society of Toxicology Global Senior Scholarship (USA) and admittance to the Fellowship of the American Academy of Toxicological Sciences. Two of his PhD students received 'best poster presentation' prizes for their presentation on some aspects of this work.

Some sectors of the general public, especially among the elite, have reservations towards herbal products and have adopted a western diet and medicines, but over time this attitude is changing. Our experience has addressed this aspect significantly, as many have become better informed through seminars, publications in journals, periodicals and newspapers and conferences.

Sustainability

One way of sustaining the experience is to preserve the *G. kola* plant species in the forest. Deforestation is rife across large parts of Africa and many important medicinal plants are becoming endangered or extinct.

The experience has also impacted the Ministry of Environment in Nigeria, which organised a national summit on *G. kola*, with the principal author invited to act as chair. It is expected that through the Minister of Environment, legislators and policy makers in the country will be better informed. In particular, the summit discussed issues relating to forest loss and availability of the raw product.

Replicability

The success of this project has alerted policy makers and created a renewed awareness among the public on the beneficial role of this seed in Nigerian society.

The active compound Kolaviron isolated from *G. kola* seed was patented some years ago in the USA by another group that worked on it. New therapeutic compounds from the same seed have been isolated by our group and we are working on the patents.

Through international collaboration with scientists in Denmark, Germany, South Korea and the USA, the innovative experience has become relevant across different regions, especially as the whole world is in search of naturally occurring, non-toxic therapeutic agents.

Lessons Learned

The main circumstance for the successful implementation of this project is the availability, accessibility and affordability of the *G. kola* seeds, the obtaining of which did not pose problem, placing the population in the region at an advantage in having access to the material.

Some individuals in the community do not like to eat the seed because of its bitter taste but through publications and engaging the press we are making efforts to ensure that the public buys into this innovation.

The major obstacles faced in the successful implementation of the project are funds and availability of state-of-the-art equipment to conduct meaningful experiments. Self-funding, University of Ibadan Senate Research grants, as well as several international fellowships and grants helped significantly in conducting experiments that resulted in meaningful conclusions.

Future Plans

Future plans include carrying out additional experiments on the interaction of the seed and isolated compounds on the bioavailability of other therapeutic drugs that may be consumed simultaneously as well as carrying out clinical trials using the product.

There are also plans to continue scientific collaborations and to collaborate further with researchers in Asia, Europe and the USA.

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