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Safety evaluation of water-soluble palm fruit bioactives

Barry S. Lynch*, Spencer West, Ashley Roberts

Intertek Scientific & Regulatory Consultancy, 2233 Argentia Road, Suite 201, Mississauga, Ontario, L5N 2X7, Canada

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ABSTRACT

Water-soluble palm fruit bioactives, derived from the aqueous stream of palm oil processing, have shown anti-diabetogenic effects in rodent models. To assess the safety of potential incorporation of this polyphenol-containing material in food, *in vitro* bacterial reverse mutation and *in vitro* chromosome aberration assays were conducted along with a 90-day subchronic toxicity study in Sprague-Dawley rats. Water-soluble palm fruit bioactives were inactive in the Ames and *in vitro* chromosome aberration assays up to the limit doses of 5000 μg/plate and 5000 μg/mL, respectively. In the 90-day feeding study, water-soluble palm fruit bioactives were administered *via* gavage at doses 0, 500, 1000 or 2000 mg/kg body weight/day. No significant effects were noted on body weight, food consumption, hematology, clinical chemistry, organ weights, and histopathological examination. The No Observable Adverse Effect Level was considered to be 2000 mg/kg body weight/day, the highest dose tested. These data provide evidence to support the safe use of water-soluble palm fruit bioactives in food or food ingredients.

1. Introduction

The potential health benefits of phenolic compounds present in fruits and leafy vegetables have long been studied (Williamson et al., 2011; Howes and Simmonds, 2014; Khalesi et al., 2014; Vuong et al., 2014; Blumberg et al., 2015; Matsui, 2015). Anti-inflammatory, anti-carcinogenic, anti-thrombotic, thermogenic, and hepatoprotective activities of polyphenolics contained in fruits and vegetables, as well as herbal products such as green tea, commonly eaten in the diet have been described (Middleton et al., 2000; Scalbert et al., 2005; Soobrattee et al., 2005, 2006).

The oil palm (*Elaeis guineensis*) fruit is known to contain lipid-soluble phytochemicals including carotenoids, tocopherols and tocotrienols. These compounds have been reported to also have substantial antioxidant activity (Sambanthamurthi et al., 2000; Abeywardena et al., 2002; Sundram and Nor, 2002; Sundram et al., 2003). While much attention has been given to the lipid-soluble phytochemicals, the water-soluble phytochemicals of the palm fruit have generally been overlooked and have only relatively recently received attention for their antioxidant and other biological activities (Sundram et al., 2003; Balasundram et al., 2005; Sambanthamurthi et al., 2008). Antioxidant activity can attenuate the effects of reactive oxygen species produced by normal metabolic and/or inflammatory processes. These reactive oxygen species in part are likely to contribute to age-related cancers, inflammation, diabetes, cardiovascular and neurological conditions (Griendling and Alexander, 1997; Ha et al., 2008; Bhattacharyya et al., 2014; McMurray et al., 2016; Rani et al., 2016).

During the production of palm oil, large amounts of aqueous liquor are produced which are typically discarded in the waste stream as palm oil mill effluent. While such effluent has been noted to be an environmental concern, Sambanthamurthi et al. (2008) developed an extraction process whereby the aqueous liquor, also known as vegetation liquor, is channeled away from the effluent stream to yield a water-soluble product rich in polyphenolics.

The aqueous extract, termed "water-soluble palm fruit bioactives" containing these polyphenols has been shown to have strong antioxidant, radical scavenging, hydrogen-donating and reducing properties in *in vitro* assays (Balasundram et al., 2005). Moreover, in animal models this water-soluble palm fruit bioactive extract, containing 18,200 mg/kg (dry weight) gallic acid equivalents of phenolics, including 10,800 and 7000 mg/kg of caffeoyl-shikimic acid and p-hydroxybenzoic acid, respectively, has been demonstrated to have potential beneficial effects on degenerative diseases (Sekaran et al., 2010; Sambanthamurthi et al., 2011a,b). These effects included reduction of blood pressure in nitric-oxide

Abbreviations: 2-AA, 2-aminoanthracene; 2-NF, 2-nitrofluorene; 4-NQ, 4-nitroquinoline-1-oxide; 9-AA, 9-aminoacridine; ARA, arachidonic acid; bw, body weight; FDA, Food and Drug Administration; FOB, functional observational battery; GLP, Good Laboratory Practice; NaN_3, Sodium azide; OECD, Organisation for Economic Co-operation and Development; S9, S9 microsomal fraction.

* Corresponding author.
E-mail address: barry.lynch@intertek.com (B.S. Lynch).

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deficient rats, reduction of plaque formation in rabbits fed an atherogenic diet, and, in the Nile rat, protection against the development and progression of metabolic syndrome and type II diabetes. The Nile rat has been used as a model to assess the spontaneous development of metabolic syndrome and diabetes in response to normal and diabetogenic carbohydrate rich diets. In addition, the water-soluble palm fruit bioactives showed anticancer activity in inbred BALB/c mice (Sekaran et al., 2010), including notable effects on gene expression as measured by microarray analysis (Leow et al., 2011, 2012; Sambanthamurthi et al., 2011b). Part of the anti-diabetic effect of water-soluble palm fruit bioactives may be related to effects mediated at the molecular level as evidenced by Illumina microarray analyses of lipid catabolism and cholesterol biosynthesis genes (Leow et al., 2011, 2012). It was recently reported that water-soluble palm fruit bioactives down-regulated the insulin-signaling pathway, suggesting that the anti-diabetic effects may be attributable to mechanisms other than an increase in insulin secretion including increase in insulin sensitivity (Leow et al., 2016).

Leow et al. (2011) reported that water-soluble palm fruit bioactives were not toxic to mice, based on the results of hematological, biochemical and histological analyses (liver, spleen and heart), when treated at 1500 mg gallic acid equivalents/L drinking water ad libitum for a period of 6 weeks. This equates to a total dose of about 100 mg gallic acid equivalents/kg body weight/day. Teratological studies where rats were supplemented with water-soluble palm fruit bioactives at both 1500 and 2400 mg/l gallic acid equivalents/L drinking water did not result in any congenital abnormalities in the offspring observed through threegenerations (Sambanthamurthi et al., 2011a).

Further demonstration of the anti-diabetic effects, including reduced body weights, plasma glucose and lipid profiles, and reduced insulin resistance and/or enhanced insulin production, of the water-soluble palm fruit extract were shown in a series of subchronic studies conducted with the Nile rat (Bolsinger et al., 2014). Doses assessed were in the range of 170–240 mg gallic acid equivalents/kg body weight (bw)/day over periods of 4–36 weeks. The series of studies conducted by Leow et al. (2011) and Bolsinger et al. (2014) do not indicate the occurrence of any significant toxicity; however, these studies were not designed to specifically assess safety of use as a food ingredient.

Given the potential health benefits that could be associated with consumption of polyphenols contained in the palm fruit bioactive extract, and recent developments in the production of commercial grade, standardized forms of the extract, the Malaysia Palm Oil Board commissioned a series of *in vitro* genotoxicity studies and a subchronic toxicity in rats, all Good Laboratory Practice (GLP)- and Organisation for Economic Co-operation and Development (OECD)-compliant in nature, to further assess the safety of the potential application of water-soluble palm fruit bioactive extract as a food ingredient.

2. Materials and methods

All studies[1] were conducted in compliance with one or more of the United States Food and Drug Administration (FDA) regulations on Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (Title 21 of the *Code of Federal Regulations*, Part 58) (U.S. US FDA, 2013), the Organisation for Economic Co-operation and Development (OECD) Principles of GLP (OECD, 1998a). The in-life experimental procedures to be undertaken during the course of the rat toxicity studies were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (the Act).

2.1. Test materials

Water-soluble palm fruit bioactives, a cream colored powder, was provided by the Malaysian Palm Oil Board (Batch no. CL/2016-06-12-006-41). The phenolic acid content of the powder was approximately 4–5% as gallic acid equivalents as determined by the Folin Ciocalteau method (Singleton et al., 1999). The gallic acid equivalent content is composed predominantly of caffeoylshikimic acid isomers and p-hydroxybenzoic acid based on methodology presented in Sambanthamurthi et al. (2011a). The structures of these three compounds are shown in Figs. 1–3.

The results of standard proximate analyses testing determined the overall composition of the material to include carbohydrates (~60%), total sugars (~10%), protein (~10%), moisture (~8%) and ash (~20%). The product was determined to be stable (based on GAE analysis using the Folin Ciocalteau method) over the course of time that the experiments were conducted.

The palm fruit bioactive extract was prepared by applying only physical refining processes (*i.e.* heat exchange, filtration, centrifugation, ultrafiltration and reverse osmosis) to the aqueous liquor from the production of palm oil (Sambanthamurthi et al., 2008). The physical refining removes undissolved solids, oleaginous parts, colloids and higher weight molecules from the vegetation liquor to give an aqueous fraction containing phytochemicals (*e.g.* flavonoids, phenolic acids, hydroxyl acids, shikimic acid, and soluble fibre). The results of the genetic toxicity studies, and of the subchronic toxicity study in the rat, are reported herein.

Positive controls for the *in vitro* bacterial reverse mutation assay included: 2-Aminoanthracene (2-AA), 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), 4-nitroquinoline-1-oxide (4-NQ), and sodium azide (NaN_3) Dimethyl sulfoxide and sterile distilled water were used as solvents. The positive controls for the *in vitro* chromosome aberration assay included mitomycin C and cyclophosphamide and the solvents used (negative controls) included dimethylsulfoxide and distilled water. The S9 microsomal fraction (S9) used as the metabolic activation system in both *in vitro* genetic toxicity assays was prepared by and purchased from Harlan Laboratories UK. The S9 was prepared from male Sprague-Dawley rats induced with phenobarbital/5,6-benzoflavone.

2.2. Bacterial reverse mutation assay (Ames test)

This study was conducted in conformance with the following internationally accepted guidelines and recommendations: ENV/MC/CHEM (98) 17, OECD Guidelines for the Testing of Chemicals (OECD, 1997). Genetic Toxicology: Bacterial Reverse Mutation Test, Guideline 471, and FDA. Toxicological Principles for the Safety Assessment of Food Ingredients (Redbook, 2000): IV.C.1.a. Bacterial Reverse Mutation Test.

The study was conducted according to the plate incorporation (Ames et al., 1975) and the pre-incubation methods (Maron and

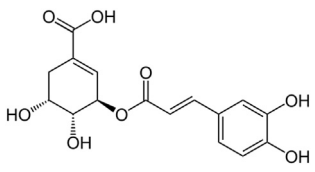

Fig. 1. Caffeoylshikimic acid.

[1] The animal study was conducted at Envigo CRS at Eye, Suffolk UK and the genotoxicity studies were conducted at Envigo CRS, Alcobury, Cambridgeshire, UK.