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Review

Safety assessment of kola nut extract as a food ingredient

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ABSTRACT

Kola nut extract is used in the food industry as a flavoring ingredient. Kola nut extract is derived from the seeds of primarily two tropical *Cola* species (*Cola nitida* (Vent.) Schott *et* Endl. or *Cola acuminata* (Beauv.) Schott *et* Endl.) of the Family, Sterculiaceae. Present day consumption of kola nut extract is 0.69 mg/kg/day. Caffeine and theobromine are two important constituents of kola nuts. Although limited biological data are available for kola nut extract specifically, the published data of the major constituents of kola nuts suggest the pharmacological/toxicological properties of kola nut extract, parallel to those of a roughly equivalent dose of caffeine. Frank developmental/reproductive effects have not been reported and changes in offspring cannot be extrapolated to humans. A NOEL/NOAEL cannot be defined for repeated oral exposure to kola nut extract from available data. Notwithstanding the foregoing, U.S. consumers have a history of safe consumption of cola-type beverages containing kola nut extract that dates at least to the late 19th Century, with a significant global history of exposure to the intact kola nuts that date centuries longer.

1. Introduction

Kola nut extract (CAS No. 68916–19-8) consists of an extract of the nuts (or seeds) from the pods of primarily two species of the *Cola* plant (Family, Sterculiaceae). In the food industry, kola nut extract is used as a flavoring ingredient and is approved for food uses by the Council of Europe (CoE), US Food and Drug Administration (FDA), the Flavor and Extract Manufacturers' Association (FEMA), and the International Organisation of Flavor Industries (IOFI). This review evaluates the safety-in-use of kola nut extract as a food ingredient.

1.1. Historical perspective

There is evidence that intra-African trade in kola nuts dates back to at least the 14th Century, with firm written records of African exports to England and the U.S. which date to the mid-19th Century. It was in 1886 that the druggist, John S. Pemberton, from Atlanta, Georgia, invented the popular soft drink, Coca-Cola®, by combining coca and kola extracts for use as a headache and hangover remedy (Kiple and Ornelas, 2000b). In its 1977 survey of industry on food additives in the U.S., the US National Academy of Sciences (NAS) indicated that the first reported use of kola nut extract was in 1935 (NAS, 1979).

The kola nut's primary social and economic significance lies in its being a very concentrated source of the central nervous system (CNS) stimulant, caffeine, with one nut containing more caffeine than two large cups of American coffee (Kiple and Ornelas, 2000b). Kola nuts have been, by one author's expression, "...the indispensable tonic of the west (*sic*) African people from time immemorial" (Arctander, 1960). Even today, chewing of fresh kola nut remains a 'social lubricant', with strong cultural significance for many indigenous West African people, particularly those of Islamic faith who are forbidden to consume alcohol (Blumenthal, 2000).

1.2. Description, natural occurrence and sources

Kola nut extract is an aqueous or ethanolic extract of the dried cotyledons (*i.e.*, embryo leaves from the seeds contained in a seed or nut pod) of the *Cola* plant (Family, Sterculiaceae). Referred to generically in some references by the Latin, '*Colae semen*', the nuts for the commercial extract are derived, almost exclusively, from two species of *Cola*, either *Cola nitida* (Vent.) Schott *et* Endl. or *Cola*

Abbreviations: CAS, chemical abstracts service; CFR, code of federal regulations; CoE, Council of Europe; DINFO, daily intake via natural food occurrence; EC, European Community; EU, European Union; FCC, Food Chemicals Codex; FEMA, Flavor and Extract Manufacturers' Association; FDA, Food and Drug Administration; GRAS, generally recognized as safe; IARC, International Agency for Research on Cancer; IOFI, International Organization of Flavor Industries; MRCA, Market Research Corporation of America; MSDI, maximum survey-derived daily intake; NACGM, National Association of Chewing Gum Manufacturers; NAS, National Academy of Sciences; NOEL/NOAEL, no observed (adverse) effect level; OTC, over-the-counter; PADI, possible average daily intake; PMDI, possible maximum daily intake; RIFM, Research Institute for Fragrance Materials; TAMDI, theoretical added maximum daily intake; USDA, United States Department of Agriculture.

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acuminata (Beauv.) Schott *et* Endl; three additional species of minor importance are listed in the table (Table 1). Cola extract may be fluid, soft, or dried, and may be produced as tannin free. It should be noted that in the literature, 'Kola' and 'Cola' are frequently interchanged; the spelling and name, 'Kola nut extract', will be used in this review (Bradley, 1992; Burdock, 1997; Blumenthal, 2000; Burdock, 2005). Synonyms and other descriptive characteristics of kola nut extract are provided in Table 2.

The *Cola* species of interest originated in western Africa (the plant is indigenous of Togo, Sierra Leone and Angola), but is now extensively cultivated in other tropical areas of Africa and Central and South America. Most sources describe the source plant as an evergreen tree of moderate height (40–60 feet), with greenish-yellow or white flowers spotted with purple, and leaves that are six to eight inches long, elliptoid to ovate and pointed at both ends. The star-shaped fruits (Fig. 1) consist of follicles, or green wrinkled pods, that contain 1–10 bean-like seeds (PDR Herbal, 2004). The cotyledons, or cola nuts, comprise the bulk of the seed; they generally are two in number, but may be as many as five. The cola nuts are brownish, about the size of a chestnut, and have a bitter, astringent taste when fresh. After drying, however, the taste becomes milder and faintly aromatic, with an odor that hints of nutmeg. Of the two species, *C. nitida* is reported to be the most important and valuable, though the literature provides no more basis for this assessment than that this species grows cola nuts that are large in comparison to the small cola nuts of *C. acuminata* (Felter and Lloyd, 1898; Arctander, 1960; Grieve, 1971; Burdock, 1997; Blumenthal, 2000; Kiple and Ornelas, 2000a; Burdock, 2005).

In addition to being a significant source of caffeine, as noted above, kola nuts also contain modest amounts of the related methylxanthine, theobromine. Flavonoids, anthocyanins, and tannins (all of which are various permutations of plant phenolic compounds) have also been reported to be present in significant amounts (Harborne et al., 1999a,b,c; Burdock, 2005). Some specific chemical constituents of the *C. acuminate* kola nut that has been quantified and/or identified were reported by Duke (1992) (Table 3); some representative structures are provided in (Fig. 2). In fresh kola nuts, the caffeine is largely complexed through hydrogen bonding to the abundant catechins and tannins; whereas, in the dried nuts, oxidative degradation and polymerization of low molecular weight polyphenolics results in liberation of the caffeine unless the material is stabilized (Bradley, 1992; Evans, 1996).

1.2.1. Specifications

Specifications of kola nut extract for use as an ingredient for addition to food have not been published by any relevant authoritative body. Herbal medicinal preparations of kola nut extract are typically standardized for methylxanthine (*e.g.*, caffeine and theobromine) content, usually 1.5–2.5% (Bradley, 1992; Blumenthal, 1998). However, reports of caffeine content can vary between 1.5% and 3.8%, depending on the variety of nut characterized, as well as the treatment of the kola nut. Treatments include fresh (raw) nuts, cured nuts (6 months), sun-dried nuts (sun dried for 40 days), and, milled and stored nuts (sun-dried nuts milled and stored for 12 months) (Atawodi et al., 2007).

1.2.2. Economic uses

Kola nut extract is used in the food industry as a natural flavoring agent (Ash and Ash, 1995; Burdock, 1997). The extract also finds use as a natural herbal preparation for the treatment of mental and physical fatigue (Bradley, 1992; Blumenthal, 1998). The nut itself is also exported worldwide for extraction and is used in the manufacture of methylxanthine-based pharmaceuticals (Blumenthal, 2000).

1.2.3. Regulatory history

Kola nut extract has been approved for use in food by CoE, FDA, FEMA, and the IOFI (Table 4). CoE has classified kola nut extract as Category 4, which is defined as "plants, animals and other organisms, and parts of these or products thereof, and preparations derived therefrom, not normally consumed as food items, herbs or spices in Europe, which contain defined 'active principles' or 'other chemical components' requiring limits on use levels" (CoE, 2000). The FDA and FEMA have determined kola nut extract to be GRAS for use as a flavoring ingredient. IOFI has classified the extract as, "natural flavor isolated by physical methods" (Clydesdale, 1997). The FDA has also approved kola nut extract for use as an inactive ingredient in certain oral and rectal pharmaceutical preparations (FDA, 2005). Kola extract is approved by German Commission E (Blumenthal, 1998).

Certain chemical constituents of kola nuts have also been approved in pure form for use in foodstuffs. Caffeine is regulated by FDA as GRAS for use in cola-type beverages up to a maximum use level of 0.02% (0.2 mg/mL) (21 CFR § 182.1180). Both caffeine (Flavouring Substances Registry, No. 16.016) and theobromine (No. 16.032) have been approved by the European Commission as flavoring substances for use in or on foodstuffs (EC, 2002a,b). Caffeine is also regulated as a drug by FDA, specifically as the only active ingredient approved for use in over-the-counter (OTC) stimulant drug products (21 CFR § 340); pre-existing uses in OTC weight control drug products have been disallowed as part of the Agency's ongoing OTC Drug Review (21 CFR § 310.545).

FEMA (Burdock, 2005) and the National Association of Chewing Gum Manufacturers (NACGM, 1977) reported food uses of kola nut extract which are provided in Table 5.

1.3. Consumption

Several methods can be applied to estimate the consumption of a substance in the diet (refer to Burdock and Carabin (2009) for a detailed explanation). For example, the *per capita* estimate of intake is based on "disappearance data" (i.e., production data) used for food and the differences between the two sources of data (NAS versus FEMA) are based on an estimate of how much is reported versus actual value (refer to Table 6). Production value estimated by NAS is 489,000 lbs for the year 1987 (NAS, 1989) for a per capita consumption of 41.42, or 0.69 mg/kg/day (for an average individual weighing 60 kg) and a possible methylxanthine intake of 0.6–1.5 mg/day. A production value of 48,400 lb reported by FEMA (Lucas et al. 1999) yields a per capita extract consumption of 2.88 or 0.05 mg/kg/day and a possible methylxanthine intake of 0.04–0.10 mg/day.

In contrast to the *per capita* method of estimation, the Theoretical Added Maximum Daily Intake (TAMDI) values such as FEMA

Table 1
Cola spp. (http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic = TSN&search_value = 500687).

Kingdom	Plantae – plants
Subkingdom	Tracheobionta – vascular plants
Superdivision	Spermatophyta – seed plants
Division	Magnoliophyta – flowering plants
Class	Magnoliopsida – dicotyledons
Subclass	Dilleniidae–
Order	Malvales –
Family	Sterculiaceae – cacao family
Genus	*Cola* Schott & Endl. – cola
Species	*Cola nitida* (Vent.) A. Chev. – ghanja kola
Species	*Cola acuminata* (Beauv.) Schott & Endl.– abata cola
Species	*Cola anomala* Schumann – anomalous cola
Species	*Cola pachycarpa* Schumann – cola
Species	*Cola verticillata* (Thonn.) Stapf ex A. Chev – cola

Table 2
General description of kola nut extract.

	Description	References
Botanical source	*Cola nitida* (Vent.) Schott *et* Endl. and/or *Cola acuminata* (Beauv.) Schott *et* Endl.	Bradley (1992)
Botanical family	Sterculiaceae	Burdock (2005)
Other names of botanical source	Kola nut, bissy nut, guru nut	McGuffin et al. (1997); PDR Herbal (2004)
Synonyms	*Cola* (genus), ext.; Cola extract; Cola nut extract; Kola extract; Kolas nut extract (*Cola acuminata* Schott *et* Endl.)	ChemIDplus (2008b)
Functionality in food	Flavoring ingredient	Burdock (1997)
CAS No.	68916–19-8	ChemIDplus (2008a)
CAS No. (alternative)	89997–82-0; 977024–83-1	Burdock (1997), ChemIDplus (2008c)
CoE No.	149	CoE (2000)
EINECS No.	272–824-0	ECB (2005)
FEMA No.	2607	Hall and Oser (1965)
NAS No.	2607	NAS (1989)

CAS = chemical abstracts service; CoE = Council of Europe; EINECS = European Inventory of Existing Chemical/Commercial Substances; FEMA = Flavor and Extract Manufacturers' Association; NAS = National Academy of Sciences.

FIG. 176.—Cola (Kola Nut). Showing longitudinal section of fruit × ⅓; cross-section of red seed × ½; longitudinal section of red seed showing embryo × ½; cross-section of red seed × ½; longitudinal section of white seed. (*After Kohler.*)

Fig. 1. Drawing of the *Cola* plant and nut (from Sayre (1917)).

Possible Average Daily Intake (PADI) and Possible Maximum Daily Intake (PMDI) are calculated based on the theoretical consumption of food to which the ingredient has been added. The FEMA PADI of 36.49 or 0.61 mg/kg/day is the mean consumption of kola nut extract that is based on an approved "usual" level of use as a flavoring by FEMA (providing a daily intake of methylxanthines from 0.5 to 1.3 mg/day). The PMDI can be calculated using the mean consumption of food as above and the maximum levels approved by FEMA (Table 3). The PMDI-calculated theoretical value for kola nut extract, when used as a flavor ingredient, was 88.23, or 1.47 mg/kg/day (yielding a possible daily intake of methylxanthine from 1.3 to 3.3 mg/day).

Table 3
Reported chemical constituents of the *Cola* plant (*Cola acuminata* (Beauv.) Schott *et* Endl.) (Duke, 1992).

Constituent	Level (ppm)	Plant part
Ascorbic acid	540–1456	Seed
Betaine	2500	Seed
Caffeine	10,000–25,000	Seed
B-carotene	1	Seed
D-catechin	3000–4000	Seed
DL-catechin	NR	NR
Colalipase	NR	NR
Colaoxydase	NR	NR
L-epicatechin	NR	NR
Kolanin	NR	Seed
Kolatein	NR	Seed
Kolatin	NR	Seed
Phlobaphene	NR	Seed
Tannin	NR	Seed
Theobromine	1000	Seed

NR = Not reported; for level, constituent was detected but not quantified.

2. Biological data

Some limited biological data are available which are specific to the extracts of the kola nut, per se, and these data will be discussed as appropriate in the following sections. However, while the important biological properties – in particular, the pharmacological/toxicological properties – of kola nut extract have frequently been reported to be those of caffeine (Grieve, 1971; NMCD, 2008), as noted above, specifications for kola nut extract have not been published and, therefore, a standardized caffeine content of the food-use material has not been defined. In addition, the published literature on caffeine is – in contrast to that for kola nut

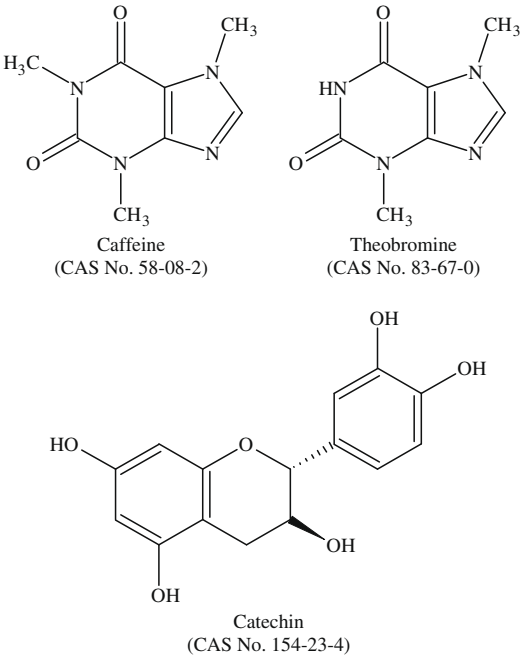

Fig. 2. Structures of representative kola nut constituents.

extract – vast and has been extensively reviewed elsewhere (Stavric, 1988; Mandel, 2002a; Nawrot et al., 2003). Thus, while reference to data specific to individual constituents of kola nuts, such as caffeine or theobromine, will be made in some of the following sections, it is beyond the scope of this review to address these data in significant detail, and this review should not be interpreted as being an evaluation of the safety of food uses of anything other than kola nut extract.

2.1. Biochemical/pharmacological effects

Seeking to explain the prevalence of peptic ulceration among indigenous African people, Osim et al. (1991) investigated the relative effect on gastric acid secretion in cats of kola nut extract (*Cola nitida alba*) and an equivalent dose of caffeine. Cats (15 male and 10 female) were fasted overnight, after which the stomachs were surgically cannulated under anesthesia and perfused with 0.9% saline. The jugular vein was also cannulated for the introduction of blocking drugs. Once stomachs were clear of solids and perfusate was clear, continuous perfusion was maintained with an infusion pump and baseline gastric acid secretion rate was established (control). Test samples (adjusted to pH 7.0) consisted of a filtrate of 6 g of ground dry kola nuts and 100 mg of caffeine, both dissolved in 200 mL of 0.9% saline. The infusion rate was maintained at 1 mL/min. Perfusate samples were collected at 10-min intervals for up to 3 h after dosing and titrated for total acidity. Treatment with kola nut extract resulted in a greater than 6.5-fold increase in gastric acid secretion over baseline. Acid secretion in response to treatment with caffeine alone peaked at roughly 42% of that seen with kola nut extract. The acid secretion response to kola nut extract treatment was blocked almost completely by intravenous administration (one hour after kola nut extract) of either atropine (0.2 mg/kg bodyweight) or cimetidine (12 μmoles/kg bodyweight), which block, respectively, muscarinic and histaminic receptors. Based on these findings, the authors speculated that caffeine is not the only constituent in kola nuts acting to stimulate gastric acid secretion, and that the overall response to the extract is mediated, at least in part, *via* cholinergic and histaminergic pathways. The authors do not discuss whether there might be other etiological factors (*e.g.*, *Helicobacter pylori* infection) involved in the observed incidence of gastric ulceration (Osim et al., 1991).

French investigators (Daels-rakotoarison et al., 2003) evaluated, in both cell-free and cellular in vitro systems, the ability of decaffeinated kola nut extracts (*C. nitida*) to mitigate the inflammatory effects of polymorphonuclear neutrophilic-derived elastase via protection of α-1-proteinase inhibitor. The extracts were prepared from five-year-old kola nuts and under rather stringent extraction conditions [three 24-h macerations at 4 °C in acetone:water (60:40 v/v), followed by decaffeination with dichloromethane and partitioning with ethyl acetate/chloroform]. The resulting extract primarily contained phenolic compounds. It was reported to inhibit both elastase release and activity, presumably through its antioxidant properties.

Kola nuts, when marketed or used as a herbal medicinal remedy, have been the subject of a number of precautionary warnings to consumers, primarily related to the caffeine content of the nuts. For example, the American Herbal Products Association includes *C. acuminata* and *C. nitida* on its lists of herbs that may cause irritation to the GI tract, that may induce nervous system stimulation, and that should not be used in pregnancy unless otherwise directed by a qualified expert (Class 2b) (McGuffin et al., 1997). The potential for kola nuts and their extracts to interact pharmacologically with a number of drugs included, but not limited to ephedrine, phenelzine, monoamine oxidase inhibitors, adenosine, clozapine, benzodiazepines, propranolol and metoprolol, phenylpropanolamine and quinolone antibiotics has been noted (Brinker, 2001; NMCD, 2008; The Pharmacogenetics and Pharmacogenomics Knowledge Base, 2008).

In regard to caffeine specifically, the spectrum of biological/pharmacological effects most likely to be observed includes diuresis, CNS stimulation, cardiac muscle stimulation, smooth muscle relaxation, stimulation of gastric acid secretion, and an increase in free fatty acid and glucose levels in plasma. The underlying mechanistic basis for these effects is generally regarded to be a selective blockade of adenosine receptors via competitive inhibition, which are present in brain, blood vessels, kidneys, heart, gastrointestinal tract, and respiratory passageways (Stavric, 1988; Mandel, 2002b; Nawrot et al., 2003).

2.2. Toxicological studies

2.2.1. Subchronic toxicity studies

Male Wistar albino rats (12/dose group) were administered distilled water (control) or kola nut extract (*C. nitida*) (0.5 mL, equivalent to 57 mg/kg bodyweight) via oral gavage, every other day, for 18 weeks (Ikegwuonu et al., 1981). The extract consisted of the supernatant resulted when fresh kola nuts were ground to the consistency of flour and suspended in hot distilled water (100 g/200 mL), and then allowed to sit undisturbed at room temperature overnight. No analysis or further characterization of the extract was undertaken. Food and water were available ad libitum throughout the study duration. Animals were weighed at study initiation and weekly thereafter; animals were also monitored for overt signs of toxicity. At study termination, rats were killed by decapitation and a gross pathological evaluation was performed. Liver, kidneys, brain, testis, and serum were harvested, and homogenates of the organ tissues were prepared. From these homogenates, total protein, RNA, and DNA levels of tissues were assessed, as were B-glucuronidase and B-galactosidase activities. Liver function was assessed via measurement of serum phosphomonoesterases, bilirubin, and cholesterol levels. No microscopic histopathology was performed.

Restlessness, excitement, irritability, loss of hair and appetite, and diuresis were observed in animals receiving kola nut extract over the course of the study. Treatment with kola nut extract

Table 4
Regulatory status of kola nut extract.

Agency	Identification	Permitted functionality	Use limits (ppm)	Reference
CoE	-*Cola acuminata*, CE No. 149; category 4 (with limits on caffeine). source materials and preparations are not considered to constitute a risk to health in the quantities used provided that the limits set for the "active principles" or the "other chemical components" are not exceeded.- *Cola nitida*, CE No. 2041; category 4 (as above)	Flavouring	Baked goods (377.8), frozen dairy (445.8), soft candy (370), gelatin, puddings (370), nonalcoholic beverages (104.5), alcoholic beverages (133.8), hard candy (117.8)	CoE (2000)
FDA	Substances generally recognized as safe. subpart A – general provisions. essential oils, oleoresins (solvent-free), and natural extractives (including distillates)	Flavoring agent or adjuvant	cGMP	21 CFR § 182.20[1]
FEMA	2607	Flavoring ingredient	See Table 5	Hall and Oser (1965)
IOFI	Natural flavor isolated by physical methods	Flavoring agent or adjuvant		Clydesdale (1997)

CFR = code of federal regulations; cGMP = current good manufacturing practices; CoE = Council of Europe; FDA = US Food and Drug Administration; FEMA = Flavor and Extract Manufacturers' Association; IOFI = International Organization of the Flavor Industry.
[1] Title 21 of the US Code of Federal Regulations (CFR), section 182.20, 2004.

Table 5
Approved food uses of kola nut extract by FEMA[†] (Burdock, 2005)[‡] and NACGM (1977).

Food category	Use level (ppm)		Food category	Use level (ppm)	
	Usual	Max		Usual	Max
Alcoholic beverages	71.90	133.82	Hard candy	80.52	117.88
Baked goods	158.11	377.89	Hard candy[*]	63.39	721.00
Frozen dairy	54.13	445.88	Nonalcoholic beverages	68.98	104.52
Gelatins, puddings	148.00	370.00	Soft candy	144.00	370.00

[‡] Compilation of GRAS lists and related information.
[†] FEMA-approved uses are for flavor ingredients; FEMA = Flavor and Extract Manufacturers' Association.
[*] NACGM = National Association of Chewing Gum Manufacturers.

Table 6
Consumption of cola nut extract.

	FEMA *per capita* low-intake value		NAS *per capita* high–intake value	
	(mg/day)	(mg/kg/day)	(mg/day)	(mg/kg/day)
Added Ingredient	2.88	0.05	41.42	0.69

FEMA = Flavor and Extract Manufacturers' Association; NAS = National Academy of Sciences.

induced a gradual loss of body weight in exposed animals – mean body weights of treated and control animals were approximately 240 g at study initiation, whereas at the conclusion of treatment, mean body weight for control animals was approximately 375 g, as compared to that for treated animals which was approximately 150 g. In contrast, absolute weights for liver, kidney, brain, and testis were all significantly increased in treated animals than in controls, while total protein, RNA, and enzyme activity levels were all decreased in each of these organs from treated animals. Fat deposition was also evident around the organs of treated animals at autopsy. Serum alkaline and acid phosphatase activities and total cholesterol were increased in animals receiving kola nut extract, while serum bilirubin levels (total and conjugated) were decreased. The authors suggest that many of these findings are consistent with the previous reports of methylxanthine toxicity, but they acknowledge that limitations of the study design preclude a complete interpretation of the results (Ikegwuonu et al., 1981).

2.2.2. Teratogenicity/reproduction toxicity

Ajarem and Ahmad (1994) studied the effects of an extract of fresh kola nuts (*C. nitida*) on the post-natal development and behavior of mice. Pregnant Swiss-Webster albino mice (8/dose group) were exposed via the drinking water to kola nut extract at reported concentrations of 0, 8, 16, or 32 mg/L, from gestation day 0 (the day the vaginal plug was detected) until weaning (day 24 post partum). Food and drinking water (treated or control water was the only source of fluid) were available ad libitum. Parameters evaluated included: pup body weights (measured every fourth day), day of eye opening and hair appearance, and assessments of pup behavior, such as locomotion and tube-restraint response. The authors claim that consumption of the prepared dosing solutions resulted in exposures of the dams to approximately 2.5, 5.0, or 10 mg/kg/day of kola nut extract.

The results of the study indicate that the high-dose (32 mg/L) dosing solution induced a significant decrease in pup body weights from day 4 onward; the mid- and low-dose effects were less pronounced, not evident until the final week before weaning, and did not attain statistical significance. Both eye opening and hair appearance occurred earlier in all three exposed groups compared to controls. Some effects on locomotional behavior were observed in treated offspring, but the responses appeared to differ between males and females, and there was no clear dose response. The results of the 'tube restraint test', which is purported to measure fear-induced attack via biting on a signal wire protruding from

the end of the restraining tube, suggested a decreased latency to first bite in all treated males, but not in females. The number of bites to the wire was also increased in some treated animals, but not in a dose-responsive manner (Ajarem and Ahmad, 1994).

The study described above (Ajarem and Ahmad, 1994), suffers from several limitations, including not meeting FDA Redbook core standards (FDA, 2000). A key reason for this is that there was no attempt to measure or control the content of the dosing solutions. Further, the solutions were prepared simply by drawing off the supernatant from a defined suspension of kola nuts in water that sat undisturbed overnight. Finally, the behavioral tests employed are insufficiently described, and their general acceptance among the scientific community does not appear to have been established.

2.2.3. Neurotoxicity

Scotto et al. (1987) conducted a behavioral study of rats exposed for two weeks to either fresh kola seed (i.e., nuts) extract (*C. nitida*) or pure caffeine. The rationale for the study derived from reports in the literature dating to the late 19th and early 20th Centuries indicated that the behavioral effects of dried kola seeds were closely akin to those of pure caffeine, whereas the response to fresh seeds was more moderated and longer lasting. The fresh kola seed extract employed in the present study was standardized to contain 6.2% caffeine, 0.9% theobromine, and 15% catechine. Male Wistar-AF rats (10/dose group) were administered distilled water (control), caffeine (20 mg/kg), or fresh kola seed extract (320 mg/kg, containing 20 mg/kg caffeine) via oral gavage each morning for two weeks. Behavioral observations (5 min/observation) were performed one hour after dosing on days 1, 4, 9, and 14 of treatment, as well as on days 3 and 7 following treatment. The behavioral parameters assessed included number of squares crossed on a locomotion grid, rearing behavior, resistance to capture, and reaction to tail tapping and to suspension on an elevated wire. The results reinforce earlier assessments that the neurostimulatory effects of kola seed extract are similar, but more gradual as compared to those of pure caffeine. The extract may also have an effect on muscle tone not evident with caffeine alone (Scotto et al., 1987).

As an extension to the studies described above (Scotto et al., 1987), and in parallel with pharmacokinetic studies (see Section 2.1), Vaille et al. (1993) evaluated the effects of kola nut extract on electroencephalogram readings in male Wistar-AF rats. Animals (10/dose group) were administered distilled water, pure caffeine (20 mg/kg/day), or kola nut extract (320 mg/kg/day, containing caffeine equal to 20 mg/kg/day), via oral gavage, for 15 consecutive days. Test article administration commenced ten days after surgical implantation of five recording electrodes into the skull of each animal. EEG recordings were taken prior to any treatment (baseline), at one hour after dosing on days 1, 5, 10, and 15 of treatment, and also on the seventh day following treatment cessation. A frequency analysis was also conducted on five rats from each group on the 12th day of treatment. The authors conclude that the effects of kola nut extract on cortical activity are consistent with what is known from the literature of the corticostimulatory effects of caffeine on EEG patterns, with the caffeine and the kola nut extract patterns being largely similar. The differences are mainly characterized by a broadening and increase in complexity of the spectral patterns of kola nut extract-treated animals. The authors note this to be consistent with the pharmacokinetic findings indicative of the caffeine in kola nut being slowly released from complexation with catechins in the nut (Vaille et al., 1993).

Ajarem (1990) studied locomotion behavior in male mice following intraperitoneal injection of kola nut extract (*C. nitida*). The extract consisted of the clear supernatant drawn off from a suspension of a paste of ground fresh kola nuts in normal (0.9%) saline that had sat undisturbed overnight. Male Swiss-Webster mice (6/dose group) were injected intraperitoneally with 0, 2.5, 5, or 10 mg/kg bodyweight of this supernatant extract, adjusted to a uniform dose volume of 0.1 mL. No analysis or control of any chemical constituents of this extract was conducted. At defined intervals following dosing of 15, 30, 60, or 120 min, the animals were placed in an enclosed arena for behavioral observations. These observations were 300 s in duration each and consisted of quantification of the number of floor grid squares crossed, rearing behavior, duration of locomotion, and immobility. The report does not state whether all six animals of each dose group were subjected to observation at each observation interval. The author concluded that while the mid-dose group (5 mg/kg) exhibited significantly increased locomotor activity, the high dose had a depressant effect, and the low dose had no effect. However, again, as with the related report described above (see Section 2.2.2) (Ajarem and Ahmad, 1994) from the same laboratory, interpretation of this study (Ajarem, 1990) is hindered by generally insufficient description of its design and conduct, and by an apparent lack of adherence to FDA standards (FDA, 2000), thus limiting its utility for risk assessment purposes.

2.2.4. Genotoxicity

Ishidate et al. (1984) reported the results of mutagenicity screening of 190 synthetic food additives and 52 food additives derived from natural sources, all of which were in use in Japan at that time. Among the additives from natural sources that were tested was a substance identified only as 'Cola extract'. This substance (in physiological saline) was tested for chromosomal aberrations in the Chinese hamster fibroblast cell line, CHL, at concentrations of up to 16.0 mg/mL. The results were reported as negative, indicating that the total incidence of cells with aberrations (including gaps) was 4.9% or less.

3. Discussion

Kola nut extract is used in the food industry as a flavoring ingredient. It is regulated as GRAS by FDA, declared GRAS by FEMA, and is approved for use by the CoE. Kola nut extract is derived from the seeds (nuts) of primarily two tropical Cola species (*C. nitida* (Vent.) Schott et Endl. or *C. acuminata* (Beauv.) Schott et Endl.) of the plant family, Sterculiaceae. Caffeine and theobromine are two important constituents of kola nuts. Kola nuts are not a food, per se, but chewing them for their stimulant/appetite suppressant properties has a social and cultural history among indigenous African people which dates at least to the 14th Century. The extract has been used in cola-type beverages in the U.S. since the late 19th Century. Present day consumption of kola nut extract is 0.69 mg/kg/day.

Published toxicity data that are specific to kola nut extract are very limited and do not meet FDA Redbook standards (FDA Redbook, 2000) . In general, the available data suggest that the pharmacological/toxicological properties of kola nut extract parallel to those of a roughly equivalent dose of caffeine. Adverse developmental/reproductive effects did not include frank terata and the reported behavioral effects are difficult to interpret in terms of equivalent effects in humans. A NOEL/NOAEL cannot be defined for repeated oral exposure to kola nut extract from the available data.

A key limitation of the available published toxicity data on kola nut extract is that they contain little or no detailed analytical characterization of the tested extract. Couple this with the fact that food ingredient specifications for kola nut extract have not been established, or at least published, and it becomes impossible to determine a quantitative estimate of a tolerable daily intake for kola nut extract, per se. Nevertheless, U.S. consumers have a his-

tory of safe consumption of cola-type beverages containing kola nut extract that dates at least to the late 19th Century, with a significant global history of exposure to the intact kola nuts that date centuries longer. And, to the extent that caffeine is the chemical constituent primarily, if not solely, responsible for any pharmacological/toxicological effects experienced from consumption of kola nut extract, there is an even longer history of safe global human consumption of caffeine-containing foods and beverages, such as coffee, tea, and chocolate. All of this history speaks to an understanding of, and tolerance for, the various desirable and adverse effects of caffeine that is widely ingrained across many different cultures and ethnicities.

4. Conclusion

Given this global safe history of use and existing regulatory constraints on added caffeine levels to food and beverages, it is scientifically reasonable to conclude that consumption of kola nut extract as an added food ingredient is safe at present use levels.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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