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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Cola nitida* (Vent.) Schott et Endl. and its varieties and *Cola acuminata* (P. Beauv.) Schott et Endl., semen

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Cola nitida* (Vent.) Schott et Endl. and its varieties and *Cola acuminata* (P. Beauv.) Schott et Endl., semen
Herbal preparation(s)	Powdered herbal substance Liquid extract (1:1, 60% ethanol) Tincture (1:5, 60% ethanol)
Pharmaceutical form(s)	Herbal preparations in liquid dosage forms for oral use. Powdered herbal substance in solid dosage forms and as herbal tea for oral use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.
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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

According to the European Pharmacopoeia, Colae semen is the whole or fragmented dried seeds, freed from the testa, of *Cola nitida* (Vent.) Schott et Endl. (*C. vera* K. Schum.) and its varieties, as well as of *Cola acuminata* (P. Beauv.) Schott et Endl. (*Sterculia acuminata* P. Beauv.). Content: minimum 1.5 % of caffeine (dried drug) (Ph. Eur. 7.0).

Cola seed consists of the endosperm freed from the testa of various *Cola* species Schott et Endlicher, particularly *C. nitida* (Ventenat), belonging to the family of the Sterculiaceae. The drug contains at least 1.5% methylxanthine (caffeine, theobromine) (Blumenthal, 1998).

According to *Bruneton*, the French Pharmacopoeia (10th Ed.) indicates that the part to be used is the seed, devoid of tegument and dried, of *C. acuminata (*Bruneton, 1998).

According to the definition of the British Herbal Pharmacopoeia 1974, Cola (syn. Cola nuts, Cola seeds) consists of the dried cotyledons of *Cola nitida* A. Chev. and of *C. acuminata* Schott & Endl. with a caffeine content not less than 1.25% (British Herbal Pharmacopoeia 1974). In the British Herbal Pharmacopoeia 1996 the definition is as follows: dried cotyledons of *C. nitida* (Vent.) Schott et Endl. or *C. acuminata* (Beauv.) Schott et Endl (British Herbal Pharmacopoeia 1996).

The fruits comprise two to six lignified, voluminous (8-12x4-8 cm) follicles gathered into a star; they are generally collected before maturity. The follicles are opened and the seeds (5-10/follicle) are recovered and left for several days in a pile or immersed in water. Next, the pulpy tegument, which has disintegrated, is eliminated (Bruneton, 1998).

The seeds, incorrectly called Cola nuts, comprise two cotyledons (*C. nitida*) or 3-6 cotyledons (*C. acuminata* (Niemenak, 2008). The colour of the fresh seeds varies, those of *C. acuminata* being white or crimson and *C. nitida* either red or white (Evans, 2002).

Macroscopical description: Reddish brown, plano-convex pieces about 2.5 cm long and 2 cm wide but frequently somewhat distorted, hard and solid, fracture difficult but short, fractured surface smooth and pale brown (British Herbal Pharmacopoeia 1996).

Microscopical description: Outer layer of thick-walled cells containing brown tannin, inner portion of thin-walled polyhedral, parenchymatous cells 40-50 micrometer wide containing simple, ovoid or spherical starch granules 5-10 micrometer and 20-30 micrometer in diameter with a protuberance and a two to three radiate hilum (British Herbal Pharmacopoeia 1996).

Odour and taste: Cola seeds have no odour. The herbal substance has a bitter, astringent taste when fresh. After drying, the taste becomes milder and faintly aromatic, with an odour suggestive of nutmeg.

Botanical characteristics of Cola seeds:

The *Cola* genus comprises about 140 species and the most commonly consumed are *C. acuminata* and *C. nitida*. (Bruneton, 1998) Other species frequently used in commerce include *C. verticillata* and *C. anomala* (Blumenthal, 2000).

The early records did not distinguish between the two commercial species, *C. nitida* and *C. acuminata*. The major centres for *C. nitida* were Sierra Leone, Benin, Ghana and Ivory Coast. By the middle of the

20th century the cultivation of the species had spread westwards to the southern border of Senegal and Gambia, eastwards into Zaire and also overseas to the Caribbean islands, especially Jamaica. *C. acuminata* has its original area of distribution stretching from Nigeria to Gabon and it has been extensively planted in other parts of West Africa (Adeyeye, 1994).

Synonyms of Cola (according to Seitz, 1992):

- *Cola nitida* (Vent.) Schott et Endl.: *C. vera* K. Schum., *Sterculia nitida* Vent., *C. acuminata* var. *latifolia* Schum.
- *Cola acuminata* (P. Beauv.) Schott et Endl.: *Sterculia acuminata* P. Beauv., *C. pseudoacuminata* Engl.

Phytochemical characteristics of Cola seeds:

Purine alkaloids

Cola seeds contain purine bases chiefly represented by caffeine, ranging from 1.5 to 3.2% (2.5% on average in the dried drug) (Bruneton, 1998), traces of theobromine, which ranges from 0.02 to 0.08% and theophylline (Morton, 1992). The composition of *C. nitida* and *C. acuminata* seeds differ only quantitatively. The purine alkaloid content of *C. nitida* is higher (Seitz, 1992).

In a systematic analysis of different Cola accessions the caffeine and theobromine content of *C. acuminata* was determined as 4725-16138 mg/kg and 76-1277 mg/kg, respectively and 8624-19302 mg/kg and 37-1570 mg/kg for *C. nitida*. The quantity of caffeine by the sum of the means was 21 fold higher than theobromine (Niemenak, 2008).

Phenolic compounds

The tannin content of Cola seeds is 5-10%. Other notable constituents are the flavan-3-ol type polyphenols: (+)-catechin,(-)-epicatechin, and proanthocyanidin dimers of group B (Bruneton, 1998). Tannins also include colatin, colatein, colanin (Heinrich, 2004).

The total phenolic content of 0.1 M HCl extract of Cola seed was determined as 49.36±2.75 and 31.36±4.83, respectively in *C. nitida* and *C. acuminata* (expressed in mg equivalent of chlorogenic acid per g of fresh weight). Catechin was the major compound and represented 48.93±2.5% and 51.18±2.2% of total soluble phenols (Boudjeko, 2009).

White and pink Cola *acuminata* seed (90% methanol extract) contains 3.37 and 4.17 mg/100 g fresh weight total phenol, respectively. The same value for white, pink and red *C. nitida* semen was 4.45, 6-12 and 9.09 (Odebode, 1996).

Phenolic compounds identified from 90% methanol *Cola* extract using authentic phenolic compounds as reference, Solvent B.A.W.(4:1:5) were as follows:

Authentic phenolic compounds	***C. nitida***	***C. acuminata***
Chlorogenic acid	++	++
Quinic acid	++	++
Tannic acid	+++	+++
Catechin	+++	+++
Epicatechin	+++	+++
Gentisic acid	-	-
Rubutin	-	-

In a systematic analysis of different Cola accessions the total polyphenol content of *C. acuminata* was determined as 56.3-105.2 mg/g and 39.0-70.3 mg/g for *C. nitida*. Catechin was the predominant flavonoid in the seeds. The catechin content was 7-24 mg/g in *C. acuminata* and 2-10 mg/g in *C. nitida*. (Niemenak, 2008).

Two condensed proanthocyanidins have been isolated from the fresh fruit of *C. acuminata* (Adeyeye, 1994).

Caffeine forms a molecular association with the catechin derivates, and therefore, the proportions of free and combined caffeine vary depending on whether the drug is fresh, dry, or stabilized (Bruneton, 1998). It has been suggested that the differences in the stimulatory action between fresh and dried seeds may be due to the formation of a caffeine-catechin complex in the latter (Evans, 2002).

It is also considered that, in fresh Cola seeds, an unstable complex occurs as colatin and caffeine glycosides. This complex oxidises and hydrolyses to form ”Cola-red” and free caffeine under the influence of enzymes, when the seeds are drying out. If these enzymes are inactivated prior to drying the seeds, for instance with heat treatment, then this process does not occur and the dried seeds a said to retain their physiological action. It has been stated that caffeine occurs partly free and partly in the above-mentioned complex (Adeyeye, 1994).

Mineral contents

The major elements of Colae semen were Ca (0.07-0.09%), Na, K (1.01-1.47%), Mg (0.2-0.27%) and Fe. Other trace elements determined were Zn, Co, Mn, Cu and Cr. The determination of Pb is to screen for environmental pollution. Phosporus was also determined (0.1-0.2%). The highest mineral concentrations are Na, K and P in that order. *Cola acuminata* is rich in most of the essential minerals and could therefore serve as additional sources of such minerals (Adeyeye, 1994).

Other

Three secondary amines (dimethylamine, pyrrolidine and piperidine) and four primary amines (methylamine, ethylamine, isobutylamine and isopentylamine) were detected in one or more Cola seed varieties. *C. acuminata* contained the highest average amounts of dimethylamine (4 mg/kg), pyrrolidine (7.4 mg/kg) and ethylamine (13 mg/kg). Piperidine and isobutylamine were not detected. Methylamine content was 1.2 mg/kg, isopentylamine was 0.4 mg/kg (Atawodi, 1995).

Thiamine and other B-vitamins (Heinrich, 2004), betaine (2500 ppm), ascorbic acid (540-1456 ppm), β-carotene (1 ppm) are also present in the herbal substance.

Cola seed is good source of protein (8-12%), fat (1.6%), fibre (8.1-9%), total ash (3.4-3.5%) and carbohydrate (52.4-53.1%).

Proximate composition (%) of *C. acuminata* (Adeyeye, 1994):

Parameter	Mean	±SD
Moisture	3.39	0.16
Dry matter	96.62	0.16
Crude protein	11.95	0.35
Total ash	3.95	0.07
Ether extract	15.72	0.69
Crude fibre	14.80	0.28
Carbohydrate	50.20	1.56

Amino acid composition (mg/g crude protein) (Adeyeye, 2007):

Amino acid	*C. acuminata*
Lysine	22.28
Histidine	11.87
Aginine	26.79
Aspartic acid	43.88
Threonine	11.09
Serine	16.37
Glutamic acid	92.06
Proline	10.60
Glycine	17.58
Alanine	15.64
Cystine	4.52
Valine	13.27
Methionine	5.69
Isoleucine	11.04
Leucine	28.65
Tyrosine	7.75
Phenylalanine	17.16

- Herbal preparation(s)

See sections 1.2, 2.2 and 2.3.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	See below
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	See below
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Powdered herbal substance as registered product - see below
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products authorised or registered with Cola as monocomponent

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

Note: this regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

Italy

The following herbal substances/preparations are in the list of ingredients that may be used in food supplements updated on 1 April 2009 with the following indications:

Plant	**Plant part**	**Indications related to:**
Cola acuminata Schott et Endl.	semen	semen: Tonic; physical and mental tiredness.
Cola nitida Schott et Endl.	semen	semen: Tonic; physical and mental tiredness. Stimulation of metabolism.

Poland

Although Embryo Colae and Extractum colae siccum are not present in the current list of authorised products in Poland, there is one combination product described in Polish Pharmacopoeia VI and authorised in Poland, Guttae Cardiaceae cum Colae Extractum Fluidum, containing: Convallariae tinctura titrata 27 parts; Crataegi tinctura 20 parts; Valerianae tinctura 20 parts; Colae extractum fluidum 14 parts; Ethanolum (608 g/l) ad. 100 parts.

Also authorised is the following combination product containing herbal components and caffeine: Crataegi cum Valerianae tinctura (1:4-4.5), ethanol 70% (v/v) 40 g; Convallariae tinctura titrata (1:4-4.5), ethanol 70% (v/v) 27 g; Colae extractum fluidum (1:2), ethanol 70% (v/v) 14 g; Coffeinum 0.072 g; Ethanol 70% (v/v) ad. 100 g.

Spain

One registered product containing Colae semen as single ingredient and 2 multicomponent products.

Preparation: powdered herbal substance as registered product.

Since when is on the market: 24/10/1994.

Pharmaceutical form: capsule (330 mg Colae semen/capsule).

Posology: 2 or 3 capsules 2 times a day.

Indication: tonic, fatigue, asthenia.

1.2. Search and assessment methodology

Handbooks as well as publications (PubMed, SciFinder, Web of Science, ToxNet) were used as sources. Keywords: *Cola acuminata*, Colae semen.

2. Historical data on medicinal use

Cola seeds are therapeutically and industrially important because of their polyphenol and caffeine contents. Without Cola seeds, traditional hospitality, cultural and social ceremonies are considered incomplete in Nigeria and many West African countries (Atawodi, 1995).

In Africa, Cola seeds are traditionally used as masticatory agents for their stimulating effect. It has been claimed by Cola seed consumers that Cola seeds suppress hunger, thirst and sleep. It is also said that Cola seeds strengthen dental gums and suppress gout and related diseases. Some of the seeds are used as a source of dye. The Cola pod is used in making jams and preservatives as well as fertilizer and feeding stuff for animals. The pod husk, mixed with certain ingredients, is used in traditional concoctions to reduce pain. The following products are obtainable from Cola testa: decaffeinated powder and Cola chocolate; caffeine used in pharmaceutical and food preparations; tannins, food colours and dyes; fertilizer and feeding stuff (Adeyeye, 1994).

In Trinidad and Tobago, *C. acuminata* is used for childbirth and fertility and unspecified female problems and to treat diabetes and hypertension (Lans, 2007) (Lans, 2006).

C. acuminata is used as a masticatory when fresh, while the dried seeds are used for beverages and pharmaceutical purposes in Europe and North Africa (Adeyeye, 2007).

The dried seed is utilised in African folk-medicine as a tonic, mild stimulant antiemetic, appetite supressant, and remedy for dysentery. Powdered seeds are applied to cuts to stop bleeding (Morton, 1992). It is thought that Cola increases energy and strength, and dispels drowsiness. These properties could be attributed to the richness of the seeds in purine alkaloids, polyphenols and sugar (Niemenak, 2008).

Cola seeds have also been used in African traditional medicine for the treatment of various ailments including parasitic diseases.

The seeds are also used in treatment of sexual impotence and erectile dysfunction in Western Uganda. They have been in use for centuries in treating or managing conditions of the male reproductive organs. *C. acuminata* is frequently utilised and is already under sale for treating these conditions. The preparation from the fruit can involve roasting, pounding or chewing and the mode of administration is oral in tea, porridge or milk as a beverage (Kamatenesi-Mugisha, 2005).

C. acuminata fruits are mixed with other plants in Benin to treat primary and secondary sterility. The fruits are also said to be diuretic and laxative when administrated orally (Kamatenesi-Mugisha, 2005).

In Nigeria Cola seed is commonly used against emesis gravidarum and migraine (Seitz, 1992).

Dried Cola seeds are shipped all over the world for pharmaceutical use, for extraction of caffeine, for the preparation of dry or aqueous extracts or concentrates to be used as flavourings in carbonated soft drinks, particularly with extracts of de-cocainized *Coca* leaves in 'Cola' beverages (Morton, 1992).

Cola seeds are also used in liqueurs, ice creams, confectionery and baked goods. In Europe, they are used mainly in flavouring various beverages, mineral waters, wine and pharmaceutical products (Morton, 1992). Cola seed is on the Generally Recognized as Safe (GRAS) list for food additives in the United States without any limitation (FDA, 2010).

http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm

Cola seed is approved for food uses by the Council of Europe, the Flavour and Extract Manufacturers' Association and the International Organisation of Flavour Industries (Burdock, 2009).

Specifications for Cola seed extract for use as an ingredient for addition to food have not been published by any relevant authoritative body. Herbal preparations of Cola seed extract for medicinal use are typically standardised on the methylxanthine content, usually 1.5-2.5%. The caffeine content can vary between 1.5% and 3.8%, depending on the variety of seed characterised, as well as the treatment of the Cola seed. Treatments include fresh (raw) seeds, cured seeds (6 months), sun-dried seeds (sun dried for 40 days), and milled and stored seeds (sun-dried seeds milled and stored for 12 months).

The Council of Europe has classified Cola seed extract as 'Category 4', which is defined as "plants, animals and other organisms, and parts of these or products thereof, and preparations derived there from, 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" (Burdock, 2009).

Certain chemical constituents of Cola seeds have also been approved in pure form for use in foodstuff. 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 and theobromine have been approved by the European Commission as flavouring substances for use in or on foodstuff (Burdock, 2009).

Caffeine is also regulated as a drug by FDA, specially 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). In 2005, the FDA has also approved Cola seed extract for use as an inactive ingredient in certain oral and rectal pharmaceutical preparations (Burdock, 2009).

According to Adeyeye, Soreat (1971) had proposed a combination of naturally occurring amino acids (essential and non-essential) and a fresh stabilised Cola seed extract. The proposed composition afforded food products containing 0.5 and 4% essential or non-essential amino acids and 0.4-5% freshly stabilised Cola seed extract (which contains 2-15% caffeine) which can be used in the form of syrups, pellets or powders and can be added to soda water, soluble coffee powders etc. (Adeyeye, 1994).

2.1. Information on period of medicinal use in the Community

See sections 1.2, 2.2 and 2.3.

2.2. Information on traditional/current indications and specified substances/preparations

There is evidence that intra-African trade in Cola seeds dates back to at least the 14th century, with firm written records of African exports to England and the United States which date back to the mid-19th century. It was in 1886 that the druggist John S. Pemberton invented the popular soft drink, Coca Cola, by combining coca and Cola extracts for use as a headache and hang-over remedy. The United States National Academy of Sciences indicated that the first reported use of Cola seed extract was in 1935 (Burdock, 2009).

In Europe, the dried seeds have been used as strong stimulant, in addition to use for the treatment of migraine, neuralgia, diarrhoea, and as a stimulant or cardiotonic, for loss of appetite, and as an antidepressant and for melancholy (Kamatenesi-Mugisha, 2005).

Literature data support the traditional use of Cola seed as medicinal product. Several monographs (Commission E, British Herbal Compendium, British Herbal Pharmacopoeia) have described Cola seed as a medicinal product for the treatment of mental and physical fatigue and for some other indications (Blumenthal 2000) (Bradley 1992) (British Herbal Pharmacopoeia 1996).

In the British Herbal Pharmacopoeia 1974, published in 1971, and British Herbal Compendium Volume 1, published in 1992, the following preparations of Cola seeds are listed (British Herbal Pharmacopoeia 1974):

- Powdered Cola
- Liquid extract (1:1, 60% ethanol)
- Tincture (1:5, 60% ethanol)

The indications of the listed preparations are as follows: depressive states, melancholia, atony, exhaustion, dysentery, atonic diarrhoea, anorexia migraine. A specific indication is also given: depressive states associated with general muscular weakness (British Herbal Pharmacopoeia 1974). In the British Herbal Compendium Volume 1, the indications are short-term fatigue, both mental and physical; depressive states (Bradley, 1992).

In the Commission E monograph published in 1991, the following herbal preparations are included:

- Cola nut (powdered)
- Extract (Erg.B.6) (extracting solvent 90% ethanol v/v, caffeine+theobromine content mininum 12%)
- Liquid extract (Erg.B.6) (percolate with 70% ethanol v/v, caffeine+theobromine content minimum 1.2%)
- Tincture (Erg.B.6) (DER 1:5, 70% ethanol, caffeine+theobromine content minimum 0.25%)
- Cola wine (Erg.B.6) (Cola liquid extract, Xeres wine, sugar sirup 50:850:100)

The approved indication for the herbal preparations listed above is mental and physical fatigue (Blumenthal, 1998).

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

In the British Herbal Pharmacopoeia 1974 (British Herbal Pharmacopoeia 1974) and British Herbal Compendium Volume 1 (Bradley, 1992), the following posologies are listed for the different preparations:

- Powdered Cola: 3x1-3 g (or by decoction)
- Liquid extract (1:1, 60% ethanol): 3x0.6-1.2 ml
- Tincture (1:5, 60% ethanol): 3x1-4 ml

In the Commission E monograph (Blumenthal, 1998), the following posologies (daily dose) are contained:

- Cola nut: 2-6 g Cola nut
- Dry extract (Erg.B.6): 0.25-0.75 g
- Liquid extract (Erg.B.6): 2.5-7.5 g
- Tincture (Erg.B.6): 10-30 g
- Cola wine (Erg.B.6): 60-180 g

The available marketing status overview shows that only one monocomponent Cola seed product exists on the European market as a medicinal product. However, several other medicinal products containing Cola seed are marketed in combination with other herbal ingredients. There are also products marketed as foods or food supplements.

The following herbal substances and herbal preparations have been on the European market for a period of 30 years and are included in the monograph on traditional use:

- Powdered herbal substance
- Powdered herbal substance as herbal tea (decoction)
- Liquid extract (1:1, 60% ethanol)
- Tincture (1:5, 60% ethanol)

Based on the confirmed traditional use and the plausible effects, and taking into account the Community herbal monograph on Mate folium, the following uses are considered appropriate for traditional use: symptoms of temporary fatigue and sensation of weakness.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Caffeine is a mild stimulant and has diuretic properties and this is the main basis of the use of Cola seed. Cola extracts are also astringent and anti-diarrhoeal due to the tannin content. Cola extracts are ingredients of many tonics for depression and tiredness and to stimulate the appetite (Heinrich, 2004).

The pharmacodynamic properties of Cola seed may be interpreted on the basis of its caffeine content. Caffeine itself is sometimes given in conjunction with other analgesics to produce stronger and quicker pain-killing actions, but there is no information of similar application of Cola seeds. Methylxanthines (caffeine, theobromine and theophylline) are used to treat pre-term infant apnoea, chronic obstructive pulmonary disease, and especially asthma. They relax bronchial smooth muscle, stimulate cardiac muscle, and are diuretic (Blumenthal, 2000), however no such application is documented for Cola. The effects of the xanthine derivates on acid secretion have also been reported. The pattern of their effects depends upon the species and conditions employed. Humans are relatively sensitive, and moderate oral or parenteral doses of caffeine cause the secretion of both acid and pepsin. Theophylline was reported to be as potent as caffeine in this respect (Ibu, 1986).

Caffeine increases the free fatty acid and glucose level 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 (Burdock, 2009). From a therapeutic point of view, the most important effect of Cola is the central nervous system stimulatory activity, due to its caffeine content.

The following actions were confirmed in animal experiments for Cola seed: analeptic; stimulates production of gastric acid; lipolytic and increases motility. Compared to other methylxanthines, caffeine is a weaker diuretic and positively chronotropic (Blumenthal, 2000).

Central nervous system effects

Scotto (1987) conducted a behavioural study of rats exposed for two weeks to either fresh Cola seed (i.e., nuts) extract (*C. nitida*) or pure caffeine. The fresh Cola seed extract employed in the 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 Cola seed extract (320 mg/kg, containing 20 mg/kg caffeine) via oral gavage each morning for two weeks. Behavioural observations (5 minutes/observation) were performed 1 hour after dosing on days 1, 4, 9, and 14 of treatment, as well as on days 3 and 7 following treatment. The behavioural parameters assessed included number of squares crossed on a locomotion grid, rearing behaviour, 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 Cola 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, 1987; Burdock, 2009).

Vaille (1993) evaluated the effects of Cola seed extract on electroencephalogram readings in male Wistar-AF rats. Animals (10/dose group) were administered distilled water, pure caffeine (20 mg/kg/day), or Cola seed 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 1 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 Cola seed 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 Cola seed extract patterns being largely similar. The differences are mainly characterised by a broadening and increase in complexity of the spectral patterns of Cola seed extract-treated animals. The authors note this to be consistent with the pharmacokinetic findings indicative of the caffeine in Cola seed being slowly released from complexation with catechins in the seed (Vaille, 1993; Burdock, 2009).

Ajarem (1990) studied locomotion behaviour in male mice following intraperitoneal injection of Cola seed extract (*C. nitida*). The extract consisted of the clear supernatant drawn off from a suspension of a paste of ground fresh Cola seeds in normal (0.9%) saline, left undisturbed overnight. Male Swiss-Webster mice (6/dose group) were injected intraperitoneally with 0, 2.5, 5, or 10 mg/kg body weight 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 minutes, the animals were placed in an enclosed arena for behavioural observations. These observations were 300 s in duration each and consisted of quantification of the number of floor grid squares crossed, rearing behaviour, 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 (Ajarem, 1990).

The comparative effects of chronic [28 days] consumption of Cola seed and its active constituent, caffeine diets on locomotor behaviour and body weights in mice were investigated by *Umoren*. Thirty adult Swiss white mice [15-30 g/body weight], were used for the study. The open field-maze was employed for the evaluation of locomotor behaviour. Mice in the control group [n=10] were fed normal

rodent chow, mice in the Cola seed-fed group [n=10] were fed Cola diet [25 % w/w of rodent chow] while those in the caffeine-fed group [n=10] were fed caffeine diet [0.66% w/w of rodent chow] for 4 weeks. All animals were allowed free access to clean drinking water. Daily food intake, water intake and body weight change were also measured. Daily food intake in the Cola seed and caffeine-fed group of mice was significantly [P<0.001 respectively] lower than the control. There was also a significant [P<0.001] decrease in daily water intake in the caffeine-fed group compared to the control whereas, the apparent decrease of water intake in the Cola seed-fed group was not significantly different from the control. Body weight change was also significantly [P<0.001 and P<0.05 respectively] lower in the Cola seed and caffeine-fed groups of mice when compared to the control. The frequency of rearing in the open field was significantly [P<0.01] lower in the caffeine-fed group of mice when compared to the control. The frequency of grooming was also significantly [P<0.05] lower in the caffeine-fed group of mice when compared to the control. There was also a significant [P<0.05] decrease in the frequency of light-dark transitions in the light/dark transition box for the caffeine-fed group when compared to the control. The results showed that chronic consumption of Cola seed and caffeine diets caused decrease in food intake and body weight. Consumption of caffeine-diet also significantly decreased water intake and locomotor activity. The effect of Cola seed-diets on water intake and locomotor activity was not significant. Hence, the effect of Cola seed on locomotor behaviour and water intake may not be due to caffeine only (Umoren, 2009).

Antioxidant activity

The effectiveness of certain antioxidant substances (among them Cola extracts) in the protection of red cells from oxidation and degradation with respect to their window times of survival has been analysed using UV-visible spectrophotometry. In the presence of an oxydizing agent (potassium ferricyanide), lysis of red cell membrane, oxidation of exposed haemoglobin and methemoglobin formation were observed for 12 hours. 70% methanol extracts *C. acuminata* and *C. nitida* were effective as antioxidants in red cell survival and viability. The order of antioxidative potency was as follows: *C. acuminata* (white)>*C. nitida* (pink)>*C. nitida* (red) (Atolaye 2009).

Anti-inflammatory activity

Daels-Rakotoarison evaluated, in both cell-free and cellular *in vitro* systems, the ability of decaffeinated Cola seed extracts 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 Cola seeds and under rather stringent extraction conditions, followed by decaffeination with dichloromethane. The resulting extract primarily contained phenolic compounds. It was reported to inhibit both elastase release and activity, presumably through its antioxidant properties (Daels-Rakotoarison, 2003).

Trypanocidal activity

The ethanol extracts from Cola seeds exhibited a considerably high trypanocidal activity (Kubata, 2005).

A new lead anti-trypanosomal compound was isolated from *Cola acuminata*. A proanthocyanidin oligomer was purified by chromatographic procedures and its homogeneity and structure was confirmed by NMR and MALDI-TOF mass spectrometry. *In vitro*, this compound potently induced growth arrest and lysis of bloodstream form trypanosomes in a dose- and time-dependent manner. In a mouse model, it exhibited a trypanostatic effect that extended the life of infected, treated animals up to 8 days post-infection against the 4 days for infected, untreated animals. The proanthocyanidin showed a low cytotoxicity against mammal cells, whereas treated-bloodstream form showed massive enlargement of their flagellar pocket and lysomose-like structures caused by an intense formation of multi-vesicular bodies and vesicles within these organelles. The observed ultrastructural alterations

caused rupture of plasma membranes and the release of cell contents, indicative of a necrotic process rather than a programmed cell death. The proanthocyanidin acted against the bloodstream form but not the procyclic form trypanosomes. This new anti-trypanosomal compound should be further studied to determine its efficacy and suitability as an anti-trypanosomal drug and may be used as a tool to define novel specific drug targets in bloodstream form trypanosomes (Kubata, 2005).

Cultures of bloodstream form trypanosomes treated with 100 µg/ml of ethanol extract of Cola seed showed dying cells as early as 1 hour after the drug addition. A change in the morphology of drug-treated dying cells during incubation was also observed. The Cola seed ethanol extract (100 µg/ml) exhibited a potent trypanocidal activity that completely inhibited the *in vitro* growth of bloodstream form *T. brucei* after 12 hours of exposure.

In the culture of procyclic trypanosomes, the anti-trypanosomal activity of *C. acuminata* extract was observed only after 24 hours treatment with the respective drugs.

The results revealed that *in vivo* Cola seed proanthocyanidin exhibited a trypanostatic rather than a trypanocidal effect. The anti-trypanosomal activity decreased with increasing concentrations of BSA (serum albumin) in the culture medium. The proanthocyanidin appeared to bind to BSA in a dose-dependent manner. This result suggested that the limited *in vivo* action may in part be due to the binding of the drug to mouse serum albumin, which presumably would reduce the bioavailability of the drug within the bloodstream, resulting in a trypanostatic effect. Cola seed proanthocyanidin seemed to act directly on the parasites rather than by the activation of host macrophages to produce microbicidal cytokines (Kubata, 2005).

Cytotoxicity

Cytotoxic studies on human epidermoid carcinoma (KB 3-1) cells revealed that, whereas a proanthocyanidin isolated from *C. acuminata* caused a complete inhibition of the bloodstream trypanosomes (10^6 cells) at a concentration of 50 µg/ml, the anti-trypanosomal compound showed no cytotoxicity toward mammalian cells at these concentrations. Cytotoxic effects of the compound were only observed at higher dosages (250 µg/ml), i.e. four-five times the amount of proanthocyanidin needed to completely inhibit the growth of 10^6 trypanosomes. The pure proanthocyanidin as well as the ethanol extract had no effects on various strains of *Escherichia coli*, demonstrating that this new anti-trypanosomical drug is highly specific to *T. brucei* (Kubata, 2004).

The purpose of the study of *Fontenot* was to characterise the putative phytoestrogenic compounds present in *Cola acuminata* for oestrogenic-like activity. As an initial step, five extracts (E1 - hexane, E2 - ether, E3 - acetone, E4 - methanol and E5 - water) were sequentially generated using solid-liquid phase extraction and their bioactivity was examined in MCF-7, MDA-MB-468 and LNCaP cancer cell models. MTT cell viability, dye exclusion, caspase activity and microscopic assessment of apoptotic cells demonstrated that extracts of Cola were cytotoxic to MCF-7, MDA-MB 468 and LNCaP cells. In MCF-7 cells, the acetone extract (E3) at 100 ppm elicited a potent cytotoxic response with a growth-inhibitory concentration (GI_{50}) of 67 ppm. In contrast, E3 stimulated growth in LNCaP cells. The ether extract (E2) showed a dose-dependent cytotoxic response with a GI_{50} of 13 ppm in the LNCaP cell line. Examination of the apoptotic response induced by E2 and E3 paralleled the level of cell cytotoxicity observed in both cell lines. The methanol extract (E4) was the only extract that showed a time-, dose-, and oestrogen-receptor-dependent stimulation of pS2 gene expression. On the other hand, the acetone extract (E3), which showed the highest degree of cytotoxicity, showed no transcription stimulation of pS2 in MCF-7 cells (Fontenot, 2007).

Cardiovascular effects

Investigation of the effect of aqueous extracts of different Cola seeds (*C. acuminata*, *C. nitida* subsp. *rubra*, *C. nitida* subsp. *alba*) on the rhythmic activity of mammalian heart and metabolic rate was carried out using male albino rats. Low concentrations of Cola seed extract stimulated the heart by increasing rate and force of contraction as well as metabolic rate. Higher concentrations reduced rate and amplitude of heart beat resulting, at even higher concentrations, in heart failure. The extract was added in increasing quantities of 0.2 ml each time. The calculated doses for animals of different body weights were diluted to the following range of concentrations: 2, 4, 8 and 10 mg/ml. As the concentration of the extracts was increased, the rate of metabolism also increased up to a certain limit. The increase in basal metabolic rate at high concentrations (8-10 mg/ml) was smaller than that at lower concentrations. The dose-response curves were similar for *C. acuminata* and *C. nitida* rubra (*C. nitida alba* was not tested). *C. acuminata* increased the force of contraction by 40-60% and rate by 30-47%. As the quantity of the Cola seed extract injected was increased there was a corresponding decrease in both force of contraction and rate. *C nitida rubra* increased the force by 13-20% and rate by 3%, while *C. nitida alba* increased the rate by 10-16% and force by 2%. The force of heart contraction was always the first to be affected, its decrease occurring before that of the heart rate. At even higher concentrations, the force of contraction was so severely reduced that heart failure resulted. Prior to heart failure, the rate was reduced by 60-70% and the force by 80-90%. The xanthines stimulate the mammalian myocardium directly. In isolated mammalian heart, both the rate of beat and force of contraction are increased by caffeine and theophylline (Chukwu, 2006).

Gastrointestinal effects

The use of Cola beverages has been implicated in the pathogenesis of peptic ulcer and in the management of the ulcer. The administration of caffeine to animals causes pathological changes in the gastrointestinal tract and ulcer formation. The exact mechanism by which Cola induces gastric acid secretion is not known. However, there is increasing evidence that cyclic AMP is implicated in gastric acid secretion caused by the alkaloids. The induction of gastric acid secretion by Cola could be entirely due to the presence in Cola of the xanthines or it may involve other gastric secretagogues in Cola not yet identified (Ibu, 1986).

Male and female albino rats of Wistar strain were used for the experiment. The rats were starved for 12 hours before use. The Cola was extracted in normal saline (pH 7). The test animals were divided into 2 groups. One group had the Cola by intravenous injection; the other had the extract by continuous gastric perfusion. The stimulatory dose used was 10 mg/100 g body weight. By perfusing the rats' stomach with Cola extract, the acid output increased from a mean basal value of 0.88 ± 0.08 mMol/l/h to 1.5 ± 0.77 mMol/l/h. This represents a percentage increase of 69.3%. By intravenous route the acid secretion increased from 1.27 to 2.10 mMol/l/h, which represents an increase of 65.3%. So through the oral route, the stimulation of acid secretion is slightly higher than the intravenous route. This may be due to a direct action of the Cola on the parietal cells in the stomach as no absorption is involved (Ibu, 1986).

Osim (1991) investigated the relative effect on gastric acid secretion in cats of Cola seed 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) consisted of a filtrate of 6 g of ground dry Cola seeds and 100 mg of caffeine, both dissolved in 200 ml of 0.9% saline. The infusion rate was maintained at 1 ml/minute. Perfusate samples were collected at 10 minutes intervals for up to 3 hours after dosing and titrated for total acidity. Treatment with Cola

seed 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 Cola seed extract. The acid secretion response to Cola seed extract treatment was blocked almost completely by intravenous administration (1 hour after Cola seed extract) of either atropine (0.2 mg/kg body weight) or cimetidine (12 µM/kg body weight), which block, respectively, muscarinic and histaminic receptors. Based on these findings, the authors speculated that caffeine is not the only constituent in Cola seeds 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, 1991) (Burdock, 2009).

Hormonal activities

The study of *Solipuram* characterized the androgenic and chemopreventative properties of the *C. acuminata* using androgen receptor positive and negative cell lines. Exposure of prostate cells to the ether extract of *C. acuminata* resulted in a growth inhibition (GI_{50}) of 15 ppm in LNCaP cells and 3.6 ppm in DU145 cells. The extract elicited a 2-fold increase in the mRNA of the anti-apoptotic gene Bcl2, with a 10-fold increase in that of the proapoptotic gene Bax. A 2.4- to 7.5-fold change in apoptotic cells was observed in both cell lines. The ether extract at 10 ppm elicited a time- and dose-dependent stimulation of both the protein and mRNA levels of several androgen-regulated genes. The extract caused a 36% decrease in PSA secretion and a significant increase in PSA mRNA. The relative binding affinity (IC_{50}) of the extract for androgen receptor (AR) was 2-5-fold lower than that of the synthetic androgen R1881. The ether extract was found to be a specific ligand for the AR in that the natural ligand, DHT, and the anti-androgen, flutamide, displaced the extract bound to AR and inhibited Biz-2-induced transcription and PSA secretion (Solipuram, 2009).

The oestrous cycles of rats treated with hydro-alcohol extracts (90 mg/kg intraperitoneal) of *Cola nitida*, were blocked at the dioestrous II stage. Only 50% of the cycles of rats treated with *Cola nitida* were disrupted. The extract contains weak antioestrogen-like activity that provokes a blockage of female rat ovulation and oestrous cycle by acting on the hypophysis and/or hypothalamus secretion. This effect was mediated by oestrogen receptors (Benie, 2003).

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data with regard to absorption, distribution, metabolism, elimination are available. According to older studies, the absorption of caffeine is slower from the dry drug than from the caffeine-catechine complex of the fresh plant material, however this needs further clarification (Seitz, 1992).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Because caffeine and methylxanthines are principal components of Cola seeds, at least some of the potential toxicities could be due to them. Methylxanthine toxicity has been assessed extensively elsewhere (Eteng, 1997) and is not presented here.

Acute toxicity

There are no studies available to evaluate acute toxicity of Cola seed extracts.

Subchronic toxicity

Male Wistar albino rats (12/dose group) were administered distilled water (control) or Cola seed extract (*C. nitida*) (0.5 ml, equivalent to 57 mg/kg body weight) via oral gavage, every other day, for 18 weeks. The extract consisted of the supernatant obtained when fresh Cola seeds were ground to the consistency of flour and suspended in hot distilled water (100 g/200 ml), then allowed to sit undisturbed at room temperature overnight. No analysis or further characterisation 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 Cola seed extract over the course of the study. Treatment with Cola seed extract 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 compared to control animals, 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 Cola seed 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, 1981) (Burdock, 2009).

Teratogenicity

Ajarem and *Ahmad* (1994) studied the effects of a water extract of fresh Cola seeds (*C. nitida*) on the post-natal development and behaviour of mice. Pregnant Swiss-Webster albino mice (8/dose group) were exposed via the drinking water to Cola seed extract at reported concentrations of 0, 8, 16, or 32 mg/l (roughly equivalent to the doses of 0, 2.5, 5 or 10 mg/kg body weight), from gestation day 0 (the day the vaginal plug was detected) until weaning (day 24 postpartum). 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 behaviour, 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 Cola seed 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 behaviour 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, 1994) (Burdock, 2009).

Genotoxicity

Ishidate reported the results of mutagenicity screening of 190 synthetic food additives and 52 food additives derived from natural sources. 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 (CHL) fibroblast cell line, at concentrations of up to 16 mg/ml. The results were reported as negative, indicating that the total incidence of cells with aberrations (including gaps) was 4.9% or less (Ishidate, 1984) (Burdock, 2009).

The mutagenicity studies for caffeine have inconclusive results (Genetox, 2010).

Carcinogenicity

There are no studies on carcinogenicity of Cola preparations. However, there has been a concern on the formation of nitrosatable components under certain physiological conditions, because many nitrosamines are powerful carcinogens (see below).

Nitrosatable amines and nitrosatable components formation in C. acuminata

Three secondary amines (dimethylamine, pyrrolidine and piperidine) and four primary amines (methylamine, ethylamine, isobutylamine and isopentylamine) were detected in one or more Cola seed varieties. *C. acuminata* contained the highest average amounts of dimethylamine (4 mg/kg), pyrrolidine (7.4 mg/kg) and ethylamine (13 mg/kg). Piperidine and isobutylamine were not detected. Methlyamine content was 1.2 mg/kg, isopentylamine was 0.4 mg/kg. Consumption of Cola seeds in habitual chewers generally varies from 50 g to over 200 g fresh seeds per day. On the basis of these figures, average daily exposure to aliphatic amines and, possibly, nitrosamides have been estimated in the following table:

Secondary amines	**Intake microgram / day**	**Primary amines**	**Intake microgram / day**
Dimethylamine	200-800	Methylamine	60-240
Pyrrolidine	370-1480	Ethylamine	650-2600
Piperidine	Not detected	Isobutylamine	Not detected
Total	570-2280	Isopentylamine	20-80
Total			730-2920

The methylating activity of Cola seeds is the highest ever reported for any fresh plant product that is consumed raw without processing. However the methylating activity was lowest in *C. acuminata* compared to other types of Cola.

Possible *in vivo* formation of direct-acting mutagens, including nitrosourea, nitrosourethane and nitrosoguanidine, has been intensively investigated and DNA methylation in the digestive tract of rats chronically exposed to *N*-nitroso-*N*-methylurea or precursors has been demonstrated. Earlier reports have suggested primary amines and creatinine as possible precursor compounds for *in vivo* formation of nitrosamides from foods and drugs. However, recent evidence indicates that caffeinated materials like Cola seeds, caffeidine and caffeidine acid, both decomposition products of caffeine, might be the precursor compounds. Preparation of appropriate dilutions of Cola extracts for determination of nitrite and nitrate proved difficult. However, reduction of nitrate to nitrite has been suggested as the most effective contributor of nitrite to the human body. In habitual chewers, the mouth is hardly ever free of Cola seed extracts. Therefore, the possibility of amines ingested from chewed Cola seeds undergoing nitrosation in the oral cavity under the influence of a resident bacterial population should also be

considered. The Cola seed is usually chewed throughout life in habitual chewers since it contains caffeine which is an addictive compound. Moreover, most Cola chewers are also cigarette smokers. Therefore, it may be expected that an increased concentration of thiocyanate in the saliva, due to smoking, could catalyse the nitrosation of amines ingested from Cola seeds. Complex interactions might occur *in vivo* between carcinogens and carcinogenic precursors contained in or generated from both tobacco and Cola seeds (Atawodi, 1995).

3.4. Overall conclusions on non-clinical data

The traditional use of Cola seed for physical and mental tiredness, symptoms of fatigue and sensation of weakness can be explained by the caffeine content. The caffeine content of Cola seed is at least 1.5% (Ph. Eur. 7.0). The maximum daily dose of Cola seed (9 g) is equivalent to 135-288 mg caffeine. At such a dose there is plausibility for the pharmacological effects of caffeine to be exhibited in the human organism.

Although there are many articles on the pharmacokinetics of caffeine, the main active component of Cola, no data are available on that of the herbal substance or the herbal preparations, therefore, no conclusion can be drawn.

The non-clinical toxicological data for Cola are limited. However, taking into account the long-standing safe use of the herbal substance in therapeutic doses, toxic effects can be excluded.

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

For the herbal substance and its preparations, no data are available.

However, the effect of caffeine on mental and physical tiredness has been convincingly confirmed.

The aim of the study of *Glaister* was to examine the effects of caffeine supplementation on multiple sprint running performance. Using a randomised double-blind research design, 21 physically active men ingested a gelatin capsule containing either caffeine (5 mg/kg body mass) or placebo (maltodextrin) 1 hour before completing an indoor multiple sprint running trial (12 x 30 m; repeated at 35-s intervals). Venous blood samples were drawn to evaluate plasma caffeine and primary metabolite concentrations. Sprint times were recorded via twin-beam photocells, and earlobe blood samples were drawn to evaluate pre-test and post-test lactate concentrations. Heart rate was monitored continuously throughout the tests, with Rate of perceived exertion (RPE) recorded after every third sprint. Relative to placebo, caffeine supplementation resulted in a 0.06-s (1.4%) reduction in fastest sprint time (95% likely range = 0.04-0.09 s), which corresponded with a 1.2% increase in fatigue (95% likely range = 0.3-2.2%). Caffeine supplementation also resulted in a 3.4-bpm increase in mean heart rate (95% likely range = 0.1-6.6 bpm) and elevations in pre-test (+0.7 mmol/l; 95% likely range = 0.1-1.3 mmol/l) and post-test (+1.8 mmol/l; 95% likely range = 0.3-3.2 mmol/l) blood lactate concentrations. In contrast, there was no significant effect of caffeine supplementation on the RPE. The results of the study show that caffeine has ergogenic properties with the potential to benefit performance in both single and multiple sprint sports (Glaister, 2008).

Hogervorst carried out a study to examine the effects of ingesting a performance bar, containing caffeine, before and during cycling exercise on physical and cognitive performance. Twenty-four well-

trained cyclists consumed the products [a performance bar containing 45 g of carbohydrate and 100 mg of caffeine (CAF), an isocaloric noncaffeine performance bar (CHO), or 300 ml of placebo beverage (BEV)] immediately before performing a 2.5 hours exercise at 60% VO_2 max followed by a time to exhaustion trial (T2EX) at 75% VO_2 max. Additional products were taken after 55 and 115 minutes of exercise. Cognitive function measures (computerised Stroop and Rapid Visual Information Processing tests) were performed before exercise and while cycling after 70 and 140 minutes of exercise and again 5 minutes after completing the T2EX ride. Participants were significantly faster after CAF when compared with CHO on both the computerised complex information processing tests, particularly after 140 minutes and after the T2EX ride ($P < 0.001$). On the BEV trial, performance was significantly slower than after both other treatments ($P < 0.0001$). There were no speed-accuracy tradeoffs ($P > 0.10$). T2EX was longer after CAF consumption compared with both CHO and BEV trials ($P < 0.05$), and T2EX was longer after CHO than after BEV ($P < 0.05$). No differences were found in the ratings of perceived exertion, mean heart rate, and relative exercise intensity (%VO_2 max; $P > 0.05$). Caffeine in a performance bar can significantly improve endurance performance and complex cognitive ability during and after exercise. These effects may be salient for sports performance in which concentration plays a major role (Hogervorst, 2008).

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

For the herbal substance and its preparations, no data are available.

Caffeine is rapidly and completely absorbed by the gastrointestinal tract and is readily distributed throughout all tissues of the body. Absorption is complete within approximately 1 hour after ingestion (typically 99% of the ingested dose is absorbed within 45 minutes) and seems to be dose-independent, at least for doses usually consumed by humans, i.e. up to 10 mg/kg. Peak plasma concentrations after normal consumption are usually around 50 µM, and the elimination half-life is between 2.5 and 10 hours. The toxic range in humans is considered to be above 200 µM. No significant splanchnic first-pass effect occurs after oral caffeine ingestion, and it has been estimated that a dose of 1 mg/kg (considered equivalent to one cup of coffee) produces peak plasma concentrations of 5 to 10 µM. Caffeine is sufficiently hydrophobic to pass through all biological membranes and is readily distributed throughout all tissues of the body. The parent compound is extensively metabolised in the liver microsomes to more than 25 derivatives, while considerably less than 5% of the ingested dose is excreted unchanged in the urine. The elimination half-life of caffeine from the plasma compartment for doses lower than 10 mg/kg, ranges from 2.5 to 10 hours in humans, and plasma clearance rates are approximately 1 to 3 ml/kg/minutes. There is, however, considerable inter-individual variability in the handling of caffeine by the body, due to both environmental and genetic factors (Magkos, 2005).

4.2. Clinical Efficacy

4.2.1. Dose response studies

For the herbal substance and its preparations, no data are available.

4.2.2. Clinical studies (case studies and clinical trials)

For the herbal substance and its preparations, no data are available.

4.2.3. Clinical studies in special populations (e.g. elderly and children)

For the herbal substance and its preparations no data are available. Use in children and adolescents under 18 years of age is not recommended because data are not sufficient.

4.3. Overall conclusions on clinical pharmacology and efficacy

Although Cola seed has been used for the relief of mental and physical fatigue and some other indications, clinical trials supporting these uses are lacking. Well-established use of Cola preparations as medicinal products is not possible due to the lack of clinical evidence. However, the maximal daily dose (9 g) is equal to at least 135 mg caffeine, which, taking into account the doses of caffeine used in clinical trials, makes the efficacy of Cola seed in the specified indications plausible.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

5.2. Patient exposure

Although limited biological data are available for Cola seed extract especially, the published data on the major constituents of Cola seed suggest the pharmacological/toxicological properties of Cola seed extract, are roughly equivalent to those of caffeine. Frank developmental/reproductive effects have not been reported and changes in offspring cannot be extrapolated to humans. A 'no observed effect level' (NOEL)/'no observable adverse effect level' (NOAEL) cannot be defined for repeated oral exposure to Cola seed extract from available data. Notwithstanding the foregoing, consumers of the United States have a history of safe consumption of Cola-type beverages containing Cola seed extract that dates back at least to the late 19th century, with a significant global history of exposure to the intact Cola seeds that dates centuries longer (Burdock, 2009).

Based on the data reviewed by *Nawrot*, it was concluded that for the healthy adult population, moderate daily caffeine intake at a dose level up to 400 mg/day (equivalent to 6 mg/kg body weight/day in a 65-kg person) is not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behaviour, increased incidence of cancer and effects on male fertility (Nawrot, 2003).

5.3. Adverse events and serious adverse events and deaths

Sleep disorders, over-excitability, nervous restlessness and gastric irritations may occur (Blumenthal, 2000). The following contraindications result from these known adverse events including a benefit-risk assessment: gastric and duodenal ulcers, cardiovascular disorders such as hypertension and arrhythmia, hyperthyroidism.

Contraindications are gastric and duodenal ulcers (Blumenthal, 2000).

3-10 g of caffeine can be lethal. The acute lethal dose of caffeine in adult humans has been estimated to be 10 g/person (calculating with Cola seed containing 1.5% caffeine, this would mean the indigestion of 667 g Colae semen). Death has been reported after ingestion of 6.5 g caffeine, but survival of a patient who allegedly ingested 24 g caffeine was also reported (Nawrot, 2003).

There is a preponderance of oral tumours and gastrointestinal malignancies especially in Northern Nigeria, where Cola seed chewing is most common (Atawodi, 1995).

5.4. Laboratory findings

No data for the herbal substance or herbal preparations are available.

5.5. Safety in special populations and situations

Potential for interactions

The potentiation of the action of psychoanaleptic drugs and caffeine-containing beverages is mentioned in the literature (Blumenthal, 2000).

Caffeine interactions: Drug interactions and/or related problems of caffeine intake: Monoamine oxidase (MAO) inhibitors, including furazolidone, procarbazine and selegiline (large amounts of caffeine may produce dangerous cardiac arrhythmias or serve hypertension because of the sympathomimetic side effects of caffeine; concurrent use with small amounts of caffeine may produce tachycardia and mild increase in blood pressure) (Thomson Micromedex, 2007)

The potential for Cola seeds 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 (Burdock, 2009).**Overdose**

No case of overdose is known in the literature.

Pregnancy and lactation

The American Herbal Products Association includes *C. acuminata* on its lists of herbs that may cause irritation to the GI tract, that may induce nervous system stimulation, and should not be used in pregnancy unless otherwise directed by a qualified expert (Class 2b) (Burdock, 2009).

Taking Cola seed during pregnancy has no effect on the birth weight or on the size of the head and breast of the newborn (Seitz, 1992). However, no studies are available on the effect of Cola seed consumption in pregnancy. For caffeine, several human studies are available.

Based on available evidence, it is suggested that women of childbearing potential should consume </=300 mg caffeine per day (equivalent to 4.6 mg/kg body weight/day for a 65-kg person) while children should consume </=2.5 mg/kg body weight/day (Nawrot, 2003).

Boylan conducted a prospective longitudinal observational study designed to examine the association of maternal caffeine intake with foetal growth restriction. The study included 2635 low risk pregnant women recruited between 8 and 12 weeks of pregnancy. Investigations quantifying the total caffeine intake from 4 weeks before conception and throughout pregnancy were undertaken with a validated CAT (caffeine assessment tool, a detailed questionnaire,). Caffeine half-life (proxy for clearance) was determined by measuring caffeine in saliva after a caffeine challenge. Foetal growth restriction, as defined by customised birth weight centile, adjusted for alcohol intake and salivary cotinine concentrations. Caffeine consumption throughout pregnancy was associated with an increased risk of foetal growth restriction [odds ratios 1.2 (95% CI: 0.9–1.6) for 100–199 mg/day, 1.5 (1.1–2.1) for 200–299 mg/day, and 1.4 (1.0–2.0) for >300 mg/day compared with <100 mg/day; test for trend P < 0.001]. Mean caffeine consumption decreased in the first trimester and increased in the third. The association between caffeine and foetal growth restriction was stronger in women with a faster compared to a slower caffeine clearance (test for interaction, P = 0.06). The authors concluded that caffeine consumption during pregnancy was associated with an increased risk of foetal growth restriction and this association continued throughout pregnancy. Sensible advice would be to reduce caffeine intake before conception and throughout pregnancy (Boylan, 2008) (Kuczkowski, 2009).

Bech conducted a randomised double blind controlled trial designed to estimate the effect of reducing caffeine intake during pregnancy on birth weight and length of gestation. The study included 1207 pregnant women drinking at least three cups of coffee (caffeinated or decaffeinated instant coffee) a day, recruited before 20 weeks gestation. Data on birth weight were obtained for 1150 live born singletons and on length of gestation for 1153 live born singletons. No significant differences were found for mean birth weight or mean length of gestation between women in the decaffeinated coffee group (whose mean caffeine intake was 182 mg lower than that of the other group) and women in the caffeinated coffee group. After adjustment for length of gestation, parity, pre-pregnancy body mass index and smoking at entry to the study, the mean birth weight of babies born to women in the decaffeinated group was 16 g (95% CI: 40-73) higher than those born to women in the caffeinated group. The adjusted difference (decaffeinated group–caffeinated group) of length of gestation was 1.31 days (95% CI: 2.87-0.25). The authors concluded that a moderate reduction in caffeine intake in the second half of pregnancy has no effect on birth weight or length of gestation (Bech, 2007) (Kuczkowski, 2009).

Weng conducted a population-based prospective cohort study designed to examine whether the risk of miscarriage is associated with caffeine consumption during pregnancy after controlling for pregnancy-related symptoms. An increasing dose of daily caffeine intake during pregnancy was associated with an increased risk of miscarriage, compared with no caffeine intake, with an adjusted hazard ratio (aHR) of 1.42 (95% CI: 0.93–2.15) for caffeine intake of <200 mg/day, and aHR of 2.23 (1.34–3.69) for intake of 200 or more mg/day, respectively. Nausea or vomiting during pregnancy did not materially affect this observed association, nor did the change in intake pattern of caffeine during pregnancy. In addition, the magnitude of the association appeared to be stronger among women without a history of miscarriage (aHR 2.33, 1.48–3.67) than that among women with such a history (aHR 0.81, 0.34–1.94). The authors concluded that high doses of caffeine intake during pregnancy increase the risk of miscarriage, independent of pregnancy-related symptoms (Weng, 2008) (Kuczkowski, 2009).

Because of the public health importance of this question, *Signorello* and *MacLaughlin* reviewed the results of 15 epidemiologic studies on this topic, with particular attention to the specific methodologic problems that would generate biased findings. These include selection and recall bias, confounding, several issues pertaining to exposure measurement, and the failure to account for foetal karyotype, caffeine metabolism, the timing of foetal demise, and the possibility that an effect of caffeine may be gestational age-specific. All the studies reviewed suffer from important methodologic limitations that hinder both the interpretation of each study individually and the comparison of results across studies. Despite the fact that most epidemiologic studies have observed a positive association between maternal caffeine intake and the risk of spontaneous abortion, it was concluded that the evidence must be considered to be equivocal, given the biases likely present and the fact that most of the potential biases would tend to overestimate any association (Signorello, 2004).

The amount of caffeine found in breast milk after the consumption of a known amount can vary considerably due to individual differences in absorption and elimination. Peak levels of caffeine are found in breast milk approximately 60 minutes after ingestion. It is estimated that the average dose to breastfed infants after heavy maternal caffeine intake (750 mg/day or 6-8 cups of coffee per day) is 0.6-0.8 mg/kg/day. Newborns metabolise caffeine very slowly, the half-life of caffeine being 97.5 hours in a neonate, 80 hours in a newborn and 2.6 hours in a six-month-old. The hepatic cytochrome P450 enzyme system is involved in the metabolism of caffeine, and as noted previously, some component of breast milk is believed to inhibit this system. This potential delay in elimination by the infant could result in accumulation of significant amounts of caffeine in the infant. Breastfeeding counsellors often report mothers who consume high volumes of strong tea, coffee or Cola complaining that the baby is jittery, colicky, constipated and generally unsettled. Caffeine may also be associated with a poor milk supply and implicated in recurrent mastitis. Caffeine has also been shown to have an

effect on the composition of breast milk. When a woman drinks more than three cups of coffee a day during pregnancy and the early phases of breastfeeding, her breast milk contains one-third less iron than that of a mother abstaining from coffee. A decrease in haemoglobin and haematocrit in mothers drinking coffee and their babies has also been observed. Caffeine is approved by the American Academy of Paediatrics for use by breastfeeding mothers. The current recommendation concedes that while occasional use appears to have little effect on the breastfeeding infant, it would seem advisable to restrict caffeine consumption to less than 300 mg/day whilst breastfeeding (Liston, 1998).

Effect on fertility

There are no data available on the effect of Colae semen on male or female fertility.

However, several articles have been published on the correlation of caffeine intake and fecundability. Taking into account the maximal posology of Colae semen (9 g daily) and the caffeine content of the herbal substance (1.5-3.2% in Colae semen (Bruneton, 1998)), the upper level of caffeine content in the daily dose of Colae semen preparations is 288 mg.

Leviton and *Cowan* published a review of the literature relating caffeine consumption by women to their risk of reproductive hazards, published up to 2000 (Leviton, 2002). They concluded that apart from a first report (suffering from several limitations) of a relationship between caffeine consumption and delayed conception, claiming that consumption of as little as 100 mg of caffeine was associated with delayed conception, 11 additional studies were published. Some provide no support for this hypothesis, whereas others show an association in a subsample. The key issue for this outcome is the repeated finding that cigarette smoking appears to place a woman at risk of subfecundity. Residual confounding might explain all the associations reported between coffee/caffeine consumption and subfecundity.

In 2010, an update of this overview was published by *Peck et al* (Peck, 2010), i.e. the review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption, between 2000 and 2009. This assessment included articles on fecundability and semen quality as well. Of the nine publications reviewed in order to assess the effect of caffeine consumption on fecundability, one evaluated multiple outcomes associated with fertility treatment, one considered self-reported ovulatory infertility, three addressed time to conception, and four assessed the relationship between caffeine and semen parameters. The only study to assess the effect of caffeine on endpoints of assisted reproductive technology reported no influence of previous or current caffeine intake on oocyte retrieval, fertilization, embryo transfer or the occurrence of a clinical pregnancy.
Time to conception: Exposure measurement errors are a primary concern for the few recent studies addressing time to conception and ovulatory infertility. Potential recall bias and exposure misclassification may explain the modest association reported for coffee and tea consumption and increased time to pregnancy. No support for an association with infertility due to ovulation disorders was provided, but exposure measurement error was likely introduced as a result of the timing of exposure assessments.
Semen quality: Evaluations of semen quality have consistently failed to observe adverse effects associated with caffeine intake. Most of the studies of male reproductive outcomes have suffered from lack of detailed reporting of caffeine exposure assessment, potential exposure misclassification for the relevant etiologic window, no or limited control for confounders, potential selection bias, or restriction to fertile men, which limited the ability to detect caffeine-related abnormalities. In summary, consistent relationships between caffeine intake and measures of subfecundity have not been observed as the result of the assessment of the available studies (Peck, 2010).

According to the report “Optimizing natural fertility” by the Practice Committee of the American Society for Reproductive Medicine, in collaboration with the Society for Reproductive Endocrinology and

Infertility, moderate caffeine consumption (one to two cups of coffee per day or its equivalent) before pregnancy has no apparent adverse effects on fertility (Practice Committee, 2008).

Taking account the caffeine content of the maximal daily dose of Colae semen, based on the available literature/data, the effect of the caffeine content of cola seeds on fertility cannot be assumed.

5.6. Overall conclusions on clinical safety

Based on empirical and experimental (partly on caffeine) evidence, the use of Cola seed at therapeutic dose is safe. However, due to the lack of data confirming the safety, the use during pregnancy and lactation and in children and adolescents is not recommended.

6. Overall conclusions

Cola seed has been used in traditional medicine for centuries in Africa and at least for 150 years in Europe. There is sufficient data available to establish a Community herbal monograph on the traditional use of *Cola nitida* and *Cola acuminata*, semen in an indication suitable for self-medication. The long-standing use and the significant caffeine content of Cola seeds make the therapeutic effect of the herbal substance and its preparations for symptoms of temporary fatigue and sensation of weakness plausible. Based on the scientific data, it can be assumed that the therapeutic use of Cola seed is safe.
In the absence of adequate data on genotoxicity, a Community list entry cannot be established.

Annex

List of references