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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Piper methysticum* G. Forst., rhizoma

Final

Based on Article 10a of Directive 2001/83/EC (well-established use)

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Piper methysticum* G. Forst., rhizoma
Herbal preparation(s)	Not applicable
Pharmaceutical form(s)	Not applicable
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1. Introduction

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

- Herbal substance(s)

Different definitions regarding the plant part used can be found in literature:

According to DAC, 1998[1] the herbal substance consists of dried rhizomes, usually free from roots and sometimes scraped, of *Piper methysticum* G. Forst. (Piperaceae). It contains not less than 3.5% of kavalactones calculated as kavain ($C_{14}H_{14}O_3$; Mr=230.2) (DAC, 1998).

According to definition from BHP (1993) kava-kava is the peeled, dried rhizome of *Piper methysticum* Forst. a plan indigenous to, and cultivated, that contains about 5% of a resin composed of a number of closely related 5,6-dihydro-α-pyrones.

Description: The dried rhizome consists of irregular, transverse and longitudinal pieces, varying considerably in size and shape: 3–20 cm in length and 1–5 cm in diameter. The outer surface is light yellowish or greyish-brown, longitudinally wrinkled, with large, whitish, circular root scars. The fracture is coarsely fibrous, the inner surface is yellow-white, with thin bark, radiate xylem, and large pith (WHO, 2004) Constituents (BHP,1993; Singh, 1992; Lebot *et al*., 1992; Parmar, 1997; ESCOP, 2003, Gruenwald *et al*., 2004; Bruneton, 2003)

The main active constituents (kavalactones) consist of a group of structurally related lipophilic lactone derivatives with an aryl-ethylene-alpha-pyrone skeleton. They are typically 4-methoxy-2-pyrones with phenyl or styryl sustituents at the 6-position and represent 3–20% of the dried rhizome depending on age of the plant and specific cultivar.

At least 18 kavalactones have been isolated from kava rhizome, of which six compounds are present in the highest concentrations and account for approximately 96% of the total kavalactones: kavain (dextro-isomer), 5,6-dihydrokavain, yangonin, desmethoxyyangonin, methysticin, and dihydromethysticin. Kavalactones are not water soluble but soluble in ethanol 95% or acetone.

Other constituents of dried rhizome are starch (43%), fibres (20%), sugars (3,2%), proteins (3,6% including peptides such as glutathione) and 3.2% minerals (potassium, calcium, magnesium, sodium, aluminum, and iron), dihydrochalcones (flavokavins A, B and C) and pipermethystine (an alkaloid).

A complete list of organic compounds (soluble in ethanol 95% or other organic solvent) isolated from kava was published by WHO, 2007 and includes two classes:

Kavalactones: 11-hydroxy-12-methoxydihydrokavain; 7,8-dihydro-5-hydroxykavain; 11,12-dimethoxydihydrokavain; methysticin; dihydromethysticin; kavain; 7,8-dihydrokavain; 5,6-dehydromethysticin; 5,6-dehydrokavain; yangonin; 5,6,7,8-tetrahydroyangonin; 5,6-dihydroyangonin; 7, 8-dihydroyangonin; 10-methoxyyangonin; 11-methoxyyangonin; 11-hydroxyyangonin; 5-hydrokavain; 11-methoxy-12-hydroxydehydrokavain.

Others: flavokavin A; flavokavin B; flavokavin C; dihydrokavain-5-ol; cuproic acid; cinnamalketone methylenedioxy-3,4-cinnamalketone; 4-oxononanoic acid; benzoic acid; phenyl acetic acid; dihydrocinnamic acid; cinnamic acid; pipermethvstine; 1-(meta-methoxycinnamoyl)pyrrolidine and 1-cinnamoylpyrrolidine.

[1] no longer available

The presence of pipermethystine in kava herbal substance (roots and leaves), kava preparations (acetonic and ethanolic) and finished products from the German market was investigated by Lechtenberg *et al.*, 2008. Results showed that pipermethystine was absent from all root and retain samples and extracts, with a limit of quantification of 45 ppm, but was present in leaves, with an amount of 0.2%.

Small amounts (1 mg/4 kg herbal substance) of cepharadione A (aporphine-type) were also isolated (Jaggy & Achenbach, 1992).

Variation of the composition

According to Whitton *et al*., 2003 who investigated the localization of the kavalactones within the different root structures, the greatest concentration of kavalactones is in the bark, with relatively lower concentrations in the parenchyma and sclerenchyma tissues. In the cross-sections of the roots, the kavalactone concentration is higher in the younger root, this reflecting the lower amounts of parenchyma and sclerenchyma tissues compared with the bark.

Analysis of the quality of different kava varieties

It was reported that there are significant differences between the composition of kava plants from different islands.

'Noble' kava varieties from Vanatu contains substantially less flavokavin B than 'non-noble' kava varieties, but a higher level of kavain (Lebot & Lévesque, 1996; Simeoni & Lebot, 2002; Lebot & Legendre, 2016).

Adulteration

a. With other parts of the plant: stem peelings may be included as raw material in kava commerce due to the high demand for the rhizome; pipermethystine is present in stem peelings (traces up to 0.85%); 3,4α-epoxy-5β-pipermethysticin (0.93%) was also isolated from stem peelings of one cultivar, but was absent from 10 other cultivars; 7,8-dihydrokavain, 7,8-dihydromethysticin and 5,6,7,8-tetrahydroyangonin are present in stem peelings.

b. With other species: the main adulteration species are *P. auritum* and *P. aduncum* (Singh, 1992; IARC, 2015).

Contaminants

Just a few data are published regarding kava contamination with mycotoxins (Teschke & Lebot, 2011). A study on ochratoxin A contamination found concentrations of 3.0 ng/g in one sample of kava root (Trucksess *et al*., 2006). The level of contamination with aflatoxin B1 in four samples of ground kava was 0.5 ng/g (Weaver & Trucksess, 2010).

- Herbal preparation(s)

In the literature there are mentioned different types of preparations:

a. Traditional non EU kava preparations (beverage) - these are prepared by maceration of finely ground roots in a water and coconut milk solution (Norton & Ruze, 1994) or only water (Simeoni & Lebot, 2014).

b. Extracts of kava: the solvents used are either ethanol (60% V/V or above) or acetone (60% V/V or above) in order to specifically extract the kavalactones. These are extracts that have been

concentrated and contain a certain amount of a particular component (e.g. total kavalactones)(WHO, 2007).

The processing techniques and specifically the extraction solvent and the ratio between solvent/plant material may have considerable influence on the chemical composition of the extracts. For example, even that pipermethystine is soluble in acetone 100%, it was not detectable in some commercial acetonic kava extracts (Teschke & Lebot, 2011).

Whitton *et al.*, 2003 analyzed the composition of different extracts and concluded that extraction with 96% ethanol resulted in 100% kavalactone extracted from the rhizoma, while extraction with 25% ethanol gave only 15% kavalactones and the water extract contained <3% of kavalactones present in the herbal substance. The same author revealed that the ratio between kavalatone and glutathione in the extracts also depends on the extraction solvent.

Kava lactone:glutathione ratio in extracts prepared according to commercial preparations of *P. methysticum* roots[a]

Sample	Solvent	Kava lactone:glutathione
Kava standardised extract powder (30% kavalactones)	25% ethanol, 75% water	1:0
82% ethanol extraction of kava root	None (this was already a liquid preparation)	1:0.017
Tincture extraction (1 part root to 3 parts solvent)	25% ethanol, 75% water	1:1.15
Fluid extract extraction (1 part root to 1 part solvent)	25% ethanol, 75% water	1:2.2

[a] Data are the means from ten replicate samples of each type.

Wang *et al.*, 2015 determined contents of kavalactones in kava beverages prepared from roots and rhizomes of two varieties from Hawaii (Isa-declared as "non noble" and Mahakea- declared by authors as "noble") and the extraction efficiency of five different solvents including hexane, acetone, methanol, ethanol and ethyl acetate. The method used to obtain the preparations included a first step of water extraction, then the aqueous extract was frozen dried and further extracted with different solvents.

The contents of kavalactones in the acetonic, ethanolic and methanolic extracts did not differ significantly. Ethanol had the highest extraction efficiency for the six major kavalactones whereas hexane gave the lowest extraction efficiency:

Solvents	Mahakea variety		Isa variety	
	Total kavalactone content		Total kavalactone content	
	Root	Rhizome	Root	Rhizome
Acetone	0.0819±0.0005	0.0513±0.0002	0.1764±0.0018	0.0870±0.0002
Ethanol	0.0948±0.0003	0.0569±0.0002	0.1782±0.0023	0.0590±0.0001
Ethyl acetate	0.0754±0.0002	0.0513±0.0003	0.1442±0.0020	0.0416±0.0003
Methanol	0.0797±0.0003	0.0482±0.0001	0.1763±0.0015	0.0514±0.0002
Hexane	0.0338±0.0004	0.0286±0.0002	0.0737±0.0004	0.0442±0.0003

Meissner & Haberlein (2005) analysed the amount of flavokavins A, B and C in an ethanolic kava extract (DER 14.5:1, extraction solvent ethanol 96% V/V) using an HPLC method. Flavokavins contents were: 0.62 mg flavokavin A /100 mg ethanolic extract, 0.34 mg flavokavin B /100 mg ethanolic extract and 0.14 mg flavokavin C/100 mg ethanolic extract.

DiSilvestro *et al.*, 2007 also analysed the amounts of flavokavins in two extracts obtained by the authors: an ethanolic extract (DER 13-20:1, extraction solvent: ethanol 96% m/m) and an acetonic extract (DER 11-20:1, extraction solvent: acetone 75% m/m). The results revealed a level of 0.31% flavokavin B and 0.28% flavokavin A in acetone extract and 0.35% flavokavin B and 0.34% flavokavin A in the ethanolic extract. Xuan *et al*., 2008 compared the content and composition of different extracts obtained using different solvents. The preparations were obtained by sonicating ground kava root with six different solvents followed by filtration and dried under vacuum.

The amount of total lactones (assessed by GC-method) in the aqueous extract (108.6 mg/g) was almost similar to that in chloroformic extract (106.2 mg/g) and much higher than that found in the methanolic, ethanolic and hexane extracts (45.6, 22.5, and 26.3 mg/g, respectively):

Table 2 Quantity of seven major kava lactones and glutathione in kava roots (mg/g extract)

Chemicals	Water	Acetone	Chloroform	Methanol	Ethanol	Hexane
Methysticin	0.0	5.5	14.4	0.0	0.0	1.2
Dihydromethysticin	31.5	51.9	18.9	5.4	3.2	3.6
Kavain	36.9	41.5	14.7	6.9	3.3	4.7
7,8-Dihydrokavain	3.8	55.1	23.0	18.6	9.4	10.1
Dihydro-5,6-dehydrokavain (DDK)	22.9	27.1	4.7	4.7	2.1	1.9
Desmethoxyyagonin	6.7	21.0	7.6	4.3	2.1	2.7
Yagonin	6.8	84.1	22.9	5.7	2.4	2.1
Total lactones	108.6	286.2	106.2	45.6	22.5	26.3
Glutathione	26.3	0.0	0.0	0.0	0.0	0.0

Also the amounts of flavakavins were investigated, results showing that flavokavin B was 0.1% present in aqueous extract and 0.5 in acetonic extract, but had not been detected in ethanolic extract.

"Standardised" extracts

In the published literature the extracts used were characterised only by the content in kavalactones; DER but also the extraction solvent were missing. In some articles, even old ones (from the 1990s), these preparations are called "standardised extracts". So, it seems that the term "standardised" was used a long time before the actual "standardisation" was defined and included in the Ph. Eur.

According to the published literature, the methods used for the "standardisation"(assay) varied during the time (from TLC, IR spectroscopy to HPLC assay). The correspondence between results obtained with different methods was never declared and is difficult to be assessed.

- 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. Search and assessment methodology

Databases and other sources used to research available pharmaceutical, non-clinical and clinical data on kava-kava or its relevant constituents:

- Relevant articles and references retrieved from databases: PubMed and Toxline. Search term: [kava], [kava-kava], [Piperis methysticum] and [Piperis methysticum rhizoma] combined with ‘human’, ‘clinical trial’, ‘randomised controlled trial’ and ‘review’; Publication year: up to January 2016. In summary more than 1700 publications were listed.
- Textbooks, pharmacopoeias and monographs.

Additionally, the European Commission’s databases on cosmetic ingredients (CosIng) were searched in August 2015 for information on [piper methysticum root or root extract].

Data was also provided by interested parties during a call for data and public consultation on the EMA website.

The EudraVigilance database and VigiLyze database of the World Health Organization’s were searched in May 2016 using the term [Piper methysticum].

The abstracts of the references found were screened manually and all articles identified that could have a possible impact on the assessment report and monograph were included. This assessment report is based on the summary of the most relevant scientific literature.

In the list of references the articles found to be relevant for assessment are included. Also the references assessed but not cited in the assessment report are mentioned in the LoR.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products.

Active substance	Indication	Pharmaceutical form	Regulatory Status
Dry extract from Piperi methystici rhizoma(12.5-20.0:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Tablet 180-288 mg corresponding to 120 mg kavalactones >12 years: 1 tablet 1-2 times daily (corresponding to 120-240 mg kavalactones per day)	1997-1999,DE, WEU according to Article 10a of Directive 2001/83/EC*
Dry extract from Piperi methystici rhizoma(12.5-20.0:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Tablet 180-288 mg corresponding to 120 mg kavalactones >12 years: 1 tablet 1-2 times daily (corresponding to 120-240 mg kavalactones per day)	At least since 1976 up to 2002, DE, WEU according to Article 10a of Directive 2001/83/EC*
Dry extract from Piperi methystici rhizoma(11.5-21.5:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Oral liquid 1 ml oral liquid contains 33-63 mg extract corresponding to 25 mg kavalactones >12 years: 60 drops (=?mg) 2-3 times daily	At least since 1976 up to 2002,DE, WEU according to Article 10a of Directive 2001/83/EC*
Soft extract from Piperi	States of nervous anxiety, tension	Capsule, soft	2000-2002 DE, WEU according to

Active substance	Indication	Pharmaceutical form	Regulatory Status
methystici rhizoma (13-20:1), extraction solvent: ethanol 96% (V/V)	and restlessness.	152-288 mg corresponding to 120 mg kavalactones >12 years: 1 or 2 capsules 1 time daily (corresponding to 120 to 240 mg kavalactones per day)	Article 16a of Directive 2001/83/EC*
Kava-kava extractum siccum, extraction solvent acetone 75% (m/m) – information on DER is not available	Long term conditions of anxiety, fear, psychological tension and restlessness, stress, psychovegetative disorders in menopause, possible alternative to benzodiazepines in gerontology.	Hard capsules 1 capsule contains 50 mg of the extract equivalent to 35 mg of kavalactones Dosage: 1 capsule 3 times daily Not to be used for more than 3 months For adults only	From 1998 to 2003, CZ (registration was granted the old legislative frame)
Kava-kava extractum siccum, extraction solvent acetone 75% (m/m) – information on DER is not available	Long term conditions of anxiety, fear, psychological tension and restlessness, stress, psychovegetative disorders in menopause, possible alternative to benzodiazepines in gerontology.	Hard capsules 1 capsule contains 100 mg of the extract equivalent to 70 mg of kavalactones Dosage: 1 capsule 3 times daily Not to be used for more than 3 months For adults only	From 1998 to 2003, CZ (registration was granted the old legislative frame)
Kava-kava extractum siccum, extraction solvent ethanol 96% (V/V), information on DER is not available	Long term conditions of anxiety, fear, psychological tension and restlessness, stress, psychovegetative disorders in menopause, possible alternative to benzodiazepines in gerontology.	Film coated tablets 1 tablet contains 375 _ 428.57 mg of the extract equivalent to 120 mg of kavalactones Dosage: ½ to 2 tablets (equivalent to 120 – 240 mg kavalactones) in the evening after meal. Not to be used more than 3 months.	From 1996 to 2001, CZ (registration was granted the old legislative frame)
Kava-kava extractum spissum, extraction solvent ethanol 96% (V/V), information on DER is not available	Mild to moderate depressions, neuroses, anxiety, tensions, restlessness, mood disorders, vegetative or psychosomatic disorders (neurovegetative dystonia), stress	Hard capsules 1 capsule contains Kava-kava extractum spissum Dosage:1 capsule 1 to 3 times daily	From 1997 to 2003, CZ (registration was granted the old legislative frame)
Soft extract from Piperi methystici rhizoma (11.5-21.5:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Capsule 67-125 mg corresponding to 50 mg kavalactones >12 years: 1 capsule	Old product, not authorised, DE

Active substance	Indication	Pharmaceutical form	Regulatory Status
		3-4 times daily (corresponding to 150-200 mg kavalactones per day)	
Dry extract from Piperi methystici rhizoma (13-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Tablet 75.2-120.2 mg corresponding to 50 mg kavalactones >12 years: 1 tablet 1-2 times daily (corresponding to 150-200 mg kava pyrones per day)	Old product, not authorised, DE
Soft extract from Piperi methystici rhizoma (12.5-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Capsule 76-144 mg corresponding to 60 mg kavalactones >12 years: 1 capsule 4 times daily (corresponding to 240 mg kavalactones per day)	Old product, not authorised, DE
Soft extract from Piperi methystici rhizoma (13-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Capsule, soft 152-288 mg corresponding to 120 mg kavalactones >12 years: 1 capsule 2 times daily (corresponding to 240 mg kavalactones per day)	Old product, not authorised, DE
Soft extract from Piperi methystici rhizoma (11.5-21.5:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Capsule 67-125 mg corresponding to 50 mg kavalactones >12 years: 1 capsule 4 times daily (corresponding to 200 mg kavalactones per day)	Old product, not authorised, DE
Soft extract from Piperi methystici rhizoma (12.5-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Oral liquid 1 g oral liquid contains 26-42.5 mg soft extract corresponding to 17.7 mg kavalactones >12 years: 4 ml (=?g) 3 times daily	Old product, not authorised, DE
Extract from Piperi methystici rhizoma (13-20:1), extraction	States of nervous anxiety, tension and restlessness.	Oral liquid 1 ml oral liquid contains 22-105 mg	Old product, not authorised, DE

Active substance	Indication	Pharmaceutical form	Regulatory Status
solvent: ethanol 96% (V/V)		extract corresponding to 25 mg kavalactones >12 years: 3.2 ml 3 times daily	
Dry extract from Piperi methystici rhizoma (12.5-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Coated tablet 54-113 mg corresponding to 40 mg kavalactones >12 years: 2 tablets 3 times daily (corresponding to 240 mg kavalactones per day)	Old product, not authorised, DE
Soft extract from Piperi methystici rhizoma (13-20:1), extraction solvent: ethanol 96% (V/V)	States of nervous anxiety, tension and restlessness.	Oral liquid 1 g oral liquid contains 26-42.5 mg extract >12 years: 20 drops (=? mg) 3 times daily (corresponding to 60 mg kavalactones per day)	Old product, not authorised, DE

*in 2002 the marketing authorizations were revoked, but in 2015 the revocation was canceled.

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Additional data regarding products from Germany: according to BfArM, until 2002 an acetonic extract (code WS 1490) was on the market; in 2002 the company accepted the withdrawl of the authorisation.

Additional data regarding the legal actions from the EU member states:

Belgium: *Piper methysticum* belongs to list 1 of the Royal Decree on 1997, with plants considered to be too toxic for being used in food supplements. It is still included on the new list of the Royal Decree, published in the Belgian State Journal on the 10th of February 2017.

Czech Republic: WHO rapid alert was received in 2002 with information that kava-kava products were withdrawn from the German market after evaluation of kava case reports on hepatotoxicity by BfArM. Based on this document the benefit/risk of the products on the Czech market was re-evaluated. Benfit/risk was found negative and therefore it was decided that renewal of products authorised will not be approved and the products were withdrawn from the market.

UK: Kava has been prohibited in unlicensed medicines since January 2003, by the Medicines for Human Use (Kava-kava Prohibition Order 2002). Following the Prohibition Order in 2002, UK Ministers made a commitment to review the ban after it had been in force for 2 years. In February 2006, following advice from an Expert Working Group (EWG) of Committee on Safety of Medicines (CSM), it was announced that the prohibition was justified and proportionate and should remain in place.

Spain: In 2001, following the decision of the PhVWP regarding medicinal products containing kava-kava, the AEMPS withdrew the only product that was registered.

France: In 2002, the French Agency for the Safety of Health Products (AFSSAPS) suspended all preparations containing kava for the duration of one year. Prohibition was completed in March 2003. Homeopathic remedies with dilutions of 1/500 or greater are exempt from the prohibition.

Portugal: In 2002, Portugal followed France's example and suspended all kava containing products.

Germany: In 2002 the German Federal Institute for Drugs and Medical Devices (BfArM) revoked the market authorization for kava kava-containing products. After a decision by the Higher Administrative Court of North Rhine-Westphalia (2015), the revocation of marketing authorizations for ethanolic preparations was cancelled (Hüttemann, 2015).

Hungary: In 2002, following BfArM action, all kava-containing products were withdrawn.

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Not applicable

2.1.2. Information on products on the market outside the EU/EEA

On 19 December 2001, the US Food and Drug Administration (FDA) issued a letter asking healthcare professionals to report any adverse events that might link kava with hepatotoxicity to the FDA's MedWatch program. On 25 March 2002, FDA published a consumer advisory concerning the potential risk of severe liver injury and rare hepatic failure associated with the use of kava-containing dietary

supplements and posted on the FDA website (FDA, 2002).On the US market are still available food supplements containing kava (no further details are available).

2.2. Information on documented medicinal use and historical data from literature

The generic name *Piper* comes from the Latin for “pepper”, and the species name *methysticum* from the Greek meaning “intoxicant”, thus *Piper methysticum* when translated into English means “intoxicating pepper”. Other names used to refer to kava include: kava kava; kawa; ava; awa; yati; yagona; and yangona (NTP, 2012).

The term ‘kava’ and the variant "*kawa"*, is primarily used to refer to the kava plant and the drink prepared from the fresh or dried roots of that plant. The general term ‘kava’, however, is also often used to refer to other specific preparations such as acetone or ethanol extracts of the plant for use in medicinal products.

Traditional use

Kava has a very long tradition of use in the South Pacific as a tranquilizing ritual beverage. The cultural history of the use of kava has been reviewed by Singh (1992, 2004) or Lebot *et al*., 1992 that indicate the use for at least 1500 years. The traditional kava beverage is prepared by soaking the pulverized root in a bowl of water and/or coconut milk solution and filtering the mix to produce a brew in a communal bowl. The kava is then drunk from a cup, sometimes a coconut shell. In parts of Vanuatu and in other regions across the Pacific in the past, the root was pulverised through mastication (Cairney *et al*., 2002). Today the fresh kava is comminuted by scraping with stones in the traditional form or by meat mincers in the modern form in kava bars whereas the ‘Fijian method’ involves the use of dry kava powder (Simeoni and Lebot, 2014). Historically kava consumed in Vanuatu was reputed to be the "strongest" (highest kavalactones content) anywhere in the South Pacific (Singh, 1992), possibly in correlation with the method of preparation used.

Use in the European Union

Many modern pharmaceutical preparations of *P. methysticum* contain extracts. The extracts are prepared by extracting the dried herb with an ethanol-water mixture or with an acetone-water mixture. The content in kavalactones can vary from 30% up to 70%, depending on the processes involved (WHO, 2007).

According to Potter's Herbal Cyclopaedia, kava-kava is used as stimulant, relaxant and antifatigue, tonic and diuretic. The dosage indicated corresponds to 120 mg extract (Williamson, 2003).

In ‘Precis de Matiere Medicale’, kava roots are described. Following therapeutic indications are mentioned: treatment of genito-urinary tract (cystitis, blennorrhagia) and the diuretic effect. The daily dosage for hydro-alcoholic extract corresponds to 0.5 to 1 g. (Leulier & Manceau, 1946).

In Madaus (1938), the following indications are mentioned: gonorrhoea, cystitis and prostate infections; it is also mentioned one preparation with sedative effects, bactericide and diuretic action. The preparation and dosage included are: kava rhizoma: 0.1-0.3 g, multiple times daily; alcoholic extract: 0.1-1 g; 1 tablet, 3 times daily (1 tablet containing 0.125 g kava rhizoma).

In Fischer & Hartwich (1919) the indications proposed are bronchitis and catarrhal conditions. The preparation described is a liquid extract (1000 g powdered rhizoma percolated with a mixture of ethanol 91%: water (8:2)); no data regarding the posology is provided (Fischer, 1919).

In a later edition of Hagers Handbuch, the following preparations are included: extractum Rhizoma Kava-Kava siccum (extraction solvent: ethanol 94% V/V + 1% methylethylcetone; contains 31.6 to

35.4 kavalactones); Kava-Kava dry hydro-alcoholic extract (contains 30% kavalactones); Kava-Kava dry acetonic extract (contains 70% kavalactones), Kava-Kava dichlormethane extract (called "kava resin"), and kava powdered rhizome (used for water extraction: 10 g powder/100 ml water). The daily dosage proposed is 60 to 120 mg kavalactones and the indications are nervous anxiety, stress, and restlessness (Hänsel *et al.,* 1994).

The British Pharmaceutical Codex 1911 includes 2 preparations based on kava-kava: Solid *Extractum Kavae* used in gonorrhoea and catarrhal conditions of the genito-urinary organs (dose: 6-30 centigrams) and *Extractum Kavae Liquidum* (100 g kava rhizome macerated for 8 hours with ethanol 45%, then percolated and evaporated to a soft extract, then dissolved in ethanol 90% V/V), used in mixtures form with bladder sedatives and diuretics; dose: 2-4 ml.

In BHP (1993) it is mentioned as specific indication in infection of genito-urinary tract (cystitis, urethritis) and indication in rheumatism and joint pains (topical aplication). It can be used in combination with *Althaea* root, *Apium* and *Agropyron* in bladder disease or with *Menyanthes*, *Cimicifuga* and *Apium* in rheumatism. Preparation and dosage (thrice daily): Dried root: 2-4 g or by decoction; Liquid extract B.P.C: 2-4 ml.

Martindale, The Extra Pharmacopoeia (2009) indicates that kava rhizome has been used in the South Pacific “to produce an intoxicating beverage used for recreational purposes and during convalescence”. It is reported to have sedative, skeletal muscle relaxant, and anaesthetic properties. It is given in some anxiety- and stress-related disorders. It was formerly used as an antiseptic and diuretic in inflammatory conditions of the genito-urinary tract in the form of a liquid extract. Kavain has also been used for nervous disorders and as a tonic. No details regarding dosages are given (Martindale, 2009).

In the monograph published in 1990 of Piperis methystici rhizoma (Kava-Kava-Wurzelstock), the German Commission E recommended the use of kava kava for conditions of nervous anxiety, stress, and restlessness. The daily dosage recommended for comminuted rhizome and other galenical preparations for oral use corresponds to 60 to 120 mg of kavalactones for short-term use, not more than 3 months (Blumenthal, 1998).

The WHO monograph describes for kava the traditional medicinal use to induce relaxation, reduce weight and treat fungal infections and the use in short-term symptomatic treatment of mild states of anxiety or insomnia, due to nervousness, stress or tension supported by clinical data. The daily dose indicated is: crude drug and extracts equivalent to 60-210 mg kavalactones (WHO, 2004).

The ESCOP monograph indicates that kava can be used in anxiety, tension and restlessness arising from various causes of non-psychotic origin. The doses proposed in adults and elderly are: dried rhizome or extracts corresponding to 60-120 mg kavalactones, usually for 1 month at most 2 months (ESCOP, 2003).

The PDR for Herbal Medicine also included Kava-kava as drug used for nervous tension, stress and agitation. The dosage indicated are: 150-300 mg root extract, twice daily, with a daily dosage of kavalactones of 50 to 240 mg; 30 drops of tincture with water, three times daily or 1/2 cup of infusion twice daily (Gruenwald *et al.,* 2004).

The same indication (treatment of anxiety) is mentioned in Bruneton (2003) for one extract (not characterized) where the daily dose corresponds to 35 up to 120 mg kavalactones.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Comminuted herbal substance	a) Sedative effect b) Gonorrhea, cystitis and prostate infections	Oral use Single dosage 0.1-0.3 g Multiple single doses/day	Madaus, 1938
	Infection of genito-urinary tract	Oral use 2-4 g dried root or by decoction; 3 times/daily	BHP, 1993
	Nervous anxiety, stress, and restlessness	Oral use Daily dose: equivalent to 60 to 120 mg of kavalactones Duration of use: not more than 3 months	Blumenthal *et al.,* 1998
	Nervous anxiety, stress, and restlessness	Oral use: 10 g powder/100 ml water The daily dosage corresponds to 60 to 120 mg kavalactones	Hänsel *et a*l., 1994
	Nervous tension, stress and agitation	Oral use 1/2 cup of infusion twice daily (no further detail)	Gruenwald *et al.,* 2004
	Anxiety, tension and restlessness arising from various causes of non-psychotic origin	Oral use Daily dose corresponds to 60 to 120 mg of kavalactones Duration of use: usually for 1 months at most 2 months	ESCOP, 2003
	Short-term symptomatic treatment of mild states of anxiety or insomnia, due to nervousness, stress or tension	Oral use Daily dose corresponds to 60 to 210 mg of kavalactones	WHO, 2004

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Extract (no further detail)	Anxiety, tension and restlessness arising from various causes of non-psychotic origin	Oral use Daily dose corresponds to 60 to 120 mg of kavalactones Duration of use: a) usually for 1 months at most 2 months b) not more than 3 months	a) ESCOP, 2003 b) Blumenthal *et al.,* 1998
Extract (no further detail)	Anxiety	Oral use Daily dose corresponds to 35 to 120 mg of kavalactones	Bruneton, 2005
Liquid extract B.P.C (no further detail)	a) Gonorrhea, cystitis and prostate infections b) Rheumatism (joint pains-topical aplication).	2-4 ml	BHP 1993
Alcoholic extract (no further detail)	a) Sedative effect b) Gonorrhea, cystitis and prostate infections	Oral use Daily dosage: 0.1-1 g	Madaus, 1938
Hydro-alcoholic extract (no further detail)	Treatment of genito-urinary tract (cystitis, blennorrhagia) and the diuretic effect.	Oral daily dosage: 0.5 to 1 g.	Leulier *et al.,* 1946.
Solid *Extractum Kavae*	Gonorrhoea and catarrhal conditions of the genito-urinary organs	Oral use: 6-30 centigrams	British Pharmaceutical Codex 1911
Extractum Kavae Liquidum (100 g kava rhizome macerated for 8 hours with ethanol 45%, then percolated and evaporated to a soft	Gonorrhoea and catarrhal conditions of the genito-urinary organs	Oral use: 2-4 ml	British Pharmaceutical Codex 1911

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
extract, then dissolved in ethanol 90% V/V)			
Ethanolic Kava extract siccum (extraction solvent: ethanol 94% V/V; extract contains 31.6 to 35.4 kavalactones)	Nervous anxiety, stress, and restlessness	Oral use The daily dosage: 60 to 120 mg kavalactones	Hänsel *et al*., 1994
Kava-Kava dry hydro-alcoholic extract (30% kavalactones)	Nervous anxiety, stress, and restlessness	Oral use The daily dosage: 60 to 120 mg kavalactones	Hänsel *et al*., 1994
Kava-Kava dry acetonic extract (70% kavalactones)	Nervous anxiety, stress, and restlessness	Oral use The daily dosage: 60 to 120 mg kavalactones	Hänsel *et al*., 1994
Kava-Kava dichlormethane extract (no further detail)	Nervous anxiety, stress, and restlessness	Oral use The daily dosage: 60 to 120 mg kavalactones	Hänsel *et al*., 1994
Extract (no further detail)	Nervous tension, stress and agitation	150-300 mg root extract, twice daily (daily dosage of kavalactones corresponds to 50 to 240 mg)	Grunewald *et al.,* 2004
Tincture (no further detail)	Nervous tension, stress and agitation	30 drops of tincture with water, three times daily	Grunewald *et al.,* 2004

2.3. Overall conclusions on medicinal use

From the market overview (section 2.1) just one indication was identified for the products authorised in Germany as well-established use: "States of nervous anxiety, tension and restlessness" and the respective preparations are:

- Extract from Piperi methystici rhizoma (DER 12.5-20.0:1), extraction solvent: ethanol 96% (V/V) - authorized from 1976 to 2002 when the marketing authorizations were revoked, but in 2015 the revocation was canceled;

- Extract from Piperi methystici rhizoma (DER 11.5-21.5:1), extraction solvent: ethanol 96% (V/V) - authorized from 1976 to 2002 when the marketing authorizations were revoked, but in 2015 the revocation was canceled;

- Soft extract from Piperi methystici rhizoma (DER 13-20:1), extraction solvent: ethanol 96% (V/V) - authorized from 2000 to 2002 when the marketing authorizations were revoked, but in 2015 the revocation was canceled. Even that the medicinal use of kava preparations is documented in several medicinal handbooks throughout a period of at least 30 years, the medicinal products are withdrawn from the EU market since 2002 based on safety concerns. The clinical efficacy of kava preparations, based on Article 10a of Directive 2001/83/EC as amended (well-established use) is evaluated in section 4 “Clinical data”, while the safety concerns are included in section 5.

Insufficient data are available regarding characterization of herbal preparations included in literature (no DER or extraction solvent used) and posology (single dose is missing, while daily dosage is expressed only as equivalent to kavalactones).

Therefore in Table 3 are included only those preparations for which all necessary data are available.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Comminuted herbal substance*	Infection of genito-urinary tract	2-4 g dried rhizoma or by decoction; 3 times/daily	BHP 1993
Dry extract from Piperi methystici rhizoma (12.5-20.0:1), extraction solvent: ethanol 96% (V/V) Tablets	States of nervous anxiety, tension and restlessness.	Single dose: 120 mg kavalactones Daily dose: 120-240 mg kavalactones	1976-2002**, DE, WEU
Dry extract from Piperi methystici rhizoma (11.5-21.5:1), extraction solvent: ethanol 96% (V/V) Liquid dosage form	States of nervous anxiety, tension and restlessness.	1 ml oral liquid contains 33-63 mg extract corresponding to 25 mg kavalactones >12 years: 60 drops 2-3 times daily	1976-2002, 2015-**, DE, WEU
Soft extract from Piperi methystici rhizome (13-20:1), extraction	States of nervous anxiety, tension and restlessness.	Single dose: 120-240 mg kavalactones Daily dose: 120-240 mg	2000-2002, 2015-**, DE, WEU

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
solvent: ethanol 96% (V/V) Capsules		kavalactones	

*only hystorical use; ** the current status of the preparations is explained in Table 1. Overview of data obtained from marketed medicinal products

3. Non-Clinical Data

Several pharmacological studies have demonstrated that Piperis methystici rhizoma and its isolated constituents display many properties *in vivo* and *in vitro*. A systematic review of all these studies is not attempted here; rather a selection of studies with emphasis on studies with relevance for the clinical efficacy is reviewed. Only relevant data regarding synthetic compounds were included. Further findings from pharmacological and pharmacokinetic studies in humans are available and are discussed in section 4.1.

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

The extracts used in the studies are specified in the comments as far as possible. Unfortunately, in many publications correct specifications of solvent and drug-extract ratio (DER) are missing. No distinction between chemovarieties is done in the published literature and often is mentioned simply as "Kava-Kava preparation". In these cases no details can be given, if the extract could not be identified otherwise.

3.1.1. Primary pharmacodynamics

Neurological and sedative effects, and anticonvulsive, muscle relaxing, and spasmolytic activity of kava extracts or isolated kavalactones have been examined in several *in vitro* and *in vivo* studies, mainly in rats and mice.

(1) Interaction with neurotransmitter receptors

Kava extracts and kavalactones are extensively analyzed toward their activity on central nervous system (CNS) receptors (especially γ-aminobutyric acid (GABA) receptors) and neurotransmitters (the inhibition of monoamine uptake or the modulation of 5-HT receptors activity) as well as toward the modulation of voltage dependent Na^+ and Ca^{2+} channels. An overview of biochemical mechanisms and possible molecular targets is given in some reviews (Bilia, 2002; Rowe *et al.,* 2011).

In vitro experiments

Interaction with GABA and benzodiazepine receptors

Ethanolic extract

A hydroethanolic extract of kava (tested at concentration of 100 to 500 µM kavalactones; no further detail) concentration-dependently enhanced the binding of [3H]muscimol to $GABA_A$ binding sites in membrane fractions from different regions of the rat brain: hippocampus (HIP), amygdala (AMY), medulla oblongata (MED), frontal cortex (FC) and cerebellum (CER). The kava extract enhanced the

binding of [3H] muscimol in a concentration-dependent manner with maximal potentiation of 358% over control in HIP followed by AMY and MED (main target brain centers). Minimal stimulation was observed in CER followed by FC. In contrast, apart from CER, the potency of kavalactones was similar in the brain areas investigated with EC50 values ranging between 200 and 300 µM kavalactones. The observed effects of kavalactones were attributed to an increase in the number of binding sites (Bmax), rather than to a change in affinity. At a kavalactones concentration of 500 µM the order of enhancement in B_{max} was HIP = AMY > MED > FC > CER ($p<0.001$ vs. control in each region) (Jussofie *et al.,* 1994).

Methanolic extract

Different methanolic leaf and root extracts (no further detail) were tested on binding affinities to CNS receptors including $GABA_A$ (GABA and benzodiazepine binding site), dopamine D2, opioid, serotonin (5-HT_6 and 5-HT_7) and histamine (Dinh *et al.,* 2001). The most potent binding inhibition was observed for leaf extracts to $GABA_A$ receptors with IC_{50} values of approximately 3 mg/ml, whereas root extracts were less active with IC_{50} values ranging from 5 mg/ml (Nene) to 87 mg/ml (Mahakea).

Table 2 Effects of *Piper methysticum* Forst. extracts on binding of specific radioligands to selected CNS receptors

IC_{50}-values	Benzodiaze-pine	Dopamine	$GABA_A$	Opioid		Histamine		Serotonin	
(µg/ml)*		D_2		µ	δ	H_1	H_2	5-HT_6	5-HT_7
Mahakea root extract	860 ± 60	850 ± 22	87 ± 17	592 ± 34	185 ± 61	850 ± 37	806 ± 53	>1000	492 ± 13
Mahakea leaf extract	510 ± 35	68 ± 4	4 ± 1	19 ± 5	240 ± 30	36 ± 7	4 ± 1	>1000	127 ± 32
PNG root extract	556 ± 88	101 ± 32	83 ± 15	256 ± 69	168 ± 16	603 ± 64	630 ± 59	>1000	472 ± 13
PNG leaf extract	710 ± 36	36 ± 18	1 ± 0.5	74 ± 11	161 ± 39	206 ± 33	215 ± 23	>1000	338 ± 17
Purple Moi root extract	900 ± 97	374 ± 61	23 ± 4	980 ± 79	340 ± 32	>1000	>1000	>1000	700 ± 34
Purple Moi leaf extract	860 ± 89	43 ± 16	6 ± 2	263 ± 42	71 ± 23	404 ± 91	240 ± 17	>1000	395 ± 18
Nene root extract	830 ± 89	380 ± 82	5 ± 2	424 ± 16	390 ± 33	>1000	>1000	>1000	905 ± 65
Nene leaf extract	490 ± 68	37 ± 8	3 ± 1	228 ± 22	134 ± 28	337 ± 23	374 ± 80	>1000	326 ± 38

* Values represent means of triplicates from one to three experiments ± Standard Error of the Mean.

Isolated constituents

Isolated compounds (kavain, dihydrokavain, methysticin, yangonin and tetrahydroyangonin) were tested at concentration ranging from 100 µM to 1 mM for their ability to compete with [^{3}H] diazepam binding to $GABA_A$ and benzodiazepine receptors in rat forebrain membranes. Only weak activity on $GABA_A$ binding sites was observed while no binding to $GABA_B$ was evident (Davies *et al.,* 1992).

The influence of kavalactones (yangonin, (+)-kavain, (+)-dihydrokavain, (+)-methysticin, and (+)-dihydromethysticin) on the $GABA_A$ receptor was demonstrated using radioreceptor assays. The kavapyrones have been investigated at assay concentrations between 10 nM and100 µM. All kavalactones enhanced the specific binding of [3H]bicuculline methochioride ([3H]BMC). (+)-Kavain, (+)-methysticin and (+)-dihydromethysticin showed maximal enhancements of 18% to 28% at a concentration of 0.1 µM, whereas a 100-fold concentration of (+)-dihydrokavain revealed a similar modulatory activity of 22%. In the presence of 1 µM yangonin an increase of about 21% of the specific [3H]BMC binding was observed. A structure comparison of desmethoxyyangonin and yangonin indicated that the aromatic methoxy group was of particular importance for the modulatory activity. In contrast, the substitution pattern of the aromatic ring did not influence the modulatory activity of the enolides in a decisive manner. A structure comparison of desmethoxyyangonin and (+)-kavain revealed that an angular lactone ring was an important structure requirement. Desmethoxyyangonin did not alter the binding behavior of the $GABA_A$-receptor. No inhibition of specific binding of [3H]flunitrazepam was observed, indicating that the influence on the $GABA_A$ receptor was not based on interaction with the benzodiazepine receptor (Boonen *et al.,* 1998a).

Inhibition of monoamine uptake

Ethanolic extract and isolated compounds

In vitro effects of a kava "spissum extract" (containing 67.6% kavalactones; 13.9% kavain, 15.9% dihydrokavain, 9.3% yangonin, 3.8% desmethoxyyangonin, 13.1% dihydromethysticin and 11.5% methysticin; no further detail regarding extraction solvent) and synthetic kavalactones on human platelet MAO-B was investigated and compared to amitriptyline, imipramine and brofaromine. The kava extract, tested at concentrations in the range of 0.25 µM up to 225 µM was found to be a reversible inhibitor of MAO-B in intact platelets (IC_{50} 24 µM) and disrupted platelet homogenates (IC_{50} 1.2 µM). Structural differences of kavalactones resulted in a different potency of MAO-B inhibition. The order of potency was desmethoxyyangonin > (±)-methysticin > yangonin > (±)-dihydromethysticin > (±)-dihydrokavainee > (±)-kavain. The two most potent kavapyrones, desmethoxyyangonin and (±)-methysticin, displayed a competitive inhibition pattern with mean Ki 0.28 µM and 1.4 µM, respectively. The authors suggest that the inhibition of MAO-B might be an important mechanism for kavalactone psychotropic activity (Uebelhack *et al.,* 1998).

Isolated compounds

The natural compounds (+) methysticine and (+)-kavain, and the synthetic racemate (±)-kavain, were tested at concentrations ranging from 10 to 400 µM for their ability to block *in vitro* the uptake of monoamines in synaptosomes prepared from the cerebral cortex and hippocampus of rats. (±)-Kavain and (+)-kavain were found to potently inhibit the uptake of [3H]-noradrenaline, by 70-80% of the control at 400 µM. Uptake of [^{3}H]-noradrenaline was inhibited in the following order of potency: (±)-kavain = (+)-kavain > (+)-methysticine, whereas none of the kavalactones efficiently blocked the uptake of [^{3}H]-serotonin. The results indicate a pyrone-specific non-stereo-selective inhibition of the [3H]-noradrenaline uptake which might be responsible for or, at least, contribute to the psychotropic properties of kavalactones (Seitz *et al.,* 1997).

Modulation of 5-HT receptor activity

Isolated compounds

(+)kavain and (+)dihydromethysticin at 20, 50 and 100 µM concentration-dependently reduced field potential changes induced on guinea-pig hippocampal slices by ipsapirone (a 5HT-$_{1A}$ receptor agonist), suggesting that these kavalactones modulate $5HT_A$ activity (Walden *et al*., 1997).

Recently, isolated kavalactones (7,8-Dihydrokavain, methysticin, 7,8-dihydromethysticin and yangonin) but also some synthetic compounds were tested at concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 µM for cannabinoid (CB) receptor affinity and inhibitory activity of two major metabolic enzymes of the endocannabinoid system, fatty acid amine hydrolase and monoacylglycerol lipase. Among the molecules tested, only yangonin exhibited affinity for the human recombinant CB_1 receptor with a K(i)=0.72 µM and selectivity vs. the CB_2 receptor (K(i)>10 µM). The authors concluded that CB 1 receptor affinity of yangonin suggests that the endocannabinoid system might contribute to the anxiolytic kava effect (Ligresti *et al.,* 2012).

(2) Sedative, tranquilizing and muscle relaxing effects

In vivo experiments

In vivo studies conducted on rats and mice evidenced sedative, tranquilizing and muscle relaxing effects of both the herbal preparations and isolated constituents.

Herbal preparations

Parenteral administration

Central activity of three aqueous extracts of kava (free from methysticin, dihydromethysticin and dihydrokavain) was investigated by Furgiuele, *et al*., 1965 in mice, rats and cats. At doses of 42 up to 240 mg/kg, intrapenitoneally, in mice the extracts depressed spontaneous motor activity without appreciably altering rotarod performance. These extracts also caused a marked reduction in irritability of rats having bilateral septal lesions and inhibited the rat conditioned avoidance response and blockade of EEG arousal patterns produced in cats by electrical, visual, or auditory stimuli. The authors suggested that other substances, not just methysticin, dihydromethysticin and dihydrokawain are capable of exerting marked pharmacologic actions in laboratory animal.

Dichloromethane (called "kava resin") and aqueous extracts of kava kavalactones-free (no further detail) were tested for their effect on amphetamine-induced hypermotility in mice and on conditioned avoidance response behavior in rats in a shelf-jump apparatus. Both kava extracts reduced amphetamine-induced hypermotility. Aqueous kava extract injected i.p. at doses of 30 mg/kg to 500 mg/kg had no effect on conditioned avoidance responses. At or below 100 mg/kg i.p. dichloromethane extract also failed to modify the number of conditioned avoidance responses obtained. However, 125 mg/kg of dichloromethane extract significantly reduced the number of conditioned avoidance responses by 18%. Increasing the dose to 150 mg/kg caused marked ataxia and sedation (Duffield *et al*., 1989b).

The central nervous activity of dichlormethane and aqueous extracts of kava (no further detail) was examined in Balb–c mice. The aqueous extract was injected i.p. at doses of 250, 125 and 62.5 mg/kg while the dichlormethane extract was injected in the same manner at dosages of 120, 150, 180 and 250 mg/kg (n=3). Control mice (same number per group) were similarly injected with saline (0.9% NaCl). A significant decrease of spontaneous motility was found in dosages as low as 62.5 mg/kg of the aqueous extract ($p<0.05$). All other doses showed a highly significant reduction ($p<0.001$). The maximum effect was obtained after 30 min and was maintained at this level for at least 2 hours. Concerning the dichlormethane extract, the spontaneous activity of the mice was significantly decreased (by 46%) 5 – 10 min after the 120 mg/kg injection, with progressively greater reductions at dosages of 150 and 180 mg/kg ($p<0.001$). At 180 mg/kg, most animals showed a loss of the righting reflex. As both the extracts show a very similar activity, the authors postulate that the action of kava is due to water-soluble pyrones (ESCOP, 2003).

In cats with chronically implanted electrodes, a kava extract in arachis oil (50–100 mg/kg kavalactones i.p; no further detail) and isolated compound D,L-kavain (10–50 mg/kg i.p.) were investigated. Blood pressure, the EEG of cortical and subcortical areas, the electromyogram, EEG arousal reactions, and subcortical evoked potentials elicited by central stimulation were recorded before and after administration. Only the extract exerted marked effects on the EEG; it induced high amplitude delta waves, spindle-like formations, and a continuos alpha- or beta-synchronization in the amygdalar recordings ($p<0.001$). As to the evoked potentials, the hippocampal response, following stimulation of the amygdalar nucleus, showed an increase in amplitude in the animals given D,L-kavain (50 mg/kg; $p<0.05$) and in those given the extract equivalent to 100 mg kavalactones/kg ($p<0.01$). In addition, after injection of the extract, further projections arising from the amygdala as well as the connection from the caudate nucleus to the amygdala proved to be activated (Holm *et al.,* 1991).

Sallström *et al*. (1998) investigated using *in vivo* microdialysis the effect of kava extract (containing d,l-19.5-23.8% kavain, 9.6-33.4% dihydrokavain, 19.5-22.3% methysticin, 5.5-11.7%, dihydromethysticin, 16.6-32.7% yangonin and 5.5-5.7% desmethoxyyangonin) and several kavalactones (D,L-kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin and

desmethoxyyangonin) on neurotransmitter levels in the nucleus accumbens of rats. Three different doses of the kava extract were administered (20 mg/kg, 120 mg/kg and 220 mg/kg). The D,L-kavain was administered in doses of 30 mg/kg, 60 mg/kg and 120 mg/kg and the other kavalactones were injected in a dose of 120 mg/kg. A small dose of kava extract (20 mg/kg body weight *i.p.*) caused changes in rat behaviour and concentrations of dopamine in the nucleus accumbens. Higher doses (120 mg/kg *i.p.*) increased the levels of dopamine. With respect to the individual compounds, D,L-kavain induced in low doses a decrease in dopamine levels and in higher amounts either an increase or no change in dopamine concentrations. Yangonin resulted in a decrease of dopamine levels to below the detection limit and desmethoxyyangonin in an increase of dopamine levels. Dihydrokavain, methysticin and dihydromethysticin did not produce any significant changes of dopamine levels. D,L-kavain caused a decrease in 5-HT concentrations. Some of the other kavalactones affected 5-HT levels as well. The results suggest that the relaxing and slightly euphoric actions may be caused by the activation of the mesolimbic dopaminergic neurones. Changes of the activity of 5-HT neurones could explain the sleep-inducing action.

Anxiolytic properties for kava extract and isolated kavalactones (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin) have been documented in an experimental model of anxiety, the chick social separation–stress procedure. In a series of experiments, kava extracts (containing 12.8 to 100.0% total kavalactones -Experiment 1) and fractions containing 1–6 kavalactones of varying concentrations (0.1–67.5%; Experiment 2–3) were screened for activity and compared against a 5.0 mg/kg dose of chlordiazepoxide (Experiment 3). Dependent measures were ventral recumbency latency (sedation), distress vocalizations, and a measure of stress-induced analgesia (in Experiment 1 and 2 only). Kava extracts samples attenuated distress vocalizations in a concentration-dependent manner. The kava fraction that contained the highest concentration of dihydrokavain attenuated distress vocalizations in a manner equivalent to that of chlordiazepoxide. The extract samples and fractions that possessed anxiolytic properties did not possess the sedative properties found in chlordiazepoxide. These findings suggest that at least 15% dihydrokavain may be necessary and sufficient for the anxiolytic activity of kava extract (Feltenstein *et al.,* 2003).

Assessor's comments: the model used is not a standard animal model to investigate the anxiolytic effect. Garrett el al. (2003) investigated the anxiolytic and sedative effects of kava ethanolic extract (20 mg powdered root extracted with 200 ml of 95% ethanol) in well established quantitative murine behavioral assays and compared with diazepam-induced behavioral changes. Diazepam was administered at doses from 0.0316 to 5 mg/kg and kava extract was administered at doses from 32 to 316 mg/kg intraperitoneally to BALB/cByJ inbred mice. Behavioral changes were measured in the mirrored chamber avoidance assay and elevated plus-maze assay. Reduced latency to enter and increased time spent in a normally avoided environment operationally defined anxiolysis. Kava extract produced statistically significant dose-dependent anxiolytic-like behavioral changes in both assays of anxiolysis. ED_{50} values for kava-induced increases in time spent inside the mirrored chamber and on the open arms of the plus maze were 125 mg/kg and 88 mg/kg, respectively. Kava extract also caused a profound decrease in locomotor activity (ED_{50} of 172 mg/kg). Flumazenil, a competitive benzodiazepine receptor antagonist, blocked both the anxiolytic and sedative effects of diazepam, but had no effect on kava's behavioral actions.

In mice, kava extract (containing 7% kavalactones; no further detail) at doses of at least 50 mg/kg (by intraperitoneal injection) reduced spontaneous motility to a greater extent than did control. The effect was enhanced by the addition of (+)-kavain (ratio of kava extract to (+)-kavain, 1:0.12), although this compound had no sedative effect when administered alone. In another experimental model, kava extract 100 mg/kg and (+)-kavain 12 mg/kg, each administered alone, had no sedative effect, whereas a combination of the two substances significantly reduced amphetamine (5 mg/kg subcutaneously)-induced hypermotility (Barnes, 2007).

Oral administration

Sedative effects have also been demonstrated for an ethanolic extract of kava (containing 50% kavalactones; extraction solvent ethanol 96%) at a single dose of 100 mg/kg administered by gastric tube in the amphetamine-induced hypermotility test(reduction with 47%) and barbiturate-induced sleeping time, respectively (sleep duration was prolonged by 45.5%) (Capasso & Sorrentino, 2005).

Isolated constituents

Parenteral administration

Kavaine, methysticin, dihydromethysticin and yangonin showed a strong centrally caused muscle-relaxing activity on the skeletal muscle tone of unanaesthetized rabbits after intravenous administration. Yangonin proved to be the most potent compound almost completely depressed the electromyographically impulses at 5–10 mg/kg; the other kavalactones required doses 2–3 times larger to show the same effect. From simultaneous obtained cortical EEGs, high voltage synchronised waves developed with relaxing doses of kavalactones. The authors concluded that the sedative effect of kava is probably a result of both depression of muscle tone and depression of the cortical activation system and limbic area by kavalactones (Kretzschmar *et al.,* 1971).

Dihydrokavain (180 mg/kg) and dihydromethysticin (90 mg/kg) also prolonged hexobarbital anesthesia in mice (Meyer, 1962).

Oral administration

In mice dihydrokavain (120 mg/kg) and dihydromethysticin (at doses up to 200 mg/kg) produced sedation, hypotermia and a corresponding reduction in total oxygen consumption (Meyer, 1962)

The effects of a single oral dose of 100 mg (+)-dihydromethysticin/kg body weight on striatal and cortical tissue concentrations of dopamine, serotonin, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid, as well as the dopamine and serotonin turnover were tested in rats. Additionally, other rats were fed with a (±)-kavain containing food (0.48 g/kg, leading to an intake of ca. 10.8 mg kavain/day) over a period of 78 days in order to evaluate the influence of a chronic treatment with kavalactones on the neurotransmitters. The results clearly demonstrate that neither (+)-dihydromethysticin in a high single dose, nor (±)-kavain chronically administered, altered the dopaminergic or serotonergic tissue levels in rats significantly (Boonen, 1998b).

Table 4: Overview of the main non-clinical data - studies

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Preparations				
Hydroethanolic extract of kava (no further detail)	200-500 µM kavalactones	*In vitro*	Jussofie *et al.,* 1994	Enhanced the binding of [3H]muscimol to GABAA binding sites in membrane fractions from different regions of the rat brain (EC_{50} 200 - 300 µM kavalactones)
"Spissum extract" (containing 67.6% kavalactones; 13.9% kavain, 15.9% dihydrokavain, 9.3% yangonin, 3.8% desmethoxyyangonin, 13.1% dihydromethysticin and 11.5% methysticin; no further detail regarding extraction solvent)	0.25 µM up to 225 µM	*In vitro*	Uebelhack *et al.,* 1998	The extract was reversible inhibitor of MAO-B in intact platelets (IC_{50}= 24 µM) and disrupted platelet homogenates (IC_{50}= 1.2 µM)
Ethanolic extract of kava (containing 50% kavalactones; extraction solvent ethanol 96%)	p.o. 100 mg/kg	*In vivo* Rats	Capasso, 2005	Sedative effects in the amphetamine-induced hypermotility test (reduction with 47%) and barbiturate-induced sleeping time (sleep duration was prolonged by 45.5%)
Kava extract (containing 7% kavalactones; no further detail) with or without kavain	i.p. 50 or 100 mg/kg (±)-kavain 12 mg/kg	Mice *In vivo*	Barnes, 2007	At 50 mg/kg kava extract reduced spontaneous motility; the effect was enhanced by the addition of (±)-kavain; at 100 mg/kg in combination with kavain significantly reduced amphetamine induced hypermotility

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Ethanolic extract of kava (20 mg powdered root extracted with 200 ml of 95% ethanol)	32 to 316 mg/kg	*In vivo* BALB/cByJ mice	Garrett el *al.,*2003	Kava extract produced dose-dependent anxiolytic-like behavioral changes in both assays of anxiolysis (ED_{50} =125 mg/kg and 88 mg/kg, respectively). Kava extract also caused a profound decrease in locomotor activity (ED_{50} =172 mg/kg)
Kava extract (containing D,L-19.5-23.8% kavain, 9.6-33.4% dihydrokavain, 19.5-22.3% methysticin, 5.5-11.7%, dihydromethysticin, 16.6-32.7% yangonin and 5.5-5.7% desmethoxyyangonin) and isolated compounds	i.p. Kava extract: 20 mg/kg, 120 mg/kg and 220 mg/kg D,L-kavain: 30 mg/kg, 60 mg/kg and 120 mg/kg the other kavalactones:120 mg/kg.	*In vivo* Rats	Baum *et al.*, 1989	Kava extract at 20 mg/kg caused changes in rat behaviour;higher doses (120 mg/kg) increased the levels of dopamine. D,L-kavain induced in low doses a decrease in dopamine levels and in 5-HT concentrations. Desmethoxyyangonin increased dopamine levels.
Three aqueous extracts of kava (free from methysticin, dihydromethysticin and dihydrokavain)	i.p. 42 mg/kg up to 240 mg/kg	*In vivo* mice, rats and cats	Furgiuele, *et al.*, 1965	Exhibited central activity (depressed spontaneous motor activity, reduced irritability of rats having bilateral septal lesions and inhibited the rat conditioned avoidance response and blockade of EEG arousal patterns produced in cats by stimuli)
Kava extract (no further detail) and isolated compound (D,L-kavain)	i.p. kava extract: 50–100 mg/kg kavalactones	*In vivo* Cats	Holm *et al.*, 1991	Only the extract exerted marked effects on the EEG. The hippocampal response showed an increase in amplitude in the animals given D,L-

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	D,L-kavain: 10–50 mg/kg			kavain (50 mg/kg; $p<0.05$) and in those given the extract equivalent to 100 mg kavalactones/kg ($p<0.01$)
Dichlormethane and aqueous extracts of kava (no further detail)	i.p. Aqueous extract: 250, 125 and 62.5 mg/kg Dichlormethane extract: 120, 150, 180 and 250 mg/kg	*In vivo* Balb–c mice	ESCOP, 2003	A significant decrease of spontaneous motility was found in all dosages of both extracts
Dichloromethane and aqueous extracts of kava (no further detail)	i.p. 30 mg/kg to 500 mg/kg aqueous extract 100 to 150 mg/kg dichloromethane extract	*In vivo* Rats and mice	Duffield, 1989b	Both extracts reduced amphetamine-induced hypermotility in mice. Aqueous kava extract at doses of 30 mg/kg to 500 mg/kg had no effect on conditioned avoidance responses in rats. At or below 100 mg/kg dichloromethane extract had no effect, but at 125 mg/kg significantly reduced the number of conditioned avoidance responses by 18%. Increasing the dose to 150 mg/kg caused marked ataxia and sedation.
Isolated compounds				
kavain, dihydrokavain, methysticin, yangonin and tetrahydroyangonin	100 µM to 1 mM	*In vitro*	Davies *et al*., 1992	Only weak activity on $GABA_A$ binding sites was observed while no binding to $GABA_B$ was evident
yangonin, (+)-kavain, (+)-dihydrokavain, (+)-methysticin,	10 nM up to 100 µM and	*In vitro*	Boonen *et al.,* 1998a	All kavalactones enhanced the specific binding of [3H]bicuculline

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
and (+)-dihydromethysticin				methochioride but no inhibition of specific binding of [3H]flunitrazepam was observed
(+) methysticine and (+)-kavain, and the synthetic racemate (±)-kavain	10 to 400 µM	*In vitro*	Seitz *et al.,* 1997	(±)-kavain and (+)-kavain were found to potently inhibit the uptake of [3H]-noradrenaline, by 70-80% of the control at 400 µM. Uptake of [^{3}H]-noradrenaline was inhibited in the following order of potency: (±)-kavain = (+)-kavain > (+)-methysticine
(+) kavain and (+) dihydromethysticin	20, 50 and 100 µM	*In vitro*	Walden *et al*., 1997	Concentration-dependently reduced field potential changes induced on guinea-pig hippocampal slices by ipsapirone
7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin and yangonin	0.1, 0.5, 1.0, 5.0, and 10.0 µM	*In vitro*	Ligresti *et al.,* 2012	Yangonin exhibited affinity for the human recombinant CB_1 receptor with a K(i)=0.72 µM and selectivity vs. the CB_2 receptor (K(i)>10 µM)
kavain, methysticin, dihydromethysticin and yangonin	i.v. yangonin 5–10 mg/kg The other compounds: 10–30 mg/kg	*In vivo* rabbits	Kretzschmar *et al.,* 1971	All compounds showed a strong centrally caused muscle-relaxing activity; Yangonin proved to be the most potent compound
dihydromethysticin	p.o.	*In vivo*	Boonen & Hberlein, 1998b	No influence on neurotransmitters

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
(±)Kavain	100 mg/kg, as a single dose 10.8 mg/day, 78 days	rats		
dihydrokavain, dihydromethysticin	p.o. 120 mg/kg up to 200 mg/kg	*In vivo* mice	Meyer, 1962	Sedation, hypotermia and a corresponding reduction in total oxygen consumption
dihydrokavain and dihydromethysticin	i.p. Dihydrokavain(180 mg/kg) Dihydromethysticin (90 mg/kg)	*In vivo* mice	Meyer, 1962	Prolonged hexobarbital anesthesia

3.1.2. Secondary pharmacodynamics

(1) Anticonvulsive activity

In vitro experiments

Methysticin anticonvulsant and neuroprotective properties were tested on different *in vitro* seizure models using extracellular recordings in rat temporal cortex slices containing the hippocampus and the entorhinal cortex. Elevating [K^+] induced seizure-like events with tonic and clonic electrographic phases in area CA1. Lowering [Ca^{2+}] caused recurrent seizure like episodes with large negative field potential shifts. Lowering Mg^{2+} induced short recurrent discharges in area CA3 and CA1 while ictaform events lasting for many seconds were induced in the subiculum, entorhinal and temporal neocortex. In the hippocampus the activity stayed stable over a number of hours. In contrast, the ictaform events in the subiculum, entorhinal and temporal cortex changed their characteristics after one to two hours to late recurrent discharges. In a concentration-range from 10 to 100 microM methysticin reversibly blocked all these types of epileptiform activity. Decreases in [Ca^{2+}] and associated slow field potentials evoked by repetitive stimulation of the stratum radiatum or the alveus remained almost unaffected by methysticin. A paired pulse stimulus paradigm used to test for effects of methysticin on synaptically evoked transient field potentials in normal medium revealed interference with mechanisms involved in frequency potentiation. While responses to alvear stimulation were largely unaffected, the responses to a paired pulse stimulus to stratum radiatum were depressed over the whole range of tested stimulus intervals. According to the authors, the findings suggest that methysticin has effects on different patterns of epileptiform activity possibly by interfering with processes responsible for frequency potentiation (Schmitz *et al*., 1995).

The anticonvulsive action of synthetic (±)–kavain on veratridine-stimulated increase in intrasynaptosomal Na^+concentration ([Na^+]i) of cerebrocortical synaptosomes of rats was investigated. Veratridine (5 µmol/l) enhanced basal [Na^+]i 6.6–fold from 11.3 to 74.1 mmol/L Na^+. Incubation of synaptosomes for 100 sec with (±)–kavain was sufficient to dose-dependently reduce the stimulated increase of [Na^+]i with an IC_{50} value of 86.0 µmol/l, and almost complete inhibition of Na^+ channels was attained with 400 µmol/l (±)–kavain. The reference compounds, procaine (400 µmol/l) and tetrodoxin (TTX, 10 µmol/l) reduced veratridine-elevated [Na^+]i to 30.4% and 7.9% of control. Post-application of 400 µmol/l (±)–kavaine immediately diminished veratridine-elevated [Na+]i to nearly basal levels with a half life of 69.7 sec. To study the influence of (±)–kavaine on non stimulated synaptosomes, an increase in [Na^+]i was induced by 200 µmol/l ouabain, which enhanced [Na^+]i hyperbolically with an initial rate of 18.4 mmol Na+/1 min. Preincubation of synaptosomes with 400 µmol/l (±)–kavain or 10 µmol/l TTX prevented Na^+-influx to the same extend for both compounds, approximately 57% of the control. The authors came to the conclusion that the data presented indicates a fast and specific inhibition of voltage-dependent Na^+ channels by (±)–kavain (Gleitz *et al.*, 1995).

The same authors investigated the interaction of (±)-kavain with epitopes of voltage-dependent Na^+ channels and the actions of (±)-kavain on 4–aminopyridine-stimulated synaptosomes as a model of firing neurons from cerebral cortex. [^{3}H]saxitoxin and [^{3}H]batrachotoxin were used for radioligand-binding assays performed with synaptosomal membranes.(±)-kavain failed tocompete with [3H]saxitoxin up to 400 µmol/l but dose-dependently suppressed binding of [^{3}H]batrachotoxin with an IC_{50} value of 88 µmol/l (Ki = 72 µmol/l) although displacement of [^{3}H]batrachotoxin was restricted to 33% of the control at 400 µmol/l (±)–kavain. In stimulated synaptosomes, 5 mmol/L 4–aminopyridine provoked an increase in [Na^+]i and [Ca^{2+}]i. 400 µmol/l (±)–kavain suppressed the increase in [Na^+]i and [Ca^{2+}]i to 38 and 29% of control, respectively. Consistent with the increase in [Na^+]i and [Ca2+]i, 4–aminopyridine provoked glutamate release, which was dose-dependently diminished to 60% of the

control by 400 µmol/l (±)–kavain. KCl depolarization provoked an increase in [Ca^{2+}]i and glutamate release almost identical to the response elicited by 4–aminopyridine but 400 µmol/l (±)–kavain suppressed only the rate of glutamate release by 9% of the control. The authors concluded that the data suggests an interaction of (±)–kavain with voltage-dependent Na^+ and Ca^{2+} channels (Gleitz *et al*, 1996).

In a radiological binding assay using rat cerebrocortical synaptosomes, (+)kavain, (±) kavain, (+) dihydrokavain, (±) dihydrokavain and dihydromethysticin significantly decreased the apparent total number of binding sites (Bmax) for [^{3}H]-batrachotoxinin-A 20α-benzoate (control: 0.5 pmol/mg protein, kavalactones: 0.2—0.27pmol/mg protein) with little change in the equilibrium constants for [^{3}H]-batrachotoxin-A 20α-benzoate (control: 28.2 nM, kavalactones: 24—31 nM). The results indicated that kavalactones non-competitively inhibit [^{3}H]-batrachotoxinin-A 20α-benzoate binding to receptor site 2 of voltage-gated Na^+ channels (Friese *et al.,* 1998).

There are other numerous *in vitro* studies on synthetic racemic (±)kavain that investigated its mechanism of action but the results have limited value as HMPC agreed that cannot be extrapolated to the natural L(-) kavain.

In vivo experiments

Several studies investigated of the capability of kava extracts and kavalactones to antagonize chemically or otherwise induced convulsions.

Klohs *et al.* (1959) investigated the anticonvulsive action of the ground root of kava, a chloroform extract (5 kg roots/30 L chloroform) and of isolated compounds (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin) as determined by their ability to antagonize clonic strychnine convulsions and death in mice. The crude extract, methysticin and dihydromethysticin were particularly effective in affording protection against the lethal effects of strychnine, while yangonin was practically ineffective. An indication of synergistic action was found by testing a mixture of kavain (19.5%), dihydrokavain (33.4%), methysticin (19.5%), dihydromethysticin (5.5%), yangonin (16.6%) and "compound A" (5.5%; chemical structure unknown). These compounds were combined in the ratio in which they were isolated from the crude extract. The mixture showed an ED_{50} of 100 mg/kg, indicating a potency at least as good as that of dihydromethysticin:

	Strychnine ED50 w. 95% cl. in mg/kg	Roller cage		Sleeping time	
		dose, mg/kg	result	dose, mg/kg	sl. time in % of controls
Dihydrokawain	340 (270–430)	300	no effect	160	150
Yangonin	no protection at 1,000	300	no effect	160	150
Kawain	215 (160–290)	300	no effect	160	235
Compound A	no protection at 200	300	no effect	160	130
Methysticin	160 (110–232)	300	no effect	160	250
Dihydromethysticin	115 (97–152)	300	no effect	60	413
Chloroform extract	140 (121–162)	300	12/18 fall-out	160	340
Ground root	1,700 (1,400–2,100)	10,000	12/18 fall-out	10,000	400
Meprobamate	no protection at 700	ED50 = 165 (122–201)		160	250

Another study investigated the qualitative and quantitative effect of dihydromethysticin and dihydrokavain administered intraperitoneal on electroconvulsion and compared the results to the effects of anticonvulsive substances (phenobarbital, primidone, diphenylhydantoin, ospolote; chlorpromazine). The lowest effective dosage of dihydromethysticin was 25 mg/kg and produced a

convulsive threshold elevation of 33.1%. The lowest effective dosage of dihydrokavain was found to be 60 mg/kg, which is 2.4 times higher. With this dosage the convulsive threshold was elevated by only 18.8% ($p<0.01$). After the application of 40 mg/kg dihydromethysticin the elevation was 100%. The same effect was demonstrated by 150% dihydrokavain. No dosage, not even dosages lower than 25 and 60 mg/kg, or doses above 60 and 150 mg/kg, lowered the threshold, thereby contrasting the effect of chlorpromazine. The effect of dihydromethysticin and dihydrokavaine was comparable to that of the known anticonvulsive substances. However, the threshold doses of those substances were 5 – 10 mg/kg lower than those of the two kavalactones (Hänsel, 1968).

The same authors also investigated of the effect of dihydromethysticin and dihydrokavain on chemically induced convulsions in mice (by pentetrazol, bemegride, picrotoxin and strychnine). Dihydromethysticin and dihydrokavain were administered intraperitoneal 30 min before injection of the respective convulsant. Pre-treatment with 30 mg/kg of dihydromethysticin inhibited of spasms induced by dosages of up to 2.5 mg strychnine; the SD_{50} for tonic spasms was significantly raised by about 50%. No effect was shown against 3 mg/kg strychnine. The clonic threshold as well as intensity and duration of spasms induced by picrotoxin, bemegride and pentetrazol were not influenced by dihydromethysticin. There was no clear effect by dihydromethysticin on the tonic phase induced by picrotoxin. In contrast, the same dosage of dihydromethysticin increased tonic extension spasms induced by bemegride and pentetrazol; the SD_{50} was lowered by 19.6 and 14.2%, respectively. Dihydromethysticin did not reduce lethality of tonic spasms induced by picrotoxin, bemegride and pentetrazol. The effect was dose-dependent: 45 and 30 mg/kg, respectively, had exclusively anticonvulsive against tonic picrotoxin and strychnine induced spasms. Dosages of 60 mg/kg and higher have a similar effect against the tonic phase of all four convulsants, after application of 90 mg/kg no maximal extension spasm was inducible. The influence on the clonic phase was different, there was no dose-dependent influence of dihydromethysticin on the convulsants (Hänsel, 1996).

Another studies in mice and rabbits also demonstrated the anticonvulsive action of methysticin, kavain, dihydromethysticin administered i.p. or i.v. in maximal electroshock model or strycnine-induced tetanus (Kretzschmar *et al*., 1969, 1970).

The anticonvulsive effect of methysticin, kavain, yangonin, and desmethoxyyangonin was also tested using maximal electroshock and pentylenetetrazol-induced convulsions (115 mg/kg *s.c.* or 50 mg/kg *i.v.*). Further comparative studies with phenobarbital, diphenylhydantoin, mephenesin, and procaine were made. With regard to the duration of action and the influence on the seizure pattern, the anticonvulsant activity of methysticin, dihydromethysticin, kavaine, and dihydrokavaine against maximal electroshock as well as against the pentylenetetrazol convulsions resembled that of local anaesthetic compounds and differs from the action of phenobarbital and diphenylhydantoin. The anticonvulsant activity of the lactones was characterized by an inhibition of the maximum tonic extensor seizure response elicited by maximal electroshock and by pentylenetetrazol as well as by an intensification of the clonic seizure response to pentylenetetrazol. 150 mg/kg of kavain and dihydrokavaine, 70 mg/kg of methysticin and dihydromethysticin, and 750 mg/kg of yangonin p.o. produced a maximum protection of 60 – 80%. At lower dosages the kavalactones, like procaine, had a weak facilitating effect on the tonic extensor phase of pentylenetetrazol-induced convulsions. The anticonvulsive action of yangonin and desmethoxyyangonin against maximal electroshock resembled that of the other pyrones. In convulsions induced by pentylenetetrazol, yangonin also inhibited the tonic extensor phase but there was no intensification of the clonic seizure phase in high doses of yangonin, rather a small inhibition. When administered i.p. and p.o., the anticonvulsant activity decreased in the following sequence: methysticin > dihydromethysticin kavaine > dihydrokavaine >> desmethoxyyangonin > yangonin (Kretzschmar & Meyer, 1965).

(2) Spasmolytic activity

Isolated compounds

Dihydromethysticin inhibited hystamine, acetylcholine, 5-HT and barium chloride- induced spasms in isolated guinea-pig ileum with ED_{50} values (2.6-7.2 x 10^{-6}g/ml) of the same order as those of papaverine (Hänsel, 1968).

Synthetic (±)-kavain (1 µM –1 mM) dose-dependently reduced contractions of guinea-pig ileum evoked by carbachol (10 µM), by BAY K 8644 (0.3 µM), or by substance P (0.05 µM). (±)-kavain also inhibited the contractile responses induced by raising the extracellular K^+ concentrations from 4 to 20 mM and by blocking the K^+ channel by barium chloride (1 mM) or 4-aminopyridine (0.3 mM). After pre-incubation with 1 µM nifedipine, carbachol (1 µM) evoked 18.2 ± 14.3% of contraction at control (i.e. prior preincubation with nifedipine). This remaining response was completely abolished by high concentrations of (±)-kavaine (400 µM). After treatment of the longitudinal ileum strips with pertussis toxin (PTX), carbachol (1 µM) evoked 27.0 ± 6.2% of the control response in untreated ileum. These contractions were also blocked by (±)-kavaine in a concentration of 400 µM. However, (±)-kavain had no effect on the caffeine induced (20 mM) contractions of ileum strips, which were permeabilized with digitonin or β–escin. Moreover, it failed to affect Ca-evoked contractions of skinned muscles. These results suggest that the (±)-kavain may act in a non-specific musculotropic way on the smooth muscle membrane (Seitz *et al*., 1997).

Synthetic (±)-kavain and (+)-methysticin at concentration of 1-400 µM exerted a rapid and reversible inhibition of voltage-dependence of Na(+)-channels in rat CA1 hippocampal neurons (Magura *et al.,* 1997).

(3) Neuroprotective effect

Backhauß (1992) investigated the neuroprotective effects of an kava extract WS 1490 (70% kavalactones) and isolated kavalactones (kavain, dihydrokavain, methysticin, dihydromethysticin and yangonin) in rodent models focal cerebral ischemia in mice and rats. Ischemia was induced by microbipolar coagulation of the left middle cerebral artery (MCA). The effects of the kava extract and its constituents were compared with those produced by the typical anticonvulsant, memantine. The kava extract, methysticin and dihydromethysticin produced effects similar to those of the reference substance memantine. The kava extract (150 mg/kg, 1 h before ischemia) diminished the infarct area ($p<0.05$) in mouse brains and the infarct volume ($p<0.05$) in rat brains. Methysticin, dihydromethysticin (both 10 and 30 mg/kg, 15 min before ischemia) and memantine (20 mg/kg, 30 min before ischemia) significantly reduced the infarct area in mouse brains. The authors concluded that the neuroprotective activity of kava extract was probably mediated by its constituents methysticin and dihydromethysticin.

Recent studies on isolated kavalactones (methysticin, kavain, and yangonin) have revealed other neuroprotective mechanisms of action, such as the activation of Nrf2 (NF-E2-related factor 2) that can explain the kavalactone-mediated protection against amyloid-induced neurotoxicity in neuronal and glial cell lines or the stimulation of ERK1/2 phosphorylation in PC12 cells that is responsible for the subsequent activation of Nrf2 (Tzeng & Lee, 2015).

(4) Analgesic activity

Antinociceptive activity of dihydrokavain and dihydromethysticin was investigated in mice against heat induced pain and compared with other analgesic compounds. Dihydrokavaine and dihydromethysticin were suspended in arachis oil (1%) and injected intraperitoneally in doses of 80, 100 and 180 mg/kg.

The reference substances morphine (2.5 mg/kg), aminopyrine (100 mg/kg), acetylsalicylic acid (200 mg/kg) were injected s.c. The minimal effective dose of dihydrokavain was 100 mg/kg ($p<0.001$), the effect was maximal 25 min after application and the duration of diminished excitability 1 hour. 120 mg/kg were even more effective: 15 min after application a significant result was obtained, the maximum effect was reached after 35 min, and the duration was 2.5 hours. Dihydromethysticin was a little less effective: 100 mg/kg did not produce a clear effect on reaction time but 140 mg/kg induced a significantly prolonged reaction time. Maximum efficacy was reached after 1 hour, the effect lasted for 2 hours. 140 mg/kg dihydromethysticin were less effective than 120 mg/kg dihydrokavaine. The dose-effect-relationship of dihydrokavain and dihydromethysticin was calculated to be 1.3:1. In comparison to the substances with known analgesic effects it was demonstrated that 120 mg/kg dihydrokavain had about the same analgesic effect as 100 mg/kg aminopyrine and was superior in duration of effect. The data on the combined administration of dihydrokavain with the antipyretic drugs aminopyrine or acetylsalicylic acid indicated that there is an additive synergism in the analgesic potency of these mixtures (Hänsel, 1968).

Antinociceptive activity *in vivo* (mice) has been investigated also in other two distinct tests (the tail immersion method and the abdominal constriction procedure using acetic acid) for dichlormethane extract "kava resin" (150 mg/kg; no further detail) , aqueous extract (250 mg/kg; no further detail) and for the individual kavalactones, such as dihydrokavain, dihydromethysticin, kavain and methysticin at doses of 150, 275, 300 and 360 mg/kg), yangonin and desmethoxyyangonin(at doses up to 1 g/kg) and compared with controls. For the tail immersion test all drugs were injected intraperitoneal while for the acetic acid-induced writhing test only aqueous extract was administered intraperitoneally. In the tail immersion test the dichlormethane extract (150 mg/kg) had a marked antinociceptive action, which was evident at the first test time of 10 min after injection and lasted for about 80 min ($p<0.005$), while the aqueous extract had a less pronounced antinociceptive effect ($p<0.05$). Kavain, dihydrokavain, methysticin and dihydromethysticin have potent analgesic properties. The peak analgesic effect was similar, but the duration of action markedly differed. The action of dihydrokavain was very rapid but short lived (20 – 30 min), that of kavain a little more prolonged (up to 2 hours), while the actions of methysticin and dihydromethysticin were considerably more persistent (3.5 – 4 hours). Yangonin and desmethoxyyangonin had no analgesic action at all in doses of up to 1 g/kg. Both aqueous and dichlormethane extracts were effective in inhibiting the number of writhes induced by acetic acid injection. Oral administration of 200 mg/kg dichlormethane extract effectively reduced writhing ($p<0.001$). Aqueous extract is inactive orally, but dramatically reduced writhing when is administered intraperitoneally in a dose of 250 mg/kg 55 min before acetic acid (Jamieson & Duffield, 1990a).

(5) Other activities

In vitro pre-treatment of human platelets with (+)–kavain 5 min before the addition of arachidonic acid dose-dependently suppressed the aggregation (IC_{50} 78 µmol/l), diminished the release of ATP (IC_{50} 78 ± 45 µmol/l) as well as the formation of PGE2 (IC_{50} 115 µmol/l) and suppressed the generation of TXB2 (detected as a representative of TXA_2) dose-dependently with an IC_{50} of 71 µmol/l. According to the authors, the similarity of the IC_{50} values suggest an inhibition of cyclooxygenase by (+)–kavain as primary target, thus suppressing the generation of TXA_2 which induces aggregation of platelets and exocytosis of ATP by its binding on TXA_2-receptors (Gleitz *et al*., 1997).

The effect of isolated compounds (kavain, dihydromethysticin, methysticin yangonin) on COX-1 and COX-2 isoenzymes was determined by measuring the rate of oxygen uptake in a cyclo-oxygenase inhibitory assay. Three non-steroidal anti-inflammatory drugs (NSAIDs) were included as positive controls. Naproxen was the most effective in each case, resulting in approximately 32% inhibition of COX-1 and approximately 28% inhibition of COX-2. All kava compounds tested at 100 or 50 µg/ml

demonstrated better or similar COX-1 inhibition activities as compared to ibuprofen, aspirin and naproxen. Dihydrokavain showed the highest COX-1 inhibitory activity (approximately 58%) and yangonin showed the highest COX-2 inhibitory activity (approximately 34%) at 100 µg/ml. The minimum kavalactone inhibition for each COX enzyme was 25% approximately (Wu *et al*., 2002).

The fungistatic principles of the kava rhizomes are the 4-methoxy-α-pyrones, like dihydrokavain. Experiments with dihydrokavain showed that it inhibits Aspergillus niger completely in a concentration of 0.5 mg/ml; bacteria did not seem to be inhibited (Hänsel, 1968).

Antifungal or antitrypanosomal activities were also tested on aqueous kava extracts (Blaszcyk *et al*., 1997; Xuan *et al*., 2006) or other isolated compounds apart from dihidrokavain (Otoguro *et al*., 2012).

There are numerous *in vitro* studies that revealed that isolated compounds (expecially flavokavin A and B) induced apoptosis on different cell lines (Kuo *et al*., 2010, Tang *et al*., 2010a, Zhao *et al*., 2011, Lin *et al*., 2012, Eskander *et al*., 2012, Hseu *et al*., 2012, Sakai *et al*., 2012, Abu *et al*., 2014, Abu, 2015c, Jeong *et al*., 2015).

There are also *in vivo* studies in animal cancer models (such as A/J mice or genotyped UPII-SV40T mice) that indicate the inhibition of tumor growth, especially NNK-lung adenoma (Abu *et al*., 2015a, Abu 2015 b, Tang *et al*., 2010b, Johnson *et al*., 2011, Lin *et al*., 2012, Triolet *et al*., 2012, Liu *et al*., 2013, Leitzman *et al*., 2014, Narayanapillai *et al*., 2014b). *In vivo* studies are conducted mainly on isolated compounds (as flavokavins B, A, dihidrokavain or dihydromethysticin) and on ethanolic extract (containing 150 mg/ml total kavalactones) and its polar/non-polar fractions (Leitzman *et al*., 2014, Triolet *et al*., 2012).

Assessor's comment: The composition of this ethanolic extract (marketed as food supplement) is unknown, therefore these results have only informative value.

3.1.3. Safety pharmacology

Tolerance

The development of tolerance to the aqueous extract of kava (no further detail) and to the lipid soluble extract (kava resin; no further detail) was tested in mice. Tolerance developed very rapidly, in the aqueous extract given parenterally. A minimally effective daily dose (50 mg/kg) of the aqueous extract for 3 days was sufficient to produce tolerance to a test dose of 150 mg/kg, which is close to the ED_{50}. As tolerance was evident at the first test period it can be assumed to be physiological tolerance. Kava resin decreased spontaneous motility and caused a loss of muscle control. A minimally effective daily dose of kava resin (100 mg/kg) did not produce tolerance to the above effects of a weekly test dose of kava resin (166 mg/kg) within 7 weeks. In a further experiment the dose was raised to 150 mg/kg twice daily and this schedule caused partial tolerance to occur within 3 weeks, but very little further tolerance developed over the ensuing 2-week period. To try to induce learned (behaviourally acquired) tolerance a dose of 166 mg/kg kava resin was injected daily and animals were tested each day while under the influence of the drug. However, even under these conditions, there was no tolerance evident within 3 weeks, when the experiment was terminated. It appears difficult to induce the development of physiological or learned tolerance to kava resin in mice (Duffield & Jamieson, 1991).

Cytotoxicity and hepatotoxicity

Direct hepatotoxicity of kavalactones and kava-extracts has been assessed in numerous *in vitro* studies yielding different and partly inconsistent results.

Tang *et al*., 2010 studied the *in vitro* toxicity of kavain, methysticin and yangonin in HepG2 cells using lactate dehydrogenase (LDH) release and ethidium bromide assays. Toxic effects were observed for

methysticin and kavain at 100 and 200 µM, respectively. For yangonin, pronounced decrease of cell viability to 40% at 25 µM in the ethidium bromide assay was detected. Furthermore, the mode of cell death was elucidated using acridine orange/ethidium bromide dual staining. Early and late apoptotic cells were detected after a treatment with 200 µM methysticin and 25 µM yangonin but not with 200 µM kavain. Glutathione levels were not decreased by kavalactone treatment so glutathione depletion may not be the cause of the observed toxicity.

These findings differ from other *in vitro* studies, which analyzed the effect of individual kavalactones and kava ethanolic extract on ATP levels in primary human hepatocytes. At 100 µM concentrations, kava ethanolic extract, methysticin, and desmethoxyyangonin decreased ATP approximately up to 50%. Methysticin and dihydromethysticin were found to be the most toxic and, surprisingly, yangonin was the least toxic kavalactone (Zou *et al*., 2004).

Nerurkar *et al.*, 2004 investigated *in vitro* the toxicity of desmethoxyyangonin, dihydromethysticin and pipermethystine in HepG2 cells. In preliminary experiments the kavalactones failed to show any toxic potential in HepG2 at concentrations less than 0.5 µM for up to 2 weeks. Based on these observations, the authors tested dihydromethysticin and desmethoxyyangonin and pipermethystine at 1, 25, 50, 100, and 200 µM concentrations for 48 h. At 200 µM pipermethystine caused cell death within the first 6 h, while 50 and 100 µM pipermethystine caused significant (65 and 90%, $p<0.001$) cell death within 24 h, as measured by the release of LDH into the medium. Higher concentrations of the dihydromethysticin and desmethoxyyangonin (200 µM) caused only 30% cell death after 48 h ($p<0.01$), while lower concentrations of dihydromethysticin and desmethoxyyangonin (100 and 50 µM) did not show any toxicity at 24 h and up to 8 days of treatment.

Zhou *et al.*, 2010 demonstrate that flavokavin B is a potent hepatocellular toxin, inducing cell death in HepG2 (LD_{50}=15.3 ± 0.2 µM) and human hepatocyte cell line L-02 (LD_{50}=32 µM). Hepatocellular toxicity of flavokavin B is mediated by induction of oxidative stress, depletion of reduced glutathione (GSH), inhibition of IKK activity leading to NF-κB transcriptional blockade, and constitutive TNF-α-independent activation of mitogen-activated protein kinase (MAPK) signaling pathways. Furthermore, pretreating hepatocytes with exogenous GSH normalizes NF-κB and MAPK signaling and rescues hepatocytes from flavokavin-induced toxicity.

Lüde *et al.*, 2008 compared the hepatocellular toxicity of three different kava extracts (a methanolic and an acetonic root and a methanolic leaf extract), and investigated their toxicity on HepG2 cells and isolated rat liver mitochondria. Methanolic and acetonic root extracts contained approximately 80% kavalactones and 0.011% pipermethysticine whereas the methanolic leaf extract had 24% kavalactones and 1.34% pipermethysticine. All three extracts showed cytotoxicity starting at a concentration of 50 µg/ml (lactate dehydrogenase leakage) or 1 µg /ml (MTT test). The mitochondrial membrane potential was decreased (root extracts starting at 50 µg /ml) and the respiratory chain inhibited and uncoupled (root extracts) or only uncoupled (leaf extract) at 150 µg/ml, and mitochondrial beta-oxidation was inhibited by all extracts starting at 100 µg/ml. The ratio oxidized to reduced glutathione was increased in HepG2 cells, whereas the cellular ATP content was maintained. Induction of apoptosis was demonstrated by all extracts at a concentration of 150 µg /ml. These results indicate that the kava extracts are toxic to mitochondria, leading to inhibition of the respiratory chain, increased ROS production, a decrease in the mitochondrial membrane potential and eventually to apoptosis of exposed cells.

Martin *et al.,* 2014 measured the cytotoxicity of different US extracts towards human lung adenocarcinoma A549 cancer cells. Aqueous extracts from those products showed no toxicity at any concentration measured up to 500 mg/ml, while ethanol extracts from the same commercial sources varied greatly in their relative cytotoxicity at all concentrations measured 37.5, 75 and 150 mg/ml. The authors observed a moderate correlation between the concentrations of flavokavin A and B & and the

relative cytotoxicity across the sampled kava products. High concentrations of the flavokavins generally mirrored lower relative cell viability.

Not only the *in vitro* findings but also *in vivo* toxicity testing leads to different results concerning kavalactones or kava extracts toxicity. Both aqueous and organic extracts as well as isolated kavalactones have been investigated.

Sorrentino *et al.,* 2006 assessed the hepato-toxicity of an ethanolic kava extract (ethanolic extractum spissum, containing kavain 12.4%, dihydrokavain 8.8%, methysticin 11.8%, dihydromethysticin 6.0%, yangonin 5.0%, desmethyoxy-yangonin 3.4%) in rats. Wistar rats of both sexes were fed 7.3 or 73 mg/kg body weight of ethanolic kava extract for 3 and 6 months. The animals were examined for changes in body weight, hematological and liver parameters, and macroscopical and microscopical histological changes in the major organs. No signs of toxicity could be found. In addition, no behavioural or physiological changes were observed on discontinuation of kava-extract feeding after 3 months.

Singh *et al.*, 2003 investigated the effects of an aqueous kava extract (extracted with water at room temperature; no further detail) on liver function in rats (daily dosages of 200 or 500 mg kavalactones/kg) for 2 up to 4 weeks and compared with control groups. The serum levels of ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) were almost twice as much in the control groups after 2 weeks compared with 4 weeks. After 2 weeks, all enzymes were lower with both doses of kava extract. The changes were significant ($p<0.05$) at 500 mg/kg for ALT and AST and at 200 mg/kg for ALP. The reduction in ALT was also significant ($p<0.05$) after 4 weeks with 500 mg/kg.

Clayton *et al.,* 2007 examined the effects of an organic kava extract (containing yangonin 42.76%, 7,8- dihydrokavain 34.69%, kavain 8.87%, 7,8-dihydromethysticin 4.03%, methysticin 3.23% and 5,6-dehydrokawain 2.42%) administered in corn oil by gavage F344 in rats at concentration of 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg/day, 5 days per week for 14 weeks. Time- and dose-dependency adverse effects were observed Increased gamma-glutamyl-transpeptidase (GGT) activities were observed in the 2.0 g/kg males and 1.0 and 2.0 g/kg females, as well as increased serum cholesterol levels in males and females at 0.5 g/kg and higher. Increases in incidence and severity of hepatocellular hypertrophy were noted in males at 1.0 g/kg and females at 0.5 g/kg and higher, as well as increased liver weights. Immunohistochemical analyses of the expression of cytochrome-P450 (CYP) enzymes in liver of the control and 1.0- and 2.0-g/kg-treated groups indicated decreased expression of CYP2D1 (human CYP2D6 homolog) in 2.0 g/kg females and increased expression of CYP1A2, 2B1, and 3A1 in 1.0 and 2.0 g/kg groups of both sexes. The no-observed adverse effect level (NOAEL) corresponds to 0.25 g/kg in both genders, based on increases in GGT, cholesterol, liver weight, and hypertrophy and decreases in body weight.

Another study conducted in rats investigated the effect of an acetonic extract (DER 11–20:1, extraction solvent: acetone 75%, m/m) and an ethanolic extract (DER 13–20:1, extraction solvent: ethanol (96%, m/m) at three different oral doses 31.25, 62.5 and 133 mg/kg diet, for 3 months. The tested doses did not cause any liver injury based on serum markers of liver damage (sorbitol dehydrogenase activities, bile acid concentrations, and β-glucuronidase activities) and serum lipid peroxide readings. Moreover, for these same parameters, kava feeding did not enhance the effects of the hepatotoxin galacatosamine (500 mg/kg ip); the dose of 133 mg/kg (for both kava preparations) plus 62.5 mg/kg dose of ethanolic extract even showed modest protection against liver injury. Liver histology analysis showed no signs of kava causing or enhancing liver injury (Di Silvestro, 2007).

A methanolic extract (containing 30% kavalactones) was administered orally for 14 weeks in Fischer rats and B6C3F1 mice, at doses up to 2 g/kg. There was not a clear sign of liver toxicity, but a

dramatic induction of gene expression Cyp1a1, Cyp3a1 in a dose-dependent manner (Guo 2009, 2010).

No signs of hepatotoxicity could be found in rats treated for 12 days with ethanolic extract (150 mg/ml total kavalactones) of its polar and non-polar fractions, at doses of 6 g/kg, in diet (Triolet *et al*., 2012) or in mice after 22 weeks of administration of the same ethanolic extract at doses up to 10 mg/g in diet (Johnson *et al*., 2011).

Isolated compounds

Fu *et al.,* 2008 investigated the effect of kavain on liver ultrastructure and function by infusing 10 µg/mL kavain solution for 2 h in isolated rat livers. After standard fixation and tissue preparation, the samples were examined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and light microscopy (LM). LM, SEM, and TEM examinations indicated kavain-treated rat livers displayed severe vascular and endothelial damage manifesting as vasoconstriction, gaps and loss of endothelial and subendothelial liver tissue integrity compared to control livers.

Zhang *et al.*, 2012 investigated the hepatotoxicity of kavain and methysticin at concentration of 43.3 µM in perfused livers of rats, which were either pretreated or not with the macrophage intoxicant gadolinium chloride. An extensive damage was observed in kavalactone-perfused livers whereas the damage was significantly lower with a gadolinium chloride pretreatment. The authors suggested that the activation of liver macrophages may be a key factor for observed hepatotoxicity of kavalactones.

The hepatotoxic potential of flavokavin B was investigated *in vivo* in mice that received daily oral doses of 25 mg/kg flavokavin B or vehicle (0.5% methyl-cellulose) for 1 week. Histological analysis revealed massive liver damage with hepatocellular swelling and vesiculated cytoplasm indicating inflammatory infiltration. Inflammatory infiltration was also evident predominantly in the periportal area. Consistent with histologically observed liver damage, serum AST and AKP levels were also increased in mice treated with flavokavin B (Zhou *et al.,* 2010).

Assessor's comments: the amounts of flavokavin B in marketed EU products is unknown; Teschke R et al., 2011 used the published data to predict that in ethanolic extracts 0.54–7.06 mg flavokavin B are found equating to 120 mg kavalactones. Based on this assumption, the tested dose in vivo is 250 times higher than the predicted level of flavokavin B. This observation has limited value as is not based on real results from the marketed products.

Narayanapillai *et al.*, 2014a evaluated the toxicity of kava ethanolic extract (containing 150 mg/ml total kavalactones) as a single entity (long-term study) or in combination with acetaminophen (APAP) in C57BL/6 mice (short-term study). In the long-term study, mice in the kava group were given 500 mg/kg/day ethanolic extract via gavage, 6 days a week, for 14 weeks, while control group was treated with PEG-400. At the tested dose kava extract did not affect mouse growth. There were also no statistically or biologically significant differences between control and kava- treated mice with respect to ALT and AST. The short-term combination studies were designed to evaluate the potential synergism of kava and its chemicals to APAP-induced hepatotoxicity. C57BL/6 mice were treated with PEG-400 (control group), kava ethanolic extract (500 mg/kg), dihydromethysticin (37.5 mg/kg) or Flavokavins A (8, 16, 32 mg/kg) and Flavokavins B (11.5, 23, 46 mg/kg), daily via oral gavage for 2 days. On the third day, mice in the respective groups were coadministered with APAP (800 mg/kg). Kava pretreatment potentiated APAP-induced hepatotoxicity, resulted in an increase in serum ALT and AST (3- fold increase relative to APAP alone), and increased severity of liver lesions. Histopathological analyses of the liver tissues revealed no lesions in control and kava treated mice confirming the lack of hepatotoxicity by kava treatment alone. Flavokavins A and B together at all three doses tested did not

induce any changes on serum ALT and AST but combined with APAP, dose-dependently potentiated the increase in ALT and AST (2-3 times) induced by APAP; dihydromethysticin had no such effect.

Pipermethystine (at 50 µm or higher) caused cytotoxicity and apoptosis under *in vitro* conditions using HePG2 cells assays (Nerurkar, 2004), but *in vivo* studies in rats treated with high doses of 10 mg/kg/day pipermethystine failed to reveal any experimental liver injury (Lim *et al.,* 2007).

Assessor's comments: the level of pipermethystine in kava-rhizoma extracts is lower than the detection limit, therefore the evidence for pipermethystine as cause for human kava hepatotoxicity is questionable.

3.1.4. Pharmacodynamic interactions

Prolongation of barbiturate-induced sleeping time

Klohs *et al*., 1959 demonstrated the ability of kava chloroform extract (5 kg roots/30 L chloroform) and kava isolated components and to potentiate sodium pentobarbital-induced sleeping time in mice. Dihydromethysticin appeared to be the most potent agent; at a dosage of 60 mg/kg sleeping time was prolonged by 413%. The chloroform extract at doses of 60, 160 and 250 mg/kg prolonged sleeping time by 134, 250 and 440%, respectively. Methysticin, kavain, dihydrokavaine and yangonin were administred in dosages of 160 mg/kg, but only prolonged sleeping time by 150 (yangonin, dihydrokavaine) to 250% (methysticin; kavain: 235%). In additional experiments, varying doses of dihydromethysticin further demonstrated its potency. 10 mg/kg caused a prolongation by 152 ± 30%, 20 mg/kg prolonged sleeping time by 240 ± 27%, 40 mg/kg: 457 ± 43%, 60 mg/kg: 896%, 160 mg/kg: 1800%.

Another study on male mice demonstrated that premedication with dihydrokavain or dihydromethysticin prolonged and deepened sodium hexobarbital anaesthesia. The lowest effective dose of dihydromethysticin, 20 mg/kg, doubled hexobarbital induced sleeping time. The lowest effective dose of dihydrokavain was 60 mg/kg. Dihydromethysticin proved to be at least twice as effective as dihydrokavain over the entire dose range. However, none of the drugs reached the efficacy of chlorpromazine (Hänsel, 1968).

An ethanolic extract of kava (extraction solvent ethanol 96%) at a single dose of 100 mg/kg (corresponding to 50 mg kavalactones) administered by gastric tube in mic prolonged barbiturate-induced sleeping time, by 45.5% (Capasso & Sorrentino, 2005).

Interaction with ethanol

An intraperitoneally dose of 200 mg/kg of lipid soluble kava extract (no further detail) caused a highly significant ($p<0.001$) increase in the sleeping time of mice injected with 3.5 g/kg or 4 g/kg of ethanol. Increasing the kava extract dosage to 300 mg/kg further prolonged the hypnosis, but proved lethal to three of the six mice treated with 4 g/kg ethanol, indicating that toxicity as well as hypnosis was increased. A dosage of 1 g/kg ethanol did not alter the kava induced sleeping times of mice when injected with either 350 or 450 mg/kg of the extract but 2 g/kg ethanol greatly prolonged the mean sleeping time produced by 350 mg/kg kava extract (Jamieson and Duffield, 1990b).

3.1.5. Conclusions

Several *in vitro* and *in vivo* studies were conducted on kava extracts, isolated kavalactones or synthetic kavalactones in order to investigate neurological and sedative effects, anticonvulsive, muscle relaxing and spasmolytic activity or other activities (eg. antifungal, apoptotic).

Because *in vitro* studies were performed with different extracts (aqueous, methanolic, dichlormethane or ethanolic) not always well characterised, the relevance of the results is questionable. The same comment regarding the heterogeous type of extracts tested is applicable for the *in vivo* studies, where the results are not consistent and mainly obtained after parenteral administration According to the existing non-clinical data, HMPC considered that kavalactones may contribute to the anxiolytic activity, but the clinical efficacy of these componds is not demonstrated.

The results obtained with synthetic racemic (+) kavain cannot be transfered to the natural L(-)kavain, therefore their value is limited.

Regarding safety pharmacology, direct hepatotoxicity of isolated kavalactones and kava extracts has been assessed in numerous *in vitro* studies yielding different and partly inconsistent results. Also *in vivo* toxicity leads to different results concerning its intrinsec potential; some studies on rats revealed an increased serum level of transaminases and hepatocellular hypertrophy while other studies were negative. There are some *in vivo* studies on hepatotoxic potential of flavokavin B, but the tested doses are much higher than the usual daily uptake. Therefore further studies are needed to confirm the mechanism of action on the target (liver).

These results should be correlated with NTP- carcinogenicity findings (see 3.3.4. Carcinogenicity).

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

Herbal preparations

In vitro

Bioavailability of kavalactones in ethanolic and aqueous extracts was studied *in vitro* using Caco-2 cell monolayers (Matthias *et al.*, 2007). The extracts showed only minor differences in the ratio of kavalactones but there was a difference in the total amount (204 mg/ml in ethanolic and 103 mg/ml in aqueous extracts). Good bioavailability permeability (Papp) calculated from uptake data from 10 to 90 min was for all > 40 x 10^{-6} cm/s. Complete intestinal absorption is considered for Papp>1 x 10^{-6} cm/s. Yangonin was potentially retained in Caco-2 cells as recovery on the apical side was only 40%. Permeability of purified kavain was significantly lower compared to kavain uptake from extracts. Bioavailability was not affected by the extraction method.

Zenger *et al.*, 2015 investigated *in vitro* the metabolism of flavokavins A, B, and C (FKA, FKB and FKC) using human liver microsomes. Phase I metabolism and phase II metabolism (glucuronidation) as well as combined phase I+II metabolism were studied. Major phase I metabolites were generated by demethylation in position C-4 or C-4′ and hydroxylation predominantly in position C-4, yielding FKC as phase I metabolite of FKA and FKB, helichrysetin as metabolite of FKA and FKC, and cardamonin as metabolite of FKC. To an even greater extent, flavokawains were metabolized in the presence of uridine diphosphate (UDP) glucuronic acid by microsomal UDP-glucuronosyl transferases. For all flavokavins, monoglucuronides (FKA-2′ -O-glucuronide, FKB-2′ -O-glucuronide, FKC-2′ -O-glucuronide, FKC-4-O-glucuronide) were found as major phase II metabolites. The dominance of generated glucuronides suggests a role of conjugated chalcones as potential active compounds *in vivo*.

In vivo

When " *crude kava resin*" (120 mg/kg, containing 44 mg of kavain, 23 mg of dihydrokavain, 18 mg of yangonin and 16 mg desmethoxyyangonin; no further detail) was given intraperitoneally to male Balb/c mice, the maximum concentrations of kavain and yangonin in brain markedly increased (2 and

20 times, respectively) relative to the value measured fron their individual injection while the concentrations of 7,8-dihydrokavain and desmethoxyyangonin were similar to those measured after they were injected separately (Keledjian *et al*., 1988).

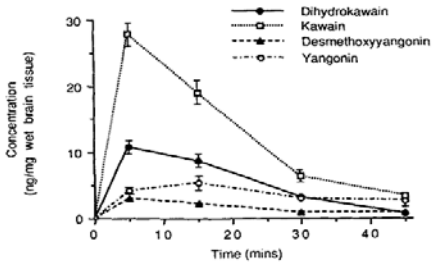


Figure 2—*Kava constituents measured in mouse brain after a single ip dose of kava resin (120 mg/kg). Each point represents the average of 6–9 analyses. Unless indicated to the contrary, error bars fell within the symbol area. Analysis of the kava resin is given in Table II.*

After oral administration of a kava extract (no further detail) to mice at 100 mg/kg, maximum plasma concentration of individual kavalactones (kavain, dihydrokavain, methysticin, and dihydromethysticin, but not yangonin) ranging between 300-900 ng/ml, were attained within 5 minutes; the elimination half-life was approximately 30 minutes. When mice and rats were treated orally with 100 mg/kg of kava extract formulation (suspended in 0.2% agar), bioavailability clearly increased, with maximum plasma levels of kavalactones in mice reaching 1.7-2.5 µg/ml (except yangonin, 0.3 µg/ml) 0.5 hours after administration; in rats, however, two absorption peaks of lactones were observed, at 15 minutes and approximately 2 hours. Surprisingly, kavalactones levels in the brain showed peak concentrations (1.1-2 µg/g of brain) at the same time as in the plasma. Elimination half-life in mouse plasma and brain were approximately 1 hour and even longer in the rat (Biber, 1992).

The same researchers investigated in mice the bioavailability of the kavalactones after oral administration (in 0.2% agar) of either 100 mg/kg extract WS 1490, or 100 mg/kg of the same extract as a formulation (WHO, 2007). In addition pure (+)-kavain was administered to mice at a dose of 14.4 mg/kg, which was the corresponding dose of kavain in the extract. Dogs received an oral dose of 10 mg/kg WS 1490 as the formulation as well as the pure lactones. In both species bioavailability increased in the order pure compound, extract and extract formulation. The authors concluded that clinical data from one preparation or formulation cannot simply be transferred to other formulations without corresponding biopharmaceutical characterisation.

Guo *et al.*, 2009 investigated the changes in gene expression of drug metabolizing enzymes in the livers of Fischer 344 male rats administered kava extract in corn oil (no further detail) by gavage at doses of 0.125, 0.25, 0.5, 1.0, or 2.0 g/ kg/ day, 5 days per week for 14 weeks. Analysis of 22, 226 genes revealed that there were 14, 41, 110, 386, and 916 genes significantly changed in the 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg treatment groups, respectively. There were 16 drug metabolizing genes altered in all three high-dose treatment groups, among which seven genes belong to cytochrome P450 isozymes. While gene expression of CYP1A1, 1A2, 2C6, 3A1, and 3A3 increased; CYP 2C23 and 2C40 decreased, all in a dose-dependent manner.

Isolated compounds

Oral route

Kavain is rapidly absorbed from the gastrointestinal tract, distributed to tissues, and eliminated.

In male F344 rats given kavain at a single oral dose of 100 mg/kg, the maximum blood concentration of kavain was measured at 0.88 hours, after which plasma concentrations declined with a mean

terminal half-life of 1.3 hours. The mean oral bioavailability of kavain in F344 rats was about 50% (Mathews *et al.,* 2005).

In male F344 rats given kavain orally for 7 days, kavain was primarily excreted in the urine, with about 77% recovered during the 72 hours after the last dose. Faecal excretion accounted for about 14% of the administered dose. Only 0.4% of the kavain was retained in the tissues, and kavain did not accumulate preferentially in any particular tissue. In addition, there were no differences in the pharmacokinetics of kavain when administered as a single dose or as repeated doses (Mathews *et al.,* 2005).

The absorption of 6 kavalactones (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin) administered orally in a peanut oil solution was investigated in mice. Kavain and dihydrokavain were rapidly absorbed from the gastrointestinal tract (with a peak at 10 minutes), followed by methysticin and dihydromethysticin (30–45 minutes). Yangonin and desmethoxyyangonin were poorly absorbed, and rapid elimination occurred (Singh, 1992; Robinson *et al.,* 2009).

Parenteral route

In male F344 rats given an intravenous injection of kavain at a dose of 7 mg/kg bw, kavain was rapidly eliminated from the systemic circulation, with a terminal half-life of 0.63 hours. Systemic clearance and volume of distribution were 89 ml/minutes per kg and 2.70 L/kg, respectively, indicating that a significant amount of kavain was rapidly distributed out of the plasma into tissues and quickly cleared from the body (Mathews *et al.,* 2005).

Keledjian *et al.*, 1988 observed a peak concentration at 5 minutes in brain for kavain and 7,8-dihydrokavain; the compounds were rapidly eliminated after intraperitoneal administration (100 mg/kg bw) of individual kava constituents in male Balb/c mice. The maximum concentrations of kavain and 7,8-dihydrokavain were 64.7 and 29.3 ng/mg wet brain tissue, respectively, and were rapidly eliminated. In contrast, desmethoxyyangonin and yangonin had poorly defined maxima corresponding to concentrations of 10.4 and 1.2 ng/mg wet brain tissue, lower than those of kavain or 7,8-dihydrokavain but were more slowly eliminated from brain tissue.

Metabolism and excretion

In vitro

Fu *et al.*, 2009 and 2012a studied the rat microsomal metabolism of kavain, desmethoxyyangonin and methysticin (each at concentration of 10 µg/ml). P-hydroxykavain, m,p-dihydroxykavain, and p-hydroxyyangonin were identified as primary metabolites. Moreover, cytochrome P450 isoforms responsible for kavalactone metabolism were examined. CYP3A1/3A23 was found to be responsible for kavalactone metabolism in female rats, CYP3A2 in male rats while the roles of CYP1A2, -2 C6, -2 C9, -2E1 and -3A4 are limited. For desmethoxyyangonin CYP2C6 and CYP2C11 were involved in males and CYP2C12 in females. CYP3A1/3A23 may also be involved in females.

The disposition profiles of three kavalactones (kavain, methysticin and desmethoxyyangonin) and their respective metabolites werealso examined in the perfusate and bile of the isolated perfused rat liver (Fu *et al.*, 2012b). The rat livers were exposed to kavain, methysticin and desmethoxyyangonin for 120 min. Metabolism was found to be of first-order nature with similar half-lives of decay (1.2 – 3 h). p-hydroxykavain and m,p-dihydroxykavain were found as metabolites. Biliary excretion of kavalactones was negligible.

In vivo

Rasmussen *et al.*, 1979 and later NTP (2012) investigated the metabolism of five kavalactones (kavain, dihydrokavain, methysticin, yangonin, and dihydroyangonin) in male albino rats. The individual kavalactones were administered orally (400 mg/kg bw) or intraperitoneally (100 mg/kg bw), the metabolites and the recovered parent substrate in the urine were then identified:

Dihydrokavain: Approximately half of an oral dose of dihydrokawain (400 mg/kg) was recovered as metabolites in the urine in 48 hours. Nearly a 2:1 ratio between hydroxylated and ring-opened products was seen. There were three mono- and three di-hydroxlated derivatives, of which *p*-hydroxydihydrokawain was the most abundant. The remaining third consisted of metabolites formed by scission of the 5,6-dihydro-a -pyrone ring and included hippuric acid. Small amounts of unchanged dihydrokawain were found in the feces. No metabolites were identified in feces or 0-22 hour bile samples.

Kavain: Although lower amounts of urinary metabolites were excreted following kavain administration, both hydroxylated and ring-opened products were formed. Metabolites identified included p-hydroxybenzoic acid; 4-hydroxy-6-phenyl-5-hexen-2-one; hippuric acid; 4-hydroxy-6-hydroxyphenyl-5-hexen-2-one;*p*-hydroxydihydrokavain; hydroxykavain; *p*-hydroxykavain; and *p*-hydroxy-5,6-dehydrokavain. Two metabolites were unidentified. Large amounts of unchanged compound were identified in the feces.

Methysticin: Methysticin gave rise to only small amounts of two urinary metabolites formed by demethylenation of the methylenedioxyphenyl moiety (*m,p*-dihydroxykawain and *m,p*-dihydroxydihydrokawain). Unchanged methysticin was identified in feces.

7,8 – Dihydroxyyangonin: The major urinary metabolite of 7,8-dihydroxyyangonin was *p*- hydroxy-5,6-dehydro-7,8-dihydrokawain, two minor metabolites were hydroxylated derivatives of this compound. No ring-opened products were detected.

Yangonin: Relatively small amounts of yangonin metabolites were detected in the urine. The three metabolites identified were formed via *O*-demethylation; the major metabolite was *p*-hydroxy-5,6-dehydrokawain. No ring-opened products were detected.

Enzyme interactions

Yamazaki *et al.,* 2008 investigated the effects of administration of kava preparations on gene expression of hepatic CYP1A isoforms in rats. A high dose (380 mg kavalactones/kg/day) of two different types of kava products (Kava A: kava root extract (kavalactones: 80% or greater) and Kava B (unfiltered juice of whole lateral root of kava, freshly freeze-dried) for 8 days significantly increased liver weights. CYP1A2 mRNA expression was moderately increased (2.8–7.3 fold). More importantly, the high dose of kava markedly enhanced CYP1A1 mRNA expression (75–220 fold) as well as ethoxyresorufin O-deethylase activities and CYP1A1 immunoreactivities.

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

3.3.1. Single dose toxicity

Herbal preparation

The LD50 values of an acetone-water extract of kava (DER 11-20:1, 70% kavalactones) in mice were: 380 mg/kg (intraperitoneal) and 1800 mg/kg (oral); in rats: 370 mg/kg(intraperitoneal) and 1600 mg/kg (oral). Acute reactions were dose-dependent and manifested by reduced spontaneous motility,

ataxia, and sedation, lying on their sides with reduced reflex excitability, unconsciouness and death from respiratory paralysis (Duke, 2002).

Isolated compounds

The acute toxicity of isolated compounds as dihydrokavain and dihydromethysticin was studied in mice, rabbits, cats and dogs. The compounds were dissolved in arachis oil and administered i.p. and p.o. After i.p. administration, the LD_{50} of dihydrokavain was 325 ± 4.5 mg/kg in mice, 350 mg/kg in rabbits, >250 mg/kg in cats, and >200 mg/kg in dogs. The LD_{50} of dihydromethysticin after i.p. administration was 420 ± 20.0 mg/kg in mice, 300 mg/kg in rabbits, and >200 mg/kg in dogs. After p.o. administration the LD_{50} was 920 ± 52.0 mg/kg for dihydrokavaine and 1050 ± 67.0 mg/kg for dihydromethysticin in mice. The first symptoms occur only few minutes after i.p. or p.o. administration of a toxic dose. In mice, a reduction of spontaneous motility is followed by an ataxic state. After that the mouse turns to a side position, which is combined with muscle relaxation and reduced excitability. The side position continues for 3 – 7 hours, the mouse finally dies of respiratory paralysis. Cats and dogs regularly reacted by vomiting before assuming the side position, even with i.p. administration (Meyer, 1962).

The same working group again studied the LD_{50} of dihydrokavaine and dihydromethysticin after i.p. application in male mice. This time, dihydrokavaine had a LD_{50} of 490 ± 15.2 mg/kg and a minimal neurotoxic dosage of 170 mg/kg. The LD_{50} of dihydromethysticin was confirmed; its minimal neurotoxic dosage was 165 mg/kg (Meyer & Meyer-Burg, 1964).

A further study summarized the oral, intraperitoneal and intravenous LD_{50} values of six kava lactones in mice: kavain (1130 mg/kg, 420 mg/kg and 69 mg/kg); dihydrokavain (980 mg/kg, 490 mg/kg and 53 mg/kg); methysticin(> 800 mg/kg, 530 mg/kg and 49 mg/kg); dihydromethysticin (1050 mg/kg, 420 mg/kg and 67 mg/kg); demethoxyyangonin (> 800 mg/kg, >800 mg/kg and 55 mg/kg) and yangonin (> 1500 mg/kg, >1500 mg/kg and 41 mg/kg)((Kretzschmar & Meyer, 1969).

3.3.2. Repeat dose toxicity

Herbal preparation

Data should be read in conjunction with section 3.1.3 Safety pharmacology (cytotoxicity and hepatotoxicity)

Following studies were conducted and reported by NTP (2012):

2-week studies (rats and mice)

In one study rats were treated with kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 16 days. One female rat administered 2.0 g/kg kava extract died on day 3 of the study. Mean body weights of all dosed groups of rats were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in the 2.0 g/kg groups of males and females and ataxia and lethargy in the 1.0 g/kg group of females. Liver weights were significantly increased in 1.0 and 2.0 g/kg males and in 0.5 g/kg or greater females compared to the vehicle controls. Minimal hepatocellular hypertrophy occurred in all 2.0 g/kg males and in all females administered 0.25 g/kg or greater.

In another study mice were treated with kava kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) in corn oil by

gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 17 days. In the 2.0 g/kg group of males, one died on day 2 and one died on day 3. Mean body weights of all dosed groups of mice were similar to those of the vehicle controls. Clinical findings included abnormal breathing, ataxia, and lethargy in males and females in the 1.0 and 2.0 g/kg groups. Liver weights of 2.0 g/kg males and females were significantly increased. The incidence of hepatocellular hypertrophy in 2.0 g/kg female mice was significantly greater than that in the vehicle control group.

3-month studies (rats and mice)

Rats were treated with kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 14 weeks. Deaths attributed to kava kava extract administration included three males and four females in the 2.0 g/kg groups and one female in the 1.0 g/kg group. One 0.25 g/kg male and one vehicle control female also died before the end of the study. The mean body weights of males in the 1.0 and 2.0 g/kg groups and females in the 2.0 g/kg group were significantly less than those of the vehicle controls. Ataxia and lethargy were observed in males and females in the 1.0 g/kg groups during week 1 and in the 2.0 g/kg groups throughout the study. Increased γ-glutamyltransferase activity in 1.0 g/kg females and 2.0 g/kg males and females may represent enzyme induction. However, the hepatocellular hypertrophy observed in the 2.0 g/kg females may have contributed to the increased γ-glutamyltransferase activity. The liver weights of 0.25 g/kg or greater males and 0.5 g/kg or greater females were significantly increased compared to the vehicle controls. The kidney weights of 0.5 g/kg or greater males and females were significantly increased compared to the vehicle controls. The incidence of hepatocellular hypertrophy in 2.0 g/kg females was significantly greater than that in the vehicle controls.

In another study mice were treated with kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) in corn oil by gavage at doses of 0, 0.125, 0.25, 0.5, 1.0, or 2.0 g/kg, 5 days per week for 14 weeks. Four male and three female 2.0 g/kg mice died during week 1; these deaths were attributed to kava kava extract administration. One additional 2.0 g/kg female died during week 6 due to a gavage accident. The mean body weights of dosed males and females were similar to those of the vehicle controls. Ataxia and lethargy occurred in males and females in the 1.0 and 2.0 g/kg groups during week 1. The liver weights of 2.0 g/kg males and 1.0 and 2.0 g/kg females were significantly increased compared to those of the vehicle control groups. The incidences of centrilobular hypertrophy in the liver of 0.5 g/kg or greater males and 1.0 and 2.0 g/kg females were significantly greater than those in the vehicle controls.

Isolated compounds

The toxicity of the demethoxyyangonin was evaluated in male ICR mice and male Wistar rats. Mice received oral demethoxyyangonin doses of 30, 100, or 300 mg/kg twice a day for 14 days or 100 mg/kg twice a day for 9 weeks; rats were treated with 30, 100, or 300 mg/kg daily for 3 months. In all groups, histological and hematological findings were negative. In rats, demethoxyyangonin lowered cholesterol in the first two months but increased cholesterol after three months (Hsu *et al.*, 1994).

Male FVB/N mice were fed with diet supplemented with 0.6% (6 g/kg food) flavokavin A or 0.6% commercial kava root extract (no further detail) for three weeks. Dietary feeding of flavokavin A did not affect food consumption and body weight, whereas that dietary feeding of kava extract increased both wet liver weight and liver to body weight ratio. Macroscopical examination of the livers revealed no signs of hepatotoxicity among all experimental groups. Pathological analysis demonstrates a regular structure of normal liver parenchyma with small portal tracts, regular reticulin network and low

variation in hepatocellular nuclei size in both control and flavakavin A treatment groups. There is no identifiable inflammation or steatosis. However, dietary feeding of 0.6% kava extract resulted in a significant appearance of proliferating nodules consisting of closely packed hepatocytes in the liver. The cytotoxicity profile showed flavokavin A had minimal side effects on bone marrow and small intestinal epithelial cells compared with adriamycin. In addition, oral feeding of flavokavin A increased activities of both glutathione S-transferase and quinone reductase in the liver, lung, prostate and bladder tissues of mice (Li *et al*., 2014).

A/J mice exposed to (+)-dihydromethysticin at a dose of 0.5 mg/g of diet for 17 weeks did not present with any adverse effects in the following parameters: average weekly body weight increase, average weekly food intake, clinical chemistry analyses of serum samples collected at Week 8 and 17, hematology analysis at Week 17, final body weight, relative weight of heart, lung, liver, kidney and spleen at Week 17, and the pathology of heart, lung, liver, kidney, spleen and pancreas at Week 17. (Narayanapillai *et al*., 2014a).

3.3.3. Genotoxicity

Herbal preparation

Kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) was not mutagenic in *Salmonella typhimurium* (strains TA97, TA98, TA1535 or TA100) or *Escherichia coli* strain WP2 uvrA pKM101 with or without metabolic activation (rat liver S9) at concentration up to 10 000 µg/plate (NTP, 2012).

Two types of lipid soluble extracts of kava (containing 150 mg kavalactones/g extract) at concentrations up to 200 and 400 µg/ml, respectively and 6 isolated compounds (dihydromethysticin, desmethoxyyangonin, methysticin, dihydrokawain, yangonin, D-Kavain and DL-Kavain) were evaluated in L5178Y mouse lymphoma cells. The maximum dose levels tested for each kavalactones were 300 µg/ml dihydromethysticin; 150 µg/ml methysticin; 200 µg/ml dihydrokavain; 160 µg/ml DL-kavain and 160µg/ml D-kavain. Neither the kava extracts nor the kavalactones induced a mutagenic response in the L5178Y mouse lymphoma mutation assay with the addition of human liver S9 activation. (Whittaker *et al*., 2008).

The only report of positive mutagenic activity with n-butanol fraction of kava leaves (two positive results, but six negative results) involved the *umu* point mutation assay. Further investigations using bioassay-directed isolation and analysis indicated that 2 C-glycoside flavonoid compounds accounted for the positive mutagenic results. Two isolated compounds were identified as 2''-O-rhamnosylvitexin and schaftoside (Jhoo *et al*., 2007). These data are considered by IARC not useful, because the results were not analysed statistically. Moreover, the results cannot be extrapolated to kava rhizoma.

In vivo, in male or female mice given kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7%dihydrokavain, 4% yangonin, 3% dihydromethysticin) at a dose of up to 2.0 g/kg/day by gavage for 3 months, there was no increase in the frequency of micronucleated normochromatic or polychromatic erythrocytes in blood (NTP, 2012).

3.3.4. Carcinogenicity

Herbal preparation

Data should be seen in conjunction with section 3.1.3 Safety pharmacology (cytotoxicity and hepatotoxicity).

In one study in B6C3F1 mice kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) was given orally, by gavage, at a dose of 0 (corn oil vehicle, 10 ml/kg), 0.25, 0.5, or 1.0 g/kg/day, 5 days per week, for 105 weeks. In males, the mean body weight of the dosed groups was similar to that in the control group. In females, the mean body weight of the group at 1.0 g/kg was 11% less than that in the control group after week 21. The mean survival time of male and female mice in the dosed groups was similar to that of the controls.

The main findings are related with male mice. In males, the incidence of hepatoblastoma was significantly higher in the groups receiving the intermediate and highest dose, and had a significant positive trend (0/50, 4/50, 9/50, 12/50).

The incidences of all benign or malign neoplasms of the liver in male and female mice can be found in the NTP report:

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Male				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hypertrophy[a]	0	34** (1.0)[b]	30** (2.0)	39** (2.0)
Eosinophilic Focus	28	32	42**	43**
Angiectasis	3 (1.0)	6 (1.0)	7 (1.1)	10* (1.7)
Necrosis	3 (1.7)	10* (2.0)	7 (2.0)	13** (2.0)
Hepatocellular Adenoma, Multiple	13	19	19	23*
Hepatocellular Adenoma (includes multiple)[c]	27	32	29	35
Hepatocellular Carcinoma, Multiple	4	3	7	5
Hepatocellular Carcinoma (includes multiple)[d]	20	18	26	20
Hepatoblastoma, Multiple	0	0	2	3
Hepatoblastoma (includes multiple)[e]				
Overall rate[f]	0/50 (0%)	4/50 (8%)	9/50 (18%)	12/50 (24%)
Adjusted rate[g]	0.0%	9.4%	20.1%	26.4%
Terminal rate[h]	0/34 (0%)	2/33 (6%)	8/34 (24%)	8/36 (22%)
First incidence (days)	—[i]	687	679	582
Poly-3 test[j]	P<0.001	P=0.057	P=0.002	P<0.001
Hepatocellular Carcinoma or Hepatoblastoma (includes multiple)[k]				
Overall rate	20/50 (40%)	21/50 (42%)	30/50 (60%)	25/50 (50%)
Adjusted rate	42.7%	46.8%	61.7%	53.3%
Terminal rate	11/34 (32%)	14/33 (42%)	19/34 (56%)	16/36 (44%)
First incidence (days)	455	431	455	574
Poly-3 test	P=0.136	P=0.426	P=0.046	P=0.205

Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Kava Kava Extract

	Vehicle Control	0.25 g/kg	0.5 g/kg	1.0 g/kg
Female				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hypertrophy	0	20** (1.0)	48** (1.9)	49** (2.0)
Eosinophilic Focus	9	7	16	26**
Hepatocellular Adenoma, Multiple	0	4	6*	1
Hepatocellular Adenoma (includes multiple)[l]	8	11	14	5
Hepatocellular Carcinoma, Multiple	1	1	1	0
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	3/50 (6%)	13/50 (26%)	8/50 (16%)	8/50 (16%)
Adjusted rate	6.7%	28.1%	16.5%	17.2%
Terminal rate	3/38 (8%)	9/34 (27%)	6/45 (13%)	3/37 (8%)
First incidence (days)	729 (T)	701	534	604
Poly-3 test	P=0.337	P=0.007	P=0.126	P=0.109
Hepatocellular Adenoma or Carcinoma (includes multiple)[n]				
Overall rate	10/50 (20%)	21/50 (42%)	20/50 (40%)	13/50 (26%)
Adjusted rate	22.1%	45.1%	41.2%	28.0%
Terminal rate	9/38 (24%)	16/34 (47%)	18/45 (40%)	8/37 (22%)
First incidence (days)	560	669	534	604
Poly-3 test	P=0.542	P=0.015	P=0.036	P=0.338
Hepatoblastoma	0	0	1	0

The incidence of squamous cell hyperplasia of the forestomach was significantly higher in the groups receiving the lowest or intermediate doses (Behl *et al.,* 2011; NTP, 2012).

In male and female F344/N rats kava extract (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin) was given orally, by gavage, at a dose of 0 (corn oil vehicle, 5 ml/kg), 0.1, 0.3, or 1.0 g/kg/day, 5 days per week, for 104 (male rats) or 105 (female rats) weeks. The mean body weight of the groups at 1.0 g/kg bw was 10% less than that of the control group after week 65 in males and after week 41 in females. The mean survival time for rats in the dosed groups was similar to that of controls for both sexes. In males, the incidence of testis interstitial (Leydig) cell adenoma was significantly higher in the groups at the intermediate or highest dose, and had a significant positive trend. The incidence of this tumour in controls was low (76%) compared with that in historical controls (corn oil vehicle controls: range, 76–94%; all routes: range, 54–98%).The incidence of renal pelvis transitional cell hyperplasia was significantly higher in the group receiving the highest dose. In females, the incidence of renal pelvis transitional cell hyperplasia was significantly higher in the groups at the highest or intermediate dose. There was no significant increase in the incidence of any neoplasm in females (Behl *et al.,* 2011; NTP, 2012).

NTP concluded that "there is sufficient evidence in experimental animals for the carcinogenicity of kava extract, but is inadequate evidence in humans, therefore is classiffied as possible carcinogenic to humans (Group 2 B)". The reported carcinogenicity in animals is most probably mediated through nongenotoxic mechanisms.

There are no studies conducted on other type of preparations.

Assessor's comment: The extract used by NTP in the repeated dose, genotoxicity and carcinogenicity studies is characterised only by its kavalactones pattern but not by other parameters, such as DER or extraction solvent. Because the phytochemical comparability with other preparations is not demonstrated, the results are attributed only to this preparation.

3.3.5. Reproductive and developmental toxicity

There are no studies on herbal preparations.

NTP, on its website (http://ntp-server.niehs.nih.gov; accessed on January 2016) in its toxicity profile for kava-kava, refers to an old study conducted or rats and rabbits treated orally with a mixture of 40 percent kavain, 40 percent dihydrokavain, and 20 percent yangonin. The mixture was administered orally at doses of 100 or 500 mg/kg on days 6-15 of gestation. The mixture was not teratogenic or embryotoxic in Wistar rats. The mean fetal weight in treated animals was lower than in the controls, although it still fell within physiological limits. The possibility of an effect of the compounds on fetal weight could not be excluded. The mixture was also negative for teratogenic activity in New Zealand strain rabbits when administered orally at doses of 20 or 200 mg/kg on days 6-18 after mating. There was a significant dose-related reduction of fetal weight in treated rabbits (NTP, 1998).

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Extensive toxicological data are publised by NTP regarding one kava preparation. Repeated dose studies on rats and mice revealed increased weights of liver and kidney and hepatocellular hypertrophy.

The same preparation was used in the carcinogenicity studies conducted on two species (mice and rats), treated by gavage. In mice the preparation caused a dose-depending increase in the incidence of hepatoblastoma in males.

This is a rare kind of tumor in mice which is also found in humans mainly in children/adolescents. Each animal species has its own timing (and basis) to develop hepatoblastoma - some in young ages, others in older animals only. The mechanism of action of the kava preparation is unknown.

There was no hepatoblastoma (0/50) in the control group (corn oil), but it is not unusual as this is a rare kind of tumor. Actually historical control data exist and the incidence for this rare tumor vary between 1-4/50. Even using the historical controls to assess the effects, the increase of hepatoblastoma would still be dose-depending. All the other liver tumors were seen in vehicle control group but also and in historical controls.

Not only the type of tumor but also the target organ (liver) is important for the final assessment. Since in humans the target organ seems to be the liver the findings of NTP points probably into the right direction.

In conclusion, there is sufficient evidence in experimental animals for the carcinogenicity of this kava extract. The relevance of such findings for humans cannot be excluded, especially if the study is mainly seen as evidence for such neoplastic mechanisms in the target organ. The same extract was neither mutagenic *in vitro* on *Salmonella typhimurium* (strains TA97, TA98, TA1535 or TA100) or *Escherichia coli* strain WP2 uvrA pKM101 with or without metabolic activation, not *in vivo* in the micronucleus test.

The extract used by NTP in the repeated dose, genotoxicity and carcinogenicity studies is characterised only by its kavalactones pattern but not by other parameters such as DER or extraction solvent. Because the phytochemical comparability with other preparations is not demonstrated, the results are atributed only to this preparation.

There are no adequate reproductive and developmental toxicity studies on kava preparations. The only study is related with a mixture of isolated compounds, therefore its relevance is limited.

3.4. Overall conclusions on non-clinical data

Results from *in vitro* and *in vivo* studies with extracts and isolated constituents should be carefully interpretated because the studies are conducted with heterogeous extracts, not always well characterised and the administration is mainly via the parenteral route. HMPC considered that kavalactones may contribute to the anxiolytic activity in animals, but the clinical efficacy of these compounds is not demonstrated (see Section 4. Clinical data).

Non-clinical information on the safety suggests that kava preparations may have hepatotoxic potential, but further studies will be needed to clarify the mechanism(s) of action.

The assumption that liver is the target organ should be correlated with the NTP study that provided sufficient evidence in experimental animals for the carcinogenicity of one kava preparation (containing 27% kavalactones with 3% methysticin, 6.3% kavain, 7% dihydrokavain, 4% yangonin, 3% dihydromethysticin). In mice, the preparation caused a dose-dependent increase in the incidence of hepatoblastoma in males. Based on these findings, NTP included this preparation into group 2B,

meaning sufficient evidence in experimental animals. Even that the evidence in humans is inadequate, the relevance of such findings for humans cannot be excluded and constitute a cause for safety concerns.

The same extract gave negative results in several standard bacterial assays for mutation in the absence or presence of metabolic activation, therefore the reported carcinogenicity of kava extract is most likely mediated through a nongenotoxic mechanism.

There are no studies conducted on other type of preparations.

There are no adequated reproductive and developmental toxicity studies on kava preparations. The only study is related with a mixture of isolated compounds, therefore its relevance is limited.

4. Clinical Data

The extracts used in the trials are specified in the comments as far as possible. Unfortunately, in some publications correct specifications of solvent and drug-extract ratio (DER) are missing. In these cases no details can be given, if the extract could not be identified otherwise.

The extracts used in the studies are specified in the comments as far as possible. A special extract "WS1490" was used in some clinical trials. Regarding this preparation, the manufacturing process and the content in kavalactones changed during time; the extraction solvent used was either acetone or ethanol. Therefore, if in the cited references the extraction solvent was not explicitly declared, the type of preparation is considered unknown.

4.1. Clinical pharmacology

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

(a) Herbal substance

No data

(b) Herbal preparations

Cognitive performance

The effects of oxazepam and a kava extract (WS1490), were investigated for reaction time and event-related potentials (ERPs) in a visual search paradigm on 12 healthy young males (age: 24 – 37) using a double-blind design. Three types of medication were administered: placebo, 3 x 200 kava extract mg/day for 5 days, and oxazepam 1 x 15 mg on the day before testing, 75 mg on the morning of the experimental day. Participants took one capsule three times daily for five days prior to the experimental session. Significant effects were obtained with oxazepam in a number of psychometric tests as well as search time and quality. Several ERP components of different latency, topography and functional significance were affected by the medication. Oxazepam led to a reduction of the amplitude of the parietal N1, frontal N2, posterior contralateral N2, and occipital P3 components. Kava extract was associated with a greater posterior N1, posterior contralateral N2, and occipital P3. The authors value those findings as evidence of a positive effect of kava extract on the allocation of attention and processing capacity (Münte *et al*., 1993).

The effects of kava-kava on alertness and speed of access to information from long-term memory were investigated in a single-blind study using letter-match task; two groups, each consisting of 9

caucasian(5 male) undergraduates, completed two identical experimental sessions 2-6 days apart. The no-kava group consumed no kava prior to either session. The low-dose kava group drank a 250-ml of aqueous preparation of kava (30 g of kava root powder soaked in water, then filtered) 30 min prior to testing on the second occasion. An additional group of 9 Caucasian (5 male) consumed a 500-ml aqueous preparation of kava (made up to a strength of 1 g/kg body weight) 1 h prior to the test. Kava aqueous preparation was found to have no discernible effect on cognitive performance (Russell *et al*., 1987).

Vigilance

A single blind pilot study with healthy volunteers (2 male, 4 female, age: 24 – 47) was carried out in order to determine the neurophysiologic efficacy (quantitative EEG, evoked potentials) and the effects of the kava extract WS 1490 (containing 70% kavalactones) on emotional and general variables concerning personality, on the subjective state as well as on various cognitive parameters. The volunteers received 300 mg or 600 mg/day WS 1490 for one week. The quantitative EEG showed an increase in the β-/α-index typical for the pharmaco-EEG profile of anxiolytics. The increase in the β-activity was most pronounced in the β2-range. Kava extract showed no sedative-hypnotic effects after administration of 600 mg. The results of the evoked potential studies indicate that information processing may be improved in the cortical areas studied, i.e., vigilance is increased. These findings correlate with the results of the psychometric tests, which indicate increased activity and an improvement in emotional stability (Johnson *et al.,* 1991).

In a double-blind, 3-fold crossover study 12 healthy volunteers received single doses of either an ethanolic extract (containing 120 mg kavapyrones) or 10 mg diazepam or placebo. All tests were done immediately before as well as 2 and 6 hours after administration of the preparations. The washout period between cross-over was seven days. After administration of kava extract and diazepam the EEG showed an increase in the relative intensity of slow waves, which was recorded in the occipital area for both preparations, and in the frontal area only for kava extract. Maximum effects were often recorded 2 hours after administration of diazepam. However, the kava preparation, showed the most distinct effects after 6 hours. A benzodiazepine-specific increase in beta-activity was not recorded for the kava preparation. During the observation period the placebo group showed a time-dependent decrease of the relative intensity in the zone of the alpha-waves. The marked beta-activity in the diazepam group is the result of frequent significant differences between the two test preparations. In psychophysiological tests the critical flicker frequency was more distinctly reduced by kava extract and diazepam than by placebo. As opposed to these results, the volunteers showed significantly better results in the mental performance test-PAULI test ($p<0.001$), the simple reaction time test and the complex multiple-choice reaction test 2 hours after administration of kava-extract ($p<0.01$). For diazepam and placebo an improvement of performance could not be statistically proven (Gessner *et al.,* 1994).

A preliminary study involving 20 healthy individuals assessed the effects of a single dose of 300 mg kava extract (containing 30% kavalactones; no further detail) on cognitive performance and mood in a randomised, double-blind, placebo controlled trial. Cognitive performance was examined with the Sperling partial report and the Sternberg item recognition task, which were used as an index for visual attention and short-term memory processing. The intake of Kava extract led to an increase in state cheerfulness, while the phytopharmacon did not influence state seriousness and bad mood. The mood-elevating effects of Kava were most prominent in trait cheerful subjects, indicating that trait cheerfulness moderated the drug-induced increase in cheerful mood. Furthermore, Kava improved the accuracy and the speed of performing the partial report and the item recognition task, indicative of a beneficial effect of the phytopharmacon on visual attention and short-term memory retrieval, respectively (Thomson *et al.,* 2004).

(c) Isolated compound (kavain)

In a double-blind, placebo-controlled study the encephalotropic and psychotropic effects of D,L kavain (synthetic), as compared with clobazam, were investigated, utilising EEG brain mapping as well as psychometric and psychophysiological analyses. 15 healthy volunteers received randomised single oral doses of placebo, 200, 400 or 600 mg d,l kavain as well as 30 mg clobazam as reference compound at weekly intervals. EEG recordings, psychometric tests, evaluations of pulse, blood pressure and side effects were carried out at the hours 0, 1, 2, 4, 6, and 8. Brain maps of drug induced pharmaco-EEG changes (pharmaco-EEG maps) demonstrated that kavain exerted a significant action on the human brain function as compared with placebo characterised by a dose-dependent increase of delta, theta and alpha 1 activity, while alpha 2, beta activity and the centroid of the total activity decreased. These findings are indicative of a sedative action, which was, however, in type quite different from that of the 1,5-benzodiazepine. The latter produced a decrease of delta, theta, alpha 1 and alpha 2 and an increase of beta activity while the total centroid was accelerated. Interestingly, 200 mg kavain also induced vigilance promoting effects with a decrease of delta and beta activity and an increase of alpha activity and total power. Psychometric tests also demonstrated clear differences between the two compounds on behaviour. Compared with placebo kavain at all three dosages significantly improvement in intellectual performance (PAULI test), attention, concentration, reaction time and motor speed (rigidity test), while opposite findings were observed after 30 mg clobazam. In regard to thymopsychic variables such as drive, wakefulness, affectivity, mood, well-being, 200 mg kavain produced an improvement when compared to placebo while 600 mg kavain produced sedation, as did 30 mg clobazam. Psychophysiological tests resulted in only minimal results. Topographically, most encephalotropic effects after administration of kavain were found in the frontal area, after administration of clobazam in the central and parietal areas (Saletu *et al.,* 1989).

Sleeping pattern

In a single-blind study *was* examined the influence of the kava extract (WS 1490, containing 70% kavalactones) on sleep quality in healthy volunteers versus placebo. Two groups with 6 volunteers each received either 150 mf or 300 mg kava extract or placebo. A polygraphic sleep-EEG was recorded on nights and the quality of sleep and the subjective state were recorded daily in a questionnaire. After 150 mg or 300 mg kava extract the amount of sleep spindles and the percentage of deep sleep increased, REM-sleep did not change, sleep stage 1 and sleep latency tended to decrease. The subjective sleeping time increased. The authors conclude that the kava extract WS 1490 might have an effect similar to chemical tranquillizers concerning spindle denseness in the sleep-EEG. Furthermore, kava extract positively influences sleep in general, mainly by increasing slow wave sleep (while REM sleep remains unchanged) and decreasing sleep latency (Emser and Bartylla., 1991).

Sedative effects

Two multiple crossover studies were performed with 12 healthy female volunteers (mean age 53.7 years) to screen for acute sedative effects of eight different plant extracts, including kava extract LI 158 (DER 12-5:1; extraction solvent not known) by quantitative EEG analysis. An increase in power of both the theta and slow alpha bands was noted 2 hours after administration of a single oral dose of 600 mg kava extract. The increase in theta power was still present 3 hours after administration, while fronto-centrally a decrease in power was evident in the high frequency beta 3 band. Although the quantitative EEG can indicate drug-induced CNS changes, is not easy to conclude whether such changes are valid predictors of sedation or anxiolysis (Schulz *et al*., 1999).

Mental stress

Cropley *et al.*, 2002 performed an open, randomised study comparing the effect of valerian root, kava extract and an untreated control group in a pressure situation (psychological stress induced under laboratory conditions in a group of healthy volunteers). 54 students completed the colour/interference test with increasing speed of presentation before and after one week intake of 600 mg of valerian root extract (n=18), or 120 mg of an kava extract LI150 (no further details) (n=18) or no medication. Blood pressure and heart rate were recorded before, during and after the test situation, subjective ratings of pressure before and during the test were documented on a 7-point scale. At the second test, there was a significant decrease in systolic blood pressure responsivity in both the kava and valerian groups relative to first test, but there were no significant reductions in diastolic blood pressure. Between first and second test, the heart rate reaction to mental stress was found to decline in the valerian group but not in the kava group. Individuals taking kava or valerian reported less pressure during the task at second test relative to first test. There were no significant differences in blood pressure, heart rate or subjective reports of in the controls. Behavioural performance on the colour/word task did not change between the groups over the two time points. The authors suggest that kava and valerian may be beneficial to health by reducing physiological reactivity during stressful situations. These results lead to the assumption that the kava extract decreases subjective experience of stress (while cognitive performance is not reduced).

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

Few studies have been published on the kinetics of kava preparations or isolated compounds in humans.

Herbal preparations

Following ingestion of 1 L of kava beverage (prepared by the traditional method of aqueous extraction 450 g pulverised kava rhizome was immersed in 3 L water at room temperature) by healthy male subjects seven major, and several minor kavalactones were identified in human urine. Kavain, dihydrokavain, desmethoxyyangonin, tetrahydroyangonin, dihydromethysticin, 11-methoxytetrahydroyangonin, yangonin, methysticin and dehydromethysticin were detected unchanged in human urine; metabolic transformations observed included reduction of the 3,4-double bond and/or demethylation of the 4-methoxyl group of the kavalactone ring. The C12 hydroxyanalogue of yangonin (12-hydroxy-12-desmethoxyyangonin) was also detected, and it may have been formed by demethylation of yangonin and/or C12 hydroxylation of desmethoxyyangonin. In contrast to metabolism in rats, no dihydroxylated metabolites of the kavalactones or products from ring opening of the 2-pyrone ring system, were identified in human urine (Duffield *et al.,* 1989a).

Zou *et al.*, 2005 identified a pyrone ring-opened product, 6-phenyl-3-hexen-2-one, a proposed metabolite of kava, as its mercapturic acid adduct, in urinary samples from two kava drinkers after ingestion of 10 g kava powder with 1.3 g kavalactones. The level of the mercapturic acid adduct was low (0.6 µg/ml). This metabolite was possibly formed from enzymatic demethylation of 7,8-dihydromethysticin, followed by ring opening of the α-pyrone ring, and rearrangement.

11,12- Dihydroxy-7,8-dihydrokavain-oquinone and 11,12-dihydroxykavain-o-quinone, two electrophilic metabolites, were identified as glutathione conjugates when kava extract was incubated with human liver microsomes, but not in the urine of a human volunteer. Instead, the glucuronic acid and sulfate conjugates of these two urinary metabolites were detected in a human volunteer who ingested a single

dose of a dietary supplement containing kava extract (about 90 mg of kavalactones) (Johnson *et al.,* 2003).

Isolated compounds

After a single oral dose of 200 mg D,L-kavain in humans, approximately 80% is absorbed, of which up to 98% is metabolized, mainly to more hydrophlic p-hydroxykavain, on first pass through the liver. Maximum plasma levels of p-hydroxykavain sulfate conjugate (50 ng/ml) and kavain (18 ng/ml) are obtained within 1.7-1.8 hours. The elimination half-life of p-hydroxykavain sulfate is about 29 hours, while elimination of kavain shows a biphasic pattern with half-lives of 50 min for the first phase and approximately 9 hours for the second (Hansel & Woelk, 1995).

Ten urinary metabolites were identified when synthetic D,L-kavain was given to five healthy volunteers as an oral dose of 200 mg. The major metabolite was a hydroxy-dihydrokavain. Hydroxylation of the phenyl ring, reduction of the 7,8 double bond, hydroxylation of the lactone ring with subsequent dehydration, and opening of the lactone ring appeared to be the main metabolic pathways. The metabolites were mainly excreted in the form of their conjugates (Köppel & Tenczer, 1991).

Tarbah *et al.*, 2003 studied kinetics after administration of a single oral dose of 800 mg kavain in a self-medication study. The main metabolite of kavain is p-hydroxykavain, which was found in serum and urine in its free (~ 10% in serum) and conjugated forms (glucuronide and sulfate). Further metabolization takes place to p-hydroxy-7,8-dihydrokavain, which was only detected in urine in form of its conjugates. Opening of the lactone ring, demethylation, decarboxylation and oxidation leads to 6-phenyl-5-hexene-2,4-dione which was detected in urine after 24 h. Kavain is furthermore dehydrated to form 5,6-dehydrokavain. The latter molecule is hydroxylated and demethylated to desmethyl-hydroxy-5,6-dehydrokavain. Serum concentrations within 1-4 h after oral uptake ranged between 40 and 10 ng/ml for kavain, 300 and 125 ng/ml for p-hydroxykavain, and 90 and 40 ng/ml for o-desmethyl-hydroxy-5,6-dehydrokavain. The major metabolite p-hydroxykavain appears in serum in free and conjugated forms with a lag time of 0.25 h and peaks after 0.75 h. The half-lives of free and conjugated forms range between 0.7 and 1.9 h indicating that kavain metabolites can be found up to 10 h in serum samples.

Enzyme interactions

There are extensive *in vitro* studies have shown that kavalactones are inhibitors but also inductors of CYP 450 isoforms (Cote *et al*., 2004; Foster *et al*., 2003; Mathews *et al*., 2002; Unger *et al*., 2002; Weiss *et al*., 2005; Zou *et al.,* 2002, Ma *et al*, 2004), while *in vivo* studies excluded any type of modulation of human CYP 450 isoforms (Anke *et al*., 2006; Gurley *et al*., 2005a; Gurley *et al*., 2005b; Russmann *et al*., 2005; Gurley *et al*., 2007; Gurley *et al*., 2008a; Gurley *et al*., 2008b) The studies were performed with different preparations or isolated compounds.

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

The assessment of clinical data in this section is structured as follows:

Substance tested:

(a) herbal substance, (b) herbal preparations, (c) isolated compounds

Type of study:

Clinical trial, Meta-analysis

Endpoint / therapeutic effect:

a. Treatment option for anxiety disorders nervousness and restlessness,

b. Anxiety in the climacteric phase

Control-type of clinical trial:

Placebo controlled studies, Reference controlled studies, Non-controlled studies

Specified herbal preparation tested:

Ethanolic preparation, Acetonic extract, Other extracts

(a) Herbal substance

No data

(b) Herbal preparations

Clinical trials

Clinical trials with kava preparations have focused on the use as treatment option for anxiety disorders. Several other trials have assessed effects of isolated compounds, as kavain.

The following indications related with kava preparations were proposed by:

German Commission E monograph (1990): "used in nervous anxiety, stress and restlessness"

WHO monograph (2002): "short-term symptomatic treatment of mild states of anxiety or insomnia, due to nervousness, stress or tension"

ESCOP monograph (2003): anxiety, tension and restlessness arising from various causes of non-psychotic origin.

a. Treatment option for anxiety disorders nervousness and restlessness

According to ICD-10 and DSM-IV-TR there are different types of anxiety disorders: generalised anxiety disorder (GAD), social anxiety disorder (SAD), panic disorder, posttraumatic stress disorder, obsessive-compulsive disorder or specific fobia.

European guidelines concerning the clinical investigation of medicinal products indicated for anxiety disorders (CPMP/EWP/4284/02 for generalised anxiety disorders; CHMP/EWP/3635/03 for social anxiety disorder and CHMP/EWP/4280/02 for panic disorder) have been used as a basis for evaluation of the published clinical trials. All three guidelines can be used "*for medicinal products intended for the treatment independent of the class of product under investigation".*

To evaluate the efficacy at least 8-weeks are required for short term use (in generalised anxiety or panic disorder), and 12 weeks for social anxiety disorder. Also, in general, three-arm-studies including placebo and active comparator are requested. Deviations are accepted if are explained and discussed.

Since many years preparations from kava are discussed and used as treatment option for anxiety disorders, mainly generalised anxiety (GAD). At least 5 trials included patients with GAD.

Placebo controlled studies

Ethanolic preparation

Connor *et al.,* 2002 assessed in a randomised, double-blinded, placebo trial the efficacy and safety of an ethanolic extract (no further data) in treating generalized anxiety disorder (GAD). 38 adults (31 female, 7 male; aged 31-75 years, mean 52 years) meeting DSM-IV criteria for GAD and having HAMA score $\geq$ 16 were randomly assigned, following a 1-week placebo run-in, to 4 weeks of treatment with kava ethanolic extract (n=19) corresponding to 2 x 70 mg of kavalactones daily in the first week and dose 2: 2 x 140 mg of kavalactones daily for the last 3 weeks or a matching placebo (n=19). Weekly efficacy assessments [Hamilton Anxiety Rating Scale (HAMA), Hospital Anxiety and Depression Scale (HADS), Self Assessment of Resilience and Anxiety (SARA)] and safety evaluations (side effects, withdrawal symptoms) were conducted. Improvements were observed with both treatments, with response rates ($\geq$ 50% reduction in baseline HAMA score) of 35% and 50% respectively, but no differences were found in the primary outcomes. Post-hoc analyses revealed significant differences based on baseline anxiety severity, whereby kava was superior on the SARA in low anxiety and placebo was superior on the HADS and SARA in high anxiety.

Safety: both treatments were well tolerated (no evidence of withdrawal or sexual side effects). Three subjects treated with kava experienced slight elevations in ALT, compared with no subjects in the placebo group, but these changes were not clinically significant. Side effects reported in kava group: diarrhea (2 cases), dry mouth (2 cases), rash (2 cases), nausea (2 cases); in placebo group: headaches, heart pounding, swelling, trembling (2 cases for each side effect).

Assessor's comment: no differences between verum and placebo; the authors concluded that further studies were needed.

Acetonic extract

The efficacy and tolerability of Kava extract WS1490 (DER 11–20:1; extraction solvent: acetone 75% in water) were investigated by Gastpar *et al.*, 2003 in a randomized, placebo-controlled, double-blind multicenter study in patients suffering from neurotic anxiety (DSM-III-R diagnoses 300.02, 300.22, 300.23, 300.29, or 309.24). 141 adults, male and female out-patients received 150 mg/day kava-extract (corresponding to 105 mg kavalactones; n=71) or placebo (n=70) for four weeks, followed by two weeks of observation without study-specific treatment. The primary outcome measure for treatment efficacy was the average total score of the Anxiety Status Inventory (ASI) at the end of randomized treatment (week 4). 14 (9 in the verum group and 5 in the placebo group) were withdrawn from the study, but in 6 of these patients premature termination was unrelated to the investigational treatment. Without baseline correction, the ASI total score means (with 95% confidence interval) at

treatment end were 39.0 (36.6; 41.3) points for verum and 40.6 (38.3; 43.0) points for placebo. The U-test for the difference between the treatment groups was not significant (p>0.05). But an exploratory analysis of variance across the differences between treatment end and baseline, with center as a second factor, showed superiority of the verum over placebo (p<0.01, two-sided). 73% of the patients treated with verum exhibited ASI score decreases >5 points versus baseline, compared to 56% for placebo. Significant advantages for verum were also evident in a structured well-being self-rating scale (Bf-S) and the Clinical Global Impressions (CGI), while the Erlangen Anxiety, Tension and Aggression Scale (EAAS) and the Brief Test of Personality Structure (KEPS) showed only minor treatment group differences.

Safety: Neither physical examination nor vital signs assessment indicated any adverse effects. The same applied to the results of the safety laboratory examination (liver function tests in particular- GOT, GPT, γ-GT, alkaline phosphatase) where no systematic or individual changes towards abnormal values were observed.

Assessor's comment: Mixed anxiety population together with the short duration of the trial and too short follow-up phase limit the conclusions that can be drawn from this report.

In a multicenter, randomized, placebo-controlled, double-center trial Lehrl (2004) investigated the efficacy and safety of kava special extract (WS 1490, containing 70% kavalactones, DER: 11-20:1 extraction solvent: acetone-water) in 61 patients with sleep disturbances associated with anxiety and restlessness states of non-psychotic origin. The patients included were diagnosed with GAD, agoraphobia, social phobia or adaptation disorders (according to the DSM-III-R: 300.02, 3000.23, 300.29, 309.24), with a total score on HAMA of not less than 15 points and at least 2 points on HAMA item "insomnia". The patients received 200 mg of kava extract (corresponding to 140 mg of kava lactones; n=34), or placebo (n=23) once a day for 4 weeks. Main outcome measures were the SF-B, the HAMA scale, the Bf-S self-rating scale of well-being and the CGI scale. Double-blind treatment was followed by a 2 weeks of phase without study medication. The confirmatory analysis of the two primary efficacy variables, the differences of sleep questionnaire SF-B sub-scores 'Quality of sleep' and 'Recuperative effect after sleep' after 4 weeks of double-blind treatment compared to baseline, demonstrated statistically significant group differences in favor of verum group (p=0.007 and p=0.018, respectively). Superior effects of kava extract were also present in the HAMA psychic anxiety sub-score (P=0.002), but the HAMA total score had a significant higher decrease in placebo group in comparison with verum group (12.6 vs. 11.24). More pronounced effects with respect to Bf-S and CGI also indicated superior therapeutic efficacy of kava extract:

Table 2
Primary efficacy variables' score differences between baseline and treatment end (means and medians with their 95% confidence intervals; intention-to-treat analysis)

SF-B sub-score	WS® 1490 (n=34)	Placebo (n=23)	P-value*
Quality of sleep	0.60 0.79 (0.35; 0.93)	0.36 0.36 (0.09; 0.86)	0.007
Recuperative effect after sleep	0.80 0.81 (0.50; 1.13)	0.64 0.63 (0.00; 1.00)	0.018

* Exact stratified one-tailed U-test.

Assessor's comments: Even that statistically significant group differences in favor of verum group were calculated for the sub-scores 'quality of sleep' and 'recuperative effect after sleep' the confidence intervals are overlapping, therefore only a minor plausibility for the determined significance can be found in the given information. This should be correlated with other weaknesses of the study, such as

mixed anxiety population, higher decrease of HAMA total score of the placebo group in comparison with verum group (12.6 vs. 11.24) and the short duration of the trial.

Other extracts (not fully characterised)

In a randomized, placebo-controlled, double-blind outpatient trial the efficacy and safety of kava extract WS1490 was investigated for 4 weeks in 50 patients (39 women and 11 men); aged 51–90 years (mean age 76 years) suffering from non-psychotic anxiety (Geier *et al.*, 2004). During the treatment patients received 150 mg (3 x 50 mg) kava extract (containing 70% of kavalactones; n=25) or placebo (n=25). Treatment period as followed by a two week safety observation period. Inclusion criteria were the presence of non-psychotic anxiety (according to the DSM-III-R criteria agoraphobia, specific phobia, GAD and adjustment disorder with anxiety), a HAMA total score of at least 18 and a minimum score of 12 in the multiple choice vocabulary test (MWT-B). Primary outcome criteria were the HAMA total score which was determined upon inclusion in the one-week run-in phase (without study medication), at the start of the treatment and after 2, 3 and 4 weeks of the treatment. Secondary efficacy variables were the HAMA subscales with the dimensions 'somatic' and 'psychic anxiety', the Erlanger anxiety, tension and aggression scale (EAAS) and GCI. For the primary outcome variable and the intention to treat analysis, a tendency of superiority over the course of treatment was observed with verum (p=0.1). Due to the erroneous inclusion of 5 patients (with a total HAMA score of less than 18) and 3 very early dropouts in verum, a per protocol analysis was performed. In this analysis a statistically and clinical relevant advantage of 4.7 points in favor of the kava treatment was observed after 4 weeks (p=0.03):

Table 1. HAMA total score

	Intention-to-treat analysis			Per-protocol analysis		
	WS 1490 (*n* = 25)	Placebo (*n* = 25)	*p*-value *U*-test one-tailed	WS 1490 (*n* = 18)	Placebo (*n* = 20)	*p*-value *U*-test one-tailed
Before treatment	25.6 (21.6; 29.5)	27.6 (23.8; 31.5)	0.36[a]	26.6 (23.9; 29.3)	29.9 (26.2; 33.5)	0.18[a]
After 2 weeks of treatment	18.8 (14.8; 22.8)	21.0 (17.3; 24.7)	0.1	17.9 (15.4; 20.4)	22.2 (18.6; 25.8)	0.03
After 4 weeks of treatment	14.8 (10.5; 19.1)	16.8 (13.3; 20.4)	0.1	12.4 (10.1; 14.7)	17.1 (13.6; 20.6)	0.03

[a] Two-tailed.
Mean of HAMA total score with 95% confidence interval for the intention-to-treat and the per-protocol analysis respectively.

For the HAMA subscales "somatic anxiety" and psychic anxiety a statistically significant advantage of verum was also detectable (p=0.03 and 0.04). For the further secondary outcome variables a trend in favor of the kava extract was observed, but none of them reached significance. But on item I (severity of illness) of the Clinical Global Impression (CGI) scale the number of patients graded as "at least markedly ill" was twice as high (p=0.08, chi-square test) in the placebo group (12 out of 21 patients) compared to the verum group (6 out of 22 patients), at the end of the treatment. At the beginning of treatment in both groups 16 out of 25 patients had been rated "at least markedly ill". No adverse events related to the study medication were observed and none of the patients showed withdrawal symptoms during follow up phase.

Some errors were observed by the authors: a higher HAMA score of the placebo group at baseline, differences between groups in previous medication (the number of patients receiving centrally active substances before the start of the study was twice as high in the active treatment group), significant dropouts in verum.

Assessor's comments: The significance of results could only be shown in the per-protocol analysis and not in the decisive intent-to treat analysis. Taking into account also the errors observed by the authors the conclusions that can be drawn from this report are limited.

In a randomised double-blind study (Bhate *et al*., 1989; Bhate and Gerster, 1992) , 56 hospitalized patients (aged 25-81 years) undergoing surgery under epidural anaesthesia were premedicated at 9.00 pm on the evening before the operation and again 1 hour before operation with 300 mg kava extract (no further detail) corresponding to 60 mg kavalactones (n=28) or placebo (n=28). Assessments were made on sleep quality, psychological status, blood pressure and pulse rate, evaluation of the course of narcosis, postoperative blood pressure and pulse, and patients' questioning (anxiety scale). By dividing the patients into four groups with a planned operation duration of a) less than 40 min, b) 40 – 80 min, c) 80 – 120 min, and d) more than 120 min, verum and placebo turned out to have nearly the same good results in the first group. In the second and third group, the kava extract proved to be of doubtless advantage compared to placebo. Anxiety was significantly reduced ($p<0.05$). In the fourth group the results were nearly equal.

Assessor's comments: The results are of dubious clinical relevance due to the brief duration of treatment (2 doses) and the nature of the results (nonstandard scoring scale, relatively small differences between the treatment groups); the extract is not fully characterised (DER, extraction solvent).

Lehmann (1988) examined in a randomized, placebo controlled, double blind study the anxiolytic effect of a dry extract (150 mg extract corresponds to 47.5 -52.5 mg kavalactones; no further detail) in 20 women with acute anxiety concerning suspected breast cancer. The patients were treated eather with kava -kava extract with 3 x 50 mg/day, orally for 7 days (n=10) or placebo (n=10). The outcomes investigated were: two self-rating scales (State trait anxiety scale and 60 item characteristic word list) and one observed-rated scale (State trait anxiety inventory). Tests were conducted before and after 3 and 7 days. A significant reduction of anxiety compared with placebo was seen after 7 days, based on combined scores from the rating scales mentioned above. In addition a significant increase was noted in alertness and a lessening of fatigue, introverted behavior and excitability as well a reduction in levels of depression under kava treatment over the observation period. In none of the cases examined did any undesirable side effect occur.

Assessor's comments: Inclusion and exclusion criteria and dropouts are missing; small groups and no follow-up phase limit the conclusions that can be drawn from this report; the extract is not fully characterised (DER, extraction solvent).

Reference controlled studies

Ethanolic preparations

Connor *et al*., 2006 analyzed the efficacy and safety of kava ethanolic extract in GAD. Data were analyzed from three randomized, double-blind, placebo-controlled trials of kava, including one study with an active comparator (venlafaxine), in adult outpatients with DSM-IV GAD. The first trial was a 4-week evaluation of kava vs placebo in patients who fulfilled DSM-IV criteria for GAD, using a modified duration requirement of 1 month (see also Connor *et al*., 2002) and with a minimum baseline HAMA score of 16. A second trial was conducted using similar entry criteria, including patients with milder anxiety symptoms (baseline HAM-A score of 12–20). The third study was a randomized trial of patients meeting DSM-IV criteria for GAD (including minimum symptom duration of 6 months) of moderate severity (minimum baseline HAM-A score of 18) who were randomized to 8 weeks of treatment with kava, venlafaxine-XR, or placebo). The pooled sample (n=64) included the following number of participants: kava, n=28; placebo, n=30; and venlafaxine, n=6. Study medication used in the first two

trials was identical and a corresponded product was used for the third study. The dose in each trial was initiated at 140 mg kavalactones (KAV) per day (70 mg kavalactones twice daily) for 1 week and then increased to 280 mg kavalactones per day (140 mg kavalactones twice daily). In the third trial, a double-dummy design was employed, with placebo (PBO) matched for kava and for venlafaxine-XR (VEN), with the dose of venlafaxine-XR started at 37.5 mg/day and titrated to a maximum daily dose of 225 mg. Clinical assessments were virtually identical in the three studies and included the HAM-A Scale, the Hospital Anxiety and Depression Scale (HADS) and the Sheehan Disability Inventory (SDI). Safety assessments conducted in each trial included liver function tests, which were performed at screening and at the completion of treatment. Given the comparability of the study designs, the data comparing kava and placebo were then pooled for further efficacy and safety analyses. No significant differences were observed between the treatment groups in any of the trials. In the pooled analyses, no effects were found for kava, while a significant effect in favor of placebo was observed in participants with higher anxiety at baseline.

Individual studies: In study 1, a significant effect was observed in favor of placebo on the SDI ($p<0.03$). In study 2, a trend was found in favor of KAV on the HAM-A ($p=0.05$). No other differences were observed between the treatment groups on any of the other continuous outcome measures or in the rates of treatment response, which were as follows: KAV, 0–50%; PBO, 29–60%; and VEN, 50%. Further, no treatment differences were noted in terms of remission rates, which were as follows:

study 1, KAV 24% (n=4), PBO 22% (n=4); study 2, KAV 50% (n=3), PBO 29% (n=2); and study 3, KAV 0% (n=0), PBO 0% (n=0), and VEN 33% (n=2):

Table 2 Symptom severity on outcome measures before and after treatment by study and treatment group (median Q1, Q3)

	Study 1		Study 2		Study 3			Pooled data	
	KAV	PBO	KAV	PBO	KAV	PBO	VEN	KAV	PBO
HAM-A	*n*=17	*n*=18	*n*=6	*n*=7	*n*=5	*n*=5	*n*=6	*n*=28	*n*=30
Before	21 (18, 21)	18 (16, 21)	17 (16, 18)	14 (13, 20)	32 (24, 34)	24 (20, 28)	29 (28, 32)	20.5 (18, 22)	18 (16, 21)
After	13 (9, 21)	10 (8, 13)	7.5 (3, 12)	10 (4, 17)	20 (20, 26)	12 (10, 20)	16 (6, 22)	13.5 (8, 20.5)	10 (8, 14)
HADS	*n*=17	*n*=18	*n*=6	*n*=7	*n*=5	*n*=5	*n*=6	*n*=27	*n*=30
Before	17 (12, 19)	16 (12, 20)	18 (14, 19)	15 (14, 20)	21 (18, 23)	14 (11, 16)	17.5 (16, 19)	17 (14, 21)	16 (12, 17)
After	17 (12, 20)	13.5 (9, 17)	10.5 (9, 15)	14 (12, 16)	18 (14, 18)	12 (11, 17)	14 (13, 15)	15.5 (10, 18.5)	13.5 (11, 17)
SDI	*n*=17	*n*=18	*n*=6	*n*=7	*n*=5	*n*=5	*n*=5	*n*=27	*n*=30
Before	10 (5, 13)	12 (9, 17)	5 (0, 12)	14 (5, 15)	8 (7, 18)	1 (1, 10)	10 (0, 12)	9 (5, 13)	12 (6, 16)
After	10 (4, 12)	8.5(4, 13)	2 (0, 4)	7 (3, 15)	6 (3, 14)	2 (2, 2)	6 (2, 12)	5 (1, 12)	7 (2, 13)
Response rate[a]	*n*=6, 35%	*n*=9, 50%	*n*=3, 50%	*n*=2, 29%	*n*=0, 0%	*n*=3, 60%	*n*=3, 50%	*n*=9, 32%	*n*=14, 47%

HAM-A, Hamilton Anxiety Scale; HADS, Hospital Anxiety and Depression Scale; SDI, Sheehan Disability Inventory; KAV, kava; PBO, placebo.
[a]Response: ≥ 50% reduction in HAM-A score from baseline.

Pooled sample: Significant effects in favor of placebo were found on the HAM-A ($F=4.45$, d.f. 1, $p<0.04$) and the HADS ($F=4.15$, d.f. 1, $p<0.05$). A significant effect was also observed for study on the HAM-A ($F=15.96$, d.f. 2, $p<0.0001$), and this was explained by the authors by a lower HAM-A entry criterion in study 2. When the groups were compared by baseline anxiety level, a significant treatment by baseline anxiety level interaction was found on both the HADS and the SDI Scales. Tukey's tests between means showed an effect in favor of placebo in high anxiety on both the HADS and the SDI Scales. Remission rates in the pooled samples were as follows: KAV, 25% (n=7); PBO 20% (n=6); and VEN 33% (n=2) (NS):

Table 3 Mean change from baseline on the HADS and SDI at endpoint by baseline anxiety level

	Kava		Placebo		
	n	Mean (SD)	*n*	Mean (SD)	*P*
HADS[a]					
Low anxiety	11	−4.9 (6.0)	19	−2.0 (3.8)	NS
High anxiety	15	0.8 (3.9)	12	−2.9 (4.2)	<0.05
SDI[b]					
Low anxiety	11	−4.6 (3.7)	19	−2.2 (5.1)	NS
High anxiety	15	0.1 (3.7)	12	−6.2 (4.5)	<0.001

HADS, Hospital Anxiety and Depression Scale; SDI, Sheehan Disability Inventory; low anxiety: baseline HAM-A ≤ 19; high anxiety: baseline HAM-A>19.
[a]Tukey's test: F=7.70, d.f. 1, 53, $P<0.01$.
[b]Tukey's test: F=13.20, d.f. 1, 53, $P<0.001$.

Safety: Assessment of changes in hepatic enzymes (ALT, AST, alkaline phosphatase) and total bilirubin showed no evidence that either kava or placebo was associated with significant alteration in liver function. Slightly elevated alanine aminotransferase values were observed in three patients on kava, but these changes were determined to be not clinically significant.

The authors concluded that findings from these three controlled trials do not support the use of kava in DSM-IV GAD. Several limitations were also acknowledged: lack of adequate statistical power to detect a treatment difference, abnormal placebo effect (possible type II error), different durations of the studies (4 weeks for studies 1 and 2 and 8 weeks for study 3), the average age of participants > 50 years.

In a randomised clinical study, Mittmann (2000) compared the acute sedative and anxiolytic activity of a viscous ("*spissum")* kava extract (extraction solvent: ethanol-water; no further detail) with those of benzodiazepines as pre-medication in women (n=53, average age 68.3 years), waiting to undergo vaginal hysterectomy under regional anaesthesia. After randomisation, pre-medication was performed as follows: on the evening before the operation both groups received 25 mg promethazin p.o., whereby group I (n=27) also received flunitrazepam 1-2 mg p.o., and group II (n=26) 2 capsules of kava extract (corresponding to 100 mg kavalactones). On the morning of the operation both groups received 0.5-1 mg atropine i.m. (dose corrected to body weight). Group I also received 10 mg diazepam i.m., while group II received kava extract equivalent to 100 mg kavalactones p.o. Factors in the evaluation included extent of anxiety, physician and patient evaluations of medication induced sedation (3-step scale), blood pressure, pulse frequency and blood oxygen saturation values; these were evaluated before and after application, as well as during and after operation. Physician and patient assessment of the anxiety and the quality of medication induced sedation, together with blood pressure, pulse frequency and blood oxygen saturation values showed comparable efficacy between kava extract and benzodiazepines. Significantly higher systolic blood pressures (p=0.029) were recorded in the benzodiazepines group.

Safety: Adverse events of nausea and vomiting were at the same level (5 cases) in both groups but could not be attributed to the medication.

Assessor's comments: The clinical relevance is limited due to the brief duration of treatment (2 doses combined with other CNS medications such as promethazin), small groups and the nature of the results (non-standard scoring scale).

Boerner *et al.*, 2001 investigated the effect and safety of Kava-Kava LI 150 (containing of 30% kavalactones, DER: 13-20:1 extraction solvent: ethanol 96%) in GAD in an 8-week randomized, reference-controlled, double-blind, multi-centre clinical trial. 129 out-patients (107 females, 20 males; age:20-65 years) received either 400 mg Kava LI 150 extract, containing 120 mg kavalactones

(n=43), 10 mg Buspirone (n=43) or 100 mg Opipramol (n=43) daily for 8 weeks. At week 9, subjects were seen to check for symptoms of withdrawal or relapse. Primary outcome measures comprised the HAMA scale and the proportion of responders at week 8. Secondary measures were the Boerner Anxiety Scale (BOEAS), SAS, CGI, a self-rating scale for well-being (Bf-S), a sleep questionnaire (SF-B), a quality-of-life questionnaire (AL) and global judgments by investigator and patients. The average duration of existing illness was 40 months and 62% of the patients had not previously been treated. After 8 weeks, about 76.7% of patients in kava group, 76.2% in the opipramol group and 73.8% in the buspirone group were classified as responders (defined by a reduction in HAMA score of 50% or to less than 9 points). Drop-out rate in kava group was 5%, while in the comparators group not one patient withdrew; In 127 patients no significant differences could be observed between the three treatments, regarding all seven secondary parameters, including CGI:

Table 3. Summary of efficacy measures – 95% confidence intervals of differences of means between treatment groups (ITT population).

Measure*	Kava vs. Buspiron		Kava vs. Opipramol		Buspiron vs. Opipramol		p-value**
(Baseline)	(n = 43)	(n = 42)	(n = 43)	(n = 42)	(n = 42)	(n = 42)	
HAMA (total score)	–3.99	2.43	–4.43	1.58	–3.91	2.62	0.49
BOEAS (total score)	–2.46	1.93	–1.83	1.82	–1.96	2.48	0.98
SAS (Index)	–7.61	2.70	–8.21	1.02	–6.39	4.11	0.29
Bf-S (total score)	–7.37	6.85	–8.55	3.70	–8.94	4.61	0.71
AL (total score)	–12.5	12.3	–16.1	7.6	–17.8	9.6	0.83
SF-B (factor SQ)	–0.69	0.28	–0.64	0.27	–0.47	0.52	0.50
(factor GES)	–0.70	0.29	–0.56	0.34	–0.38	0.56	0.68
(factor PSYA)	–0.70	0.16	–0.54	0.24	–0.30	0.53	0.48
(factor PSYE)	–0.48	0.19	–0.28	0.36	–0.14	0.52	0.74
(factor PSS)	–0.32	0.18	–0.38	0.10	–0.31	0.17	0.43
(Week 8)							
CGI (severity)	–0.45	0.65	–0.23	0.86	–0.34	0.77	0.57
CGI (improvement)	–0.43	0.56	–0.14	0.80	–0.23	0.75	0.34
Global judgement of efficacy (investigator)	–0.42	0.39	–0.12	0.62	–0.16	0.69	0.26
Global judgement of efficacy (patient)	–0.52	0.31	–0.30	0.47	–0.23	0.61	0.64
Response yes/no (HAMA reduction >50%)	–	–	–	–	–	–	0.95
Remission yes/no (HAMA < 9)	–	–	–	–	–	–	0.98

* HAMA – Hamilton Anxiety Scale; BOEAS – Boerner Anxiety Scale; SAS – Self-Rating Anxiety Scale; Bf-S – Self-Rating Scale for Well-Being according to von Zerssen; SF-B – Self-Rating Sleep Questionnaire; CGI – Clinical Global Impressions

** P-values of Kruskal-Wallis-Test, except for "Response" (Chi-Square-Test)

Safety assessment: A total number of 57 emergent adverse events have been documented during 8 week treatment, with one serious event occurring in the kava group (panic attack). Regarding laboratory values, outside the normal range at week 8, only a GGT increase in an opipramol patient was rated to be of clinical importance and was reported as an adverse event. Slight increases of transaminases to values above the upper limit of the normal range were in fact documented for two kava patients, three buspirone patients and two opipramol patients (no details given). Of these cases, one subject in the kava group (buspirone: three; opipramol: one) had displayed transaminase values slightly above normal range already at baseline:

Table 4. Frequencies of treatment emergent adverse events* during 8-week treatment phase by treatment group.

	Kava Kava (n = 43)	Buspiron (n = 42)	Opipramol (n = 42)
No. of Adverse Events	27	16	14
No. of Patients reporting Adverse Events (%)	14 (33%)	10 (24%)	11 (26%)
No. of Adverse Events rated to be "probably" related to Medication	1	3	1
Nos. of specific Adverse Events			
Common Cold, Pharyngitis, Bronchitis	5	3	3
Nausea/Emesis	3	1	–
Diarrhea	1	1	1
Other Gastro-intestinal disorders	1	2	2
Weight Changes	2	1	4
Skin Affections	2	3	–
Tachycardia	2	–	–
Sedation	–	1	–
Others	10	4	4

* Adverse Events that have not been pre-existing at screening or baseline visit

Assessor's comments: The dosing regimen of buspirone (10 mg) and opipramol (100 mg) used in the trial would be considered to be sub-therapeutic (according to Moller et al., 2001), given the therapeutic dosing ranges of 15–60 mg/day for buspirone and up to 200 mg/day for opipramol in GAD, therefore the clinical significance of the results reported in this trial is questionable. The comparators used are not the first-line agents in GAD treatment. Moreover, the lack of a placebo arm further limits the conclusions that can be drawn from this report.

Aqueous dry preparations

Sarris *et al*., 2009c investigated the combination of St. John's wort (SJW) and Kava for the treatment of major depressive disorder (MDD) with comorbid anxiety in a randomised controlled trial. Twenty-eight adults with MDD and co-occurring anxiety were recruited for a double-blind RCT. After a placebo run-in of 2 weeks, the trial had a crossover design testing SJW (1 tablet contains dry ethanolic extract standardised to 990 µg hipericin and 1500 µg floavone glycosides, 3 times/day) and Kava(1 tablet contains dry aqueous extract containing 50 mg kavalactones, 3 times/day) against placebo over two controlled phases, each of 4 weeks. The primary analyses used intention-to-treat and completer analyses. On both intention-to-treat (p=0.047) and completer analyses (p=0.003), SJW and Kava gave a significantly greater reduction in self-reported depression on the Beck Depression Inventory (BDI-II) over placebo in the first controlled phase. However, in the crossover phase, a replication of those effects in the delayed medication group did not occur. Nor were there significant effects on anxiety or quality of life. The authors concluded that was some evidence of antidepressant effects using SJW and Kava in a small sample with comorbid anxiety. Possible explanations for the absence of anxiolysis may include a potential interaction with SJW, the presence of depression, or an inadequate dose of Kava extract.

The same authors (Sarris *et al*., 2009a, b) investigated in a 3-week placebo-controlled, double blind trial the effect of aqueous kava extract (250 mg of kavalactones/day) on 41 patients (18–65 years) with GAD. The aqueous extract of Kava reduced participants' Hamilton Anxiety Scale score in the first controlled phase by −9.9 (CI = 7.1, 12.7) vs. −0.8 (CI =−2.7, 4.3) for placebo and in the second controlled phase by −10.3 (CI = 5.8, 14.7) vs. +3.3 (CI = −6.8, 0.2). The pooled effect of Kava vs. placebo across phases was highly significant (p<0.0001), with a substantial effect size (d = 2.24, h2 p=0:428). Pooled analyses also revealed highly significant relative reductions in Beck Anxiety Inventory and Montgomery–Asberg Depression Rating Scale scores.

The authors also noted the limitations to the current study such as short length of the intervention phases, mixed anxiety patients (only 66% of participants did satisfy DSM-IV/CIDI-Auto criteria for a diagnosis of GAD). The aqueous extract was found to be safe, with no serious adverse effects and no

clinical hepatotoxicity. Only one minor adverse effect led to withdrawal in a Kava phase: a mild case of nausea that commenced at the start of the phase and resolved within a day of discontinuation. Four other minor adverse effects occurred during the trial. One case of dizziness, stomach discomfort, and flu-like symptoms started in the initial placebo phase and worsened during the Kava treatment; one case of mild dizziness occurring within the first day of Kava resolved by the second day (the participant continuing with no further problem); one case of constipation in a placebo phase; and one mild case of infrequent nausea in a Kavaphase.

The same authors (Sarris. *et al*., 2012) also conducted on 66 patients a study comparing the acute neurocognitive, anxiolytic, and thymoleptic effects of dry aqueous extract of kava to a benzodiazepine in a three-arm, placebo-controlled, double-blind, crossover trial. Twenty-two moderately anxious adults aged between 18 and 65 years were randomized to receive an acute dose of kava extract (180 mg of kavalactones), oxazepam (30 mg), and placebo 1 week apart in a crossover design trial. After exposure to cognitive tasks, a significant interaction was revealed between conditions on State–Trait Anxiety Inventory-State anxiety ($p=0.046$, partial $\eta^2= 0.14$). In the oxazepam condition, there was a significant reduction in anxiety ($p=0.035$), whereas there was no change in anxiety in the kava condition, and there was an increase in anxiety in the placebo condition. An increase in Bond–Lader "calmness" ($p=0.002$) also occurred for the oxazepam condition. Kava extract was found to have no negative effect on cognition, whereas a reduction in alertness ($p<0.001$) occurred in the oxazepam condition. The authors concluded that this particular kava cultivar do not provide anxiolytic activity.

Safety: Liver function tests revealed no significant change on any parameter (e.g., gamma-glutamyl transferase, alkaline phosphatase, aspartate aminotransferase, bilirubin) between baseline and after kava or oxazepam administration. In all conditions marked fatigue occurred, 12/22 (kava), 10/22 (oxazepam), and 10/22 (placebo). After oxazepam treatment, 3/22 experienced headaches and 5/22 dizziness, although a similar outcome occurred in the placebo condition: headaches 4/22 and dizziness 2/22.

A total of 75 participants with GAD and no comorbid mood disorder were enrolled in a 6-week double-blind trial of a dry aqueous extract of kava (120/240 mg of kavalactones per day depending on response) versus placebo (Sarris *et al*., 2013a and b). Reduction in anxiety was measured using the HAMA as the primary outcome. Intention-to-treat analysis was performed on 58 participants who met inclusion criteria after an initial 1week placebo run-in phase. Results revealed a significant reduction in anxiety for the kava group compared with the placebo group with a moderate effect size ($p=0.046$, Cohen $d = 0.62$). Among participants with moderate to severe diagnosed GAD, this effect was larger ($p=0.02$; $d = 0.82$). At conclusion of the controlled phase, 26% of the kava group were classified as remitted (HAMA ≤ 7) compared with 6% of the placebo group ($p=0.04$).

Safety: Kava was well tolerated, and a side from more headaches reported in the kava group ($p= 0.05$), no other significant differences between groups occurred for any other adverse effects. No participant in either group developed clinical signs of hepatic abnormality. Furthermore, the mean values of the liver function tests at all time points for both groups were well within standard range. The only trend for difference occurred for gamma- glutamyl transpeptidase being slightly raised in the kava group compared with placebo (baseline to study end point), with an increase of 3.8 in the kava group versus a reduction of 1.6 points in the placebo group ($p=0.08$). Overall, aspartate aminotransferase showed the opposite trend for differences between the groups with the placebo group being raised over time ($p=0.07$).

No differences in withdrawal or addiction were found between groups. The authors concluded that standardized aqueous kava preparations may be a moderately effective short-term option for the treatment of GAD.

The authors also mentioned some limitations of the study like: (1) the lack of synthetic comparator such as an SSRI; (2) the sample size was not adequately powered to strongly confirm this finding; (3) although participants were randomly assigned to groups, baseline HAMA anxiety for the kava group was by chance 2 points higher; (4) because the advertising detailed the study of a ''herbal treatment'' for anxiety, this may have encouraged participation of biased individuals; (5) the long-term efficacy and safety effects (i.e., 96 months) of kava use is missing.

Other extracts (not fully characterised)

Malsch & Kieser (2001) conducted a 5-week randomized, placebo-controlled, double-blind trial to investigate the efficacy of kava-kava WS1490 extract (no further detail) on non-psychotic nervous anxiety, tension and restlessness states, following pretreatment with benzodiazepines. 40 Patients (25 male, 15 female) suffering from agoraphobia (n=2), simple or social phobia (n=14), GAD (n=12) or adaptation disturbances (n=1) according to the DSM-III-R had been included in this trial. Further inclusion criteria have been a maximum score of 14 in the HAMA scale and a minimum history of 14 days (mean duration 21 months) of uninterrupted treatment with benzodiazepines (lorazepam, bromazepam, alprazolam, or oxazepam) prior to the study inclusion. Study medication was either 50 mg of dried kava extract (corresponding to 35 mg of kavalactones; no further details) or placebo. During the first week the daily dose was increased from 50 mg (1 capsule) up to 300 mg (3x 2 capsules). Simultaneously, the preexisting benzodiazepine treatment was tapered off at a steady rate over the first two weeks of double-blind treatment (at least 50% reduction at day 7). These three weeks of initial treatment were followed by 3 weeks of anxiolytic treatment with the study medication alone. The treatment was followed by a three-week follow-up-phase, at the end of which the patients were reexamined. Primary outcome measures of the trial were the differences in the overall scores of the Hamilton Anxiety Scale (HAMA) and the "Befindlichkeits-Skala" (Bf-S — subjective well-being scale) and the incidence of benzodiazepine withdrawal symptoms during the double-blind treatment phase. The results of the primary outcome measures showed a clear statistical significant superiority of verum compared to placebo (HAMA: p=0.01; Bf-S: p=0.002) at the end of the study. In the kava-group the HAMA total score improved with a median of 7.5 points between baseline and treatment end, with a beneficial treatment effect already visible after one week. In contrast, no comparable improvement was found in the placebo-group, in which the median HAMA total score varied around baseline level (maximum improvement: 1 point). There were 60% responders (defined as a reduction of the HAMA total score by at least 50%) in the kava- group and 20% in the placebo group. Furthermore, secondary variables measured on the Erlangen Anxiety and Aggression Scale (EAAS) and Clinical Global Impressions Index (CGI) do support the results of the primary outcome measures. After 3 weeks, 14 patients whose HAMA scores had improved while taking kava extract in the 5-week study received placebo; 9 of these patients showed a recurrence of the basic symptoms of anxiety disorder:

Table 2 HAMA total score change during double-blind treatment (ITT population; medians with 95% confidence intervals; *P*-values: *U*-test, one-tailed)

HAMA difference	WS®1490 (*n*=20)[a]	Placebo (*n*=20)[a]	*P*-value
Day 1 to day 8	1 (0; 2)	–0.5 (–1; 1)	0.01
Day 1 to day 15	2 (0; 3)	–1 (–3; 1)	0.07
Day 1 to day 22	4.5 (1; 6)	1 (0; 3)	0.04
Day 1 to day 29	6 (3; 8)	0 (–1; 1)	0.01
Day 1 to day 36[b]	7.5 (0; 8)	–1 (–4; 0)	0.01

[a] Positive values indicate symptom improvement
[b] Confirmatory analysis

Assessor's comment: Mixed anxiety population, with only mild severity status (HAMA scores between 10 and 14) correlated with the short duration of the trial and too short follow-up phase limit the

conclusions that can be drawn from this report; the extract is not fully characterised (DER, extraction solvent).

In a randomised, placebo-controlled double blind multicenter study 101 outpatients (average age: 54 years, 74 female, 27 male) suffering from anxiety of non-psychotic origin (DSM-III-R criteria: agoraphobia, specific phobia, generalized anxiety disorder, and adjustment disorder with anxiety) were treated daily for with either 3 x 100 mg of WS1490 dry extract (no further detail) containing 70 mg kavalactones (n=52) or placebo (n=49) (Volz *et al*., 1997). The trial duration was 25 weeks after a one-week single-blind placebo washout period. After a 24-week random treatment period, a one-week placebo washout was performed. The following ratings were performed at the beginning of the placebo washout period and at weeks 0, 12, and 24: HAMA scale (main outcome criterion), self-report symptom inventory 90 items - revised (SCL–90–R), CGI, Adjective Mood Scale (Bf-S), and registration of adverse events according to an open, non-leading questionnaire. Additional HAMA ratings and adverse event checks were performed at weeks 4, 8, 16, and 20. The HAMA total score showed a pronounced decrease in both groups. The verum group was superior on all assessment days during the treatment phase. The difference was statistically significant at week 8 (p=0.02) and increased later in the treatment period (week 12: p=0.002, weeks 16, 20, 24: p<0.001). The HAMA sub-scores showed a statistically significant advantage for the verum starting at week 8 (p=0.02). The GCI also showed a very clear result; the patients treated with verum had a statistically significant advantage over those taking placebo (p=0.001 after 12 weeks). For the self-rating scales the results are very similar (p<0.05); in the case of the Bf-S, the result was borderline significant at week 24 (p=0.08):

	HAMA-total score			SCL-90-R (GSI)			BF-S-total score		
week	WS 1490	Placebo	p	WS 1490	Placebo	p	WS 1490	Placebo	p
0	30.7 (7.2)	31.4 (9.2)	.98	1.7 (0.6)	1.7 (0.6)	1.00	39.6 (11.0)	40.0 (12.5)	.46
4	23.3 (9.2)	24.2 (8.6)	.23						
8	17.1 (9.1)	20.3 (7.6)	.02						
12	13.4 (9.6)	18.0 (9.1)	.002	1.0 (0.6)	1.3 (0.6)	0.01	20.5 (15.4)	29.2 (15.4)	.01
16	10.9 (9.1)	16.4 (9.1)	<.001						
20	9.8 (9.8)	15.5 (9.1)	<.001						
24	9.7 (9.9)	15.2 (9.6)	<.001	0.7 (0.6)	1.0 (0.6)	.04	16.3 (13.8)	27.5 (15.5)	.08

Assessor's comment: Mixed anxiety population with depressive comorbidity, poor description of methodology; no data regarding % responders; confidence intervals not mentioned; possible differences between the centres are not discussed; the extract is not fully characterised (DER, extraction solvent).

A randomized, placebo-controlled, double-blind trial Kinzler *et al*., 1991 included 58 patients (43 female; 15 male) with anxiety syndrome of non psychotic origin (according to ICD 9). They were treated for a period of 4 weeks either with 3 x 100 mg of an WS1490 dry extract (no further detail), containing 70 mg kavalactones (n=29) or placebo (n=29). Patients included in the trial showed a minimum total score of 18 on the HAMA scale. Outcome measures were examined at the start of the trial (day 1), day 7, 14 and at the end of the study (day 28). The total score of HAMA served as the main outcome measure to prove the effects of the therapies. From the first week on the verum group (mean total score = 16.2/SD = 7.1) showed significantly better results in the reduction of HAMA total scores than the placebo group (mean total score = 21.8/SD = 7.8). The reduction of HAMA total score increased during the treatment up to the end of the trial in the verum-group (mean total score = 12.6/SD = 8.6) while only a slight reduction of HAMA total score was observed in the placebo-group (mean total score = 21/SD = 10.1). The superiority of the kava extract compared to placebo was statistically significant on all three measurement points (p<0.01). Similar results have been observed in the secondary outcome measures, as the subscales of the HAMA (somatic anxiety and phychological

anxiety) and the Clinical Global Impression Index (CGI). At the end of the trial 6 patients (10%), 4 in the verum-group and 2 in the placebo-group, dropped out without further explanation. During the 4-weeks treatment, the patients had reported no adverse effects (Kinzler *et al*., 1991).

Assessor's comment: Mixed anxiety patients and small groups; short duration of the treatment no follow-up phase; no data regarding % responders and incomplete statistical analysis (no confidence intervals); the extract is not fully characterised (DER, extraction solvent).

In a reference-substance-controlled double-blind multicenter study over a period of 6 weeks the efficacy of the kava extract WS1490 (each capsule contains 100 mg dry extract containing 70 mg kavalactones; no further detail) on patients with conditions of anxiety, agitation, and tension of non-psychotic origin was compared to that of oxazepam and bromazepam (Woelk *et al*., 1993).
172 patients (18 – 65 years; both sexes) from 12 medical practices were assigned randomly to three groups. One group received kava extract (3 x 100 mg/daily, n=57), and the other two groups received either oxazepam (3 x 5 mg/ daily, n=59) or bromazepam (3 x 3 mg/daily, n=56). The data was objectified by the HAMA scale and CGI. All three types of treatment led to a significant decrease in anxiety. Statistical comparison of the variables of the three groups respectively did not produce a relevant difference between the three types of treatment with respect to a decrease in anxiety and concomitant variables (comparison of HAMA total score bromazepam / WS 1490 after six weeks: p=0.0925, oxazepam/WS 1490 after six weeks: p=0.6198). Bromazepam led to a slightly more pronounced decrease in anxiety, but only to a minor extent as compared to the other two groups (Woelk *et al.,* 1993).

Assessor's comments: The statistical analysis focused on the degree of differences rather than the demonstration of equivalence; heterogenous (mixed) anxiety patients included because inclusion criteria were not sufficiently rigorous; the lack of a placebo arm further limits the conclusions that can be drawn from this report; the extract is not fully characterised (DER, extraction solvent).

Jacobs *et al*., 2005 performed a randomized, double-blind, placebo-controlled trial using a novel Internet-based design to determine if kava is effective for reducing anxiety. E-mail recruitment letters and banner advertisements on websites were used to recruit a large pool of interested participants from 45 states over an 8-week period. Participants were first asked to read study information, complete an online informed consent process, and undergo electronic identity verification. In order to be eligible for the study, participants were required to have 1) anxiety as documented by scores of at least 0.5 standard deviations above the mean on the State-Trait Anxiety Inventory State subtest (STAI-State) on 2 separate occasions, and 2) insomnia, defined as a ''problem getting to sleep or staying asleep over the past 2 weeks.'' The authors randomly assigned 391 eligible participants that received a 28-day treatment according to the following protocol: 1) Kava group (n=121): 1 kava capsule (containing 100 mg of total kavalactones; 30% total kavalactones in extract) 3 times daily, and 2 placebo-valerian capsules 1 hour before bedtime. 2) Valerian group (n=135): 2 valerian capsules (containing 3.2 mg of valerenic acids; 1% valerenic acid in extract) 1 hour before bedtime, and 1 placebo-kava capsule 3 times daily or double placebo (n=135). The primary outcome measures were changes from baseline in anxiety (STAI State questionnaire) and insomnia (Insomnia Severity Index [ISI]) compared with placebo. Participants receiving placebo had a 14.4 point decrease in anxiety symptoms on the STAI-State score and an 8.3 point decrease in insomnia symptoms on the ISI. Those receiving kava had similar reductions in STAI-State score (2.7 point greater reduction in placebo compared with kava; 95% confidence interval [CI], - 0.8 to +6.2). Those receiving valerian and placebo had similar improvements in sleep (0.4 point greater reduction in the placebo than the valerian group; 95% CI, -.3 to +2.1). Results were similar when limited to the 83% of participants who adhered to study compounds for all 4 weeks. Neither kava nor valerian relieved anxiety or insomnia more than placebo.

Safety: Adverse events occurred with similar frequency between active and placebo groups. The only significant difference was a more frequent report of diarrhea among those receiving valerian (18%) compared with those receiving placebo (8%) (p=0.02). This study was conducted before the safety warnings about kava-related hepatotoxicity. Subsequent to these warnings, all 121 study participants receiving Kava were contacted by e-mail and postal mail, and none reported adverse events related to liver injury.

Non-controlled studies

52 outpatients (15 male, 37 female; average age: 49 ± 15) suffering from anxiety of non-psychotic origin with (n=26) or without (n=26) concomitant depression, were included in an open, observational, multicentric study (Scherer *et al*., 1998). Patients were treated with capsules of unit dose 100 mg dry extract (no further detail), corresponding to 50 mg kavalactones. The dosage varied from 2 to 6 capsules (15 patients received one capsule twice a day; 28 patients were given one capsule three times a day; and 9 patients were asked to take two capsules three times a day) and the mean treatment duration was 51 days. Global improvement was rated on a five-point scale as “slightly worse”, “no change”, “slightly improved”, “much improved” and “very much improved”. Target symptoms of “anxiety”, “tension”, and “restlessness” were rated by physicians on a four-point scale: “not present”, “mild”, “moderate”, and “severe”. On a global five-point improvement scale, 42 patients (80.8%) rating the treatment as “very good” or “good”. The target symptoms of anxiety, restlessness, and tension all showed a pronounced decrease from baseline. Before therapy, 22 patients (42.3%) rated their anxiety as “severe”, 16 (30.8%) as “moderate”, and 7 patients (13.5%) as “mild”; in 7 patients (13.5%), no anxiety was present at baseline. At study end, 6 patients (11.5%) described “moderate” anxiety and 26 patients (50.0%) “mild” anxiety symptoms. No patient had “severe” anxiety, and 20 patients (38.5%) did not report any anxiety at all. Before therapy, tension was rated “severe” in 26 patients (50.0%), “mild” in 2 patients (3.8%), and non-existent in 6 patients (11.5%). By the end of the study, no patients had “severe” tension; 8 patients (15.4 %) had “moderate” tension and 29 patients (55.8%) “mild” tension. In 15 patients (28.9%), tension was no longer present. Before being treated, 21 patients (40.4%) had “severe” restlessness, 18 (34.6%) “moderate”, and 8 patients (15.4%) “mild” restlessness; in 5 patients (9.6%), this symptom was not present. By the end of the study, restlessness was “severe” in no patient, “moderate” in 6 patients (11.6%), “mild” in 28 patients (54.8%), and non-existent in 18 patients (34.6%).

Assessor’s comment: The small number of patients and the open design of the study preclude any conclusion.

1673 patients (average age: 48.84 ± 14.77; 1168 female, 503 male) suffering from anxiety (n=1421) and/or nervousness and restlessness participated to a post-marketing surveillance study and were treated daily with 3 x 133 mg kava extract KW1491 (corresponding to 120 mg kavalactones) for a minimum 4 weeks (Spree *and Croy*, 1992). After an average of 15.5 days an intermediate assessment was made followed by the final assessment after an average of 34.5 days. Clear improvements could already be seen at the intermediate assessment (done based on a questionaire). After the treatment all primary (anxiety, nervous tension, restlessness) and secondary (sleep impairment, exhaustion syndrome, climacteric complaints, muscle tension) symptoms were clearly improved or eliminated. In the category “nervous tension and restlessness” more than 60% of the patients were suffering from severe or very severe complaints. By the final assessment, only 5% of the patients had not improved to “good” or “very good”. Anxiety symptoms decreased in average intensity from 2.33 to 0.74 and nervous symptoms from 2.7 to 0.99. Concerning the anxiety states, 25% of the patients were free of complaints, and more than 50% suffered from only minor ailments. Full effectively was reached after an average of 10.98 days, in 38% of patients it was 5 days, in 22% 5 – 10 days. In 75% of the cases, the efficacy of the treatment with the kava extract was good or very good.

Assessor's comment: This is a post-marketing surveillance with a limited value.

In a very large, open, multicenter drug-monitoring trial, 4049 patients (average age: 49; 72% female, 28% male) suffering from conditions of nervous anxiety, stress, and restlessness were included (Siegers *et al.,* 1992). The major cause (55% of the cases) of nervous anxiety, stress, and restlessness was "exhaustion syndrome", followed by "anxious upset" (31%), "loss syndrome" (24%), "climacteric discomforts" (20%) and others (6%). The data of 3873 patients could be analysed. In 70% of the patients only one cause was held responsible for the symptoms, in 25% it was two causes, and in 5% it was more than two causes. They received 150 mg kava extract WS 1490 (containing 105 mg kavalactones) for 6 weeks. Assessments were made before the beginning of the treatment, twice during the trial, and at the end. Assessments were made according to the HAMA scale. By the end of treatment, based on the HAMA the total score of "psychic symptoms" had dropped from 2.54 to 0.79 and the total score for "vegetative symptoms" from 2.13 to 0.62. After the end of the study, symptoms were improved or even non-existent in more than 80% of the cases. The majority of patients (87%) judged their general quality of life improved after the end of the study. 9% felt unchanged, 1.4% evaluated their state of health as slightly worse, and 2.7% did not comment. In about 74% of the cases, physicians rated the efficacy of the kava extract as "good" or "very good", and as "satisfying" in about 18%. Poor efficacy was seen in only 6% of the cases. The incidence of adverse effects observed was 1.5%, all mild and reversible effects (gastrointestinal and skin reactions).

Assessor's comments: The open design of the study precluded any conclusion regarding efficacy; could only be partially supportive for safety of use, but liver parameters were not evaluated.

850 patients (192 men and 574 women) suffering from anxiety syndrome were treated daily with 3x100 mg kava extract WS1490 for 4 weeks in an open multicentric study. The instrument of analysis used was the modified HAMA (Neto, 1999). The HAMA overall score dropped from 30 to 9 (a reduction of 70%) after the treatment showing statistical significance ($p<0.0001$) and efficacy was considered excellent or good by 93.7% of physicians and 86.9% of patients. The tolerability was considered good and very good in 95.8% of patients. Adverse events were observed in 16.7% patients, including somnolence (2.7%), nausea (1.8%), epigastralgia (1.1%).

Assessor's comments: The open design of the study precluded any conclusion regarding efficacy; could only be partially supportive for safety of use, but liver parameters were not evaluated.

b. Anxiety in the climacteric phase

Placebo-controlled studies

There are some placebo-controlled trials and positive-controlled-studies but also open studies that investigated the effect of kava extracts in women with climacteric psychosomatic disturbances.

Extracts not fully characterised

In the first double-blind study, 40 women with climacteric syndrome (primary anxiety and vegetative dysregulation in peri- and post-menopausal phases) were treated with either a placebo (n=20) or 2 x 150 kava extract (corresponding to 2 x 30 mg kavalactones; no further detail) daily, for 12 weeks (Warnecke *et al*., 1990). At the end of 4 and 8 weeks, assessment using Kuppermann Index (severity of climateric symptoms)and Anxiety Status Index (rating of anxiety disorders) showed significant improvements ($p<0.001$) in the verum group, in which 11 patients reduced their daily dose to 1 x 150 mg kava extract in the final weeks of the study(mainly from week 9). Due to a high drop-out rate during week 6-10, primary because of lack of efficacy (2 in the verum group and 14 in the placebo group), statistical comparisons were not reliable after 12 weeks. In the verum group 5 patients

reported minor adverse effects such as lowering of vigilance of tiredness in the morning; 2 verum and 3 placebo patients reported gastrointestinal complaints (Warnecke *et al*., 1990).

In the second randomized, placebo-controlled double-blind study, 40 women (45 – 60 years) with climacteric-related symptomatology (psychovegetative symptoms as anxiety, restlessness and sleep disorders and psychosomatic symptoms) and HAMA initial score >18 were treated with the kava extract WS1490 (containing 70% kavalactones; 3 x 100 mg WS 1490/day, resulting in a daily dosage of 210 mg of kavalactones; no further detail; n=20) or a placebo(n=20) preparation for a period of 8 weeks (Warnecke, 1991) . The main outcome- the HAMA overall score – was assessed after 0, 1, 4 and 8 weeks. After 1 week of treatment the average HAMA score in the verum group had decreased by more than 50% (from 31.10 to 14.65) significantly more ($p<0.001$) than in the placebo group (decrease from 30.15 to 27.50). The differences widened by the end of week 4 and week 8 ($p<0.0005$). Other parameters such as depressive status inventory (DSI), the CGI and the climacteric symptomatology (Kuppermann menopause index and Schneider scale) also demonstrated a high level of efficacy of the kava extract over the whole treatment period. The mean score on the DSI decreased significantly from 42.5 to 24.8 ($p<0.01$), while the mean score on the Kuppermann Index also decreased significantly from 20.35 to 3.60 ($p<0.01$).

Assessor's comment: The small number of patients and the lack of follow-up phase preclude any conclusion on the efficacy of kava-kava extract; in addition the extract is not fully characterized (DER, extraction solvent).

To evaluate the efficacy of kava extract (containing 55% of kavain; no further detail) in combination with hormone replacement therapy and to compare it with hormone replacement therapy alone in the treatment of menopausal anxiety, 40 women patients in physiological or surgical menopause with GAD in accordance with DSM-IV criteria (HAMA > 19) were assigned to one of four treatments for 6 months, in a radomized trial (De Leo *et al*., 2000). Twenty-two of the 40 women were in physiological menopause and 18 in surgical menopause due to benign uterine pathology. The former were randomly assigned to one of the following protocols: HRT-K (n=13): 50 µg/day (17 β-estradiol) with progestogen and 100mg/day kava extract; HRT (n=9): 50 µg/day (17 β-estradiol) with progestogen and placebo. The patients in surgical menopause were assigned to one of the following protocols: ERT-K (n=11): 50 µg/day (17 β-estradiol) and 100 mg/day kava extract; ERT (n=7): 50 µg/day (17 β-estradiol) and placebo. HAMA score was evaluated before and after 3 and 6 months of therapy in all four groups. A significant reduction in HAMA score was observed in all four groups of women. The reduction was more significant in groups using combination therapy (HRT-K, 55.5%; ERT-K 53.3%) than in groups treated with hormones only (HRT 23.0%; ERT 25.8%). Furthermore, in both groups given combination treatment (HRT-K or ERT-K), reductions of HAMA subscores for both somatic anxiety and psychic anxiety were significantly greater ($p<0.05$) than corresponding groups treated with hormones only:

Table 2
HAMA score and subscores in the four groups of treatment before and after therapy

	HRT+K ($n=13$)		HRT ($n=9$)		ERT+K ($n=11$)		ERT ($n=7$)	
	Before	After	Before	After	Before	After	Before	After
Hama score	27 ± 5	$13 \pm 1.5^{a,b}$	26 ± 4	18 ± 2^{a}	30 ± 5	$14.5 \pm 3^{a,b}$	31 ± 6	24 ± 2^{a}
Somatic subscore	11 ± 1.3	$5 \pm 0.7^{a,b}$	11 ± 1.2	7 ± 1.3^{a}	12 ± 1.5	$5.5 \pm 1.1^{a,b}$	14 ± 1.8	11 ± 2^{a}
Psychic subscore	16 ± 1.7	$8 \pm 1^{a,b}$	15 ± 1.8	10 ± 1.3^{a}	18 ± 2.1	$9 \pm 1.5^{a,b}$	17 ± 3	13 ± 2^{a}

[a] $P<0.05$ vs. before therapy.
[b] $P<0.05$ HRT+K vs. HRT and ERT+K vs. ERT.

Assessor's comment: Although the authors report a positive outcome, this publication is considered only marginally because the amount of the contribution of each partner (hormone replacement therapy or kava extract) to the overall efficacy cannot be estimated.

Reference-controlled studies

Eighty perimenopausal women with climacteric symptoms were enrolled in a 3 months open study (Cagnacci *et al.,* 2003). Women received 1 g/day of calcium and were randomized to receive for 3 months: (1) no other treatment (control; n=40); (2) Kava extract 100 mg/day (corresponding to 55% of kavaina; no further detail; n=20); (3) Kava extract 200 mg/day (n=20). No placebo was available. Data on 68 patients (n=34; n=15 and n=19 respectively) were available for evaluation. Anxiety was evaluated by the State Trait Anxiety Inventory (STAI, 20 items), while depression was evaluated by the Zung's scale (SDS) and climacteric symptoms by the Greene's scale. Evaluations were performed at baseline and after 1 and 3 months. In the control group during the 3 months, anxiety, depression and climacteric symptoms tended to decline, but not significantly. Compared with the control group, scores for anxiety in both kava-groups declined significantly ($p<0.009$, two-factors ANOVA). Baseline values were similar to those of the control group (46.5 $\pm$1.5) but significantly declined ($P<0.0001$) after 1 and 3 months of treatment. The effect was similar for the 100 mg and the 200-mg dose. In the 100 mg group (n=15) the anxiety score decreased ($p<0.025$) from baseline values of 47.3$\pm$2.2, to 43.2$\pm$1.9 after 1 month and to 42.7$\pm$2.25 after 3 months of treatment. In the 200 mg group (n=19) the anxiety score decreased ($p<0.0003$) from baseline values of 46.6$\pm$2.1, to 43.1$\pm$1.8 after 1 month and to 41.3$\pm$1.6 after 3 months of treatment. Although scores for depression and climacteric symptoms declined more in kava-groups and were significant compared to baseline, the differences were not statistically significant compared with control group. Also the modifications of Greene's subscales that were observed during Kava treatment were not significantly different from those observed in the control group.

Safety: Side effects as nausea and gastric pain were observed in 1 subject of the control group and 6 subjects receiving kava extract (17%). Intensity of these symptoms was slight. Only in 2 cases, receiving Kava extract gastric pain induced the subjects to withdraw from the study. In all women with side effects (7 patients), the biochemical evaluation did not show any alteration, including those parameters documenting liver toxicity, but no data were provided.

Assessor's comments: Lack of statistically significant differences compared with control make this trial useless. Also the amount of the contribution of each partner to the overall efficacy cannot be estimated.

Meta-analysis

Pittler *et al.*, 2000, 2003 and later 2010 provided a systematic review and meta-analysis aimed at assessing the evidence for or against the efficacy of kava extract as a symptomatic treatment for anxiety. Only double-blind randomized placebo-controlled trials of oral treatment for the treatment of anxiety, without restrictions referring the language of publication, were included in this meta-analysis. Trials not performed using kava mono-preparations were not included. Twelve double-blind RCTs (n=700) met the inclusion criteria. Seven of the 12 trials (Lehrl 2004; Geier 2004; Connor 2002; Malsch 2001; Volz 1997; Lehmann *et al*., 1996 ; Warnecke 1991), involving a total of 380 participants, used the total score on the HAMA as their primary outcome measure, and provided data suitable for meta-analysis. The five studies not included in the meta-analysis reported statistically significant improvements for kava recipients, compared with placebo recipients, on outcomes (e.g. response rates, reduction in scores on various anxiety scales). These five studies were heterogeneous in that they involved different patient groups, such as women with anxiety associated with the perimenopausal period, individuals with preoperative anxiety, and outpatients with neurotic anxiety.

Consequently, dosage regimens of kava varied widely (e.g. equivalent to kavalactones 60 mg in the evening and 1 hour preoperatively, to 140 mg kavalactones daily for four weeks).

The result suggests a significant effect towards a reduction of the HAMA total score in patients receiving kava extract compared with patients receiving placebo (weighted mean difference: 3.9, 95% confidence interval: 0.1 to 7.7; p=0.05; n=380). All except one of these trials included participants with nonpsychotic anxiety; one study involved women with anxiety associated with the climacteric (perimenopausal period). Removing this trial and the trial that did not assess the kava extract WS-1490 from the meta-analysis indicated a statistically significant reduction in anxiety scores for kava patients compared with placebo (weighted mean difference: 3.4; 95% CI, 0.5–6.4;p=0.02).

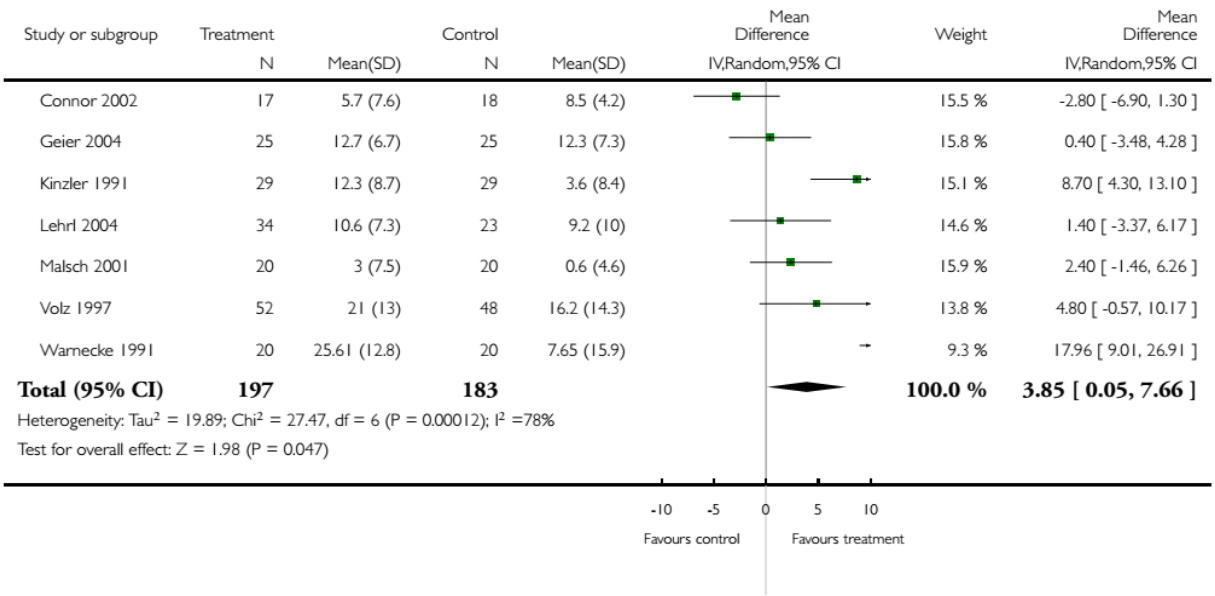
Adverse events as reported in the reviewed trials were mild, transient and infrequent as stomach complaints, restlessness, drowsiness, tremor, headache and tiredness. These are reported by patients receiving kava extract in 5 of seven trials. In two of these 7 studies no adverse effects were observed. Authors' conclusions were that compared with placebo, kava extract is an effective symptomatic treatment for anxiety although, at present, the size of the effect seems small. The effect lacks robustness and is based on a relatively small sample. The data available from the reviewed studies suggest that kava is relatively safe for short-term treatment (1 to 24 weeks), although more information is required. Rigorous trials with large sample sizes are needed to clarify the existing uncertainties. Also, long-term safety studies of kava are required:

Analysis 1.1. Comparison 1 Kava versus placebo for anxiety, Outcome 1 Improvement (HAMA-score).

Review: Kava extract versus placebo for treating anxiety

Comparison: 1 Kava versus placebo for anxiety

Outcome: 1 Improvement (HAMA-score)



Assessor's comment: This meta-analysis did not differentiate the type of extract involved. All trials were assessed separately.

Witte *et al.*, 2005 conducted a meta-analysis to assess the efficacy of the acetonic Kava-Kava Extract WS 1490 (DER 11–20:1; extraction solvent: acetone 75% (w/w) in patients with non-psychotic anxiety disorders. Six placebo-controlled, randomized trials with the kava extract WS 1490 were identified (Geier, 2004; Kinzler *et al.*, 1991*; Lehrl, 2004; Malsch, 2001; Volz, 1997; Warnecke, 1991). The endpoints were the change in HAMA during treatment (continuous and binary). No restrictions

regarding the daily dose, duration of treatment or follow-up time were imposed. The authors concluded that WS1490 has an effective success rate of OR=3.3 (95% confidence interval of 2.09–5.22) in patients with non-psychotic anxiety disorders. The continuous outcome supports this result: mean improvement with WS 1490 by 5.94 (95% confidence interval −0.86 to 12.8) points on the HAMA scale better than placebo. Kava seems to be more effective in females and in younger patient:

* Kinzler *et al*. is a duplicate of Lehmann *et al.,* 1996

Table 1. Description of included studies

Author	Number of evaluated patients		Proportion of females	Mean ± SD HAMA score before treatment		Daily dose (mg)	Treatment duration (weeks)
	WS®1490	Placebo		WS®1490	Placebo		
Geier and Konstantinowicz, 2004	25	25	78%	25.6 ± 9.6	27.6 ± 9.3	150	4
Kinzler *et al.*, 1991	29	29	74%	25.3 ± 5.6	24.4 ± 5.1	300	4
Lehrl, 2004	34	23	56%	21.8 ± 6.1	22.5 ± 5.7	200	4
Malsch and Kieser, 2001	20	20	38%	13.0 ± 1.3	12.8 ± 1.0	300	4
Volz and Kieser, 1997	52	48	73%	30.7 ± 7.2	31.4 ± 9.3	300	24
Warnecke, 1991	20	20	100%	31.1 ± 2.8	30.2 ± 4.4	300	8

All studies are randomized, controlled, double-blind trials treating patients with non-psychotic anxiety disorders.
Patients in Warnecke (1991) had anxiety disorders due to climacteric complaints which is also a non-psychotic genesis.
SD, standard deviation.

Table 2. Treatment effects observed in the included studies

Author	Continuous outcome			Binary outcome		
	Mean improvement of HAMA score		HAMA difference with CI	Success rate		OR with CI
	WS®1490	Placebo		WS®1490	Placebo	
Geier and Konstantinowicz, 2004	10.80	10.80	0.00 [−4.42 to 4.42]	52.0%	36.0%	1.93 [0.62 to 5.98]
Kinzler *et al.*, 1991	12.31	3.59	8.72 [4.21 to 13.24]	55.2%	20.7%	4.72 [1.48 to 15.0]
Lehrl, 2004	10.56	9.17	1.38 [−3.19 to 5.96]	58.8%	43.5%	1.86 [0.64 to 5.42]
Malsch and Kieser, 2001	3.00	−0.55	3.55 [−0.40 to 7.50]	60.0%	20.0%	6.00 [1.46 to 24.7]
Volz and Kieser, 1997	20.98	16.23	4.75 [−0.66 to 10.17]	73.1%	50.0%	2.71 [1.18 to 6.25]
Warnecke, 1991	25.60	7.65	17.95 [8.71 to 27.19]	85.0%	35.0%	10.52 [2.27 to 48.8]

HAMA difference is mean improvement of the disease under WS®1490 application minus mean improvement under placebo.
Success was defined as an individual improvement of HAMA score during treatment by at least 50% compared with baseline.
OR, odds ratio of WS®1490 to placebo.
Positive differences and OR > 1 favour WS®1490.
CI, 95% confidence interval.

Assessor's comments: All trials included in this meta-analysis were already assessed separately. The weaknesses of the analysis are correlated with: small size groups, significantly different HAMA baseline scores between trials that indicates different severity status, mixed anxiety disease (e.g Warnecke et al., 1991 had anxiety disorders patients due to climacteric complains) different duration of the trials (4 weeks up to 24 weeks), differences between trials regarding tested dose (from 150 mg to 300 mg extract/day), one trial with significant errors (Geier, 2004).

(c) Isolated compounds

Several randomised, double-blind, controlled trials (Möller *et al*.,1992; Möller & Heuberger 1989; Lehmann *et al*., 1989; Staedt *et al*., 1991; Lindenberg & Pitule-Schödel, 1990) involving patients with anxiety have compared the effects of the (+)-kavain administered at a dose of 200 mg three times daily for 3–4 weeks, with those of placebo or benzodiazepines, such as oxazepam. Generally, these studies have reported beneficial effects for synthetic kavain. Therefore their relevance is limited and cannot be extrapolated to the natural L-kavain.

Table 5: Clinical studies

GAD= generalized anxiety disorder; HAMA= Hamilton Anxiety Scale; HADS=Hospital Anxiety and Depression Scale; SARA=Self Assessment of Resilience and Anxiety; ASI=Anxiety Status Inventory; SDI=Sheehan Disability Inventory; Bf-S=Subjective well-being scale; CGI= Clinical Global Impressions

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance*
A. General anxiety, nervousness and restlessness							
A.1. Ethanolic extract							
Connor *et al*., 2002	Randomised placebo double-blind study	Placebo Verum: 70 mg of kavalactones in the first week and 140 mg of kavalactones for the last 3 weeks Orally 2 times daily Duration: 4 weeks	38 patients (31-75 years) Verum (n=19) Placebo(n=19)	DSM-IV GAD(HAMA>16)	Principal outcome: efficacy Assessed by HAMA, HADS, SARA	Student's t or Kruskal-Wallis tests	No difference between groups
Connor *et al*., 2006	Three randomised double-blind positive control studies	Verum: 140 mg kavalactones 1 week, then 280 mg kavalactones Control: 37.5 mg up to 225 mg venlaflaxine/day Placebo Orally Duration: 4 to 8 weeks	64 patients Verum(n=28) Control (n=6) Placebo(n=30)	Patient with GAD First trial: HAMA > 16; Second trial:HAMA (12–20); Third trial: HAMA > 18	Outcome measures: HAMA, HADS, SDI	Student's t-test	No significant difference between the treatment groups in any of the trials
Mittmann, 2000	Randomised positive control study	Verum: 100 mg kavalactones + 25 mg prometazine; next day: another 100 mg kavalactones Control: 1-2 mg flunitrazepam + 25 mg prometazine; next day: 10 mg diazepam.i.m Orally Duration: 2 doses	53 patients (average age 68.3 years) Verum(n=27) Control(n=26)	Patients undergoing vaginal hysterectomy	Primary outcomes: sedation (3-step scale),blood pressure, pulse frequency and blood oxygen saturation values	Student's t-test	Comparable efficacy

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance*
Boerner *et al*., 2001	Randomised, double-blind, reference - controlled multicenter study	Verum: 400 mg Kava LI 150 (containing 120 mg kavalactones, DER: 13-20:1 extraction solvent: ethanol 96%) Control 1: 10 mg buspirone Control 2: 100 mg opipramol Orally Duration: 8 weeks	129 patients verum(n=43) Control 1(n=43) Control 2(n=43) Drop-out: verum 5%; none in control groups	GAD	Primary outcome: HAMA score and % of responders. Secondary measures: Boerner Anxiety Scale, SAS, CGI, Bf-S	None	No differences between the groups
A.2. Acetonic extracts							
Gastpar *et al*., 2003	Randomised, double-blind, placebo-controlled multicenter study	Placebo Verum : 150 mg kava-extract (DER 11-20:1, extraction solvent: acetone 75% V/V in water) corresponding to 105 kavalactones Orally Duration: 4 weeks	141 patients Verum (n=71) Placebo(n=70) Drop-out: 9 verum; 5 placebo	neurotic anxiety (DSM-III-R diagnoses 300.02, 300.22, 300.23, 300.29, or 309.24).	The efficacy assessed by primary outcome: ASI score Secondary: Bf-S and CGI	U-test	Limited value
Lehrl, 2004	Multicenter, randomised, double-blind, placebo-controlled study	Placebo Verum : 200 mg kava-extract (corresponding to 140 mg kavalactones) Orally Duration: 4 weeks Follow-up: 2 weeks	61 patients (51-90 years) Verum (n=38) Placebo(n=23)	Patients GAD, agoraphobia, social phobia or adaptation disorders (DSM-III-HAMA $\geq$15)	Main outcome measures were the SF-B, the HAMA scale, Bf-S, CGI scale	Student's t-test	Limited value
A.3. Aqueous extracts							
(Sarris *et al*., 2013a) (Sarris *et al*., 2013b)	Randomized, double-blind, placebo-controlled	Aqueous kava extract 120-240 mg kavalactones /day Placebo Duration: 6 weeks	58 patients (dropout:10) Verum (n=27) Placebo (n=31)	Patients with GAD	The efficacy assessed by primary outcome: HAMA	Student's t-test	Verum treatment was superior to placebo
(Sarris *et al*., 2012)	Randomized, cross-over	Aqueous kava extract 180 mg kavalactones /day 30 mg oxazepam placebo Duration: 1 week	66 patients Verum (n=22) Oxazepam Placebo (n=22)	Moderate anxiety	The efficacy assessed by primary outcome: STAI	Student's t-test	No difference between groups
(Sarris *et al*., 2009a) (Sarris *et al*., 2009b)	Randomized, double-blind, placebo-controlled, cross-over	Aqueous kava extract Verum: 5 x 50(250 mg kavalactones/day) Placebo Duration: 1 week	41 patients (droupout:4) Verum(n=19) Placebo(n=22)	Patients with GAD	Outcome measures: HAMA	Student's t-test	Verum treatment was superior to placebo

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance*
A.4. Other extracts(not defined)							
Bhate *et al.*, 1989	Randomised, double-blind placebo-controlled study	Placebo Verum : 300 mg kava-extract (corresponding to 60 mg kavalactones) Orally Duration: single dose	56 patients (25-81 years) Verum (n=28) Placebo(n=28)	Patients undergoing surgery under epidural anaesthesia	The primary outcomes: sleep quality, psychological status, anxiety scale.	None	Small differences
Woelk et al., 1993	Multicenter, randomised, double-blind, reference placebo-controlled study	Verum: 3 x 100 mg kava-extract (corresponding to 70 mg kavalactones) up to 300 mg extract Control 1: 3 x 5 mg oxazepam Control 2: 3 x 3 mg bromazepam Orally Duration: 6 weeks	172 Patients (18-65 years) Verum (n=57) Control oxazepam(n=59) Control bromazepam(n=56)	Anxiety, agitation, and tension of non-psychotic origin	HAMA score, CGI	Student's t-test	No differences between the groups
Malsch & Kieser (2001)	Randomised, double-blind, placebo-controlled study	Placebo Verum : 50 mg kava-extract (corresponding to 35 mg kavalactones) up to 300 mg extract Orally Duration: 5 weeks	40 Patients (25 male, 15 female)	Non-psychotic nervous anxiety, tension and restlessness states following pretreatment with benzodiazepines	Primary outcomes:HAMA, Bf-S and the incidence of benzodiazepine withdrawal symptoms	Student's t-test	Limited value
Lehmann *et al.*, 1988	Randomised, placebo-controlled, double-blind study	Placebo Verum: 150 extract (3x 50 mg),corresponding to 47.5 -52.5 mg kavalactones/daily Orally Duration: 7 days	20 patients Placebo(n=10) Verum (n=10)	women with acute anxiety concerning suspected breast cancer	Principal outcomes: two self-rating scales - State trait anxiety scale and State trait anxiety inventory	Student's t-test	Limited value
Volz *et al.*, 1997	Randomised, placebo-controlled double blind multicenter study	Placebo Verum : 3 x 100 mg kava-extract (corresponding to 70 mg kavalactones) Orally Duration: 25 weeks	101 patients (27 male and 74 female; mean age: 54 years) Verum(n=52) Placebo (n=49)	Anxiety of non-psychotic origin	Primary outcome: HAMA score, CGI, Bf-S	U-test	Limited value
Scherer *et al.*, 1998	Open, observational, multicentric study	200 mg to 600 mg dry ethanolic extract (corresponding to 100-300 mg kavalactones)	52 outpatients (15 male, 37 female; average age: 49 ± 15)	Anxiety of non-psychotic origin with (n=26) or without (n=26) concomitant	Global improvement rated on a five-point scale	None	Limited value

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance*
		Orally Duration: 51 days		depression			
Spree *et al.*, 1992	Post-marketing surveillance study	3 x 133 mg kava extract (corresponding to 120 mg kavalactones) Orally Duration: 4 weeks	1673 patients (average age: 48.84 ± 14.77 ; 1168 female, 503 male)	Anxiety and /or nervousness and restlessness	Questionaire	None	Limited value
Lehmann *et al.*, 1996	Randomised, placebo-controlled double blind multicenter study	Placebo Verum : 100 mg kava-extract (corresponding to 70 mg kavalactones) Orally Duration: 4 weeks	58 patients (15 male and 43 female) Verum(n=29) Placebo (n=29)	Anxiety syndrome of non psychotic origin (HAMA> 18)	Primary outcome: HAMA score, GCI	Student's t-test	Limited value
Siegers *et al.*, 1992	Open, multicenter drug-monitoring trial	150 mg kava extract WS 1490 (containing 105 mg kavalactones) Orally Duration: 6 weeks	4049 patients (average age: 49; 72% female, 28% male)	Nervous anxiety, stress, and restlessness	Modified HAMA	None	Limited value
Geier *et al.*, 2004	Randomised, double-blind, placebo-controlled study	Placebo Verum : 150 mg (3 x 50 mg) kava-extract (standadized to 70% kavalactones) Orally Duration: 4 weeks	50 patients (51-90 years) Verum (n=25) Placebo(n=25)	Suffering from non-psychotic anxiety (DSM-III-R criteria; HAMA >18)	Primary outcome: HAMA total score Secondary efficacy variables: HAMA subscales with the dimensions 'somatic' and 'psychic anxiety', the Erlanger anxiety, tension and aggression scale (EAAS) and GCI	Student's t-test	Limited value
Geier *et al.*, 2004	Randomised, double-blind, placebo-controlled study	Placebo Verum : 150 mg (3 x 50 mg) kava-extract (standadized to 70% kavalactones) Orally Duration: 4 weeks	50 patients (51-90 years) Verum (n=25) Placebo(n=25)	Suffering from non-psychotic anxiety (DSM-III-R criteria; HAMA >18)	Primary outcome: HAMA total score Secondary efficacy variables: HAMA subscales with the dimensions 'somatic' and 'psychic anxiety', the Erlanger anxiety, tension and aggression scale (EAAS) and GCI	Student's t-test	Limited value

B. Anxiety in the climacteric phase							
B.1. Other extracts(not defined)							
Warnecke, 1991	Randomized, placebo-controlled double-blind study	Placebo Verum: 3 x 100 mg kava extract WS 1490 (daily dosage of 210 mg of kavalactones) Duration: 8 weeks	40 patients (45 – 60 years) Verum (n=20) Placebo(n=20)	Climacteric syndrome (HAMA> 18)	HAMA score, depressive status inventory (DSI), CGI, Kuppermann menopause index and Schneider scale	Student's t-test	Limited value
Warnecke, 1990	Double-blind, placebo controlled study	2 x 150 kava extract (corresponding to 2 x 30 mg kavalactones) Placebo Orally Duration: 12 weeks	40 patients Placebo(n=20) Verum (n=20) Drop out: Verum:2 Placebo: 14	Climacteric syndrome	Primary outcome: Kuppermann Index and Anxiety Status Index	Student's t-test	Limited value
De Leo, 2000	Randomized, placebo-controlled double-blind study	HRT-K: 50 µg/day 17 β-estradiol + progestogen + 100mg/day kava extract(containing 55% of kavain); HRT: 50 µg/day 17 β-estradiol+ progestogen + placebo ERT-K: 50 µg/day 17 β-estradiol+ 100 mg/day kava extract; ERT: 50 µg/day 17 β-estradiol + placebo Duration: 6 months	40 women HRT-K(n=13) HRT (n=9) ERT-K(n=11) ERT (n=7)	Menopausal anxiety with GAD(HAMA > 19)	HAMA score	Student's t-test	Combined treatment superior to hormonal treatment
Cagnacci, 2003	Randomised positive control study	Control: 1 g/day of calcium Verum 1: 1 g/day of calcium + 100 mg kava extract/day (corresponding to 55% of kavaina); Verum 2: : 1 g/day of calcium + 200 mg kava extract/day Duration: 3 months	80 patients Control (n=40) Verum 1(n=20) Verum 2(n=20) Drop-out: 12	Climacteric syndrome	Anxiety was evaluated by the State Trait Anxiety Inventory (STAI, 20 items), while depression was evaluated by the Zung's scale (SDS) and climacteric symptoms by the Greene's scale	Student's t-test	No differences between the groups
* should be seen in correlation with the weaknessess of the trial (see descriptive part)							

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

There are several trials that evaluated the use of kava preparations as treatment option for anxiety disorders, as generalised anxiety or anxiety in the climacteric phase. However the majority are small with methodological weakness, such as: mixed anxiety population, short duration of the trials, short follow-up phase, no data regarding percentage of responders. There were many differences in the products studied (acetonic, aqueous or ethanolic extracts, different DERs, sometimes synthetic compounds), study designs (mainly open studies) and methodology and the dosage administered that was reported either in milligrams of kavalactones or in milligrams of kavain.

Taking into account all these differences comparison of the results between products is rather difficult and it is not possible to assess correctly the impact of kava preparations on those patients.

Some meta-analysis (Pittler *et al*., 2000, 2003, 2010) that investigated the reliability and quality of some of the clinical trials did not differentiate the type of extract involved, therefore clinical improvements cannot be attributed confidently to a particular extract, while another meta-analysis (Witte *et al*., 2005) included short term studies with significantly different HAMA baseline scores between the trials, patients with mixed anxiety diseases, different doses tested. Therefore no clear conclusion on efficacy can be drawn and further studies are needed, especially on long-term efficacy and safety.

To conclude, the clinical data available for kava preparations as treatment option for anxiety disorders, are not considered sufficient to support a well-established medicinal use according to Article 10a of Directive 2001/83/EC as amended.

5. Clinical Safety/Pharmacovigilance

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

Traditional use outside Europe

Several authors stated that during the long-term use of traditional kava preparations (beverage) outside the European Union kava has not shown any severe side effects that suggested any severe liver damage. There are entire books dedicated to this topic (Lebot *et al.,* 1992; Singh, 2004). The published studies suggest only a minimal effect induced by kava aqueous preparations. These data should also be correlated with spontaneous case-reports (Russmann *et al.,* 2003, Christl *et al*., 2009).

39 healthy aboriginal users of a kava preparation (dried rhizome prepared as infusion in cold water) were compared with non-users by Matthew *et al.,*1988; 20 were classified as very heavy users (mean consumption, 440 g/week), 15 were heavy users of kava (310 g/week) and 4 were occasional users (100 g/week). Various adverse effects were observed at these high intakes, including the levels of gamma-glutamyl transferase which were highly increased: 251 UI/l in very heavy users, 312 in heavy users and 77 in occasional users, compared with < 60 UI/l cosidered as normal values. Very heavy users of kava were 20% underweight. Albumin, plasma protein, urea and bilirubin levels were decreased in kava users, and high-density lipoprotein cholesterol levels were increased.

On the same community in a cross-sectional study with 98 participants, 36 non-users and 62 kava users (of which 23 had discontinued kava at least 1 year before the study) liver function tests were

done. Continuing users had not used kava for 1 to 2 months (n=10) or 1 to 2 weeks previously (n=15) and some (n=14) had used kava within the previous 24 hr. The average quantity of kava powder consumed was 118 g/week, and median duration of use was 12 years (range, 1-18 years). Almost one-half (48%) of the kava users showed GGT above a normal reference range (OR=2.6, 1.0–6.5, p=0.034), with 37% having abnormally elevated alkaline phosphatase ALP (OR=3.4, 1.2–10.1, p=0.017) but not with alanine aminotransferase or bilirubin, which were not elevated. There was no association between duration of kava use and abnormally elevated liver enzymes. However, the quantity consumed per week was associated with an abnormally elevated ALP (p=0.009). In those who were not heavy alcohol users, only those who used kava within the previous 24 hr showed GGT levels higher than non-users (p<0.001), whereas higher ALP levels occurred only in those who last used kava 1 to 2 weeks (p=0.015) and 24 hr previously (p=0.005). The authors concluded that liver function changes in users of aqueous kava extracts at these moderate levels of consumption appear to be reversible and begin to return to baseline after 1 to 2 weeks abstinence from kava. No evidence for irreversible liver damage has been found (Clough, 2003).

Later on, Brown *et al*., 2007 investigated the effects of regular use of kava beverage on the liver function tests of 31 healthy adult kava drinkers and compared against a control group of 31 healthy adult non-kava drinkers. The liver function profile included AST, ALT, ALP, GGT, and bilirubin (total and direct). Other tests included total protein, albumin, and screens for viral hepatitis and hemochromatosis when indicated. Chronic kava beverage consumption was associated with elevation of GGT in 65% of the kava drinkers versus 26% in the controls (p=0.005). ALP was elevated in 23% of kava drinkers versus 3% in the controls (p=0.053).

Kava users more frequently showed a characteristic kava-induced skin reaction, a scaly rash that is suggestive of ichtyosis – a condition called “kava dermopathy” (pellagroid dermopathy) that appears to heavy chronic drinkers and has been attributed to niacin deficiency. Although the skin becomes yellow, the description does not suggest an underlying hepatic condition, the rash is not itchy and the condition is ameliorated without treatment if heavy use of kava is reduced (Ruze, 1990)**.**

Use in Europe

Clinical trials

Apart from the experience of the non EU-traditional use which is referring to aqueous infusions, several controlled and non-controlled trials can be referred to when evaluating the safety of kava extracts.

Old clinical trials of kava extracts generally have suffered from well-known shortcomings, such as small sample size, lack of information about type and dose of extract used, ill-defined patient population, lack of adverse event reporting, etc. Therefore WHO assessed in the document "Assessment of the risk of hepatotoxicity with kava products" only the new trials.

The adverse effects found in all clinical trials are summarised in Table 6.

Table 6: Clinical safety data from clinical trials

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
A. Ethanolic extracts						
Boerner *et al.,* 2001	Randomized, reference-controlled, double blind, multicentre	verum: 400 mg kava extract containing 30% kavalactones /day Reference group: 10 mg/day buspiron 100 mg/day opipramol Orally Duration: 8 weeks + 1 week follow-up	129 patients (25-65 years) verum (n=43) reference groups: buspiron(n=43) Opipramol (n=43) 4 drop-out (verum) 1 drop-out (reference)	Patients with GAD	Verum: slight increases of transaminases above upper limit in 2 subjects (one had already displayed values slightly above normal at baseline). One subject suffered from panic attack requiring stationary treatment. No significant hepatotoxic reactions were reported in about 330 treatment weeks in this trial Reference group: Slight increases of transaminases above upper limit in 5 subjects (4 had already displayed values slightly above normal at baseline). Only one GGT increase was rated to be of clinical relevance (opipramol).	No difference was observed between placebo and verum groups.
Cropley *et al*., 2002	Randomized, controlled trial	verum: 120 mg/day Kava LI150 valerian group: 2 x 600 mg/day Valerian LI 156 placebo orally Duration: 1 week	54 volunteer (18-30 years) Verum (n=18) Valeriana (n=18) Placebo (n=18)	Healthy volunteers	Verum: none reported Control/Reference group: none reported Placebo Group: none reported	No difference was observed between placebo/reference and verum groups.
Connor *et al*., 2002	Randomized, double-blinded, placebo-	verum: Dose 1: 140 mg/day kavalactones Dose 2: 280 mg /day	38 patients (31-75 years) Verum (n=19)	Patients with DSM-IV GAD	Verum: diarrhea (2 cases), dry mouth (2 cases), rash (2 cases), nausea (2 cases); in placebo group: headaches, heart pounding, swelling, trembling (2 cases or	No difference was observed between placebo and verum groups.

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
	controlled	kavalactones placebo orally Duration: 1 week of dose 1 followed by 3 weeks of dose 2	Placebo (n=19) 3 droup-out		each side effect)	
Connor *et al.*, 2006	Double-blind, placebo-controlled	Kava extract 70% kavalactones; 140-280 mg kavalactones/day Placebo Duration: 4 weeks	13 verum: 6 placebo: 7	Mild GAD (HAMA 12-20)	No deviation in liver function tests	No AE reported
		Kava extract 70% kavalactones; 140-280 mg kavalactones/day Placebo Venlafaxin Duration: 4 weeks	16 volunteers verum: 5 placebo: 5 venlaflaxine: 6	Moderate GAD (Hama ≥ 18)	No deviation in liver function tests	No AE reported
Mittmann *et al.*, 2000	Randomized, unblinded, diazepam controlled	Verum: 100 mg kavalactones + 25 mg promethazin evening before operation and 100 mg 60 min. before operation Reference group: 1-2 mg flunitrazepam +25 mg promethazine orally	53 patients Verum (n=26) Reference group (n=27)	Women with planned vaginal hysterectomy	Nausea and vomiting were at the same level (5 cases) in both groups but could not be attributed to the medication.	No difference was observed between reference and verum groups
Thompson *et al.*, 2004	Double-blinded randomized, placebo-controlled	verum: 300 mg kavalactones/day placebo orally Duration: 1 dose	20 patients (18-53 years) Verum (n=10) Placebo (n=10)	Healthy volunteers	None observed	No difference was observed between placebo and verum groups

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
B. Acetonic extracts						
Gastpar *et al.,* 2003	Randomized, placebo-controlled, double-blind Multicentre, parallelgroup	verum: 150 WS 1490 acetonic extract mg/day placebo orally Duration: 4 weeks+ 2 weeks observation	141 patients Verum (n=71) 9 drop-out Placebo (n=70) 5 drop-out	Patients with neurotic anxiety	Verum: Tiredness(one case) Placebo: sneezing attacks(1 case);developing a ganglion on left wrist(1 case)	
Lehrl , 2004	Prospective, randomized, placebo controlled, double blind	verum: 200 WS 1490 acetonic extract mg/day placebo orally Duration: 4 weeks	61 patients (24-72 years) Verum (n=34) Placebo (n=27) 4 drop-out	Patients with neurotic anxiety	verum: none placebo: gastrointestinal complaints and nausea (1 case)	
C. Other extracts(not characterised)						
De Leo *et al*., 2000	Randomized, placebo controlled	Verum: 100 mg extract/day placebo orally Duration:6 months All received estradiol 50µg/day with or without progestogen	40 patients Verum (n=24) placebo (n=16)	Women in physiological or surgical menopause	Verum: none Placebo: none	
Malsch *et al.,* 2001	Randomized, placebo controlled, double blind	verum: 50 mg up to 750 mg extract/day gradually increased placebo orally Duration: 5 weeks + 3 week follow-up	40 patients (21-75 years) verum (n=20) 3 drop-out placebo (n=20)	Patients with non-psychotic anxiety and pre-treatment with benzodiazepines	Verum: none Symptoms due to withdrawal of benzodiazepine (5 cases) Placebo: none Symptoms due to withdrawal of benzodiazepine (10 cases)	

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
			1 drop-out			
Geier *et al.,* 2004	Randomized, placebo-controlled, double-blind	verum: 150 WS 1490 extract mg/day placebo orally Duration: 4 weeks+ 2 weeks observation	50 patients (51-90 years) Verum (n=25) Placebo (n=25) 2 drop-out	Patients with nonpsychotic anxiety	Verum:none Placebo: nausea, retching, restlessness and sleeplessness (1 case)	
Lehmann *et al.*, 1988	Prospective, double-blinded, randomized, placebo-controlled	verum: 450 mg kavalactones /day placebo orally Duration: 7 days	20 patients Verum (n=10) Placebo (n=10)	Women with anxiety concerning suspected breast cancer	None observed	No difference was observed between placebo and verum groups
Bhate *et al.*, 1989	Randomized, double-blind, parallel-group	Kava extract 3x150 mg extract (60 mg kavalactones) Placebo Duration: 2 doses	60 Verum (n=30) Placebo(30/29)	Anxiety in the context of surgery	Verum : 2 cases of post-surgery hang-over (one case with additional medication) Placebo: 4 cases of post-surgery hang-over (three cases with additional medication)	
Warnecke *et al.*, 1990	Randomized, placebo-controlled, double-blind, parallel group	kava extract WS 1490 300 mg /day (60 mg of kavalactones) Placebo Duration: 12weeks	40 8 weeks: verum(n=20) Placebo(=20/16) 12 weeks: verum(20/18) Placebo(20/6)	Psychovegetative symptoms in patients with climacteric symptoms	Verum : 5x tiredness in the morning 2x mild gastrointestinal complaints Placebo: 3x mild gastrointestinal complaints No laboratory values	
Kinzler *et al.*,, 1991	Randomized, double-blind, Placebo-controlled	kava extract WS 1490 300 mg /day (210 mg of kavalactones) Placebo	58 verum: 29 placebo: 29	Patients with anxiety syndrome not caused by psychotic disorders	No AE	

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
		Duration: 4 weeks				
Warnecke 1991	Randomized, placebo-controlled, double-blind, parallel group	kava extract WS 1490 300 mg /day (210 mg of kavalactones) Placebo Duration: 8 weeks	40 verum: 20/18 placebo: 20/13	Peri- and postmenopausal women with psychovegetative and psychosomatic symptoms	Gastrointestinal complaints, restlessness, tiredness in both groups: K: 4 AE P: 6 AE No effect on liver function tests	
Siegers *et al*., 1992	Open	150 mg kava extract WS 1490 (105 mg kavalactones) Duration: 7 weeks	4049/3873	Nervous anxiety and tension	AE in 61 patients (1.5 %); mild gastrointestinal and skin reactions	
Spree and Croy 1992	Open(post-marketing surveillance)	Kava extract WS1491 3 x 133 mg extract(120 mg kavalactone/day) Duration: 4 weeks	1673	Anxiety with nervous tension, sleep disorders, climacteric disorders	AE in 69 patients (2.3%), all mild and reversible: 9x "*allergic reaction*" 31x gastrointestinal complaints 22x headache, nausea 11x other events (e.g. photosensitivity)	
Volz and Kieser 1997	Randomized, placebo-controlled, double-blind	Verum: 300 mg WS 1490 lipophilic extract (210 mg kavalactones)/day Placebo Duration: 25 weeks	101/73 verum: 52/49 placebo: 49/43	Anxiety of non-psychotic origin	Verum: 6 AE in 5 patients: 4x unrelated, 2x stomach upset Placebo : 15 AE in 9 patients: 12x unrelated, 2x vertigo, 1x palpitations No changes in liver function tests	
Woelk *et al*.,, 1993	Double-blind, reference-controlled parallel group	Kava extract 3 x 100 mg extract (210 mg lavalactones/day) 9 mg Bromazepam 15 mg Oxazepam	172 patients Kava: 57/55 Bromazepam: 56/52 Oxazepam: 59/57	Anxiety, restlessness and inner tension of non-psychotic origin	Bromazepam: 4x tiredness; 1x mild pruritus Oxazepam: 1x tiredness, 1x restlessness Kava: 1x gastrointestinal complaints; 1x inhibition under	

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
					stress No laboratory values	
Scherer 1998	Open	Kava extract 100 mg kavalactones (n=15)/150 mg kavalactones (n=28)/200 mg kavalactones (n=9) Duration: 50 days	52 (dropout:9)	Anxiety of non-psychotic origin	1x restlessness (unrelated) 1x stomach upset 1x bitter taste No cardiac, renal or hepatic changes.	
Singh *et al*., 1998	Double-blind, placebo-controlled	Kava extract 2 x 200 mg extract (120 mg kavalactones/day) Placebo Duration: 4 weeks	60 K: not stated P: not stated	Stress and anxiety	None mentioned	Not a full publication (an abstract for a symposium)
Neto, 1999	Open	300 mg kava extract WS1490 Duration: 4 weeks	850 patients 766 ended	Stress-related anxiety	AE in 16.7 % of patients Most common: somnolence (23 cases = 2.7 %), nausea (16 cases = 1.8 %), dizziness (10 cases = 1.1 %) and epigastric complaints (10 cases = 1.1 %).	
Cagnacci A *et al*., 2003	Randomized prospective open	Group 1: 100 mg extract /day Group 2: 200 mg extract /day Control orally Duration: 3 months All subjects received 1 g/ day of calcium during study	80 patients (47-53 years) Group 1(n=20) Group 2(n=20) Control (n-40)	Perimenopausal women.	Group 1 and 2: nausea and gastric pain (6 cases) Control: nausea and gastric pain (1 case)	

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Adverse reactions	Comments
Jacobs *et al*., 2005	Double-blind, placebo-controlled	Kava (unknown extract) 300 mg kavalactones Valeriana (daily 6.4 mg of valerenic acids) Placebo Duration: 4 weeks	verum: 121 valeriana: 135 placebo: 135	Anxiety (STAI) and insomnia (ISI)	No difference between groups with the exception of a higher incidence of diarrhoea with valerian. All verum-patients were negative for liver reactions	Negative for liver reactions were based on self-reporting(e-mail)
D. Aqueous dry extract						
Sarris *et al*., 2011 Sarris *et al*., 2013a Sarris *et al*., 2013b	Randomized, double-blind, placebo-controlled	Aqueous kava extract 120-240 mg kavalactones /day Placebo Duration: 6 weeks	58 volunteers (dropout:10) Verum (n=27) Placebo (n=31)	Patients with GAD	Headache (unrelated): 13 verum; 30 placebo Possibly related: Verum: 1 x dermatitis; 1 x stomach upset Placebo: 1 x allergy No signs of hepatic disorders Verum: 1x GGT-increase from 45 to 106 and ALT from 30 to 59	
Sarris *et al*., 2012	Randomized, cross-over	Aqueous kava extract 180 mg kavalactones /day 30 mg oxazepam placebo Duration: 1 week	66 Verum (n=22) Oxazepam Placebo (n=22)	Moderate anxiety	No change of liver function tests Marked fatigue occurred, 12/22 (verum), 10/22 (oxazepam), and 10/22 (placebo). Oxazepam: 3/22 headaches; 5/22 dizziness, Placebo: headaches 4/22 and dizziness 2/22.	No difference was observed between placebo and verum groups
Sarris *et al*., 2009a Sarris *et al*., 2009b	Randomized, double-blind, placebo-controlled, cross-over	Aqueous kava extract Verum: 5 x 50(250 mg kavalactones/day) Placebo Duration: 1 week	41/droupout:4 Verum(n=19) Placebo(n=22)	Anxiety and depression	Verum: 2 x nausea (drop-out) 1x mild dizziness Placebo: 1x dizziness, gastric discomfort and flu-like 1x constipation	No change of laboratory liver values

In these trials, kava extracts have been generally well tolerated and no severe adverse effects have been observed. Only rarely mild side effects or adverse reactions are reported in controlled clinical studies and non-controlled trials. Less than 2% of the patients complained about adverse effects; the majority of adverse reactions were gastrointestinal complaints. WHO concluded that these trials are not designed or powered to pick-up long-term adverse reactions especially rare or uncommon adverse reactions like serious liver toxicity.

WHO also emphased that the number of patients usually involved in Phase III clinical trials is typically too small to detect hepatic necrosis that occurs with an incidence of 1/10 000 and even too small to provide high assurance against risk with an incidence of 1/1000 or less. Most drugs that cause hepatic necrosis also cause an asymptomatic, but significant (>5 fold), elevation of transaminases in a larger fraction of the population treated, which can be detected in typical Phase III trials. Therefore, any drug that is found to cause a significant incidence of elevated transaminases relative to control, must undergo additional investigations into the mechanisms involved. However that smaller elevations should not be seen as forerunners of more severe liver damage.

The total number of patients involved on clinical trials was 8136 and only 509 were involved in the trials that assessed liver function. Therefore the data available is inadequate to calculate any incidence HILI rate (or even estimate). Therefore hepatotoxicity cannot be excluded.

The true incidence of hepatotoxicity related to kava preparations can only be ascertained by a proper epidemiological study. Until now this is missing.

5.2. Patient exposure

Aside from its market presence and data from clinical studies in humans, kava is used as recreational beverage and can also be found in dietary supplements.

In Arnhem Land, Australia, weekly per capita consumption was estimated as 145 g of powder for 1989–1990 and 368 g of powder for 1990–1991. In a detailed review of the literature on weekly consumption levels and possible lactone contents, the estimations encompassed a wide variation from 39 to 1840 g of kava powder consumed, and from 4.1 g to 188.6 g of lactones consumed per week (Clough *et al*., 2003).

A typical dosage of dried root or by decoction was reported to be 6–12 g per day (IARC, 2015).

Based on the information from the clinical trials 8136 adults have been exposed to oral use of kava-kava containing products.

5.3. Adverse events, serious adverse events and deaths

Clinical trials

See section 5.1.

Pharmacovigilance database (WHO)

Results of the overview of side effects collected in the WHO Vigilyse database were reviewed. In the VigiLyze database of the World Health Organization's Uppsala Monitoring Centre for the period up to May 2016, there were 94 spontaneous reports of suspected adverse drug reactions associated with the single-ingredient *Piper methysticum.* The adverse reactions declared with the highest incidence were: rash, pruritis, hepatitis. Regarding liver toxicity following adverse reactions were reported: hepatic enzyme increased (8 cases), hepatitis (7), hepatic cirrhosis (3), jaundice (3). There are no details

regarding the type of extract used; only the daily dose (if was known) is reported. Generally, the Vigilyse database may serve for the detection of signals of potential safety issues by data-mining. The causality assessment is often hampered by poor reporting. Underreporting must also be taken into consideration.

Spontaneous reporting can be illustrated by published case reports:

Case reports

No reports of hepatotoxicity associated with the use of kava extracts at therapeutic dose levels have been published until the late 1990s, when cases of severe liver damage linked to the use of kava, althrough rare, began to emerge and were increasingly reported in the literature and to regulatory authorities.

In 2007, WHO summarized all case reports up to 2002 worldwide, including also the cases already published (eg. Strahl *et al*., 1998; Campo *et al*., 2002; Humberston *et al.,* 2003, Rusmann *et al.,* 2001):

Germany

Out of 105 spontaneous adverse reports on kava, 24 were associated with impaired liver function or symptoms that could be linked to liver toxicity (including cases of cirrhosis, cholestatic hepatitis, and other types of hepatoxicity). Of the 24 cases, there was one fatality, three cases required liver transplant, and 18 cases were considered possibly or probably related to kava ingestion.

Switzerland

4 Swiss cases were reported in which severe hepatic complications resulted from the use of an acetone extract of kava. Of the 4 cases (2 severe hepatitis, 1 liver fibrosis, 1 severe liver injury), 3 were histologically confirmed, and one was a case of fulminant irreversible hepatitis requiring transplantation. In 3 of the cases, prothrombin time was increased. All 4 cases presented with jaundice.

Australia

On 16 August 2002 the Australian Therapeutic Goods Administration (TGA) initiated a recall of kava-containing products the classification of recall being class II (defects could cause illness or mistreatment).

The recall was for certain batches of one product, which had been found adulterated by the TGA.

Canada

On 26 August 2002 a summary of 11 case reports associated with kava was submitted to Health Product Safety Information Division (HPSID) of Health Canada. Four Canadian cases of liver toxicity associated with the use of kava-containing products were reported in response to a Public Advisory (issued 16 January 2002) in which health professionals were asked to report any cases of kava-related hepatotoxicity to HPSID. Two cases were considered serious.

United Kingdom

As of April 2002, the Medicines Control Agency (MCA) received 3 reports of liver toxicity suspected of being related to kava consumption.

The Committee on Safety of Medicines' (CSM) Expert Working Group (EWG) on the safety of kava was established by MHRA in March 2002 to investigate ongoing safety concerns about liver toxicity suspected to be associated with the use of herbal medicinal products containing Kava. The safety of

food products containing Kava was outside of the remit of the EWG. The EWG met on two occasions (12 March 2002 and 6 October 2005) to consider the available data. Rather than using the RUCAM scale method, in 2002 EWG used the WHO scale, developed by WHO Collaborating Centre for International Drug Monitoring. EWG considered that RUCAM criteria do not translate well to new safety signals in spontaneous case reports and is more appropriate for use within clinical studies which can accommodate the necessary testing time points. Later, due to the subjective nature of the causality assessment criteria it was agreed that the EWG would not allocate causality categories to the cases reviewed in 2005.

At the time the decision to prohibit unlicensed products containing kava was taken (2002), the MHRA had received details of 68 cases of adverse liver reactions; up to 2005, details of an additional 42 cases have been received. Details of the specific kava products have only been provided in 47% of cases (n=53).

Details of the onset of reaction were not provided on 32% of cases (n=35), although just 6% were reported to have occurred after more than nine months treatment with Kava (n=5) or after Kava had been stopped (n=2). No specific trend could be identified in terms of a predictable onset of hepatic reactions.

At the time of the prohibition, 40% (n=27/68) of the cases were reported to have recovered after treatment with kava had stopped. A further 26% were reported to be recovering after withdrawal of kava (n=5), recovered after treatment (n=2) or recovered in some cases whilst still taking Kava but in others it is unclear whether treatment with Kava had continued (n=11).

The most serious cases relate to individuals who were reported to have died after experiencing hepatotoxicity suspected to be associated with the use of kava. A total of nine case reports were received with a fatal outcome. Of these individuals, seven were reported to have died as a consequence of the hepatotoxicity and two died during or soon after receiving a liver transplant. Liver failure was listed as an adverse reaction in five of these cases, hepatic necrosis was indicated in two individuals and hepatic encephalopathy was listed in a further two cases.

An additional nine individuals received liver transplants and survived. Six of these cases reported symptoms generally associated with acute hepatitis. Two further individuals were reported to have developed necrosis and the remaining individual reported cholestatic hepatitis.

Evaluation of the safety signal by the EWG determined that kava was associated with an unacceptable risk of idiosyncratic hepatotoxicity which could not be minimised or prevented by any regulatory measures other than the removal of Kava products from the market (MHRA, 2006).

France

In France, two non-serious liver case reports were reported, but both with questionable causality for kava.

United States of America

An update of the adverse reaction reports received by the FDA (4 March 2002) revealed a total of 47 adverse reaction reports received in association with kava, 20 of which were related to the liver.

In conclusion, up to July 2002, WHO initially identified 93 cases of hepatic events associated with the use of kava preparations have been reported worldwide including cases of liver failure resulting in 14 liver transplants and 7 deaths. It also mentioned that there is the possibility of a small number of duplications:

Table 3 Hepatic events reported in association with kava

Hepatic event	Count	%
Hepatitis	22	23.7
Hepatitis cholestatic	9	9.7
Hepatitis fulminant	3	3.2
Hepatitis toxic	2	2.2
Hepatic failure	11	11.8
Hepatic necrosis	6	6.5
Jaundice	9	9.7
Hepatocellular liver injury	8	8.6
Liver injury	1	1.1
Hepatic function abnormal	18	19.4
Cirrhosis	2	2.2
Not described	2	2.2
Total	93	100.3

The causality assessment was performed using WHO scale, developed by WHO Collaborating Centre for International Drug Monitoring. Of the 93 reports, eight (8.6%) were coded as having a probable causality and 53 as possible. 28 were unassessable because of lack of data.

For a "probable" classification according to WHO report there must be good information, which would include duration to onset of the event, recovery on withdrawal of the product and the absence of other potential causes of hepatotoxicity.

For the comparisons between the different extracts- see ***Reviews of case reports*** below***.***

Cases reported after 2002

Several new case reports of liver toxicity have been published in the literature since 2002. Six cases are presented here.

Two cases of acute liver injury have been associated with ingesting traditionally kava preparations for 4-5 weeks (Russmann *et al.,* 2003):

Case 1: A 59-year-old female patient of Oceanian origin started drinking traditional kava prepared with tap water and dried kava root imported from the islands of Vanuatu. Four weeks later she presented with icterus, transaminases elevated 18 and 14 times the upper limit of normal (aspartate aminotransferase, 671 U/l; normal < 37 U/l; and alanine aminotransferase, 568 U/l; normal < 40 U/l), a prolonged thromboplastin time (Quick value, 62%; normal, 70–120%) and an eosinophilia of 10%. Total bilirubin was not measured until 2 weeks after the initial presentation, when it was 30.7 µmol/l (normal, 17 µmol/l). Abdominal ultrasound showed no dilatation of the bile ducts, and the titres for antinuclear and anti-DNA antibodies were minimally elevated (1/128 and 1/20). Hepatitis serology was positive for anti-HBc immunoglobulin (Ig)G antibodies but negative for HBs antigen and antibodies against HBs, hepatitis A and hepatitis C. The patient did not consume any alcohol. Long-term medication included lisinopril, phenobarbital and fenofibrate, which had all been taken for several months or years. Initially all drugs and kava drinking were stopped, but the formerly taken drugs were then re-started. Drinking kava was not re-started and the patient recovered, and laboratory values normalized over the following 3 months. The authors concluded: *The failure to identify other causes of liver injury, the time interval from the onset of kava consumption to presentation with symptoms and the resolution of the biochemical abnormalities after abstinence from kava strongly argue for the consumption of aqueous kava extract as the cause of liver injury*"

RUCAM score: +5 (possible adverse drug reaction).

Case 2: A 55-year-old female patient of Oceanian origin started drinking traditional kava in a quantity of about 4 cups per evening, corresponding to approximately 18 g kavalactones per week. Five weeks later, she presented with fatigue, icterus and transaminases and total bilirubin elevated 42 and 13 times the upper limit of normal, respectively (aspartate aminotransferase, 1569 U/l; alanine aminotransferase, 1666 U/l; total bilirubin, 220 µmol/l). Abdominal ultrasound showed no dilatation of the bile ducts. Antinuclear antibodies were minimally positive (1/40), autoantibodies against LKM and smooth muscle were negative. Hepatitis serology was positive for anti-HBc and anti-HBs IgG antibodies, but negative for anti-HBc IgM and hepatitis A IgG antibodies and HBs antigen. Intake of other drugs and alcohol was denied. Drinking kava was consequently stopped and the patient recovered and laboratory values normalized over the following 3 months.

The authors concluded: "The failure to identify other causes of liver injury, the time interval from the onset of kava consumption to presentation with symptoms and the resolution of the biochemical abnormalities after abstinence from kava strongly argue for the consumption of aqueous kava extract as the cause of liver injury".

RUCAM score: +6 (probable adverse drug reaction).

In the same study a survey on 27 heavy kava drinkers showed elevated gamma glutamyl transferase in 23/27 and minimally elevated transaminases in 8/27. GGT elevated levels should not be considered a sign of liver injury, particularly in the absence of other symptoms and signs of liver disease (Russmann *et al.,* 2003).

One case of icteric hepatitis was reported in Spain (Bujanda *et al*., 2002):

Case 3: A 55 years old, male, started to use for 3 months capsules containing 250 mg of an extract of kava-kava (no detail regarding the type of extract), 3 times daily after having experienced anxiety for one month. No co-medication was taken. Liver function tests were reported normal prior to the incident.

Two weeks after the start of kava the patient showed asthenia, a poor general well-being, epigastric and right hypochondriac complaints. As the duration of intake was stated with three months and the duration of anxiety until the presentation for liver complaints was stated with four months, kava must have been continued for another 2.5 months (10 weeks) despite the developing symptoms.

The patient was admitted to the hospital with the diagnosis of icterus. Liver function tests were recorded as follows:

TABLA I. **Evolución de las pruebas de función hepática**

	AST (U/l)	ALT (U/l)	FA (U/l)	GGT (U/l)	BT (µmol/l)	TP (%)
Ingreso	1.506	2.300	514	874	111,15	65%
1 mes	679	1.018	613	890	146,2	69%
2 meses	132	200	454	806	64,6	87%
4 meses	32	35	178	40	23,8	89%

FA: fosfatasas alcalinas; GGT: gammaglutamintranspeptidasa; BT: bilirrubina total; TP: tiempo de protrombina.

Alcohol intake was denied. Virus serology was negative for hepatitis B and C "as well as for the remainder of hepatotropic viruses", although no specification was made. Autoimmune hepatitis (ANA, AMA, AML and anti-LKM1) were negative, the metabolism indicated as normal. Sonography and

nuclear magnetic resonance examinations of liver/bile and pancreas did not show abnormal changes. Liver biopsy showed a centrilobular haemorrhagic necrosis without steatosis, fibrosis or cholestasis with minimal mixed diffuse inflammatory infiltrate, compatible with a venoocclusive disease.

After two weeks without kava, liver function was distinctly improved (no data given). Normal values were reached after four months.

RUCAM score +7 (probable adverse drug reaction).

In Australia a case of acute liver failure and death was described by Gow *et al*., 2003:

Case 4: A 56 year-old woman took a preparation containing 60 mg of kavalactones (no further detail), 50 mg of *Passiflora incarnata* and 100 mg of *Scutellaria laterifloria* for 3-4 months. The preparation was prescribed and provided by a naturopath for the treatment of anxiety.

In July 2002, a 56-year-old woman was admitted to the hospital for the investigation of jaundice. She had been previously well apart from a history of benign monoclonal gammopathy (IgG, 24 g/L; normal, 6.9–15.4 g/L), which had been diagnosed 12 months previously. The patient had presented to her local doctor with a two week history of fatigue, nausea and increasing jaundice. She had no risk factors for viral hepatitis, no history of liver disease and drank minimal amounts of alcohol. Over the preceding three months she had been taking a herbal supplement for anxiety, prescribed and provided by a naturopath (one tablet thrice daily, labelled as containing kavalactones 60 mg, Passiflora incarnata 50 mg and Scutellaria laterifloria 100 mg). She had also been taking some vitamin and mineral supplements but no other medications (no further details are included in the report).

Examination on presentation to hospital revealed the patient to be deeply jaundiced without stigmata of chronic liver disease.

Following relevant abnormal pathology test results were obtained:

1: Patient laboratory data on presentation to hospital and 17 days later (day of transplantation)

	Serum albumin (g/L) (normal, 35–50 g/L)	Serum bilirubin (μmol/L) (normal, < 18 μmol/L)	Serum alkaline phosphatase (U/L) (normal, 40–129 U/L)	Serum alanine aminotransferase (U/L) (normal, < 55 U/L)	International normalised ratio (normal, 1–1.2)
Presentation	34	209	190	4539	2.3
Day 17	23	607	357	438	6.6

Extensive investigations to screen for recognised causes of acute liver failure failed to reveal any cause. Assays for acute hepatitis A, B, and C viruses, Epstein Barr virus and cytomegalovirus were all negative. Serum copper and ceruloplasmin levels were normal and Kayser– Fleischer rings were not present. Antinuclear antibodies were detected at a titre of 1:160, but anti-smooth-muscle antibodies were not detected. No paracetamol was detected in the blood. An abdominal doppler ultrasound revealed a small liver with normal flow in the hepatic arteries, hepatic veins and portal veins. The paraprotein level had remained stable over the previous 12 months. A repeat bone marrow biopsy did not suggest the presence of multiple myeloma. A trans-jugular liver biopsy performed on the fifth day of admission showed non-specific severe acute hepatitis with pan-acinar necrosis and collapse of hepatic lobules. Over the subsequent week, the patient's condition deteriorated and she was urgently listed for transplantation. On day 17 of admission the patient underwent liver transplantation. The procedure was complicated by massive bleeding. The patient died of progressive blood loss, hypotension and circulatory failure. Histological examination of the explanted liver confirmed the presence of massive hepatic necrosis.

Chemical analysis of the product was done by the authors. The product contained kava and Passiflora incarnata as labelled, and a third, as yet unidentified, compound. Although the label listed Scutellaria

laterifloria as an ingredient, none was identified in the compound. The presence of some other herbs reported to be hepatotoxic has been excluded. Later, Thomsen *et al.,* 2004 used TLC and HPLC methods to investigate the composition of another batch of the product. The results revealed the absence of *Passiflora incarnata* and *Scutellaria laterifloria.*

The authors used the Naranjo Adverse Drug Reaction Probability Scale to assess the case as: "probable". RUCAM score: +8 (probable adverse drug reaction).

Case 5: Musch *et al*. (2006) reported an acute hepatitis in a previous healthy 48-year-old female. She was taking simultaneously synthetic kavain (1-3 x 200 mg/day) for 10 weeks for the treatment of psychovegetative exhaustion. Co-medication used were: valerian drops, St John's Wort extract, soy extract and zopiclone.

Six weeks after starting therapy with kavain and St. John's wort plus zopiclone the patient started developing a hepatopathia with increased transaminases (GOT up to 613U/l and GPT up to 752U/l), but no clinical correlate such as general malaise or icteric complaints. Body temperature was not elevated; abdomen, liver and spleen were normal, as were appetite and bowel functions or body weight.

The patient had undergone immunisation against hepatitis A and B. She did not drink alcohol.

All other laboratory values were in the normal range. Hepatitis C, EBV, CMV, VZV, Coxsackie virus and HSV were excluded. Antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antimitochondrial antibodies (AMA), liver-kidney microsome antibodies (LKM) and antibodies against soluble cytoplasmatic liver cell antigen (SLA) were negative. An ECG, x-ray of the chest, the ultrasound examination of the abdomen and a nuclear magnetic resonance tomogram did not show pathologic findings with the exception of a slight dilatation of the Ductus hepatocholedochus.

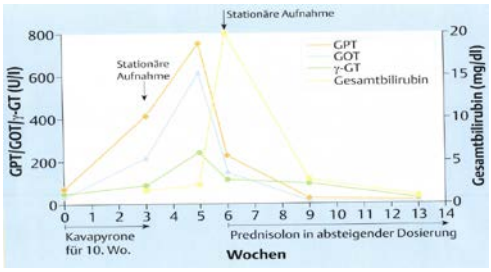
A liver biopsy showed lobular and portal necrotic-inflammatory changes with mild cholestasis, but no signs of liver cirrhosis or granulomatosis.

Liver function tests continued to increase despite a cessation of kava and despite a two-week infusion with ornithine aspartate, multivitamins, vitamin E and C and the application of silymarin.

The patient was discharged on her own wish, but returned one week later with a massive increase of total bilirubin, although the liver transaminases had started to improve.

At this point further viral or bacterial and fungal causes were excluded (Echo virus Borrelia, Candida, Chlamydia, Toxiplasmosis, Brucella abortus, Listeria monocytogenes, Mycoplasma pneumoniae, parvovirus, leptospirae). Smooth muscle autoantibodies (SMA), cytoplasmatic antibodies (c-ANCA) and perinuclear antibodies (p-ANCA) were negative. There was, however, a T cell activation with an increased CD4/CD8 ratio and increased neopterine (7.3 ng/ml; norm: 0.1-2.5) as typically observed in autoimmune hepatitis.

The patient was subsequently treated with prednisolone and azathioprine plus ursodesoxycholic acid. The transaminases normalised after three weeks with the exception of GGT and total bilirubin, which normalised within the next four weeks:



The authors consider this case as a proof for the toxicity of synthetic racemic kavain (called "kava") and concluded that was an immune-mediated reaction to kava.

The relevance of this case that involved synthetic kavain for the safety of kava-preparations is limited.

Case 6: A 42-year-old healthy male spent his 20-day honeymoon on the Samoan Islands. He presented 3 weeks later with weakness, loss of appetite, and jaundice. Although he admitted to modest alcohol consumption of 1 drink daily, he denied any medication or illicit drug use. He had florid scleral icterus and jaundice of the skin. His liver span was normal but pain was elicited on palpation. Significant laboratory abnormalities included serum AST 1602, ALT 2841, gamma GTP 121, LDH 420, and alkaline phosphatase 285. The total bilirubin 9.3 mg/dL, mostly direct, eventually rose to 31 mg/dL. He had negative serologies for hepatitis A, B, C, CMV, and EBV. The CBC, coagulation tests, and protein electrophoresis were normal, the serum ferritin of 1531µg/L was elevated, ceruloplasmin was normal but with increased urine copper excretion; genetic testing for hemochromatosis was negative. Pursuit of autoimmune causes of acute liver failure showed negative values for the following antibodies: ANA, anti-smooth muscle, anti-liver/kidney microsomal, anti-soluble liver antigen, and anti-mitochondrial antibodies. Hepatic imaging by abdominal ultrasound revealed a hyperechoic liver with normal biliary ducts and thickened gallbladder wall; one 15 mm lymph node was noted in portal area. Histopathology on the liver biopsy showed infiltration of portal fields with lymphocytes and eosinophilic granulocytes, necrosis of hepatocytes, and swollen Kupffer cells consistent with drug-induced or toxic liver injury. Upon further questioning about his activities, the patient admitted he repeatedly participated in kava ceremonies and consumed a total volume of 2-3 liters of traditional kava preparations. Laboratory tests returned to normal after 36 days (Christl *et al*., 2009).

(RUCAM score calculated by the authors): +10 (causality is highly probable).

Assessor's comments:

The mechanism(s) of hepatotoxicity has not been clearly elucidated. Also the constituent(s) involved are unknown. Human kava hepatotoxicity is considered by some authors a idiosyncratic liver injury, meaning unpredictable, with a variable latency (1 week up to 12 months), dose independency and associated low incidence in humans (Teschke et al., 2008). According to other authors it may also have features of an intrinsic injury type, but futher data are needed to confirm any hypothesis (Frenzel and Tescheke, 2016). The existence of kava HILI is confirmed by a few positive reexposure cases.

Reviews of case reports

Almost all known case reports were assessed by different researchers or expert working groups in order to find a relationship between kava exposure and hepatotoxicity (Schmidt, 2003, Stickel *et al.*, 2003; Teschke *et al.*, 2003; Gruenwald, 2004; WHO, 2007; Teschke *et al*., 2009a, Teschke *et al*., 2009b, Teschke *et al*., 2010, Teschke and Wolff, 2011). The majority of severe adverse effects associated particularly with liver toxicity are derived from the files of the BfArM in Germany.

Schmidt *(*2003) analyses 82 hepatotoxicity cases from different sources: German health authorities - BfArM): 38 reports (excluding double entries), Swissmedic: 5 reports (excluding those also listed in the German case reports), US FDA: 21 reports, UK-MHRA: 4 reports, Health Canada: 3 reports, France (AFSSAPS): 2 reports, Australia TGA: 1 report, EMEA: 1 report (excluding those already mentioned in other categories), medical literature: 5 reports (excluding those already mentioned in other categories), unconfirmed German newspaper stories: 2 reports). The author states that 20 cases are obviously not related to kava intake; in 21 case reports a potentially hepatotoxic concomitant treatment was identified. In seven cases there is considerable doubt concerning the causality of kava, whereas in 31 other cases the available data is too fragmentary for an assessment. That leaves only three cases where a likelihood of hepatotoxic effects by kava can be established, although in two of

these three there were higher dosages and longer-term treatment than recommended. In only one of these case reports was kava taken according to the dosage recommendations of the German Commission E Monograph of no more than 120 mg kavalactones per day for three months or less. Therefore only one case remains. The authors also did a thorough analysis of the hepatotoxic potential of frequently used concomitant medications and a comparison of kava with other treatments for anxiety. They concluded that the hepatotoxic effects of kava intake cannot generally be ruled out but, in comparison with pharmaceutical treatments for stress and anxiety disorders, and in relation to drug intake related hepatotoxicity in general, the risk of adverse liver effects seems to be very low.

WHO investigated the Schmidt's assessment indicating that some cases excluded by Schmidt (considered "unrelated to kava intake") are coded by WHO as "possible". WHO underlined that cases unassessable cannot be classified as "unrelated"(or related). According to Schmidt "*From the cases where a causal relationship seemed probable, an incidence rate of >0.02 cases per one million daily doses is calculated, corresponding to less then one case in 50 million days of application. This incidence calculation is far below the liver risk for diazepam with one case on 472.000 days of application*".

It is well known that the estimated incidence of adverse events based on spontaneous reporting is usually much lower than the true incidence. WHO (2007) pointed out that the true incidence of adverse events related to kava is not known, but can only be ascertained by a proper epidemiological study.

Stickel *et al.,* 2003 analyzed 29 novel cases of hepatitis along with Kava ingestion which occurred between 1990 and 2002 in addition to the seven already published case reports using a clinical diagnostic scale established for adverse hepatic drug reactions. Hepatic necrosis or cholestatic hepatitis were noticed with both alcoholic and acetonic Kava extracts. The majority of the 29 patients and the additional seven published reports were women (27 females, nine males). Both the cumulative dose and the latency to when the hepatotoxic reaction emerged were highly variable. Nine patients developed fulminant liver failure, of which eight patients underwent liver transplantation. Three patients died, two following unsuccessful liver transplantation and one without. In all other patients, a complete recovery was noticed after the withdrawal of Kava.

Another independent analysis of 19 known cases from Germany was published in the peer-reviewed literature in 2003 (Teschke *et* al., 2003). The authors conclude that only two cases were probable kava-associated hepatotoxicities. In addition, 80% of these patients took kava overdoses and or self medicated kava for longer than three months. Most patients were taking concomitant medications with known hepatotoxicity. The authors also analyse discrepancies in the evaluations of cases made by regulatory agencies in Germany (BfArM) and UK (MCA). The authors advise nevertheless, that physicians and patients should be alert to possible hepatotoxic side effects in the course of kava treatment, stop the treatment at first suspicion and begin a careful diagnostic work up ruling out all other causes.

WHO report (2007) assessed 93 case reports that involved different types of extracts. In only 54 of the cases (58%) the type of extract could be identified: ethanolic n=32; acetone n=14; water n= and synthetic n=4. The doses used, expressed as kavalactones were the following: in the acetone extract group the mean dose was 142.7 mg/day; range 70-245 mg/day; in the ethanolic extract group mean dose was 165.8 mg/day; range 30-840 mg/day. The doses for water extracts were very much higher and taking into account that kavalactones content was not known, these cases were excluded from the comparative analysis. 74 (80%) of the reports provided information on duration to onset of the event. The mean for these cases was 111 days, range 6-730 days. 80% fell within 135 days (4.5 months), while 90% fell within 195 days (6.5 months). The median time to onset was 90 days. Only 46 (62%) of known durations were 90 days or less. Hepatic events (as cholestatic and hepatocellular types of liver disorder) were described, but with many of the reports it was not possible to determine the initial type

of injury. Necrosis was described in 16 (57%) of the 28 cases, hepatocellular injury in 8 (29%), cholestatic injury in 7 (25%) and in a further 8 (28%) cases the abnormalities were described as toxic in appearance or typical of drug induced or chemical damage. The presence or absence of concomitant therapy was also included. In 57 (61.3%) of the cases other concomited drugs used might have caused or contributed to hepatic abnormalities while 15 (16%) of the patients had either no other drug, or alcohol, or no other suspect therapy. Overall there were 14 liver transplants and seven deaths. Five of the patients had a positive rechallenge. Three comparisons were made between the different products: (a) acetonic versus ethanolic extracts, (b) acetonic versus synthetic extracts and (c) ethanolic versus synthetic extracts.There was no statistically significant difference in the relative risk of hepatotoxicity between products prepared from acetonic and ethanolic extracts, but the hepatotoxicity with the product prepared from an acetonic extract occurred at approximately six times the rate of that for synthetic products while the hepatotoxicity with products prepared from ethanolic extracts occurred with a relative risk of approximately seven when compared with synthetic products. This difference is statistically significant. WHO concluded that a causal relationship between products derived from acetonic and ethanolic extracts and liver toxicity seems likely. Risk factors indicated by WHO appears to be: the use of organic extracts (that could be related with the use of incorrect plant part), the presence of contaminants (e.g ochratoxins) and the lack of quality standard for the raw materials. (WHO, 2007)

The problems linked to the quality of reports on hepatotoxicity have been considered by Teschke *et al*. in at least four reviews (Teschke *et al.,* 2008, 2009a, 2009b, 2010).

Teschke *et al.*, 2008 reassessed using the CIOMS scale the suspected 26 cases of hepatotoxicity induced by kava preparations. Causality was unassessable, unrelated, or excluded in 16 patients owing to lack of temporal association and causes independent of kava or co-medicated drugs. Low CIOMS scores additionally resulted in excluded or unlikely causality assessments (n=2), leaving a total of eight patients with various degrees of causality for kava ± comedicated drugs. The authors declared that only one out of these eight patients adhered to the regulatory recommendations regarding both daily dose (120 mg kavalactones) and duration of therapy (3 months) and experienced toxic liver injury with a probable causality for kava (CIOMS score = 9). In six cases with kava overdose and/or increased duration of kava treatment causality for kava was possible (n=3) and for kava together with the comedicated drug(s) possible (n=2) or probable (n=1). The austhors concluded that kava taken as recommended is associated with rare hepatotoxicity, whereas overdose, prolonged treatment, and co-medication may carry an increased risk.

Teschke *et al.,* 2009a reassessed the causality of hepatotoxicity by aqueous kava extracts and kava–herbs mixtures using the updated score of the quantitative CIOMS (Council for the International Organizations of Medical Sciences). Causality was established in five patients from New Caledonia, Australia, the United States and Germany for aqueous kava extracts and kava–herbs mixtures (CIOMS scores between 3 and 8). A comparison with 9 patients from Germany and Switzerland with established causality of hepatotoxicity by ethanolic and acetonic kava extracts reveals that the clinical picture in all 14 patients is similar, independently whether aqueous, ethanolic and acetonic kava extracts or kava–herbs mixtures were used. The authors concluded that kava hepatotoxicity occurs also with traditional aqueous kava extracts of the South Pacific islands and thereby independently from ethanol or acetone as chemical solvents, suggesting that the toxicity is linked to the kava plant itself with a possibly low quality of the used kava cultivar or kava plant part rather than to chemical solvents.

The same authors (Teschke *et al.,* 2009b) also analysed 20 cases of suspected kava extract hepatotoxicity reported by BfArM using the updated score of the quantitative CIOMS. The authors concluded that the regulatory information is scattered and selective, and items essential for causality

assessment, such as exclusion of kavain-dependent causes, were not, or only marginally, considered by the regulator. Quantitative causality assessment for kava was possible (n=2; CIOMS scores=3), unlikely (n=12), or excluded (n=6), showing no concordance with the regulatory ad hoc causality evaluation. Later they also assessed causality in 26 patients from Germany and Switzerland, using two structured quantitative analytical methods: the system of Maria and Victorino (MV) and that of the CIOMS. In all 26 patients (that took either ethanolic or acetonic extract), regulatory ad hoc evaluation had suggested a causal relationship between liver disease and kava use. Assessment with the MV scale resulted in no or low graded causality for kava in the 26 patients with liver disease. Causality was probable (n=1), possible (n=2), unlikely (n=7), and excluded (n=16). Causality for kava was more evident with the CIOMS scale: highly probable (n=1), probable (n=2), possible (n=6), unlikely (n=2) and excluded (n=15). The authors concluded that the results of both quantitative causality assessments are not supportive for most of the regulatory ad hoc causality assessments of the 26 patients. Grades of causality for suspected hepatotoxicity by kava were much lower when evaluated by structured quantitative causality assessment scales than by regulatory ad hoc judgements (Teschke *et al.,* 2010).

Assessor's comments: Only MV data were provided; CIOMS scores are missing.

Additional information received form National Competent Authorities (market overview)

Adverse events were also mentioned by Member States even for products not authorized anymore.

Czech Republic reported the following adverse reactions:

Kava-kava extractum siccum, extraction solvent acetone 75% (m/m): allergic skin reactions such as redness, swelling, pruritus(rare); gastrointestinal disorders (very rare); during long-term treatment, yellowish colouration of the skin and skin adnexa (nails, hair) may occur. In only one case the liver damage occurred after the use of the product, which was reversible and completely resolved after discontinuing the treatment.

Kava-kava extractum siccum, extraction solvent ethanol 96% (V/V): allergic skin reactions (rare); general allergic reactions, dyspepsia (very rare). In long term treatment, yellow colouration of skin and skin adnexa may occur.

Taking into account the case reports of hepatotoxicity, the Federal Institute for Drugs and Medical devices (BfArM) implemented the German graduated plan ("Stufenplan") since August 2015, which includes the following recommendation:

- medical prescription only for preparations containing kava-kava

- clear indications: mild to moderate severe generalized anxiety disorders; depression is not an indication

- maximum daily dose corresponing to 200 mg of kavalactones

- package size limited to 30 daily doses

- usual duration of therapy: 1 month, maximum 2 months

- determination of liver parameters (AST and ALT) before the treatment and once a week thereafter

- avoidance of concomitant medication with potentially hepatotoxic medications, especially beta-blockers, antidepressants and anti-migraine preparations. Caution in the consumption of alcohol.

Literature

ESCOP 2003: extrapiramidal side effects were reported on four patients (mechanism unknown); hypersensitivity reactions resulting in generalized rash and severe itching was reported in one-case after 3 weeks of treatment with a daily dose of 120 mg kava extract.

Gruenwald *et al*., 2004: rare cases of allergic reactions and gastrointestinal complaints; slight morning tiredness can appear at the begining of the therapy; dyskinesia and choreoathetosis of limbs, trunk, neck and facial musculature have been reported; endocrine effect (weight loss), musculoskeletal effects (minor inhibition of movement and impaired motor reflexes).

On the basis of the available data the frequency is not assessable. So the frequency is not known.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

No data available.

5.5.2. Contraindications

Kava preparations are contraindicated in patients with endogenous depression because it increases the risk of hepatotoxicity in association with antidepressants. It is also contraindicated during pregnancy and lactation (Gruenwald *et al.,* 2004, WHO 2004) and existing liver disease and alcohol abuse (ESCOP, 2003).

5.5.3. Special warnings and precautions for use

Unusual fatigue, weakness or loss of appetite and unintended weight loss, yellow discolouration of the conjuctiva or of the skin, dark urine or colourless stool can be signs of damage to the liver.

Concomitant use with beta-blockers, antidepressants and anti-migraine preparation should be avoided (Gruenwald *et al.,* 2004).

5.5.4. Drug interactions and other forms of interaction

There is limited clinical evidence regarding interactions with other drugs, even that theoretically, based on preclinical studies, kava preparations may interact with CNS depressants or psychoactive agents. The clinical evidence of drug interactions was reviewed by Anke *et al*., 2004 that found only 3 clinical case-reports and concluded that the evidence for true interactions is poor. Following interactions were summarized by Anke *et al*.: coma followed by interaction with alprazolam (one case-report; no data regarding kava dose); reduced effectiveness of levodopa (one case-report; 300 mg kava extract/day, 10 days) and rhabdomyolysis after association with caffeine (one case-report; 100 mg kava preparation as single dose).

Kava aqueous extract (1g/kg) potentiated sedation and impairment of cognition/co-ordination when combined with alcohol (Anke *et al*., 2004; Gruenwald *et al*., 2004; Foo, 1997).

Other studies (Herberg, 1992; Herberg, 1993; Herberg, 1997) revealed that preparation WS1491 (300 mg/day) is not interacting with low quantity of alcohol ingested (0.05% in blood).

5.5.5. Fertility, pregnancy and lactation

No data available.

ESCOP monograph (2003) considered that, in accordance with general medical practice, the kava preparations should not be used during pregnancy or lactation without medical advice while WHO monograph contraindicated the use during pregnancy (WHO, 2004).

Taking also into account preclinical data, kava preparations should be contraindicated in pregnant women or during lactation.

5.5.6. Overdose

The traditional ceremonial drinking of kava beverages in the South Pacific can be considered as an overdosage in relation to therapeutic doses of kava preparations. Various effects were noticed in heavy kava drinkers, including pellagroid dermatopathy, loss of body weight, up to 20% or yellow colouration of the skin and finger/toe nails (ESCOP, 2003).

Kava intoxication is characterized by specific abnormalities of movement coordination and visual attention but normal performance of complex cognitive functions (Cairney *et al.*, 2002, 2003).

Other effects reported by isolated cases of intoxication with kava traditional beverages are: (1) visual effects such as reduced near point of accommodation, increased pupil diameter and disturbed oculomotor balance were noted in a male subject (with no previous experience of kava-kava) who ingested 600 ml of kava-kava beverage in 15 minutes (Garner & Klinger, 1985); (2) rhabdomyolysis associated with ingestion of large amount of kava in a male subject of 34-years old (Bodkin, 2012); (3) an acute neurological syndrome involving generalized choreoathetosis was reported three times in the same patient as symptom of acute intoxication from excessive drinking of kava beverage (ESCOP, 2003).

There are no reported cases of overdosage with kava extracts.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

According to WHO monograph (2004) that took into account Commission E monograph (Blumenthal *et al*., 1998) when administered within the recommended dosage range, motor reflexes and the ability to drive or operate heavy machinery may be adversely affected by kava preparations. But according to ESCOP monograph (2003) kava did not impair the ability to drive or to operate machines. Recently, in a randomized, placebo-controlled, double- blind study of 22 adults aged between 18 and 65 years were tested with a driving simulator after being randomly administered a dose of kava dry aqueous extract (corresponding to 180 mg of kavalactones), oxazepam (30 mg), or placebo one week apart in a crossover design trial. No impairing effects on driving outcomes were found after kava administration compared to placebo. Results on specific driving outcome domains revealed that the oxazepam condition had significantly slower braking reaction time compared to the placebo condition ($p=0.002$) and the kava condition ($p=0.003$). The kava condition had significantly fewer lapses of concentration compared to the oxazepam condition ($p=0.033$). No significant differences were found between conditions for steering deviation, speed deviation, and number of crashes. Results were not modified by driving experience. On the Bond-Lader visual analogue sub-scale of alertness, a significant

Treatment × Time interaction (p=0.032) was found, with a significant reduction over time for oxazepam decreasing alertness (p<0.001), whereas no significant reduction was found in the kava or placebo conditions (Sarris *et al.,* 2013c).

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

The clinical trials conducted on kava preparations were not designed or powered to pick-up adverse reactions, such as rare hepatic reactions. In the trials that included the assessment of liver function the only effect observed was the raised liver enzyme levels, which cannot be considered as an evidence of hepatotoxicity. The total number of the patients involved in clinical trials is significant (more than 8000 patients), while only 509 were involved in trials that assessed of liver function. This is suggesting that the data available is inadequate to exclude hepatotoxicity. The true incidence of hepatotoxicity is unknown and can only be ascertained by a proper epidemiological study.

Some signals regarding suspected adverse reactions associated with kava use are included in the VigiLyze database. Liver toxicity (such as hepatic enzyme increased, hepatitis, hepatic cirrhosis, jaundice) is reported but the lack of the type of extract administered and the single dose used limited a possible causality assessment. Underreporting in the Vigilyse database must also be taken into consideration.

The main signals of herbal induced liver injury (HILI) are derived from single case reports. Up to July 2002 WHO identified worldwide 93 cases of suspected hepatotoxicity associated with the use of different kava preparations, including cases of liver failure resulting in 6 liver transplants and 3 deaths.

The causality assessment was performed using the WHO scale, developed by WHO Collaborating Centre for International Drug Monitoring. Of the 93 cases, eight cases (8.6%) were coded by WHO having a "probable" causality and 53 as "possible". These cases led to the withdrawal of the marketed products in Member States due to safety concerns.

Also in UK, an expert working group investigated the safety signals (in total 110 cases of adverse liver reactions of which 9 cases with a fatal outcome) and determined that kava was associated with an unacceptable risk of hepatotoxicity which could not be minimised or prevented by any regulatory measures other than the removal of kava products from the market.

After 2002 a few new case reports have been published: two cases of acute liver injury (RUCAM scores: + 5 and + 6) and one case of toxic hepatitis (RUCAM score: +10) all being associated with ingestion of traditional kava preparations, one case of icteric hepatitis associated with an unknown preparation (RUCAM score: + 7) and one case of acute liver failure and death after the use of one combination containing a kava preparation (RUCAM score: +8). Even excluding two cases (that involved unknown preparation and a combination), still there are new cases where the causality was demonstrated.

The small number of new case reports that did not involved EU preparations could be correlated with the measurements took by different EU-Member states, such as Czech Republic, France, Spain, UK, Hungary, Germany (up to 2015) and Portugal that revoked the authorisations for kava products.

There is limited clinical evidence regarding interactions with other drugs, even that theoretically, based on preclinical studies, kava preparations may interact with CNS depressants or psychoactive agents.

Kava preparations are contraindicated in patients with endogenous depression because it increases the risk of hepatotoxicity in association with antidepressants. Kava preparations are also contraindicated during pregnancy and lactation, in existing liver diseases and alcohol abuse.

6. Overall conclusions (benefit-risk assessment)

Even that the medicinal use of Piperis methystici rhizoma is documented in several medicinal handbooks, the medicinal products were withdrawn from EU national markets (in Czech Republic, France, Spain, UK, Hungary and Portugal) since 2002 based on safety concerns. BfArM also revoked in 2002 the market authorizations for kava kava-containing products, but a court decision from 2015 cancelled the revocation of marketing authorizations for the ethanolic preparations.

Carcinogenicity studies provided sufficient evidence in experimental animals for the carcinogenicity of one kava preparation. In mice, the preparation caused a significant dose-dependent increase in the incidence of hepatoblastoma in males. This preparation is included by NTP in group 2B, meaning sufficient evidence in experimental animals. The relevance of such findings for humans cannot be excluded and constitute a cause for safety concerns.

The clinical trials available for Piperis methystici rhizoma preparations as treatment option for anxiety disorders, as generalised anxiety or anxiety in the climacteric phase have methodological weaknesses, such as: mixed anxiety population, short duration of the trials, short follow-up phase, no data regarding percentage of responders. There are many differences in the products studied (acetonic or ethanolic extracts, different DERs, sometimes synthetic compounds), studies design and the dosage administered that was reported either in milligrams of kavalactones or in milligrams of kavain. An important signal of kava induced liver injury in humans came from the spontaneously reported cases. There are cases of suspected hepatotoxicity associated with the use of different kava preparations, including cases of liver failure resulting in liver transplants and deaths. These cases led to the withdrawal of the marketed products in some Member States due to safety concerns.

The HMPC/MLWP concluded that, based in the available data and for the establishment of a European Union herbal monograph on traditional or well-established herbal medicinal product containing Piperis methystici rhizoma, the benefit-risk balance for the oral use of Piperis methystici rhizoma for the treatment of anxiety disorders is unfavourable and that the following requirements are not fulfilled:

- the requirement laid down in Article 16a(1)(e) of Directive 2001/83/EC that the data on the traditional use of the medicinal product are sufficient; in particular the product proves not to be harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience.

- the requirement laid down in Article 10a of Directive 2001/83/EC that the active substance has a recognised efficacy and an acceptable level of safety and that the period of well-established medicinal use has elapsed.

In conclusion, based on the above-mentioned information, the HMPC concluded that a European Union herbal monograph on Piperis methystici rhizoma cannot be established.

If new information on clinical safety and efficacy of Piperis methystici rhizoma were to be made available, such documentation may be re-assessed by the HMPC.

Annex

List of references