

# **KAVA REPORT 2003**

## **IN-DEPTH INVESTIGATION INTO EU MEMBER STATES MARKET RESTRICTIONS ON KAVA PRODUCTS**

### **PART II B**

#### **Expert Report on the Pharmacological-Toxicological Documentation of Kava Kava (*Piper methysticum*)**

Prepared for

CDE

Centre for the Development of Enterprise

Brussels, Belgium

March 2003

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## A Profile of Kava kava

### a) Qualitative composition

According to the phytotherapeutic definition, the total extract of the rhizome of *Piper methysticum* (Piperaceae) is regarded as the active ingredient.

### b) Mode of action

*Piperis methystici* rhizoma acts anxiolytic. In animal experiments a potentiation of narcosis (sedation), anticonvulsive, antispasmodic, and central muscular relaxant effects were described. An interaction with GABA-receptors and an inhibition of platelet MAO-B is being discussed [8, 31, 50].

The effects of Kava-Kava are not immediate but become apparent after about two weeks of treatment.

The main effective components of *Piperis methystici* rhizoma are the kava pyrones. The six major kava pyrones are kavaine, dihydrokavaine, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin [3, 11, 12, 17, 21, 24, 38, 49].

### c) Preparations used in medicine

Kava is traditionally prepared by grinding and mixing the root or root bark with cold water. This makes an emulsion that is a suspension of the resinous constituents in water. The herb is also prepared as an emulsion in coconut milk. The efficiency of extraction of the active constituents, which is measured by kavalactones extraction into water, varies considerably but is higher from fresh material than from the dried plant. Kava consumed in Vanuatu is reputed to be the strongest anywhere in the South Pacific. The bioavailability of kava constituents varies substantially, depending on the method of extraction. It is thought that the increase in potency is partly the result of preparing the drink from the raw fresh roots, whereas in Fiji and elsewhere, it is made from dried rootstocks (Lebot et al., 1997). In Europe Kava is predominantly available as a so-called concentrated standardized extract that is designed to maximize extraction of kavalactones. The extraction is performed either by ethanol or acetone and the content of kavalactones used in kava containing products range from 10 to 70 %. Comparisons of traditional water-based South Pacific kava preparations with those alcoholic extracts used in Europe showed that the European preparations contain considerably lower doses of kavalactones than the traditional forms.

d) Indications

In the monograph of piper methysticum, the German Commission E recommended the use of kava kava in conditions of nervous anxiety, stress, and restlessness.

e) Dosage

Extracts kava kava are commonly standardised to its content of kavapyrone, which have been identified as its main active ingredients. In the monograph for kava radix the German Commission E recommends a daily dosage of 60 to 120 mg of kavapyrone for the treatment of conditions of nervous anxiety, stress, and restlessness. In the past also higher dosages up to 280 mg of kavapyrones were frequently used in medicinal practice.

## B Expert Report

### 1. Introduction

In 1990 the German Commission E of the former BGA (Bundesgesundheitsamt; today: BfArM) published a positive monograph on the root from *Piper methysticum* (kava) based on extensive scientific material. Since then the efficacy of extract of *Piperis methystici* rhizoma has been investigated in various pharmacological-toxicological studies. The monograph recommends *Piperis methystici* rhizoma for treatment of “conditions of nervous anxiety, stress, and restlessness”. According to Commission E, kava acts “anxiolytic. In animal experiments a potentiation of narcosis (sedation), anticonvulsive, antispasmodic, and central muscular relaxant effects were described.” [7].

In June, 2002 the BfArM (German Federal Institute for Drugs and Medical Devices) issued an official letter immediately revoking the Market authorisation of kava containing products, including homoeopathic preparations up to a concentration of D4. In this letter the BfArM declared that this measure is based upon a revised benefit-risk ratio, mainly resulting from 37 suspected cases of severe adverse effects on liver function. The institute stated that after an evaluation of research data the efficacy of kava kava cannot be regarded as approved, and that a high risk of “severe, life threatening adverse effects on the liver” has to be expected with its intake.

The purpose of this Expert Report is to critically review and objectively cite or summarize all available published relevant data concerning the pharmacology and toxicology of preparations containing *Piperis methystici* rhizoma (kava) extract. This Report carried out by the independent expert will only summarize and evaluate relevant published data concerning kava and based upon this information draw conclusions and draft recommendations. It will not comment or judge the decision taken by the officials of the German Federal Institute for Drugs and Medical Devices, or health authorities of other countries, nor it will refer to it.

In paragraph 5. (Conclusions) the expert will briefly summarize the results of the available pharmacological and toxicological data, evaluate them, and state his conclusions on the **pharmacodynamics** (paragraph 5.1.), **pharmacokinetic** (paragraph 5.2.), **toxicity** (paragraph 5.3.) of kava kava and give a brief statement of his **expert opinion** (paragraph 5.4.).

Several working groups have identified various substances that are believed to be responsible for the pharmacological actions of kava extract.

Detailed analysis began in the early sixties. However, kava preparations cannot be regarded as a new active substance because its preparations have been used traditionally for several hundred years as well as on thousands of patients for many decades. For this reason the "Proposal for a Note for guidance on non-clinical testing of herbal drug preparations with long-term marketing experience" (EMA/HMPWG/11/99) can be applied.

The Report was drawn up on the basis of published literature relevant to kava kava or kava-containing products. The results of literature searches in the database DIMDI and the private database Phytodok, Berlin, Germany, have been used up to January 2003. Articles in "peer review" journals have also been used as well as official documents not having been published in Journals, if they were of great importance.

## 2. Pharmacodynamics

### 2.1 Active ingredients

Analysis of kava root extracts has yielded a spectrum of chemical components with pharmacological activities. The leading active substances for identification and standardisation are the kavapyrones. They are considered to be the active ingredients of *Piperis methystici* rhizoma. So far 18 kavapyrones have been isolated [26], six of which constitute the major and pharmacologically important components: kavaine, dihydrokavaine, methysticin, dihydromethysticin, yangonin, and desmethoxyyangonin. The amount of the single pyrones varies according to the origin of the drug. Chemically, the six major kavapyrones belong to two slightly different categories: kavaine, dihydrokavaine, methysticin, and dihydromethysticin have only one double bond in the pyrone ring and are therefore called enolids, yangonin and desmethoxyyangonin have two double bond in the pyrone ring, which makes them dienolids. The slightly different chemical structure leads to minor differences in pharmacodynamical effect of kavaine, dihydrokavaine, methysticin, and dihydromethysticin on the one hand, and yangonin and desmethoxyyangonin on the other [3, 11, 12, 17, 20, 21, 24, 38, 49].

### 2.2 Special pharmacodynamics

#### 2.2.1 Sedative effects

The sedative action of kava was demonstrated in several animal trials: Kava was shown to decrease spontaneous activity.

One of the earliest studies on the pharmacological effects of kava investigated its sedative effects on mice and rats. Dihydrokavaine and dihydromethysticin were administered via gastric probe at dosages of 100 to 200 mg/kg. The animals fell asleep after approximately 20 min [19].

Another study substantiated the idea that the kavapyrones are responsible for the sedative action of kava. In rabbits, yangonin (10 and 15 mg/kg bw) and dihydromethysticin (20 and 30 mg/kg bw) increased the threshold of the EEG arousal reaction during electric stimulation of the *Formatio reticularis mesencephali* by 0.3 – 0.4 V (yangonin) and 0.2 – 0.5 V (dihydromethysticin), respectively. The effects had a duration of 30 – 60 min (yangonin) and 20 – 45 min (dihydromethysticin). The kavapyrones seem to be capable of reducing the excitation of the limbic system; this effect is similar to meprobamate and the benzodiazepines.

The inhibition of the limbic system activity can be regarded as an expression of the depression of emotional commotion as well as an increase in “spirit” [36].

In a further study, however, neither D,L-kavaine nor a kava extract changed the thresholds of the EEG arousal reaction. The study was conducted on unrestrained cats ( $n = 26$ ) with chronically implanted electrodes. Blood pressure, the EEG of cortical and subcortical areas, the electromyogram, EEG arousal reactions, and subcortical evoked potentials elicited by central stimulation were recorded. This was done before and after injection of D,L-kavaine (10 – 50 mg/kg i.p.) or, for comparison, kava extract in arachis oil (50 – 100 mg pyrones/kg i.p.). Only the extract exerted marked effects on the EEG; it induced high amplitude delta waves, spindle-like formations, and a continuous 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-kavaine (50 mg/kg;  $p < 0.05$ ) and in those given the extract (100 mg pyrones/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. The authors concluded from these findings that the limbic structures and, in particular, the amygdalar complex represent the preferential site of action for both D,L-kavaine and the kava extract [27].

The sedative action of a kava extract (standardized fraction of kavapyrones: 7 %) and D,L-kavaine was examined in two further experiments performed on mice using two models: evaluation of spontaneous locomotor activity and of amphetamine-induced hypermotility. Male Swiss mice (Nossan) weighing 20 – 25 g were used and divided into groups of four animals. Their spontaneous motility was recorded in an activity cage (Basile, Milan, Catalogue No. 7400). To avoid differences in behaviour resulting from the circadian rhythm of the animals, all experiments were performed in the morning and at the same time. All animals were injected i.p. with kava extract (3.1, 6.2, 12.5, 25.0, 50.0, 100.0 and 200.0 mg/kg), D,L-kavaine (12.0, 100.0 and 200.0 mg/kg) or with a mixture of these drugs in a 10 ml/kg solution (extract : kavaine = 1 : 0.12; Harras Pharma, Munich). D,L-kavaine was available in pure form, a mixture was prepared containing this compound. The compounds under study were dissolved in arachis oil. The controls received the same amount of excipients (arachis oil) by the intraperitoneal route. I.p. injections were administered immediately before the determination of spontaneous motility.

To evaluate the effect on amphetamine-induced hypermotility, the drugs under study were administered one hour prior to the measurements (kava extract: 100 mg/kg, D,L-kavaine: 12.0 mg/kg, combination extract : kavaine 100.0 : 12 mg/kg). Amphetamine (Sigma Tau, Rome) was given s.c. as sulphate in doses of 5 mg/kg.

This compound was dissolved in a 0.9 % saline solution and administered immediately before determination of motility. Motility was recorded over a period of two hours. Measurements were carried out at ten-minute-intervals.

After administration of kava extract alone, a significant reduction in spontaneous motility was seen from 50 mg/kg onward ( $p < 0.01$ ). A dose response relationship was observed with higher doses. While 200 mg/kg of D,L-kavaine failed to cause a significant sedative action, the sedation produced by the mixture of the extract and kavaine was significantly more pronounced than the effect of the extract alone: the data clearly establish that the sedative action of the kava extract had been potentiated by otherwise ineffective doses of D,L-kavaine. The results of the studies concerning amphetamine-induced hypermotility show an obvious synergism between the kava extract and D,L-kavaine. While the administration of 100 mg/kg of the extract and 12 mg/kg of kavaine alone were found to be ineffective, hypermotility was significantly reduced by 60.2 % following injection of a mixture of these drugs [5].

The same working group later conducted a very similar experiment. In order to investigate experimentally a possible synergism between a kava extract and a *Passiflora* extract, both were tested individually and in combination in a controlled pharmacological study with mice. The effects on amphetamine-induced hypermotility and on barbiturate-sleeping-time were studied. Male Swiss mice (Nossan, Corrazzana, Milano, Italy) with body weights of 20 – 25 g were used. The experiments were performed according to GLP guidelines. The animals were housed in cages, temperature:  $23 \pm 2^\circ \text{C}$ , air humidity:  $60 \pm 10 \%$ . The artificial lighting was turned on and off at 12-hour intervals. Food and water were given *ad libitum*. The dosage of the kava spissum extract (Harras-Pharma-Curarina) was 100 mg/kg, administered by gastric tube, in the test for amphetamine-induced hypermotility, and 200 mg/kg i.p. for the examination of barbiturate-sleeping-time (*Passiflora* spissum extract dosage: 50 mg/kg, administered by gastric tube, for amphetamine-induced hypermotility, 100 mg/kg i.p. for examination of barbiturate-sleeping-time; the same dosages were used for the combination). The animals were randomly divided into three treatment groups and one control group for each pharmacological test ( $n = 10$  per group). The plant extracts were dissolved in macrogol 400 (Hoechst, Germany). The volume administered in each experiment was 10 ml.

All control animals were given the same quantity of vehicle. In order to avoid differences due to circadian rhythm, all experiments were performed at the same time. 5 mg/kg of amphetamine were administered s.c. as sulphate (Sigma Tau, Rome). The substance was dissolved in a physiological saline solution. 10 ml/kg of the solution were given immediately before the measurement of motility.

The dissolved extracts were given separately or together via gastric tube one hour before the amphetamine injection in order to ensure that enteral absorption of the extracts had occurred prior to the measurements. Spontaneous motility was recorded using an activity cage (Basile, Milano, Italy). Amphetamine-induced hypermotility was registered over a two-hour period and the data was recorded every 30 min. Sodium pentobarbital (Sigma Tau) was dissolved in physiological saline and administered s.c. at a dosage of 40 mg/kg corresponding to an injection of 10 ml/kg. In order to assess effects on sleeping time, the extracts were administered i.p., separately or together, 10 minutes before administering the barbiturate. Sleeping time was determined as the time between loss and recovery of the animal's righting reflex.

Both the viscous kava extract (100 mg/kg) and the viscous *Passiflora* extract (50 mg/kg) caused a distinct decrease in amphetamine-induced hypermotility compared with the control groups. This effect was more pronounced with kava ( $p < 0.001$ ) than with *Passiflora* ( $p < 0.05$ ; 54 % and 17 %, respectively, after an observation period of 120 min). The greatest reduction was achieved when the two extracts were administered in combination (71 % after 120 min). A distinct prolongation of the barbiturate-sleeping-time was observed after administration of kava spissum extract (200 mg/kg), *Passiflora* spissum extract (100 mg/kg) and after administering the extract combination. This effect was more pronounced (prolongation of the mean sleeping time by 80 % compared to control) in the group which received the extract combination. The *Passiflora* spissum extract caused a prolongation of 40 %, the kava spissum extract caused a less pronounced prolongation of 19 %. Both increases were statistically significant compared to placebo. The kava extract, administered alone or in combination with the *Passiflora* extract, exhibited a marked sedative effect in both the amphetamine-induced hypermotility and barbiturate-sleeping-time experimental models [6].

The central nervous activity of an aqueous extract of kava was examined in mice, and compared to the effect of a lipid-soluble extract. In contrast to the lipid extract, the aqueous extract – free of pure  $\alpha$ -pyrones, the major constituents of the lipid extract – had so far received little pharmacological attention. Balb-c male mice were used, weighing 18 – 30 g with no greater than 5 g variation within each particular test.

Mice were allowed food and water *ad libitum* and all experiments were carried out at an ambient temperature of 20 – 22° C. Dried aqueous extract dissolved in saline was injected i.p. in a volume of 0.1 ml/10 g mouse, the tested dosages were 250 (n = 10), 125 (n = 10) and 62.5 mg/kg (n = 18). The lipid preparation of kava was injected in the same manner at dosages of 120 (n = 26), 150 (n = 26), 180 (n = 8) and 250 mg/kg (n = 3). Control mice (same number per group) were similarly injected with saline (0.9 % NaCl).

For the test of spontaneous motility a Varimex photocell activity meter (Columbus Instruments, Ohio) was used with a 5 min test period. A significant decrease 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 lipid-soluble kava 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 [30].

### 2.2.2 Central muscular relaxant effects

The six major components of kava, the kavapyrones, are responsible for the effect of kava extract on muscle tone.

An early study demonstrated that the kavapyrones are the pharmacologically active constituents of the kava root. The spectrum of central nervous activity of these compounds was found to be closely related to that of mephenesin type centrally acting skeletal muscle relaxants: ascending paralysis with ataxia and loss of postural and righting reflexes without loss of consciousness, abolition of the tonic extensor component of experimentally induced convulsions, blockade of spinal and supraspinal polysynaptic reflexes while monosynaptic arcs were totally resistant. The tonic stretch reflex in the unanaesthetized animal was shown to be very sensitive to the depressant action of the kavapyrones, the action of which was three times stronger than that of mephenesin [41].

To clarify the reasons for the pharmacological actions of kava the effect of the isolated pyrone constituents kavaine, methysticin, dihydromethysticin and yangonin on the skeletal muscle tone of unanaesthetized rabbits, electromyographically measured from the calf muscle during periods of a constant flexion of a paw, were studied. All kavapyrones showed a strong centrally caused muscle-relaxing activity.

Yangonin proved to be the most potent kavapyrone: 5 – 10 mg/kg i.v. almost completely depressed the EMG impulses. 2 – 3 times larger dosages of the other pyrones showed the same effect [37].

To investigate the neurophysiological effects of one of the components of kava, D,L-kavaine, a study was conducted on unrestrained cats (n = 26) with chronically implanted electrodes. Blood pressure, the EEG of cortical and subcortical areas, the electromyogram, EEG arousal reactions, and subcortical evoked potentials elicited by central stimulation were recorded.

This was done before and after injection of D,L-kavaine (10 – 50 mg/kg i.p.) or – for comparison – kava extract in arachis oil (50 – 100 mg pyrones/kg i.p.). With both D,L-kavaine and the extract, muscle tone was seen to be diminished in approx. 50 % of the experiments [27].

The central nervous activity of a pyrone-free aqueous extract of kava was examined in Balb-c male mice (18 – 30 g) and compared to the effect of a lipid-soluble extract. Mice were allowed food and water *ad libitum* and all experiments were carried out at an ambient temperature of 20 – 22° C. Dried aqueous extract dissolved in saline was injected i.p. at a volume of 0.1 ml/10 g mouse, the tested dosage was 250 mg/kg (n = 10 and n = 8 for vertical grid and rotating drum, respectively). The lipid preparation of kava was injected in the same manner at dosages of 120, 150, 180 and 250 mg/kg (all groups n = 6). Control mice (n = 10 and n = 8 for vertical grid and rotating drum, respectively) were similarly injected with saline (0.9 % NaCl). The mice were placed on a vertical grid and the number of mice unable to remain on the grid was recorded. The other mice were similarly tested on a rotation drum, and scored for their ability to remain in the equilibrium position on top of the rotating drum which rotated at 3 rev/min for 1 min. Scoring was as follows: 0 = mouse remains in equilibrium position throughout the test; 1 = mouse carried completely around on drum for 1 revolution; 2 = mouse revolves twice; 3 = mouse revolves 3 times, or revolves once and falls off once; 4 = mouse falls off twice; 5 = complete inability to cling to grid on rotation drum. All control mice could grip and remain on the grid inclined to the vertical position. The aqueous kava extract did not interfere with the ability of the mouse to grip the inclined grid and did not depress the ability of mice to remain in the equilibrium position on the rotating drum. All mice receiving 120 mg/kg of the lipid extract were able to grip the grid, of those receiving 150 mg/kg, one could not hold onto the grid. 180 and 250 mg/kg of the lipid extract significantly counteracted the efforts to cling to the grid (p < 0.005). A dose of 120 mg/kg slightly decreased the performance of mice in the rotating drum test.

When the dosage of the lipid-soluble kava extract was increased to 150 mg/kg, the ability of mice to remain in the equilibrium position was markedly impaired with mice either revolving around with the drum revolution or falling off the drum. At a dose of 180 mg/kg, most mice were unable to remain on the drum in any position and at 250 mg/kg the effect was maximal. A very steep dose-response relationship was apparent for the lipid extract. The effects appeared very rapidly (within 5 min after i.p. injection), decreased within 1 hour with full recovery of muscle tone and activity occurring within 3 hours, even at the highest dosage [30].

In a recent study the effect of the synthetic kavapyrone ( $\pm$ )-kavaine on evoked contractile activity on isolated guinea-pig ileum was investigated. ( $\pm$ )-kavaine (1  $\mu$ M – 1 mM) dose-dependently reduced contractions of ileum evoked by carbachol (10  $\mu$ M), by BAY K 8644 (0.3  $\mu$ M), or by substance P (0.05  $\mu$ M). ( $\pm$ )-kavaine 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  $\mu$ M nifedipine, carbachol (1  $\mu$ M) evoked  $18.2 \pm 14.3$  % of contraction at control (i.e. prior pre-incubation with nifedipine). This remaining response was completely abolished by high concentrations of ( $\pm$ )-kavaine (400  $\mu$ M). After treatment of the longitudinal ileum strips with pertussis toxin (PTX), carbachol (1  $\mu$ M) evoked  $27.0 \pm 6.2$  % of the control response in untreated ileum. These contractions were also blocked by ( $\pm$ )-kavaine in a concentration of 400  $\mu$ M. However, ( $\pm$ )-kavaine had no effect on the caffeine-induced (20 mM) contractions of ileum strips, which were permeabilized with digitonin or  $\beta$ -escin. Moreover, it failed to affect  $Ca^{2+}$ -evoked contractions of skinned muscles. These results suggest that the kavapyrone ( $\pm$ )-kavaine may act in a non-specific musculotropic way on the smooth muscle membrane [48].

### 2.2.3 Anticonvulsive action

Several studies were dedicated to the investigation of the capability of kava extracts and kavapyrones to antagonize chemically or otherwise induced convulsions.

One of the earliest studies concerning the anticonvulsive action of *Piper methysticum* investigated the effects of the ground root of kava, a chloroform extract obtained therefrom, and of several kavapyrones on the central nervous system 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. Male and female Carworth mice (18 – 22 g) received the materials to be tested p.o. in 10 % Tween suspension.

A single i.p. injection of 2.6 mg (LD<sub>90</sub>) of strychnine sulphate was given 15 min after medication. If the material protected the animals against strychnine-induced clonic convulsion and death, graded doses of the compound were tested and an ED<sub>50</sub> was calculated according to LICHFIELD and WILCOXON. For the preliminary testing, 10 mice were used. The ED<sub>50</sub> was determined by using at least four groups of 10 mice each, at four different dose levels. Yangonin offered no protection even at 1,000 mg/kg. The ED<sub>50</sub> of dihydrokavaine was 340 mg/kg, the ED<sub>50</sub> of kavaine was 215 mg/kg. Methysticin, dihydromethysticin and the chloroform extract had ED<sub>50</sub> values of 160, 115 and 140 mg/kg, respectively, thereby demonstrating the best results.

“Compound A” showed no protection at a dosage of 200 mg/kg. Of the ground root, 1,700 mg/kg were needed. The time of peak action was determined for dihydromethysticin and was found to be approx. 60 min. An indication of synergistic action was found by testing a mixture of kavaine (19.5 %), dihydrokavaine (33.4 %), methysticin (19.5 %), dihydromethysticin (5.5 %), yangonin (16.6 %) and “compound A” (5.5 %). These compounds were combined in the ratio in which they were isolated from the crude extract. The mixture showed an ED<sub>50</sub> of 100 mg/kg, indicating a potency at least equivalent to that of dihydromethysticin. Since the latter represented only 5.5 % of the mixture, and since the other constituents were less potent or inactive, a synergistic effect of the mixture appears to be very likely [33].

Another study investigated the qualitative and quantitative effect of kavapyrones on electroconvulsion and compared the results to the effects of convulsant and spasmolytic substances (phenobarbital, primidone, diphenylhydantoin, ospolote; chlorpromazine). Male mice (20 – 25 g) were used and given *ad libitum* access to food and water. The electric shock was initiated by a stimulation generator (NETHELER & HINZ, Hamburg, Germany). In all trials rectangular impulses with a frequency of 60 Hz were used. Dihydromethysticin and dihydrokavaine were applied i.p. in concentrations of 0.1 – 2.0 % in arachis oil. Diphenylhydantoin, primidone and ospolote were also suspended in arachis oil. Phenobarbital-Na and chlorpromazine were dissolved in physiological saline solution. The lowest effective dosage of dihydromethysticin was 25 mg/kg and produced a convulsive threshold elevation of 33.1 %. The lowest effective dosage of dihydrokavaine 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 % ( $p \approx 0.01$ ). After the application of 40 mg/kg dihydromethysticin the elevation was 100 %. The same effect was demonstrated by 150 % dihydrokavaine. 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. This substance lowered the threshold maximum to 20 mg/kg (65.5 %).

Higher doses acted anticonvulsive: after the application of 100 mg/kg chlorpromazine a maximal spasm could be induced in only 6 out of 20 animals. The effect of dihydromethysticin and dihydrokavaine was comparable to that of the known anticonvulsive substances phenobarbital, primidone, or diphenylhydantoin. However, the threshold doses of those substances were 5 – 10 mg/kg lower than those of the two kavapyrones. Nevertheless, dihydromethysticin and dihydrokavaine demonstrated a strong anticonvulsive action in the mouse electroshock model at dosages which did not seem to impair mouse behaviour [44].

The same working group also conducted a study of the effect of dihydromethysticin and dihydrokavaine on chemically induced convulsions. Again, male white mice weighing 20 – 25 g were used. All convulsant substances were diluted in physiological saline solution and injected s.c.: pentetrazol (0.5 %, n = 20), bemegride (0.2 %, n = 20), picrotoxin (0.03 %, n = 10), and strychnine (0.005 and 0.01 %, n = 10 each). Dihydromethysticin and dihydrokavaine were administered i.p. in an arachis oil suspension 30 min before injection of the respective convulsant. The animals were observed for 1 – 3 hours. Effectivity was measured as the percentage of animals demonstrating maximal tonic spasm (extension spasm of front and back legs) after a single application of the convulsant and after pre-treatment with 30 mg/kg of dihydromethysticin or dihydrokavaine. 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, exclusively anticonvulsive against tonic picrotoxin and strychnine induced spasms as well as spasms induced by electroshock (data from an earlier study by the same working group, [44]). 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.

The only exception was strychnine; animals did not show any convulsion symptoms after pre-treatment with 40 mg/kg or higher doses of dihydromethysticin. Dihydrokavaine showed very similar qualitative effect, but differed quantitatively; the effective doses were 2 – 3 times higher than those of dihydromethysticin. Further investigations into the effect of different doses of dihydromethysticin on different doses of strychnine revealed that the kavapyrone remarkably antagonizes the convulsant and lethal effects of strychnine. Convulsions were completely absent in animals protected by doses of up to 100 mg/kg; with high doses of dihydromethysticin there was a seizure syndrome of long periods of generalized clonic convulsion with delayed onset and only brief intervals. In preventing death from 4 mg/kg strychnine phenobarbital was more effective than dihydromethysticin [40].

Another study demonstrated the anticonvulsive action of the other important kavapyrones methysticin, kavaine, yangonin, and desmethoxyyangonin. The pyrones were administered i.p. or p.o. in an arachis oil suspension to male mice (20 – 27 g, n = 10 – 20; 0.2 – 0.5 ml/20 g). For the i.v. injections the pyrones were dissolved in polyethyleneglycol 300 (maximal 0.06 ml/20 g). The anticonvulsive effect was 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 pyrones 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 kavaine 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 pyrones, 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.v., the differences between the effective doses of the investigated pyrones were only slight, whereas the differences found were greater when given i.p. or p.o.

The effective dosage of desmethoxyyangonin and yangonin in particular were higher than those of the other pyrones. When administered i.p. and p.o., the anticonvulsant activity decreased in the following sequence: methysticin > dihydromethysticin ≈ kavaine > dihydrokavaine >> desmethoxyyangonin > yangonin [34].

The same working group thoroughly investigated the above-mentioned effect of kavapyrones against strychnine poisoning and local tetanus. The kavapyrones methysticin, dihydromethysticin, kavaine, and dihydrokavaine all showed a marked antagonistic effect in male albino mice (20 – 27 g, n = 10 – 30 per group) on the convulsant and lethal effect of strychnine. The convulsant was dissolved in physiological saline solutions sulphate and applied s.c. at a standard dosage of 0.4 ml/20 g. The kavapyrones were administered i.p. in an arachis oil suspension 30 min before the strychnine injection. Concentrations were 0.2 – 0.5 ml/20 g. Phenobarbital was dissolved in physiological saline solution and also administered prior to strychnine injection. Mephenesin, also dissolved in saline solution (1 %), was administered 1 min before the injection. Concerning the local tetanus, 4 rabbits (2.5 – 3.5 kg) received 1000 MLD (minimal lethal dose in mice) of tetanustoxin i.m. The results concerning the strychnine poisoning indicate that methysticin is the most potent compound as far as the minimal effective dose and the absolute limit of activity against increasing doses of strychnine are concerned. The minimal effective dose of methysticin amounted to 15 – 20 mg/kg, corresponding to approx. 1/25 of the LD<sub>50</sub>. Pre-treatment of animals with the maximal effective dose of methysticin (300 mg/kg) increased the LD<sub>50</sub> of strychnine 5 – 6 times (up to doses of 10 mg/kg s.c.). except for dihydromethysticin, the other pyrones were only a little less effective than methysticin. Dihydromethysticin had the lowest absolute limit of activity against strychnine. Kavaine and dihydrokavaine had the smallest dosage range, because their protective effect became apparent only with higher doses, close to the intrinsic toxic activity of these drugs. There was no reciprocal antagonism between the pyrones and strychnine. The pyrones had a higher protective activity against strychnine poisoning than mephenesin or non-ataxic resp. non-anaesthetic doses of phenobarbital. Except for dihydromethysticin, 2 – 4 mg/kg of strychnine were completely antagonized by the pyrones, which otherwise did not influence the behaviour of the animals. In contrast, aequi-effective doses of mephenesin and phenobarbital provoke ataxia and paralysis. Low doses of up to 150 mg/kg (methysticin, dihydromethysticin) and 200 mg/kg (kavaine, dihydrokavaine) completely prevented convulsions due to up to 4 – 5 mg/kg strychnine. Higher doses modify the convulsion due to strychnine like mephenesin and phenobarbital, producing a characteristic seizure syndrome with long periods of general violent clonic convulsions in remarkably brief intervals and with high frequency. Methysticin also has anti-tetanus activity.

Its action on the electromyographically registered muscle activity of *M. gastrocnemius* with local tetanus was found in the same range as mephenesin, but showed a longer lasting effect [35, 42].

A more recent study concentrated on the anticonvulsive properties of the kavapyrone methysticin using *in vitro* seizure models. The experiments were performed on 84 transverse temporal lobe slices containing the neocortical areas Te2 and Te3, the perirhinal cortex, the entorhinal cortex (EC), the hippocampal formation with the dentate gyrus, areas CA3 to CA1 and the subiculum. The temporal lobe slices were prepared from 130 – 180 g female Wistar rats deeply anaesthetized with ether prior to decapitation. Control experiments in male rats and in rats of both sexes anaesthetized with barbiturates did not yield different results.

Recordings were obtained with double-barrelled  $K^+$  or  $Ca^{2+}$  selective/reference microelectrodes or with 150 mM NaCl filled pipettes. The  $K^+$  selective microelectrodes were manufactured with the  $K^+$  sensitive Fluka 60031 cocktail and the  $Ca^{2+}$  sensitive microelectrodes with the FLUKA cocktail A21048. Electrode positions were usually in the entorhinal cortex layer IV/V and stratum pyramidale of hippocampal area CA1 or CA3. The epileptiform activity started 20 – 80 min after commencing with the perfusion of the seizure inducing ACSF (artificial cerebrospinal fluid). Methysticin was dissolved in DMSO and added to the epileptogenic ACSF solution to yield final concentrations varying between 1 – 100  $\mu$ M. The drugs were applied 5 – 45 min after onset of SLEs (seizure-like events) in the EC. Measurements of short recurrent epileptiform activity in area CA3 were frequently recorded as late as 2 hours after onset of epileptiform activity. The same applied to low  $Ca^{2+}$  induced seizures in the hippocampal area CA1. Tests on late recurrent discharges in the EC were made at between 20 min and 3 hours after onset of this type of activity. Elevating  $[K^+]_0$  induced seizure-like events with tonic and clonic electrographic phases in area CA1. Lowering  $[Ca^{2+}]_0$  caused recurrent seizure-like episodes with large negative field potential shifts. Lowering  $Mg^{2+}$  induced short recurrent discharges in area CA3 and CA1 while ictiform 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 ictiform events in the subiculum, entorhinal and temporal neocortex changed their characteristics after 1 – 2 hours to late recurrent discharges. In a concentration range from 10 – 100  $\mu$ M methysticin reversibly blocked all these types of epileptiform activity. Decreases in  $[Ca^{2+}]_0$  and associated slow field potentials evoked by repetitive stimulation of the stratum radiatum or the alveus remained mostly 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 affects different patterns of epileptiform activity by possibly interfering with processes responsible for frequency potentiation [47].

To further characterize the anticonvulsive action of the kavapyrones, the influence of ( $\pm$ )-kavaine, a synthetic kavapyrone, on veratridine-stimulated increase in intrasynaptosomal  $\text{Na}^+$  concentration ( $[\text{Na}^+]_i$ ) of cerebrocortical synaptosomes of adult male Wistar rats (180–220 g) was investigated.  $[\text{Na}^+]_i$  was measured spectrofluorometrically employing sodium-binding benzofuranisophthalate (SBFI) as  $\text{Na}^+$  sensitive fluorescence dye. Veratridine (5  $\mu\text{mol/l}$ ) enhanced basal  $[\text{Na}^+]_i$  6.6-fold from 11.3 to 74.1  $\text{mmol/l Na}^+$ . Incubation of synaptosomes for 100 sec with ( $\pm$ )-kavaine was sufficient to dose-dependently reduce the stimulated increase of  $[\text{Na}^+]_i$  with an  $\text{IC}_{50}$  value of 86.0  $\mu\text{mol/l}$ , and almost complete inhibition of  $\text{Na}^+$  channels was attained with 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine. The reference compounds, procaine (400  $\mu\text{mol/l}$ ) and tetrodotoxin (TTX, 10  $\mu\text{mol/l}$ ) reduced veratridine-elevated  $[\text{Na}^+]_i$  to 30.4 % and 7.9 % of control whereas the centrally acting muscle relaxant mephenesin (400  $\mu\text{mol/l}$ ) was without any effect. Postapplication of 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine or 10  $\mu\text{mol/l}$  TTX immediately diminished veratridine-elevated  $[\text{Na}^+]_i$  to nearly basal levels with a half life of 69.7 and 41.8 sec, respectively. To study the influence of ( $\pm$ )-kavaine on non stimulated synaptosomes, an increase in  $[\text{Na}^+]_i$  was induced by 200  $\mu\text{mol/l}$  ouabain, which enhanced  $[\text{Na}^+]_i$  hyperbolically with an initial rate of 18.4  $\text{mmol Na}^+/\text{1 min}$ . Preincubation of synaptosomes with 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine or 10  $\mu\text{mol/l}$  TTX prevented  $\text{Na}^+$ -influx to the same extend for both compounds, approx. 57 % of the control. The authors came to the conclusion that the data presented indicates a fast and specific inhibition of voltage-dependent  $\text{Na}^+$  channels by ( $\pm$ )-kavaine [13].

The question of how the excitability of neurons is affected during the anticonvulsive action of kavapyrones was investigated by determining the interaction of ( $\pm$ )-kavaine with epitopes of voltage-dependent  $\text{Na}^+$  channels and the actions of ( $\pm$ )-kavaine on 4-aminopyridine-stimulated synaptosomes as a model of firing neurons. The experiments were conducted with synaptosomes of adult male Wistar rat (180 – 22 g) cerebral cortex. [ $^3\text{H}$ ]saxitoxin and [ $^3\text{H}$ ]batrachotoxin were used for radioligand-binding assays performed with synaptosomal membranes.

Glutamate released from 4-aminopyridine-stimulated cerebrocortical synaptosomes and the cytosolic concentrations of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ( $[\text{Na}^+]_i$ ,  $[\text{Ca}^{2+}]_i$ ) were detected fluorometrically by using an enzyme-linked assay, sodium-binding benzofuranisophthalate (SBFI) and Fura-2, respectively. ( $\pm$ )-kavaine failed to compete with [ $^3\text{H}$ ]saxitoxin up to 400  $\mu\text{mol/l}$  but dose-dependently suppressed binding of [ $^3\text{H}$ ]batrachotoxin with an  $\text{IC}_{50}$  value of 88  $\mu\text{mol/l}$  ( $K_i = 72 \mu\text{mol/l}$ ) although displacement of [ $^3\text{H}$ ]batrachotoxin was restricted to 33 % of the control at 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine.

In stimulated synaptosomes, 5 mmol/l 4-aminopyridine provoked an increase in  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$  by 9 mmol/l  $\text{Na}^+$  and 235 nmol/l  $\text{Ca}^{2+}$ . Comparable to the reduction in [ $^3\text{H}$ ]batrachotoxin binding, 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine suppressed the increase in  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$  to 38 and 29 % of control, respectively. Consistent with the increase in  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$ , 5 mmol/l 4-aminopyridine provoked glutamate release (rate: 38 pmol/s\*mg protein), which was dose-dependently diminished to 60 % of the control by 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine. KCl depolarization (40 mmol/l) provoked an increase in  $[\text{Ca}^{2+}]_i$  and glutamate release almost identical to the response elicited by 4-aminopyridine but 400  $\mu\text{mol/l}$  ( $\pm$ )-kavaine suppressed only the rate of glutamate release by 9 % of the control. The authors concluded that the data suggests an interaction of ( $\pm$ )-kavaine with voltage-dependent  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels, thereby suppressing the 4-aminopyridine-induced increase in  $[\text{Na}^+]_i$ ,  $[\text{Ca}^{2+}]_i$  and the release of endogenous glutamate [15].

#### 2.2.4 Analgesic action

Kava has traditionally been used as an analgesic. The underlying pharmacological mechanisms have been investigated in several animal trials.

An early study with 45 male mice weighing 18 – 20 g investigated the protective action of the kavapyrones dihydrokavaine and dihydromethysticin against heat-induced pain. Heat radiation was directed to the mice's tails and the time was measured from the onset of radiation until the mouse removed the tail or twitched. Reaction times were measured at 10-min intervals 5 times before and 5 times after application of the tested substance. Untreated mice showed a medium reaction time of 5 – 6 sec. Dihydrokavaine and dihydromethysticin were suspended in arachis oil (1 %) and injected i.p. at body temperature in doses of 80 (n = 50), 100 (n = 20), 120 (n = 50) and 180 mg/kg (n = 50). The reference substances morphine (2.5 mg/kg, n = 30), aminopyrine (100 mg/kg, n = 30), acetylsalicylic acid (200 mg/kg, n = 30) and caffeine (20 mg/kg, n = 30) were dissolved in physiological saline solution and injected s.c.

The minimal effective dose of dihydrokavaine was 100 mg/kg ( $p \approx 0.001$ ), 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. However, 140 mg/kg were able to induce a significantly prolonged reaction time. Maximum efficacy was reached after 1 hour, the effect lasted for 2 hours. Concerning the whole course of time, 140 mg/kg dihydromethysticin were less effective than 120 mg/kg dihydrokavaine.

The maxima differ significantly ( $p < 0.05$ ). The dose-effect-relationship of dihydrokavaine 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 dihydrokavaine 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 dihydrokavaine with the antipyretic drugs aminopyrine or acetylsalicylic acid indicated that there is an additive synergism in the analgesic potency of these mixtures. Simultaneous application of caffeine diminished the duration of analgesic activity in both dihydrokavaine and dihydromethysticin considerably without affecting the peak effect [4].

The same working group investigated the local anaesthetic action of several kavapyrones shortly afterwards. Surface analgesia properties were studied using rabbit cornea (16 tests à 6 animals). For the infiltration analgesia studies guinea-pigs were used ( $n = 144$ ). The effects were compared with those of cocaine and procaine, which were applied in 0.9 % saline solution. The pyrones were dissolved in arachis oil, as it did not influence corneal or skin reflexes. Each concentration was tested on 6 rabbits or guinea-pigs. Concerning the rabbit cornea, kavaine and dihydrokavaine proved to be more effective than methysticin and dihydromethysticin, yangonin was completely ineffective at a 1 % solution (higher doses could not be tested due to insolubility). Kavaine acted similar to cocaine concerning the concentration that induced total anaesthesia and duration of effect. The pyrones were less effective in regard to infiltration analgesia in both intensity and duration of effect. Nevertheless, the sequence of efficacy was the same: kavaine > dihydrokavaine > methysticin > dihydromethysticin >> yangonin. All the kavapyrones tested, with the exception of yangonin, acted as local analgesics [43].

A later study conducted by the same working group confirmed the data concerning infiltration analgesia. Male and female guinea pigs weighing 175 – 400 g were used.

The kavapyrones were suspended in arachis oil and different dosages were injected i.c. (25 ml) into hairless back skin. Each concentration was tested on 6 animals. The animal's reactions to skin contact with a blunt cannula on six different regions of the urtica (short contraction of back skin muscles) were recorded 1 min after injection. The efficacy of the various concentrations was calculated from the ratio of the number of non-responses to the number of responses. The ED<sub>50</sub> of kavaine was 0.36 g%, of dihydrokavaine 0.48 g%, of methysticin 0.36 g% and of dihydromethysticin 0.58 g%. The ED<sub>50</sub> of yangonin could not be calculated due to insolubility, but must be higher than soluble concentration of 0.7 % [42].

The antinociceptive activity of a pyrone-free aqueous extract of kava was examined in Balb-c male mice (18 – 30 g). Mice were allowed food and water *ad libitum* and all experiments were carried out at an ambient temperature of 20 – 22°C. Dried aqueous extract dissolved in saline was injected i.p. in a volume of 0.1 ml/10 g mouse, the tested dose was 250 mg/kg for the hot plate test (n = 20) and the tail immersion method (n = 12). The extracts were given 10 min before testing. The effects of the aqueous extract on tail withdrawal reaction time were compared to the effects of a lipid-soluble extract. The lipid preparation of kava was injected in the same manner at dosages of 150 mg/kg (n = 8). Control mice (n = 20 for the hot plate test and n = 12 and 8, respectively, for the tail immersion method) were similarly injected with saline (0.9 % NaCl). The aqueous extract was tested using the hot plate (heated to 56°C) method with the hind limb flick as the endpoint (WOOLFE and McDONALD 1944, CARMODY 1983). If an animal failed to react within 50 sec, it was removed from the hot plate and a 50 sec score was recorded. Both aqueous and lipid extract were tested using the tail immersion method. The mouse tails were immersed to a depth of approx. 2 cm in water at 48°C, and the time from tail immersion to removal of the tail from the water was recorded (JUSZKIEWICZ-DONSBACH and LEVY 1962). If there was no reaction by 60 sec, the mouse was removed and a score of 60 sec was recorded. In the hot plate test the aqueous extract produced a significant, albeit slight (approx. 30 %), prolongation in the time to react to the hot plate from  $10.3 \pm 1.0$  sec to  $13.4 \pm 1.3$  sec ( $p < 0.05$ ). In the tail immersion test the aqueous extract also induced a significant and greater (84 %) prolongation of the time taken to react to the noxious stimuli: from  $10.0 \pm 1.1$  sec to  $18.8 \pm 2.8$  sec ( $p < 0.025$ ). The lipid extract proved to be even more effective than the aqueous extract, the withdrawal reaction time was prolonged from  $19.7 \pm 2.3$  sec to  $49.7 \pm 6.8$  sec ( $p < 0.001$ ). Two of the 12 mice that received the aqueous extract did not react within the 60 sec maximum time period allowed in this test (scored as 60 sec in statistical analysis). Five of the mice that received the lipid extract failed to remove their tails by 60 sec.

Again, the response was recorded as 60 sec for the purpose of statistical analysis, but obviously the resulting  $49.7 \pm 6.8$  sec is a considerable underestimation. No control mice failed to react within the allotted time period [30].

The same working group extended these initial observations on antinociceptive actions with a detailed study of the time course of action of the aqueous and lipid soluble extracts, and of the purified pyrones derived from the lipid soluble extract of kava. Two distinct types of antinociceptive tests involving different pathways and receptors were used: the tail immersion method (cutaneous thermal receptors) and the abdominal constriction procedure (chemical visceral receptors). Male Balb-c mice of 20 – 25 g bodyweight were used for all experiments.

For the tail immersion test the mice were held lightly, the tails immersed to a depth of 2 – 3 cm in a 48° C water bath and the time between tail immersion and removal of tail from the water was recorded (JUSZKIEWICZ-DONSBACH and LEVY 1962, [30]). If no reaction occurred within 60 sec the test was terminated and a score of 60 sec recorded. As the results for this test were found to be considerably more consistent when animals underwent a familiarization session before drug testing (including all trials with kavapyrones), the adopted protocol for the later tests was to carry out four tail immersions at 15 min intervals on one day, then test the control (vehicle-injected) and kava-treated mice for their reaction times 24 hours later. All drugs were injected i.p. in a volume of 0.1 ml/10 g bodyweight. Mice treated with the lipid kava extract or purified pyrones were first tested 10 min after injection, then at 5 – 10 min intervals during the first 40 min, and at 10 – 30 min intervals thereafter, depending upon the time course of effect of the particular component, until the analgesic effect was no longer evident. Mice given aqueous extract (250 mg/kg, n = 12) were first tested 30 min after administration, then at 15 min intervals for 90 min. For the abdominal constriction (writhing) test, mice were injected with 0.1 ml/10 g bodyweight of 0.8 % acetic acid and the number of distinct writhes, which occurred from 5 to 15 min following acetic acid administration, was recorded. As the vehicle itself inhibited writhing when administered i.p. close to the time of acetic acid injection, the lipid kava extract was administered p.o. in a dose of 200 mg/kg in a volume of 0.065 ml/10 g 1 minute prior to acetic acid injection. Control mice received an equivalent volume of the corresponding vehicle (5 % Cremefor-EL in saline). Aqueous kava, orally inactive in mice ([30]), was injected at a dose of 250 mg/kg i.p. (0.1 ml/10 g) 55 min prior to acetic acid injection.

The lipid extract (150 mg/kg, n = 20) had a marked antinociceptive action, which was evident at the first test time of 10 min after injection and lasted for about 80 min ( $p < 0.005$ ).

Because several mice in the kava group failed to respond within 60 sec for each test, these results are an underestimate. 120 mg/kg failed to produce significant analgesia at 10, 20, 30 or 40 min post-injection. The aqueous extract had a less pronounced antinociceptive effect ( $p < 0.05$ ). Of the pyrones tested it was found that kavaine, dihydrokavaine, methysticin and dihydromethysticin have potent analgesic properties. The peak analgesic effect was similar for the four active pyrones, but the duration of action markedly differed. The action of dihydrokavaine was very rapid but short lived (20 – 30 min), that of kavaine 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 the aqueous and the lipid soluble extracts were effective in inhibiting the number of writhes induced by acetic acid injection. Oral administration of 200 mg/kg lipid extract ( $n = 18$ ) effectively reduced writhing ( $p < 0.001$ ). Aqueous extract is inactive orally, but dramatically reduced writhing when given i.p. in a dose of 250 mg/kg 55 min before acetic acid ( $p < 0.001$ ). The results demonstrate that both the aqueous and lipid soluble extracts of kava have distinct antinociceptive activity, as did four of the kavapyrones from the lipid soluble extract [28].

### 2.2.5 Effect on psychosis models

Some of the effects of kava indicate a possible antipsychotic action.

The aqueous, pyrone-free extract from kava and the lipid-soluble extract were tested for their effect on conditioned avoidance response behaviour in rats in a shelf-jump apparatus. Naive female Wistar rats of approximately 150 g weight were used in these experiments. Rats were placed in the shelf-jump apparatus (Lafayette Instrument Co. Model 85200) and a protocol (similar to BEER and LENARD 1975) was followed. This schedule consisted of: 1) 5 sec – door to shelf opens; 2) 5 sec – shelf remains open; 3) 5 sec – shock, registering 2 mA, applied to foot bars; 4) 5 sec – shock off, shelf remains exposed; 5) 5 sec – door closes, rat pushed off shelf; 6) 25 sec – rest period. A total of 60 trials were carried out with each rat. Results of the first 10 trials were discarded and the subsequent 50 trials recorded. If drug treatment impaired the escape response to such a degree that a rat failed to show an escape response more than 7 times, the animal was eliminated from the experiment.

Experimental groups were set up as follows: Group 1: animals injected i.p. with 2 dosage levels of chlorpromazine (2 mg/kg and 2.5 mg/kg) 1 hour prior to testing; group 2: animals injected i.p. with 2 dosage levels of haloperidol (0.1 mg/kg and 0.5 mg/kg) 1 hour prior to testing; group 3: animals injected i.p. with aqueous kava extract at dosages of 30, 50, 90, 120, 250 and 500 mg/kg and tested 1 hour later; group 4: animals injected i.p. with kava resin at dosages of 50, 100, 125 and 150 mg/kg and tested 10 min later. Saline-treated rats were interspersed with drug-treated rats in each group to act as controls.

Prior to the testing of the kava extracts, the experimental conditions were standardized using the two classical antipsychotic drugs, the phenothiazine chlorpromazine and the butyrophenone haloperidol. It was found that chlorpromazine (2 mg/kg) inhibited the acquisition of the conditioned response in an average of  $32.4 \pm 3.5$  conditioned avoidance responses, compared to  $44.5 \pm 1.2$  (out of a possible 50) in the corresponding controls. Raising the dosage to 2.5 mg/kg produced a further reduction to  $25.5 \pm 5.3$  correct responses.

The treatment had a significant effect ( $p < 0.01$ ). Somewhat similar results were obtained for haloperidol: a dosage of 0.1 mg/kg was sufficient to depress the conditioned responses to  $31.3 \pm 4.7$  compared to  $44.0 \pm 1.9$  of the controls ( $p < 0.001$ ). The dosages administered, from 30 – 500 mg/kg of aqueous kava extract, showed that conditioned avoidance responses were not significantly affected in this dose range. The lipid kava extract was quite effective. The conditioned avoidance response was significantly affected by the administration of the extract ( $p < 0.0005$ ). At 50 and 100 mg/kg the lipid extract had no effect. When the dose was increased to 125 and 150 mg/kg, however, the treated rats showed more errors in the conditioned avoidance test than control animals. 125 mg/kg caused a slight (18 %) but significant loss of the conditioned avoidance response, with a very low (0.3 %) incidence of failure to escape ( $p < 0.05$ ). A greater effect could not be demonstrated, as it was not possible to increase the dosage without causing severe sedation and ataxia. A dose of 150 mg/kg caused such loss of motor control at 10 min that the animals were unable to react to the shock, thus severely affecting the escape response at this time. The depression in conditioned avoidance behaviour was only marginally greater (21 %, incidence of failure to escape: 0.5 %;  $p < 0.01$ ) than it was after a dose of 125 mg/kg. The authors suggest the possibility, from the range of activities produced by lipid-soluble kava extract, that an effect on conditioned avoidance response is mediated via an action on GABA or benzodiazepine receptors [10].

To reveal the neuronal functions affected by the kavapyrones, their actions on the mesolimbic reward system were recently studied using *in vivo* microdialysis. Male Wistar rats weighing 220 – 300 g were used. Under anaesthesia a guide cannula was implanted into the brain of rats mounted into a stereotaxic device (David Kopf Instruments, USA). The tip of the guide cannula was lowered into the *Nucleus accumbens*. After 72 hour recovery, individually held, the microdialysis probe was inserted through the guide cannula under a light diethylether anaesthesia and connected to a microinjection pump. This probe was continually perfused with artificial cerebrospinal fluid (aCSF) for 20 hours before the experiment commenced. The flow rate was 2.0 µl/min and 20 min fractions were collected. A small dose of kava extract (20 mg/kg 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, low doses of D,L-kavainee induced a decrease in dopamine levels and higher amounts produced either an increase or no change in dopamine concentrations.

Yangonin resulted in a decrease in dopamine levels to below the detection limit and desmethoxyyangonin in an increase in dopamine levels. Dihydrokavaine, methysticin and dihydromethysticin did not produce any significant changes in dopamine levels. D,L-kavaine caused a decrease in 5-HT concentrations. Some of the other kavapyrones slightly affected 5-HT as well. The results suggest that the relaxing and slightly euphoric actions of kava may be caused by the activation of the mesolimbic dopaminergic neurones [46].

### 2.2.6 Effect on GABA and benzodiazepine binding sites

Only a few investigations have been conducted on the neuropharmacological interactions of kava with the CNS. The results obtained are being discussed controversially.

In one of the first studies on the neuropharmacological interactions of kava with CNS receptors purified pyrones and lipid-soluble kava extract were tested for their activity on GABA and benzodiazepine binding sites in rat (Sprague-Dawley) and mouse (Balb-c) brain membranes. Only weak activity was observed on GABA<sub>A</sub> binding sites in washed synaptosomal membranes prepared from rat brain and this was abolished by extraction of the membranes with Triton X-100, suggesting that lipid-soluble components were involved. No effects were observed on GABA<sub>B</sub> binding sites in rat brain membranes *in vitro*. The kava extract and the kavapyrones exerted some weak effects on benzodiazepine binding *in vitro* but this did not correlate with pharmacological activity.

In addition, in *ex vivo* studies, no effects were observed on [<sup>3</sup>H]diazepam binding to brain membranes prepared from mice in which selected kava constituents were injected i.p., whereas similarly administered diazepam (5 mg/kg) inhibited [<sup>3</sup>H]diazepam binding by > 95 %. Similar lack of activity was observed in *in vitro* binding studies; injection of kava extract failed to influence the CNS binding of the benzodiazepine-receptor ligand [<sup>3</sup>H]Ro15-1788 injected into mice prior to being sacrificed. In conclusion, the authors are of the opinion that the pharmacological activities of lipid-soluble kava extract and kavapyrones do not appear to be explained by any significant interaction with GABA or benzodiazepine binding sites [8].

Regional differences in the modulation of [<sup>3</sup>H]muscimol binding to the GABA<sub>A</sub> receptor complex by kavapyrones were demonstrated in another study. Membrane fractions from target brain centres of kavapyrone action – hippocampus (HIP), amygdala (AMY) and medulla oblongata (MED) – , and from brain centres outside the main kavapyrone targets, such as the frontal cortex (FC) and cerebellum (CER), were used. The kava extract enhanced the binding of [<sup>3</sup>H]muscimol in a concentration-dependant manner with maximal potentiation of 358 % over control in HIP followed by AMY and MED, i.e. the main target brain centres.

Minimal stimulation was observed in the CER followed by the FC. EC<sub>50</sub> values ranged between 200 and 300 µM kavapyrones. Scatchard analysis revealed that the observed effects of the pyrones were due to an increase in the number of binding sites (B<sub>max</sub>) rather than to a change in affinity. At a pyrone concentration of 500 µM the order of enhancement in B<sub>max</sub> was HIP = AMY > MED > FC > CER. When the pyrones were administered together with pentobarbital or the pregnane steroid HPO the two components produced a more than additive, i.e. synergistic effect on [<sup>3</sup>H]muscimol binding. According to these authors, as opposed to those above, the findings suggest that one way kavapyrones might mediate sedative effects *in vivo* is through effects on GABA<sub>A</sub> receptor binding [31].

### 2.2.7 Inhibition of platelet MAO-B

To obtain more information on the mechanisms by which kava exerts psychotropic properties, the *in vitro* effects of a kava extract and pure synthetic kavapyrones on human platelet MAO-B was investigated and compared to amitriptyline, imipramine and brofaromine. The kava extract was found to be a reversible inhibitor of MAO-B in intact platelets (IC<sub>50</sub> 24 µM) and disrupted platelet homogenates (IC<sub>50</sub> 1.2 µM). Structural differences of kavapyrones resulted in a different potency of MAO-B inhibition. The order of potency was desmethoxyyangonin > (±)-methysticin > yangonin > (±)-dihydromethysticin > (±)-dihydrokavainee > (±)-kavainee.

The two most potent kavapyrones, desmethoxyyangonin and ( $\pm$ )-methysticin, displayed a competitive inhibition pattern with mean  $K_i$  0.28  $\mu$ M and 1.4  $\mu$ M, respectively. The authors suggest that the inhibition of MAO-B by kavapyrone-enriched extracts might be an important mechanism for their psychotropic activity [50].

## 2.3 General pharmacodynamics

### Neuroprotective effect

Neuronal hyperexcitation in the course of cerebral ischemia may lead to neuronal necrosis. Various studies indicate that components capable of blocking neuronal stimulation protect brain tissue against damage. Anticonvulsants reduce brain injury caused by cerebral ischemia. The components of kava, kavainee, dihydrokavainee, methysticin, dihydromethysticin and yangonin, were tested as to whether they provide protection against ischemic brain damage. A model of focal cerebral ischemia in mice and rats was applied. Male NMRI mice (Savo, Kissleg, Germany) weighing 30 – 40 g and male Fischer 344 rats (Charles River Wiga, Sulzfeld, Germany) weighing 250 – 300 g were used ( $n = 10$  per group). The animals had free access to food and water and were kept in a standard environment. Ischemia was induced by microbipolar coagulation of the left middle cerebral artery (MCA).

To quantify the size of the lesions in mice, the area of the infarct on the brain surface was assessed planimetrically 48 hours after MCA occlusion by transcatheterial perfusion of carbon black. In the rat model infarct volume was determined 48 hours after MCA occlusion by planimetric analysis and subsequent integration of the infarct areas on serial coronal slices. The kava extract (WS 1490, containing 70 % kavapyrones) was titrated with a mixture of polyethyleneglycol 400 and water (20 : 80) to give a milky emulsion which was administered p.o. Compounds (kavainee, dihydrokavainee, methysticin, dihydromethysticin, and yangonin) were dissolved in neutral oil and injected i.p. Controls received only vehicle. The effects of the kava extract and its constituents were compared with those produced by a typical anticonvulsant, memantine. Memantine was dissolved in saline and injected i.p. The kava extract, methysticin, and dihydromethysticin produced effects similar to those of the reference substance, memantine. The kava extract (150 mg/kg, 1 hour before ischemia) diminished the infarct area in mouse brains ( $p < 0.05$ ) and the infarct volume in rat brains ( $p < 0.05$ ). 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 ( $p < 0.05$ ; 10 mg/kg of methysticin:  $p < 0.01$ ).

All other compounds failed to produce a beneficial effect on the infarct area in mouse brains. The authors concluded that the neuroprotective activity exhibited by the kava extract was probably mediated by its constituents methysticin and dihydromethysticin [1].

The influences of tetrodotoxin (TTX) and ( $\pm$ )-kavainee on anoxic rat brain vesicles (adult male Wistar rats weighing 200 – 350 g) were investigated with respect to lactate synthesis, vesicular ATP content and cytosolic free  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ( $[\text{Na}^+]_i$ ,  $[\text{Ca}^{2+}]_i$ ), both of the latter determined fluometrically employing SBFI and FURA-2, respectively. Each hemisphere of brain, without the cerebellum, was homogenized in 15 ml of homogenization buffer. The homogenate was centrifuged at 450 g for 5 min and the resulting supernatant was recentrifuged at 5000 g for 5 min to obtain pellets with a protein content of 9 mg, which were stored on ice until measurement. ( $\pm$ )-kavainee was dissolved in dimethylsulphoxide (DMSO) as a 200-fold stock solution. The final concentrations of solvents and drugs amounted to 0.5 % DMSO, 500  $\mu\text{mol/l}$  ( $\pm$ )-kavainee, 10  $\mu\text{mol/l}$  veratridine (for additional stimulation) and 10  $\mu\text{mol/l}$  TTX. The  $\text{Na}^+$  channel blockers TTX and ( $\pm$ )-kavainee, if applied before anoxia, preserved vesicular ATP content, diminished anoxia-induced increases in  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$  and prevented both the veratridine-induced increases of  $[\text{Na}^+]_i$  and  $[\text{Ca}^{2+}]_i$  and the inhibition of lactate production. According to the authors, ( $\pm$ )-kavainee may be of interest as a lead compound for a new class of unchanged  $\text{Na}^+$  channel blockers directed against ischemic insults [16].

#### Antithrombotic action

(+)-kavainee was investigated regarding its assumed antithrombotic action on human platelets which was deduced from its ability to suppress arachidonic acid (AA)-induced aggregation, exocytosis of ATP, and inhibition of cyclooxygenase (COX) and thromboxane synthase (TXS) activity, the latter two effects being estimated from the generation of prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) and thromboxane  $\text{A}_2$  ( $\text{TXA}_2$ ), respectively. Platelet-rich plasma, prepared from whole blood of apparently healthy volunteers, was anti-coagulated with 1/9 volume citrate-citric acid dextrose by centrifugation at 100 g for 15 min at room temperature. Platelets of the supernatant were sedimented by centrifugation at 1000 g for 10 min. The pellet was washed twice with citrate-buffer by centrifugation at 555 g for 10 min. The final pellet was resuspended in citrate-buffer to obtain a cell titer of about  $10^9$  platelets/ml. (+)-kavaine and AA were dissolved in dimethyl sulphoxide (DMSO) to obtain stock solutions of 80 mmol/l (+)-kavaine and 20 mmol/l AA. The final concentration of DMSO in the platelet suspension amounted to 1 % (v/v). (+)-kavaine was applied to the platelet suspension 5 min before the addition of AA (100  $\mu\text{mol/l}$ ).

Challenge of platelets with AA induced an absolute aggregation of almost 90 % within approx. 3 min of its application. The application of (+)-kavaine dose-dependently suppressed the aggregation but even at concentrations of 400  $\mu\text{mol/l}$  failed to completely prevent it. (+)-kavaine dose-dependently diminished the release of ATP ( $\text{IC}_{50}$  necessary amounted to  $78 \pm 45 \mu\text{mol/l}$ ) as well as the formation of  $\text{PGE}_2$  ( $\text{IC}_{50}$  was  $115 \pm 35 \mu\text{mol/l}$ ) and suppressed the generation of  $\text{TXB}_2$  (detected as a representative of  $\text{TXA}_2$ ) dose-dependently with an  $\text{IC}_{50}$  of  $71 \pm 27 \mu\text{mol/l}$ . According to the authors, the similarity of the  $\text{IC}_{50}$  values suggest an inhibition of COX by (+)-kavaine as primary target, thus suppressing the generation of  $\text{TXA}_2$  which induces aggregation of platelets and exocytosis of ATP by its binding on  $\text{TXA}_2$ -receptors [14].

#### Fungistatic action

In an early study aqueous extracts of kava have demonstrated fungistatic activities. The highest inhibition of growth was achieved against the skin pathogen *Trichophyton ferrugineum*. The likewise pathogen species *Trichophyton tonsurans* and *Cryptococcus neoformans* were also affected by the kava extract, whereas *Candida*-species and *Fusarium solani* remained largely unaffected. The fungistatic principles of the rhizomes are the 4-methoxy- $\alpha$ -pyrones like dihydrokavaine. Experiments with dihydrokavaine showed that it inhibits *Aspergillus niger* completely in a concentration of 0.5 mg/ml. Bacteria did not seem to be inhibited by the kava extract or dihydrokavaine [22, 23].

## 2.4 Drug interactions

#### Prolongation of barbiturate-induced sleeping time

An early study with mice demonstrated the ability of kava components to potentiate sodium pentobarbital-induced sleeping time. Dihydromethysticin appeared to be the most potent agent; at a dosage of 60 mg/kg sleeping time was prolonged by 413 %. Methysticin, kavaine, dihydrokavaine and yangonin were applied in dosages of 160 mg/kg, but only prolonged sleeping time by 150 (yangonin, dihydrokavaine) to 250 % (methysticin; kavaine: 235 %). In additional experiments, varying doses of dihydromethysticin further demonstrated its potency. 10 mg/kg caused a prolongation by  $152 \pm 30 \%$ , 20 mg/kg prolonged sleeping time by  $240 \pm 27 \%$ , 40 mg/kg:  $457 \pm 43 \%$ , 60 mg/kg: 896 %, 160 mg/kg: 1800 % [33].

Another study on male mice demonstrated that premedication with dihydrokavaine 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 dihydrokavaine was 60 mg/kg.

Dihydromethysticin proved to be at least twice as effective as dihydrokavaine over the entire dose range. However, none of the drugs reached the efficacy of chlorpromazine [39].

The effect of a kava extract on barbiturate-induced sleeping time was studied in male Swiss mice (Nossan, Corrazzana, Milano, Italy) with a body weight of 20 – 25 g. The experiments were performed according to GLP guidelines. The animals were housed in cages, temperature:  $23 \pm 2^\circ \text{C}$ , air humidity:  $60 \pm 10\%$ . The artificial lighting was turned on and off at 12-hour intervals. Food and water were given *ad libitum*. The dosage of the kava spissum extract (Harras-Pharma-Curarina) was 200 mg/kg i.p. Sodium pentobarbital (Sigma Tau) was dissolved in physiological saline and administered s.c. at a dosage of 40 mg/kg corresponding to an injection volume of 10 ml/kg. The animals were randomly divided into three treatment groups and one control group for each pharmacological test ( $n = 10$  per group). The plant extract was dissolved in macrogol 400 (Hoechst, Germany). The volume administered was 10 ml. All control animals were given the same quantity of vehicle. In order to avoid differences due to circadian rhythm, all experiments were performed at the same time. In order to assess the effect on sleeping time, the extract was administered i.p. 10 minutes before administering the barbiturate. Sleeping time was determined as the time between loss and recovery of the animal's righting reflex. A distinct prolongation (19%) of the barbiturate sleeping-time was observed after administration of kava spissum extract (200 mg/kg) [6].

To investigate the neurophysiological effects of one of the components of kava, D,L-kavaine, a study was conducted on unrestrained cats ( $n = 9$ ) with chronically implanted electrodes. Cats served for polygraphic, 10-hour analyses of the sleep-wakefulness rhythm; they received – in a random sequence – 0.9% NaCl (3 ml i.p.), D,L-kavaine (28 mg/kg p.o.), pentobarbital (1 mg/kg i.m.), or the combination D,L-kavaine plus pentobarbital. The percentage duration of active wakefulness was significantly shortened by both D,L-kavaine and pentobarbital, as compared to placebo. There was likewise a significant prolongation of synchronized sleep with D,L-kavaine, pentobarbital, and the combination of both these agents. However, a potentiation of drug effects failed to occur [27].

The potentiation of barbiturate narcosis of a pyrone-free aqueous extract of kava was examined in Balb-c male mice (18 – 30 g) and compared to the effect of a lipid-soluble extract. Mice were allowed food and water *ad libitum* and all experiments were carried out at an ambient temperature of 20 – 22°C.

Dried aqueous extract dissolved in saline was injected i.p. in a volume of 0.1 ml/10 g mouse, the tested dosage was 250 mg/kg ( $n = 10$ ). Control mice ( $n = 10$ ) were

similarly injected with saline (0.9 % NaCl). The extract was injected 30 min prior to pentobarbital sodium (50 mg/kg) and the time to regain the righting reflex was recorded. Compared to control, the aqueous extract did not significantly prolong the sleeping time of mice [30].

#### Interaction with ethanol

Male Balb-c mice with 20 – 25 g bw were used in an experiment to study the interactions of a lipid-soluble kava extract. After dosing, the animals were placed in a 32° C heated chamber, and carefully observed. Sleeping times were recorded as the time from the loss of the righting reflex to regain of the righting reflex, with minimal interference from the operator during the test. The lipid-soluble extract of kava was prepared as in [30]. The extract was administered p.o., in 5 % Cremefor-EL in saline for dosages at or below 300 mg/kg. For more concentrated kava solutions (used for dosages above 300 mg/kg) 10 % Cremefor in saline had to be used as vehicle. Alcohol (ethanol, CSR, Australia) was administered i.p., diluted with saline to deliver a volume of 0.1 ml/10 g bw. Various dosages of each substance were used. For experiments where both drugs were given the kava extract was administered 2 min before the alcohol injection. Control mice received equivalent volumes of the vehicles as required. A dose of 3 g/kg of ethanol failed to cause loss in the righting reflex in five out of five mice. A dose of 3.5 g/kg produced hypnosis and the length of sleeping time increased sharply as the dosage was raised to 4 g/kg and 4.5 g/kg. The latter dosage killed one of 14 mice; above this dosage toxicity was too high to determine sleeping time. The kava extract greatly increased ethanol-induced hypnosis.

A dose of 200 mg/kg of kava extract 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. The sleeping times with these combinations were in excess of those, which could be achieved with ethanol alone. However, 300 mg/kg kava extract also 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. 2 g/kg ethanol, however, greatly prolonged the mean sleeping time produced by 350 mg/kg kava extract ( $p < 0.001$ ). This effect was further increased as the ethanol dosage was raised to 3 g/kg. Due to toxicity the interaction of ethanol and kava was difficult to study when 450 mg/kg of the extract were used to induce hypnosis.

The administration of 2 g/kg of ethanol plus 450 mg/kg of extract quickly killed three out of five mice and one further mouse showed respiratory difficulty but did regain its righting reflex at about 180 min. The remaining animal regained the righting reflex at

160 min. Decreasing the ethanol dosage to 1.5 g/kg failed to significantly increase sleeping time [29].

#### Interactions with caffeine

An early study demonstrated that caffeine greatly shortens the duration of the analgesic effect of dihydromethysticin ( $p < 0,001$ ) and dihydrokavaine ( $p < 0.01$ ), but without affecting the peak effect. Male mice ( $n = 40$  per group) received either 120 mg/kg dihydrokavaine, 120 mg/kg dihydrokavaine plus 20 mg/kg caffeine, 140 mg/kg dihydromethysticin or 140 mg/kg dihydromethysticin plus 20 mg/kg caffeine. The combination peaked 20 min (dihydrokavaine + caffeine) and 15 min (dihydromethysticin + caffeine) earlier [4].

### **3. Pharmacokinetics**

According to EMEA's "Proposal for a Note for guidance on non-clinical testing of herbal drug preparations with long-term marketing experience" (EMEA/HMPWG/11/99) pharmacological tests on pharmacokinetics are not necessary. Nevertheless, some studies on the pharmacokinetical properties of kava extracts and of single kavapyrones have been conducted.

The metabolism of several kavapyrones was studied in male Wistar rats  $250 \pm 20$  g bw). Urine and faeces were collected separately for 24 hour periods. Approximately one half of the 400 mg/kg dose of dihydrokavaine, administered p.o., was found in the urine within 48 hours. About  $\frac{2}{3}$  of this was hydroxylated metabolites (3 mono- and 3 di-hydroxylated derivatives).

The remaining third consisted of metabolites formed by scission of the 5,6-dihydro- $\alpha$ -pyrone ring and included hippuric acid (9 – 13 %). Lower amounts of urinary metabolites were excreted when kavaine was given, but both hydroxylated and open-ring products were formed. Methysticin gave rise to only small amounts of two urinary metabolites formed by demethylenation of the methylenedioxyphenyl moiety. Urinary metabolites of dihydroxyyangonin and yangonin were formed via *O*-demethylation. No open-ring products were detected [45].

In another study on the kavapyrones dihydrokavaine, kavaine, yangonin, dihydromethysticin and methysticin plasma and brain pyrone levels were determined after oral administration of 100 mg/kg of different extracts or formulations to male rats and mice, and 6 and 10 mg/kg to dogs.

Preliminary experiments in mice with 100 mg/kg extract p.o. suspended in 0.2 % agar revealed maximum plasma levels of the individual pyrones (except yangonin) already ranging between 300 and 900 ng/ml after 5 min. Elimination half-life was determined

as approx. 30 min. In further experiments with mice and rats treated orally (100 mg/kg) with a special formulation, the bioavailability clearly increased, and maximum plasma levels of 1.7 – 2.5 µg/ml were attained for dihydrokavaine, kavaine, dihydromethysticin, methysticin and of 0.3 µg/ml of yangonin 0.5 hour after administration. In rats, two pyrone absorption peaks were observed at 0.25 and approx. 2 hours. The pyrone levels in brain showed peak concentrations (1.1 – 2 µg/g brain) at the same time as in the plasma. In mice, however, the brain concentration levels decreased faster than in rats. Elimination half-lives of the pyrones in mouse plasma and brain were approx. 1 hour, and even longer for rats. In contrast to the results obtained from rodents, dogs administered an extract formulation or a pure blend of the individual pyrones showed quite obvious variations in  $t_{max}$  (0.7 – 4 hours). In this species, dihydrokavaine and yangonin could only be detected qualitatively. Maximum plasma levels of kavaine, methysticin, and dihydromethysticin ranged from 100 to < 500 ng/ml, and elimination half-lives varied from approx. 90 min to several hours. The plasma pyrone levels after administration of the blend of individual pyrones were lower than those after the extract formulation. In summary, bioavailability of a kava extract could be increased up to 10-fold by the improvement of galenic formulations after administration of a new special formulation to mice and rats [2].

In a study investigating the uptake of kava components into mouse brain, dihydrokavaine, kavaine, desmethoxyyangonin, and yangonin were administered to mice i.p. at a dosage of 100 mg/kg. At specific time intervals (5, 15, 30, and 45 min), the mice were sacrificed and the brain concentrations of these four compounds determined.

After 5 min, dihydrokavaine and kavaine attained maximum concentrations of  $64.7 \pm 13.1$  and  $29.3 \pm 0.8$  ng/mg wet brain tissue, respectively, and were rapidly eliminated. In contrast, desmethoxyyangonin and yangonin had poorly defined maximum levels, corresponding to concentrations of  $10.4 \pm 1.5$  and  $1.2 \pm 0.3$  ng/mg wet brain tissue, respectively, and these compounds were eliminated more slowly from the brain tissue. When crude kava resin was administered i.p. at a dosage of 120 mg/kg, the concentration in the brain of kavaine and yangonin markedly increased (2 and 20 times, respectively) relative to the values measured from their individual injection.

In contrast, dihydrokavaine and desmethoxyyangonin, after administration of crude resin, remained at the percentage incorporation into brain tissue established for their individual i.p. injection [32].

## 4. Toxicity

### 4.1 Acute toxicity

The acute toxicity of the kavapyrones dihydrokavaine (DHK) and dihydromethysticin (DHM) was studied in mice ( $n = 90$  for DHK and  $n = 80$  for DHM), rabbits ( $n = 12$  for DHK and  $n = 8$  for DHM), cats ( $n = 3$ ) and dogs ( $n = 2$  for both DHK and DHM). The pyrones were dissolved in arachis oil and administered i.p. and p.o. After i.p. administration the  $LD_{50}$  of DHK in mice was  $325 \pm 4.5$  mg/kg, in rabbits  $\approx 350$  mg/kg, in cats  $> 250$  mg/kg, and in dogs  $> 200$  mg/kg. The  $LD_{50}$  of DHM after i.p. administration was  $420 \pm 20.0$  mg/kg in mice,  $\approx 300$  mg/kg in rabbits, and  $> 200$  mg/kg in dogs. After p.o. administration the  $LD_{50}$  in mice was  $920 \pm 52.0$  mg/kg for DHK and  $1050 \pm 67.0$  mg/kg for DHM. 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 [39].

The same working group again studied the  $LD_{50}$  of dihydrokavaine and dihydromethysticin after i.p. application in male mice weighing 20 – 25 g. This time, dihydrokavaine had a  $LD_{50}$  of  $490 \pm 15.2$  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 [44].

A further study summarized the LD<sub>50</sub> values of all six major kavapyrones in mice [34]:

	LD <sub>50</sub> (mg/kg)		
	i.v.	i.p.	p.o.
Kavaine	69 ± 3.3 (n = 30)	420 ± 14.7 (n = 80)	1130 ± 41.3 (n = 40)
Dihydrokavaine	53 ± 1.5 (n = 38)	490 ± 15.2 (n = 80)	980 ± 52.0 (n = 45)
Methysticin	49 ± 1.7 (n = 50)	530 ± 29.9 (n = 60)	> 800 <sup>1</sup>
Dihydromethysticin	67 ± 4.2 (n = 36)	420 ± 20.0 (n = 80)	1050 ± 67.0 (n = 50)
Yangonin	41 ± 1.4 (n = 55)	> 1500 <sup>1</sup>	> 1500 <sup>1</sup>
Desmethoxyyangoni	55 ± 1.8 (n = 40)	> 800 <sup>1</sup>	> 800 <sup>1</sup>
n			

**Table 1:** Acute toxicity of the kavapyrones; (<sup>1</sup>) = not to determine due to low solubility

In a more recent study the LD<sub>50</sub> of a lipid-soluble kava extract was compared to that of an aqueous kava extract. The study was carried out on male Balb-c mice. Mouse weights were between 18 – 30 g. The extracts were administered i.p. The LD<sub>50</sub> of the lipid extract was found to be 360 mg/kg, and that of the aqueous extract was 1.5 g/kg [30].

In another study by the same working group lethality after p.o. administration in mice was greater than 50 % at and above 700 mg/kg of the lipid-soluble kava extract [29].

#### 4.2 Subacute and chronic toxicity

Studies on subacute and chronic toxicity were carried out solely with Kavaform, a geriatric preparation containing 50 mg D,L-kavaine per capsule. Additionally, one capsule contains magnesium ororate (200 mg), an extract of *Vitis vinifera* (50 mg) and several dispersing agents and excipients (570 mg). As the data is valid only for that combination, the results cannot be regarded as representative for kavaine.

In rats and dogs, the preparation proved to be non-toxic even when administered for a prolonged period at dosages of up to ten times the human therapeutic dosage, i.e. up to 100 mg/kg of the active substance (which would be up to 17 mg/kg of D,L-kavaine) [25].

#### **4.3 Fertility, embryotoxicity, and peri-/postnatal toxicity**

A mixture of three kavapyrones containing kavaine (40 %), dihydrokavaine (40 %), and yangonin (20 %), was examined for possible teratological effects in rats and rabbits. The mixture was administered p.o. as a 5 % suspension in vegetable oil. In the rat, the dosage was 100 mg/kg and 500 mg/kg, equivalent to 20 times and 100 times the human therapeutic dose, respectively. Controls received only vehicle (1 ml vegetable oil). For the assessment of all teratological effects, the alizarin skeleton-straining technique was used for rats and rabbits as well as the examination of sections of rat embryos. Sexually mature female Wistar rats (3 groups, n = 20 per group) were mated in the ratio 2 : 1. The treatment began on the 6<sup>th</sup> day of pregnancy and was continued until the 15<sup>th</sup> day of pregnancy. The males had previously been tested for fertility and they were not treated with the females. The preparation was administered p.o. by stomach tube. On the 20<sup>th</sup> day of pregnancy the females were killed and the foetuses were delivered by Caesarian section. The following observations were recorded: number of foetuses, dead and living foetuses, position in the uterus, implantation sites, resorption nodules, anomalies and development weight. Every third foetus was examined for visceral defects. The remaining  $\frac{2}{3}$  were skinned, stained with alizarin and examined for bone anomalies. Analogous trials were carried out in female rabbits (New-Zealand strain, 3 groups, n = 10 per group; 20 or 200 mg/kg of the mixture or 1 ml vehicle). Bucks were selected which had semen of good quality and which had produced good results from previous matings. The does were killed on the 28<sup>th</sup> day and foetuses were delivered by abdominal Caesarian section. The following data were recorded: number of foetuses, position in the womb, the ratio of dead to living foetuses, implantation site, resorptions, litter size and anomalies. In the rats there were no significant differences between the verum and control groups in any parameter with the exception of the distribution of weight. The statistical analysis of the weight data yielded highly significant differences between the control animals and both test groups in respect to male foetuses ( $p < 0.01$ ) and a difference between the control animals and Group 2 (500 mg/kg of the mixture;  $p < 0.1$ ). In rabbits similar effects were found. It was possible to distinguish a dose-dependent group trend concerning average weight, with weights declining from the control group through group 1 to group 2 (200 mg/kg of the mixture).

According to the authors the possibility that this reduction in foetal weight in both rats and rabbits is an effect of the preparation cannot be excluded with certainty [25].

#### **4.4 Mutagenicity and Carcinogenicity**

No data available.

#### **4.5 Local toxicity**

No data available.

#### **4.6 Immunotoxicity**

No data available.

#### **4.7 Studies conducted to clarify special problems: Tolerance**

The development of tolerance to the aqueous and to the lipid-soluble extract of kava was tested in mice. The tolerance to the unknown pharmacologically active ingredient(s) given i.p. developed very rapidly in the aqueous extract. 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<sub>50</sub>. As tolerance was evident at the first test period it can be assumed to be physiological tolerance. A minimally effective daily dose of the lipid extract (100 mg/kg) did not produce tolerance to a weekly test dose of 166 mg/kg within 7 weeks. In a further experiment, the dose was raised to 150 mg/kg twice daily, which caused partial tolerance 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 lipid kava extract was injected daily and animals were tested each day while under influence of the drug. However, even under these conditions, there was no tolerance evident within 3 weeks, when the experiment was terminated. The authors conclude that it appears difficult to induce the development of physiological or learned tolerance to the lipid-soluble kava extract in mice [9].

## 5. Conclusion

### 5.1 Pharmacodynamics

Since the 1960's a multitude of experiments was conducted to detect the pharmacological effects of kava, *Piperis methystici* rhizoma. The *in vivo* and *in vitro* studies presented in this report document this action. The investigation of kava extracts as well as single kavapyrones has yielded several pharmacodynamic effects, which refer to the anxiolytic actions of kava seen in humans. Significant sedative properties, anticonvulsive effects and central muscular relaxation were documented in major studies with experimental animals. Also effects on psychosis models and an analgesic action have been reported. The results obtained in these studies concerning dosage and consecutive effects provide the basis for the assessment of the efficacy of kava products, which can be pronounced as excellent.

Kava extracts and single kavapyrones prolong barbiturate- and ethanol-induced sleeping time. (For clinical relevance see the Expert report on the clinical documentation.)

The pre-clinical study results presented for kava extracts and for single kavapyrones were obtained following oral, intravenous and intraperitoneal administration. The animal species (mouse, rat, guinea-pig, rabbit, cat, dog) are routinely used in pharmacodynamic and toxicological studies. The methods used in these studies are standardised methods of pre-clinical testing in neurology, phyto- and neuropharmacology and reflect the state of art.

### 5.2 Pharmacokinetics

According to EMEA's "Proposal for a Note for guidance on non-clinical testing of herbal drug preparations with long-term marketing experience" (EMEA/HMPWG/11/99) pharmacokinetic studies are not required. However, some studies have been conducted on kava extracts and single kavapyrones. In mice, maximum plasma and brain pyrone levels were reached 5 min after p.o. administration. Plasma pyrone levels after administration of the single pyrones or of a pyrone blend were lower than those after the administration of the extract. Intestinal absorption was confirmed; metabolites of approximately 50 % of the administered amount of kavapyrones were found in the urine after 48 hours.

### 5.3 Toxicity

A very low order of acute toxicity is reported for kava extracts and the single kavapyrones. In acute toxicity tests the LD<sub>50</sub> of the pyrones ranged from 41 – 69 mg/kg after intravenous administration, 420 – > 1500 mg/kg after intraperitoneal administration and 980 – > 1500 mg/kg after oral administration. The LD<sub>50</sub> of the lipid-soluble extract was 360 mg/kg after i.p. administration and at least 700 mg/kg after p.o. administration. An aqueous extract had a LD<sub>50</sub> of 1.5 g/kg.

No data is available for subacute and chronic toxicity of single kavapyrones or pure extracts. A preparation containing D,L-kavaine, magnesium ororate and an extract of *Vitis vinifera* proved to be non-toxic even when administered for a prolonged period and at dosages up to ten times the human therapeutic dosage.

Studies on perinatal toxicity demonstrated a reduction in foetal weight after the administration of a very high dose of a mixture containing three of the six major kavapyrones. All other parameters were clearly unaffected.

The development of tolerance to kava is not to be expected.

Pharmacological studies on mice and rats have demonstrated the ability of kava extracts to potentiate barbiturate- and ethanol-induced sleeping time. As the effective dosages were very high, this effect is not to be expected in therapeutic dosages.

At present new investigations on the toxicity of kava preparations as well as the single kavapyrones have been done, but have not yet been published. The results of the research of Dr. Tang and Dr. Meyers indicate that, depending on the solvent and parts of the plant used for extraction, the quantitative composition of kavapyrones varies significantly. Furthermore, there are indications that methysticine may have hepatotoxic properties. Further research in this area is appreciated, to increase the knowledge of possible hepatotoxic effects of kava preparations.

### 5.4 Expert opinion

This Expert Report assesses the scientific evidence available concerning the pharmacological and toxicological properties of the extract of kava, *Piperis methystici* rhizoma. The study designs, *in vitro* and *in vivo* models, appear adequate and appropriate to establishing the pharmacological-toxicological profile. The efficacy and safety of extract of *Piperis methystici* rhizoma has been clearly established. The clinical efficacy is therefore expected to be good, and side effects are not expected following oral use.

The documentation is based on generally accessible accounts in the literature (see references to the Expert Report).

In summary preparations containing extracts of the rhizome of *Piper methysticum* can be regarded as a pharmacologically effective and safe medicine.

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## 7. Expert's CV

### Curriculum vitae

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- University Heidelberg, Diplom Biology, 1975
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- 2<sup>nd</sup> Pathological Institute, Semmelweis Medical University, Budapest, Hungary, Jan.-Apr. 1980
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##### Professional Appointments

- Research Associate, Biologische Bundesanstalt Dossenheim, FRG, 1975-1978
- Research Associate, Institute for Arteriosclerosis Research at the University of Muenster, FRG, 1978-1982
- Research Associate, Mallory Institute of Pathology, Boston University, School of Medicine, USA, 1982-1984
- Head Lab. for Cytopathology, Institute for Arteriosclerosis Research at the University of Muenster, FRG, 1984-May 1988
- Director of Clinical Research, Lichtwer Pharma GmbH, Berlin, FRG 1988-1990
- Medical Director, Lichtwer Pharma GmbH, Berlin, FRG, 1990-end of 1995
- Foundation of PhytoPharm Consulting beginning of 1996, President and CEO of PhytoPharm Consulting
- Foundation of Herbalist & Doc Gesundheitsgesellschaft mbH, beginning of 1997
- Associate Partner of Background Consultancy, beginning of 1997

##### Professional Societies

- United States Pharmacopoeia Dietary Supplements Advisory Panel on Botanicals

- German Society for Phytotherapy
- European Scientific Cooperative on Phytotherapy (ESCOP)
- International Atherosclerosis Society
- European Atherosclerosis Society
- American Heart Association, Fellow of the Council of Arteriosclerosis
- German Society for Atherosclerosis Research
- Lipid Liga

## Publications

Over 180 scientific publications in the area of phytotherapy, dietary supplements, heart disease and arteriosclerosis

- Gruenwald J, Brendler T, Jaenicke C, Scientific Editors: The PDR Family Guide to Natural Medicines & Healing Therapies, Medical Economics Company, 1999
- Gruenwald J, Brendler T, Jaenicke C, Scientific Editors: Physicians Desk Reference for Herbal Medicines, Medical Economics Company, 1998
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- Gruenwald J, Brendler T, Jaenicke C, Editors: CD-ROM Heilpflanzen - Herbal Remedies, 1<sup>st</sup> through 3<sup>rd</sup> edition, on disk, 1996-1999
- Gruenwald J, Natürlich durch die Wechseljahre, Kabel, 1997
- over 180 scientific publications

Editor of international publications:

- Advances In Natural Therapy, (Editor in Chief)
- Nutrition Business Journal (European Editor)
- AHPA Report (European Correspondent)
- Nutraceuticals World (Contributing Editor)
- Journal of Medicinal Food (Member of the Editorial Board)

## Presentations

- Speaker at over 120 scientific presentations worldwide
- Chairman or Secretary of scientific congresses, e.g. DIA Annual Meeting, Green Pharmaceuticals '98, Nutracon '99

## Honors

- Organizing Committee: Second Muenster International Arteriosclerosis Symposium, 1981
- Secretary: German study group on arteriosclerosis research, 1984-1987
- Organizing Committee: European Artery Club
- Secretary: 4th Muenster International Arteriosclerosis Symposium, 1985
- Co-Chairman: Poster session of the 7<sup>th</sup> International Symposium on Atherosclerosis, 1985
- Organizing Committee: 7<sup>th</sup> European Conference on Vascular Biology, 1986
- Co-Chairman: 7<sup>th</sup> European Conference on Vascular Biology, 1986
- Organizing Committee: 8<sup>th</sup> European Conference on Vascular Biology, 1987
- Secretary: German Society for Arteriosclerosis Research, 1987-1988
- Co-Chairman: Poster session of the Conference of the German Society for Arteriosclerosis Research, 1988
- Organizing Committee: 9<sup>th</sup> European Atherosclerosis Society, 15<sup>th</sup> Anniversary Meeting, 1989

- Member of the Board: German Society for Arteriosclerosis Research, 1988-1992
- Secretary: Second International Garlic Symposium, 1991
- Member of the International Committee BAH (German Federation of Proprietary Medicine Manufacturers), since 1991
- Member of the "Grenzgebiet Arzneimittel" (Dietary Supplements) Committee BAH (German Federation of Proprietary Medicine Manufacturers), since 1995
- Member of the Drug Research and Drug Development Committee BPI (German Federation of Pharmaceutical Industries), since 1993
- Member of Publications Committee: European Scientific Cooperative on Phytotherapy (ESCOP), since 1991
- Chairman: Seventh Muenster International Arteriosclerosis Symposium, 1993
- Chairman: "International Garlic Research" Symposium at the Congress of the German Society for Phytomedicines in cooperation with ESCOP, Berlin 1996
- Session Chairman: "Opportunities and Challenges for Heterogeneous Botanical Products", 3<sup>rd</sup> Drug Information Association Workshop on Botanicals: "Botanical Testing: Developing the Scientific Evidence to Support the Medical Use of Heterogeneous Botanical Products " in Cooperation with NIH and FDA, Washington DC, 1997
- Session Chairman: "Advantages/Disadvantages of Heterogeneous Botanicals vs Single Compounds. Drug Information Association, 33<sup>rd</sup> Annual Meeting, Montréal, Canada 1997
- Workshop Chairperson: "The Role of Botanical Supplements in Health: Research Advances and Directions" 16<sup>th</sup> International Congress of Nutrition, Montréal, Canada 1997
- Session Chairman: "The Regulation and Classification of Hypericum in Europe", First International Symposium of St. John's Wort, Anaheim, California 1998
- Session Chairman: "Hypericum as Anti-Depressant", Drug Information Association, 34<sup>th</sup> Annual Meeting, Boston 1998
- Session Chairman: "Green Pharmaceuticals '98 Conference", Vancouver, Canada, 1998
- Chairman: "Herbal Extracts As Food Ingredients, Medicines & Supplements", London, 1999
- Chairman: "Utilizing and Capitalizing on the Benefits of Herbal Extracts", Frankfurt, Germany, 1999
- Chairman: "Business Opportunities for Botanicals + Dietary Supplements in Europe, Global Business Research, Nutracon '99", Las Vegas, 1999