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Isobolographic analysis of the sedative interaction between six central nervous system depressant drugs and *Valeriana edulis* hydroalcoholic extract in mice*

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Abstract

It has been declared frequently that valerian may potentiate the effect of other central nervous system (CNS) depressant drugs, however there has been a lack of experimental data. We have evaluated the profile of the interactions between the ethanol extract of *Valeriana edulis* spp *procera* and six CNS depressant drugs using an exploratory model to test the sedative effect in mice. All the compounds tested showed a dose-dependent sedative effect with the following ED50 values: valerian 181.62, diazepam 1.21, ethanol 1938, pentobarbital 11.86, buspirone 1.04, haloperidol 0.41 and diphenhydramine 17.06 mg kg^{-1}. An isobolographic analysis was used to evaluate the sedative interaction of the intraperitoneal co-administration of 1:1 fixed-ratio combination of equi-effective doses of valerian extract with each CNS depressant drug. The ED50 theoretical (*Zadd*) and experimental (*Zexp*) for each combination were: valerian + diazepam, *Zadd* = 91.41 mg kg^{-1}, *Zexp* = 81.64 mg kg^{-1}; valerian + ethanol, *Zadd* = 1060.22 mg kg^{-1}, *Zexp* = 687.89 mg kg^{-1}; valerian + pentobarbital, *Zadd* = 96.74 mg kg^{-1}, *Zexp* = 151.83 mg kg^{-1}; valerian + buspirone, *Zadd* = 91.33 mg kg^{-1}, *Zexp* = 112.73 mg kg^{-1}; valerian + haloperidol, *Zadd* = 91.01 mg kg^{-1}, *Zexp* = 91.52 mg kg^{-1}; valerian + diphenhydramine, *Zadd* = 99.34 mg kg^{-1}, *Zexp* = 123.52 mg kg^{-1}. Neither synergistic nor attenuate effects were found in any of the combinations evaluated. We concluded that the valerian extract did not potentiate the sedative effect of commonly prescribed CNS depressant drugs as was expected. The additive effect found through the isobolographic analysis suggested that the sedative effect of *V. edulis* resulted from the activation of common mechanisms of haloperidol, diazepam, buspirone, pentobarbital, diphenhydramine and ethanol.

Introduction

In traditional medicine of many cultures the roots and rhizomes of several species of *Valeriana* genus (Valerianaceae) are used as mild sedatives and tranquilizers, and to aid the induction of sleep (Bos et al 1998; Houghton 1999). *Valeriana edulis* spp *procera* (Kunth) Meyer has been used widely for the same purposes in Mexican traditional medicine (Sánchez 1980; Martínez 1990). Mexican valerian is a commercially important species (Enciso-Rodríguez 1997). Phytochemical studies have shown that this species contains higher concentrations of valepotriates than *V. officinalis* or other *Valeriana* species (Bos et al 1998). Oliva et al (2004) demonstrated that this plant had anticonvulsant and sedative effects in mice. Also, it has been reported that children treated with *V. edulis* showed a significant reduction in sleep latencies and nocturnal time awake, lengthened total sleep time and improved sleep quality (Francis & Dempster 2002). Herrera-Arellano et al (2001), using polysomnographic recordings, demonstrated that hydroalcoholic extracts of *V. edulis* reduced the number of waking episodes, increased the sleep efficiency index, reduced morning sleepiness and did not affect the anterograde memory.

In the evaluation of new drug candidates, drug–drug interactions and drug–dietary supplements, including drug–herbal, interactions are important issues to consider. However, herb–drug interaction studies are very limited (Fugh-Berman & Ernst 2001;

Huang & Lesko 2004). There is a dearth of well-documented data in this area and there are few studies that have specifically evaluated herb–drugs interactions (Rotblatt & Ziment 2002). The drug interactions could be pharmacodynamic and pharmacokinetic. Pharmacokinetic interactions often occur as a result of activity changes of the drug-metabolizing and transporting proteins, especially cytochrome P450 (CYP) isoenzymes and P-glycoprotein (P-gp). The activity of these enzymes and drug transporters can be enhanced or inhibited by synthetic drugs as well as by natural products (Butterweck et al 2004). In-vitro, *V. officinalis* has been demonstrated to be a mild inhibitor of the 3A4 subtype of cytochrome P450 (Budzinski et al 2000). No adverse drug interactions involving valerian extracts have been reported in clinical trials, only in an unusual double-blind study in volunteers when alcohol failed to induce its predicted impairment of concentration when combined with a mixture of valepotriates from *V. officinalis* (Fugh-Berman & Cott 1999). There are some reports associated with the interaction between valerian and other medicinal herbs, prescribed drugs and alcohol (Chen et al 2002); however, these interactions have not been demonstrated completely. Animal in-vivo data have shown a prolongation of barbiturate-induced anaesthesia for some valerian species, including *V. edulis* (Oliva et al 2004).

Basic and clinical studies of the drug interactions have been performed using isobolographic analysis. This analysis offers a rigorous evaluation of the interactions between two drugs that act together to produce overtly similar effects (Tallarida 2000). The effect of the combination may be a simple addition of the individual effects (additivity). In contrast, the effect of the combination can be exaggerated or even attenuated. The exaggerated effect is termed super-additive or synergistic, whereas the attenuated effect is termed sub-additive (Tallarida et al 1997a). It has been proposed many times that the concomitant use of valerian might potentiate the sedative effect of other central nervous system (CNS) depressants drugs (Schulz et al 1998; Houghton 1999; Plushner 2000; Fugh-Berman & Ernst 2001); however there is a lack of experimental data. Therefore, this study was designed to investigate a possible synergistic pharmacodynamic interaction between the sedative effect of an hydroalcoholic extract of *V. edulis* and six CNS depressant drugs (diazepam, ethanol, pentobarbital, buspirone, haloperidol and diphenhydramine) by use of isobolographic analysis in a model to test the sedative effect in mice.

Materials and Methods

Materials

The roots of *V. edulis* spp *procera* were kindly donated by Laboratorios Mixim, México. They had been harvested on October 15, 2002, in Xochimilco D.F., México. Samples were deposited in the Herbario del Centro Medico Nacional Siglo XXI. The roots were dried in the shade at room temperature and pulverized through a 2-mm screen using a Wiley Mill.

The dried and powdered root (100 g) was extracted with 70% (v/v) ethanol (1 part drug to 5 parts solvent) in accordance with standard pharmacopoeia procedures for preparing tinctures (List & Schmidt 1989). The extract was filtered by gravity and concentrated through air current at room temperature (approximately 22°C) yielding 20.2 g hydroalcoholic extract. A 3.55% valepotriates content was detected through spectrophotometry as described by Wagner et al (1970).

Haloperidol, diphenhydramine, buspirone, valerenic acid, hesperidin and Tween 80 were purchased from Sigma Co. (St Louis, MO). Hydroxyvalerenic acid and acetoxyvalerenic acid were purchased from Apin Chemicals Ltd (Abingdon, Oxford, UK). Absolute Ethanol was of HPLC grade (Fisher Scientific) and sodium pentobarbital (Anestesal) was purchased from Pfizer S.A. de C.V. (Mexico).

HPLC/MS-ESI analysis

A high-performance liquid chromatography/mass spectrometry electro spray ionization (HPLC/MS-ESI) method was used to detect the presence of common compounds in the valerian species used. The liquid chromatograph used was an Agilent Series 1100 comprising the modular components: quaternary pump, a vacuum solvent microdegasser, an autosampler with 100-well tray and an online DAD (Diodo-array UV detector). Separation was achieved on a Luna C18 (2) column (Phenomenex, 150×3.0 mm i.d.; 5-μm particle size) and operated at 30°C. The column was equipped with a 2-cm LC-18 guard column (Supelco, Bellefonte, PA). The mobile phase consisted of water (A), acetonitrile:methanol (1:1) (B), both containing 0.05% phosphoric acid applied in the following gradient elution: from 98:02 (A:B) to 85:15 (A:B) in 12 min, to 50:50 (A:B) in 13 min, to 100 (B) in 15 min and held at that composition for 10 min. The flow rate was 0.7 mL min^{-1} and the sample size was 10 μL. The DAD was programmed to acquire the UV-spectra from 190-400 nm (step size 2 nm). The mass spectrometric analysis was performed on an Agilent Series 1100 SL instrument equipped with an ESI source. All acquisitions were performed under positive ionization mode with a capillary voltage of +4000 V. Nitrogen was used as nebulizer gas (40 psig) as well as drying gas at 13 L min^{-1} and drying gas temperature at 325°C. Data acquisition and processing was performed with the software Analyst QS.

Animals

Adult male ICR mice (25–34 g; Centro UNAM-Harlan, Harlan México, S.A. de C.V.) were used in all experiments. Procedures involving animals and their care conformed to the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) adopted in our laboratory, and in compliance with international rules on care and use of laboratory animals.

The experimental groups consisted of six (interaction study) or ten animals (calculus of ED50 of individual drug). They were maintained at constant room temperature (22 ± 2°C) and submitted to a 12-h light/dark cycle with free access to food and water. All behavioural evaluations were carried out between 10:00 and 14:00 h.

With the exception of the hydroalcoholic extract and diazepam (Roche S.A.) which were suspended in 0.5% Tween 80 in saline solution, all other compounds were dissolved in saline solution (0.9%). The drugs were freshly prepared each time and intraperitoneally injected in a volume of 0.1 mL/10 g body weight. Control animals received the same volume of vehicle (0.5% Tween 80 in saline or saline solution only).

Procedure

The apparatus consisted of a glass cylinder (16 cm in height, 11 cm in diameter, with wall of 3 mm). The cylinder was placed on filter paper in a room with constant lighting and isolated from external noise (Hiller & Zetler 1996; Oliva et al 2004).

An individual naïve mouse was put on the filter paper-covered floor of the glass cylinder; the number of rearings performed over a 5-min period was recorded. The inner side of the apparatus and floor were cleaned with alcoholic solution and the filter paper was changed between each animal test session (Oliva et al 2004). The hydroalcoholic extract and drugs were administered 30 min before testing in different doses (Table 1), with the exception of ethanol and buspirone which were administered 5 and 20 min before testing, respectively. When drugs were given in combination, valerian

Table 1 Doses used in the study of the sedative interaction between valerian extract and six CNS depressant drugs in mice

Drug	Dose (mg kg^{-1}, i.p.)			
	Drug alone[a]	Drug in combination		
		Valerian extract	CNS depressant drug	Total dose[b]
Valerian extract	10	–	–	–
	30	–	–	–
	100	–	–	–
	300	–	–	–
	560	–	–	–
	1000	–	–	–
Diazepam	0.3	22.7	0.15	22.85
	1	45.4	0.30	45.70
	2	90.8	0.60	91.4*
	4	181.6	1.21	182.81
	7.5	272.4	2.42	274.82
Ethanol	1000	22.7	242.35	265.05
	2000	45.4	484.70	530.10
	2250	90.8	969.40	1060.20*
	2500	181.6	1938.80	2120.40
	2750	272.4	2908.00	3180.40
	3000	–	–	–
	4000	–	–	–
Pentobarbital	2.5	22.7	1.50	24.20
	5	45.4	3.00	48.40
	10	90.8	5.90	96.70*
	15	181.6	11.90	193.50
	20	272.4	17.80	290.20
	40	–	–	–
Buspirone	0.15	22.7	0.13	22.83
	0.3	45.4	0.26	45.66
	1	90.8	0.52	91.32*
	3	181.6	1.04	182.64
	10	272.4	1.56	273.96
Haloperidol	0.1	22.7	0.05	22.75
	0.15	45.4	0.10	45.50
	0.3	90.8	0.20	91.00*
	1	181.6	0.40	182.00
	3	272.4	0.60	273.00
Diphenhydramine	10	22.7	2.12	24.82
	15	45.4	4.25	49.65
	17.5	90.8	8.50	99.30*
	20	181.6	17.06	198.66
	25	272.4	25.60	298.00

[a]Doses used to construct the dose–effect curves for each drug alone (n = 10 for each dose evaluated). [b]Doses used to construct the dose–effect curves for each combination (n = 6 for each dose evaluated). *These doses correspond to *Zadd*.

hydroalcoholic extracts were injected first in the right side of the peritoneum, followed by the injection of the test drug in the left side. When only one of the drugs was given, the missing drug injection was substituted with the injection vehicle. During observation, the experimenter stood next to the apparatus, always in the same place. The observations were made without prior knowledge of the experimental conditions applied to the animal. Reduced exploratory rearing showed by naïve mice after placement in an unfamiliar environment reveals a sedative effect (Rolland et al 1991; Hiller & Zetler 1996; Oliva et al 2004).

Dose–response curves were constructed to assess the sedative effect of valerian extract and the other CNS depressant drugs using ten animals at each of at least five doses (Table 1) to determine a dose–response curve. The dose that produced 50% of sedation (ED50, 50% of reduction in the number of rearings of the control group) and its associated 95% confidence intervals were calculated using standard lineal regression analysis of the log dose–response (Tallarida 2000).

Analysis of the interaction

An isobolographic analysis was performed to characterize the interaction between valerian extract with diazepam, ethanol, pentobarbital, buspirone, haloperidol or diphenhydramine. Only equi-effective doses (ED50) of each drug and their combinations, drawn from the dose–response curves, were considered for the analysis. Theoretical additive doses (*Zadd*) with their s.e.m. for each combination in the same component ratio (1:1) was computed from the equi-effective doses (ED50) of the single drugs, according to the method described by Tallarida (1992) to satisfy the following equation:

$$Zadd = f\mathrm{A} + (1-f)\mathrm{B}$$

Where A was the ED50 of valerian extract and B was the ED50 of the CNS depressant drug. For a 1:1 fixed-ratio, f in this case was 0.5 and $(1-f)$ was 0.5 also. The value $f\mathrm{A} = a$ represents the fraction of the ED50 of the valerian extract in the combination and $(1-f)\ \mathrm{B} = b$ represents the fraction of ED50 of the CNS depressant drug in the combination (Tallarida 2000). *Zadd* represents a total additive dose of the drugs theoretically providing a 50% reduction in the number of rearings made by the mice with respect to the control group. *Zexp* is an experimentally-determined total dose of a mixture of two component drugs, which was administered at a 1:1 fixed-ratio combination (Table 1) sufficient to reduce the number of rearings by 50% with respect to the control group. The *Zexp* values (with their 95% confidence limits) were determined from the respective drug–dose effect curves of combined drugs, according to standard lineal regression analysis of the log dose–response curve (Tallarida 2000), and subsequently, the 95% confidence limits were transformed into s.e.m. Statistical comparison was made of the experimentally determined (*Zexp*) with the theoretically calculated (*Zadd*) with the use of the Student's *t*-test, according to procedures previously described for Tallarida et al (1989), who proposed the use of this statistical test for analysing the data in isobolography. That was why the 95% confidence intervals of each ED50 evaluated experimentally needed to be transformed into s.e.m. *Zexp* values that were lower than the *Zadd* values, with a $P < 0.05$ for the differences in both the X and Y directions, were interpreted as a significant super-additive interaction. Values of *Zexp* that were higher than *Zadd* values, with a $P < 0.05$ for the differences in both the X and Y directions, were interpreted as a significant sub-additive interaction. When there was no statistical difference between the values of *Zexp* and *Zadd* this was interpreted as no interaction and an additive relationship (additivity) was established in the combination (Tallarida 2000).

Graphical presentations of the observed interactions in the shape of isoboles (iso-effect curves or isobologram), which is a simple form of visualization of interactions, facilitated the interpretation of interactions between valerian extract and each CNS depressant drug studied. The isobologram was constructed by connecting the ED50 of valerian extract on the abscissa with ED50 of the combined CNS depressant drug on the ordinate to obtain the additivity line (Tallarida 2000). The amounts of each component in combination (experimental (*Zexp*) and theoretical additive (*Zadd*) doses) were also plotted on the same graph. The theoretical additive point lies on a line connecting the ED50 values of the individual drugs. Experimental values that lie below and to the left of this additive line are considered to be synergistic or super-additive, whereas values that lie above and to the right of the line demonstrate an antagonist or sub-additive interaction.

To obtain a value to describe the magnitude of the interaction, a fractional analysis was performed for each combination, using the ED50 of the valerian extract, the CNS depressant drug and their combination according to:

$$a/\mathrm{A} + b/\mathrm{B}$$

Where A and B are the ED50 when each drug (valerian extract and CNS depressant drug) acts alone, and a and b are the amounts when each drug acts in the combination. This total fraction value measures the divergence between the experimental dose (*Zexp*) of the combination and the theoretical (*Zadd*), equi-effective additive dose (Tallarida 2000). Statistical difference demonstration ($P < 0.05$) of 1 for the relation $a/\mathrm{A} + b/\mathrm{B}$ was interpreted as a super-additive interaction if $a/\mathrm{A} + b/\mathrm{B}$ was < 1.0 and as a sub-additive interaction if $a/\mathrm{A} + b/\mathrm{B}$ was > 1.0; the absence of a statistical difference ($P > 0.05$) was interpreted as an additive effect (Tallarida 2000).

Results

Intraperitoneal administration of *Valeriana edulis* spp *procera* extract and the CNS depressant drugs resulted in a dose-dependent decrease of the number of rearings in the exploratory model. In this model the control group performed an average of 47.68 rearings with a standard deviation of 15.63 (n = 131 mice) in a 5-min period. The criterion to define

the time of the test was based on the time used by Hiller & Zetler (1996) and our previous study (Oliva et al 2004). Moreover, we performed an experiment in which ten animals were injected with saline solution 30 min before the test, and the number of rearings was registered each minute for 10 min (Figure 1). After 4 min there was no significant difference ($P > 0.05$) in the number of rearings compared with the following 6 min. Thus we decided to use 5 min as the time for the test. Figure 2 shows the dose–response curves for each of the tested drugs. The values of the effective dose 50 (ED50) and 95% confidence limits (CL95) for valerian extract and the other drugs appears in Table 2. The order of sedative effect was (ED50): haloperidol (0.41 mg kg^{-1}) > buspirone (1.04 mg kg^{-1}) > diazepam (1.21 mg kg^{-1}) > pentobarbital (11.86 mg kg^{-1}) > diphenhydramine (17.06 mg kg^{-1}) > valerian (181.62 mg kg^{-1}) > ethanol (1938.83 mg kg^{-1}). *Zadd* and *Zexp* values for each combination tested are given in Table 3.

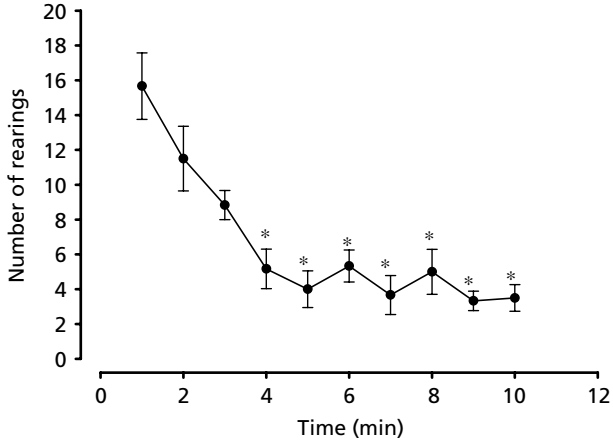

Figure 1 Rearing vs time (min) curve. *$P < 0.05$ compared with 1, 2 and 3 min, Duncan's multiple comparisons test after repeated measures analysis of variance. Mean ± s.e.m. of the number of rearings of 10 animals.

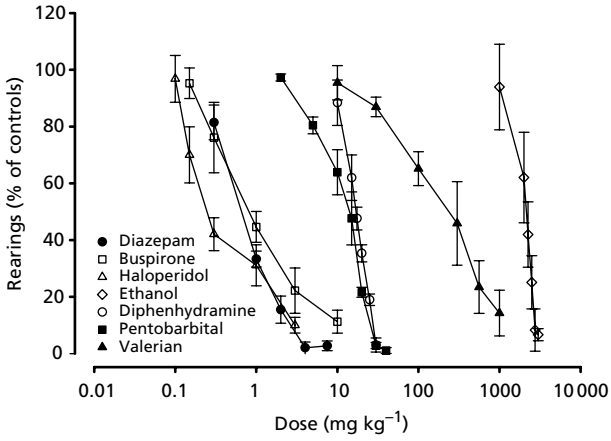

Figure 2 Dose–response curves plotting % of rearing with respect to its control represented on the y-axis for the sedative effect of haloperidol, buspirone, diazepam, pentobarbital, diphenhydramine, valerian extract and ethanol. Values are expressed as mean ± s.e.m. of ten mice. Doses (mg kg^{-1}) are represented logarithmically on the x-axis.

Table 2 ED50 values and 95% confidence limits of the sedative effect induced by valerian ethanol extract and different central nervous system depressant drugs in mice

Drug	ED50 (mg kg^{-1}, i.p.)	CL95%
Valerian extract	181.62	(158.66–207.90)
Diazepam	1.21	(0.97–1.49)
Ethanol	1938.83	(1617.65–2323.77)
Pentobarbital	11.86	(10.53–13.34)
Buspirone	1.04	(0.73–1.46)
Haloperidol	0.41	(0.30–0.55)
Diphenhydramine	17.06	(15.70–18.53)

The isobolograms of the simultaneous injection of equi-effective doses of valerian + haloperidol (Figure 3A), valerian + diazepam (Figure 3B), valerian + buspirone (Figure 3C) and valerian + diphenhydramine (Figure 3D) resulted in an additive relationship, because no statistical difference was demonstrated between the *Zadd* and *Zexp* value for each one of these combinations (Tallarida et al 1997b). In the combination valerian + haloperidol the *Zexp* value (91.52 mg kg^{-1}) displayed was similar to the *Zadd* value (91.01 mg kg^{-1}). The combination valerian + diazepam displayed a slight tendency towards super-additivity because *Zexp* (81.69 mg kg^{-1}) was lower than *Zadd* (91.41 mg kg^{-1}). The *Zexp* values for the combination of valerian + buspirone (112.73 mg kg^{-1}) and valerian + diphenhydramine (123.52 mg kg^{-1}) were higher than the *Zadd* values (91.33 and 99.34 mg kg^{-1}, respectively), however they were not statistically significant. The simultaneous injection of valerian extract plus ethanol gave an experimental point lying in the super-additive region (Figure 3E). In this case, the *Zexp* value (687.89 mg kg^{-1}) was lower than the *Zadd* value (1060.22 mg kg^{-1}), however a synergistic interaction was not statistically demonstrated (Student's *t*-test; $P > 0.05$). The isobologram for the combination valerian plus pentobarbital (Figure 3F) also resulted in an additive relationship. A sub-additive interaction may be questioned, but the difference between the *Zexp* (151.83 mg kg^{-1}) and *Zadd* (96.74 mg kg^{-1}) values were not significant ($P > 0.05$). This might have been due to the more important variability of the sedative effect with this combination (Figure 3F). The fractional analysis of the interaction between valerian with each of these CNS depressant drugs, based on the relationship $a/A + b/B$ (see Materials and Methods: Analysis of the interaction) also indicated that at a 1:1 fixed ratio, the sums of the fractional doses were not statistically different to 1.00 (Table 3), indicating additivity in all the cases tested (Tallarida 2000).

The HPLC/MS-ESI analysis identified the valtrates (valtrate/isovaltrate) as the major components of the extract. They appeared as two peaks corresponding to adductions $[M + Na]^+$ with m/z 445 and $[M + K]^+$ with m/z 461 at 34.9 and 35.2 min of retention time (RT), respectively. The absence of valerenic acid (RT = 37 min), hydroxyvalerenic acid (RT = 33.5 min), acetoxyvalerenic acid (RT = 30.8 min) and hesperidin (RT = 20 min) was demonstrated running

Table 3 Theoretical (*Zadd*) and experimental (*Zexp*) ED50 values ± s.e.m. for combinations of valerian extract with central nervous system depressant drugs and magnitude of the interaction values

Combination	*Zadd* (mg kg^{-1})	*Zexp* (mg kg^{-1})	Magnitude of the interaction[a]
Valerian:diazepam	91.41 ± 14.80	81.64 ± 10.54	0.89
Valerian:ethanol	1060.22 ± 64.88	687.89 ± 125.50	0.65
Valerian:pentobarbital	96.74 ± 14.80	151.83 ± 23.96	1.56
Valerian:buspirone	91.33 ± 14.80	112.73 ± 12.35	1.23
Valerian:haloperidol	91.01 ± 14.80	91.52 ± 21.77	1.00
Valerian:diphenhydramine	99.34 ± 14.80	123.52 ± 16.64	1.24

[a]According to the relation: $a/A + b/B$, see Materials and Methods.

under the same chromatographic conditions as the extract of *V. edulis* and the standard compounds.

Discussion

The results indicated that the co-administration of *Valeriana edulis* spp *procera* with each of the CNS depressant drugs (haloperidol, buspirone, diazepam, pentobarbital, ethanol and diphenhydramine) had an additive effect. This additive effect suggested the activation of a common mechanism between valerian extract and each one of these CNS depressant drugs (Miranda et al 2001).

Although the major constituents of valerian, including valepotriates, lignans, flavonoids and sesquiterpenes, have been isolated and pharmacologically investigated (Houghton 1999; Schumacher et al 2002; Fernández et al 2004), the molecular mechanism of the sedative action is still unclear and different compounds found in valerian have different effects on the CNS (Houghton 1999). When the mechanisms of action are not well understood, the effect of a combination of agents may be unexpected; this could be the case for valerian.

When valerian extract and haloperidol were co-administered, the experimental value of the ED50 lay exactly on the ED50 theoretical value predicted for an additive effect (Figure 3A). According to the isobolographic analysis, some components of the extract may be acting by a mechanism common to haloperidol i.e. as antagonist of D_2 dopamine receptors together with its effects on D_1, α-adrenergic and 5-HT_2 receptors. This observation could be related to the high concentrations of valepotriates present in *V. edulis* (Bos et al 1998) which have been found to bind to dopamine receptors and thus may inhibit the stimulatory effect of endogenous dopamine in the CNS (Houghton 1999).

Valerian has been demonstrated to induce sedation and hypnosis through modulation of γ-aminobutyric acid (GABA) neurotransmission and receptor function (Abebe 2002; Yuan et al 2004). Extracts from valerian have been found to inhibit the uptake and stimulate the release of GABA using the [^{3}H]muscimol and [^{3}H]GABA binding technique on synaptic membranes isolated from rat brain cortices (Carvalho et al 1994; Santos et al 1994; Ortiz et al 1999; De Feo & Faro 2003). The presence of high levels of GABA and glutamine have been demonstrated in valerian extracts, which could be metabolized to GABA, and these have been thought to be the substances causing the depressant effect (Santos et al 1994; Houghton 1999), but their absorption after oral administration is doubtful. Recently it has been suggested that the pharmacological effects of valerian extract and valerenic acid are mediated through modulation of $GABA_A$ receptor function (Yuan et al 2004). It has been demonstrated also that the flavonoid 6-methylapigenin, isolated from *V. wallichii*, is a competitive ligand for the benzodiazepine-binding site in the $GABA_A$ receptor (Wasowski et al 2002). It is known that valerenic acid is absent in *V. edulis* (Castillo et al 2002). Using HPLC/MS-ESI analysis it was verified that valerenic acid was not present in *V. edulis*, so the activity of this compound on $GABA_A$ receptor was discounted for *V. edulis*. The presence of 6-methylapigenin has not been demonstrated in *V. edulis*. However, Oliva et al (2004) proposed that the GABAergic system was the main neurotransmission involved in the anticonvulsant effect demonstrated in *V. edulis*. The participation of the GABAergic system is the major evidence in explaining the CNS depressant activity of valerian (Houghton 1999). The additive effect observed by the combination of diazepam plus valerian extract (Figure 3B) lends additional support to the suggestion that some components of *V. edulis* act on the benzodiazepine binding site of the $GABA_A$–benzodiazepine receptor complex.

The additive effect shown by the simultaneous administration of valerian extract with buspirone (Figure 3C), a 5-HT_{1A}-serotonin receptor agonist with anxiolytic properties (Briones-Aranda et al 2002), indicated that some components of the valerian extract act through the activation of 5-HT_{1A} receptors. This could be related to the presence of lignans, another group of compounds isolated from the roots of valerian (Bodesheim & Höltz 1997). One of those compounds, 1-hydroxypinoresinol, was found to exhibit an affinity to 5-HT_{1A} receptors in low micromolar concentrations (Bodesheim & Höltz 1997). However, it would be necessary to demonstrate the presence of this lignan in *V. edulis*. Even so, the results obtained with the isobolographic study for this combination suggested that the *V. edulis* extract had activity on serotonin receptors.

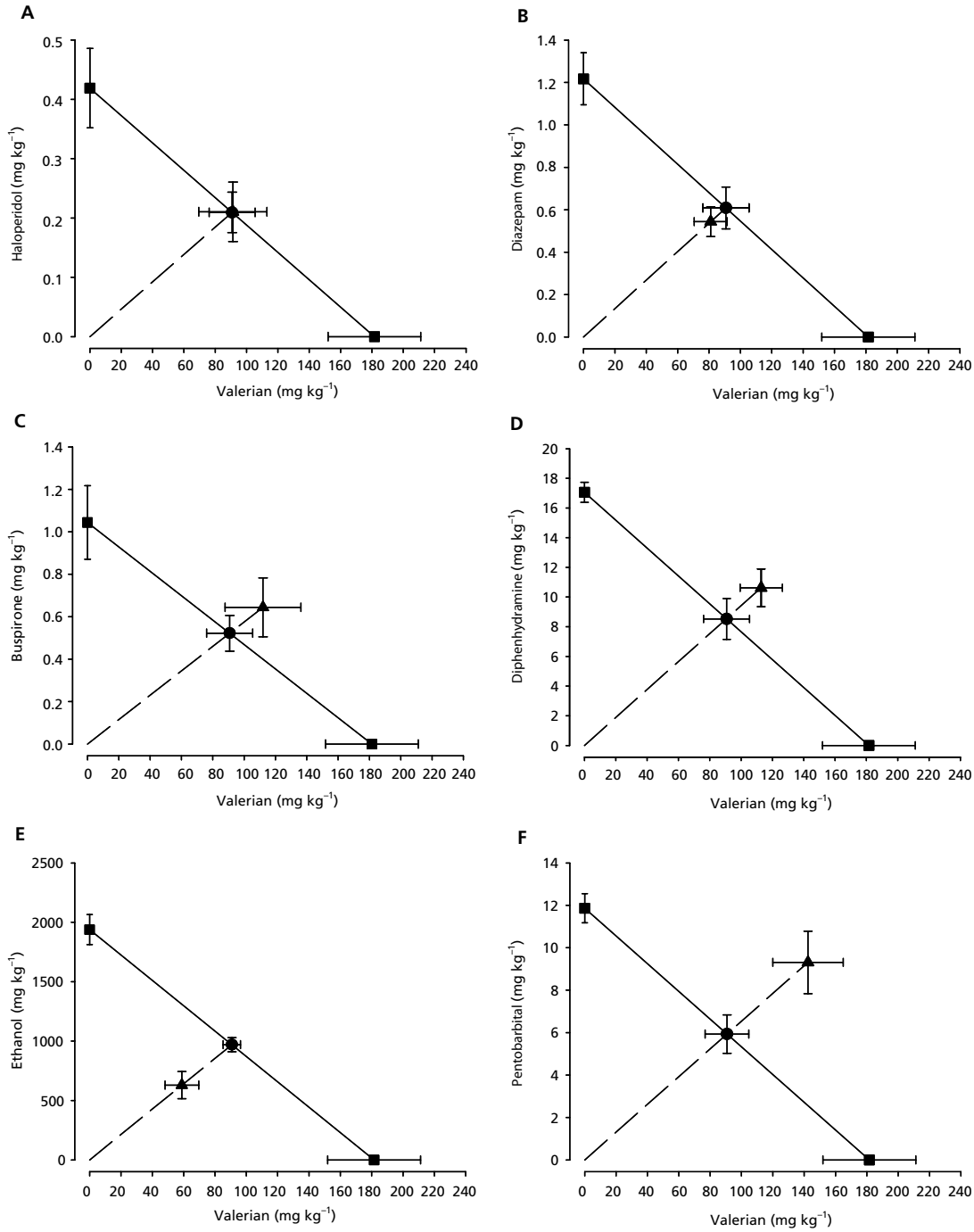

Figure 3 Isobolograms for the intraperitoneal co-administration of *Valeriana edulis* extract with central nervous system depressant drugs. A, valerian + haloperidol; B, valerian + diazepam; C, valerian + buspirone; D, valerian + diphenhydramine; E, valerian + ethanol; F, valerian + pentobarbital. The individual ED50 values in each combination (■), the theoretical calculated ED50 for an additive effect (*Zadd*) in a fixed ratio 1:1 (●), and the experimentally found ED50 values (*Zexp*, ▲) are represented in the graphs. Horizontal and vertical bars indicate s.e.m. The values of *Zexp* were close to *Zadd*, indicating an additive relationship for all the combinations studied.

Taking as a base the similarity and closer use with the first generation H_1 antihistamine OTC sleep aids, it has been proposed that *V. edulis* displays an H_1 antihistamine effect to explain the traditional use of this medicinal plant as a sleep aid (Oliva et al 2004). The results obtained with the isobolographic analysis for the co-administration of valerian extract with diphenhydramine (Figure 3D), which demonstrated an additive effect, are in agreement with this postulation. There is little information available about the antihistamine effect of valerian.

Several studies have demonstrated that valerian extract and isolated compounds of these species produced a

prolongation of the pentobarbital-induced sleeping time when they were injected 30 or 60 min before the administration of the pentobarbital (Abebe 2002; Hadley & Petry 2003). Previously in our laboratory this effect for *V. edulis* extract had been obtained, but only at high doses ($1000\,mg\,kg^{-1}$). Augmentation of the effect of pentobarbital on the motor co-ordination and myo-relaxant effect 120 min after the administration of the extract was observed also (Oliva et al 2004). An apparent contradictory effect of the co-administration of valerian and pentobarbital was observed in this study, because the *Zexp* point appeared in the subadditive region of the isobologram (Figure 3F), however there was no statistical difference either vertically or horizontally with *Zadd* (Tallarida 2000). A possible explanation to this apparent discrepancy was that in those experiments in which the valerian extract prolonged the sleep time of barbiturates, the valerian extract was administered at high doses and 30 min before the administration of pentobarbital. In this study valerian was administered simultaneously with pentobarbital. Again the additive effect observed in the combination valerian plus pentobarbital suggested the participation of the $GABA_A$ receptor complex.

There are some reports in the literature about concomitant ingestion of valerian and ethanol, but the interaction between these two substances has not been clearly demonstrated (Fugh-Berman & Cott 1999; Houghton 1999; Chen et al 2002; Hadley & Petry 2003). The additive effect observed by the co-administration of valerian and ethanol (Figure 3E) was in agreement with the information available in the literature, and suggested that the sedative effect of valerian was through the $GABA_A$ receptors.

The additive effect showed by *V. edulis* with some CNS depressant drugs indicated that the sedative effect of this medicinal plant occurred by different mechanisms, including the increase of the activity on $GABA_A$ and $5\text{-}HT_{1A}$ receptors and decrease of the activity on D_2 and H_1 receptor systems. These results might have been due to the variety and amounts of active compounds present in the extract. Equivalent results could be found from the isobolographic analysis with other valerian species and CNS depressant drugs, because they share similar uses, and similar effects and chemical composition. The additive effect may have clinical implications, because in an additive relationship, the effect of the drugs co-administration is predictable and the doses of the drug with more side effects can be decreased and the doses of the drug with less side effects can be increased to get the same desired effect (Tallarida 2000). However, it is necessary to demonstrate that these findings could be reproduced in clinical trials. In this case, the absence of synergistic or attenuate effect, found in this work, could explain the absence of reported clinical data on the interaction of valerian and commonly prescribed CNS depressant drugs. However, these combinations should be avoided.

Future scientific study on the medicinal plants of the interesting genera *Valeriana* may mean that they can be used with more confidence and could provide new knowledge on the pharmacology of CNS depressant drugs. Also, new active compounds could be found with different mechanisms of action, for example, some new lignans have been shown to have high affinity for rat and human A_1 adenosine receptor (Schumacher et al 2002). The flavanone glycosides 2S(-)hesperidin and the flavone glycoside linarin, isolated from *V. officinalis*, have shown sedative activity (Fernandez et al 2004). One topic that should be considered for future study is the pharmacokinetic herb–drug interaction for valerian, considering the mild in-vitro inhibitory effect of CYP3A4 reported for *V. officinalis* (Budzinski et al 2000). The possibility of pharmacokinetic interaction was not considered here due to the acute character of the study and that each animal received only one dose of valerian or CNS drug alone or in combination.

In conclusion, this was the first study that offered an isobolographic analysis of the co-administration of *V. edulis* spp *procera* and the CNS depressant drugs haloperidol, diazepam, buspirone, pentobarbital, diphenhydramine and ethanol in mice. In contrast to the expected synergistic interaction, frequently cited in the literature for the co-administration of valerian extract and CNS depressant drugs, additive effects were observed for these combinations. The results on the additivity observed in this work for *V. edulis* and CNS depressant drugs were in agreement with the background accumulated for several species of valerian. In addition, the additive relationships observed in this study suggested that this medicinal plant had components that might have acted on $GABA_A$, $5\text{-}HT_{1A}$, D_2 and H_1 receptor systems.

References

Abebe, W. (2002) Herbal medication: potential for adverse interactions with analgesic drugs. *J. Clin. Pharm. Ther.* **27**: 391–401

Bodesheim, U., Höltz, J. (1997) Isolierung, strukturaufkldrung und radiorezeptorassays vonalkaloiden und lignanen aus *Valeriana officinalis* L. *Pharmazie* **52**: 387–391

Bos, R., Woerdenbag, H. J., Van Putten, F., Hendriks, H., Scheffer, J. J. C. (1998) Seasonal variation of the essential oil, valerenic acid and derivatives, and valepotriates in *Valeriana officinalis* roots and rhizomes, and the selection of plants suitable for phytomedicines. *Planta Med.* **64**: 143–147

Briones-Aranda, A., Lopez-Rubalcava, C., Picazo, O. (2002) Influence of forced swimming-induced stress on the anxiolytic-like effect of 5HT(1A) agents in mice. *Psychopharmacol*ogy **162**: 147–155

Budzinski, J. W., Foster, B. C., Vandenhoek, S., Arnason, J. T. (2000) An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. *Phytomedicine* **7**: 273–282

Butterweck, V., Derendorf, H., Gaus, W., Nahrstedt, A., Schulz, V., Unger, M. (2004) Pharmacokinetic herb-drug interactions: are preventive screenings necessary and appropriate? *Planta Med.* **70**: 784–791

Carvalho, A. P., Cunha, A. P., Ferreira, F., Macedo, T., Ribeiro, C. F., Santos, M. S. (1994) Synaptosomal GABA release as influenced by valerian root extract-involvement of the GABA carrier. *Arch. Int. Pharmacodyn. Ther.* **327**: 220–231

Castillo, P., Zamilpa, A., Marquez, J., Hernandez, G., Lara, M., Alvarez, L. (2002) Comparative study of differentiation

levels and valepotriate content of *in vitro* cultures and regenerated and wild plants of *Valeriana edulis* ssp *procera*. *J. Nat. Prod.* **65**: 573–575

Chen, D., Klesmer, J., Giovanniello, A., Katz, J. (2002) Mental status changes in an alcohol abuser taking valerian and ginkgo biloba. *Am. J. Addict.* **11**: 75–77

De Feo, V., Faro, C. (2003) Pharmacological effects of extracts from *Valeriana adscendens* Trel. II. Effects on GABA uptake and amino acids. *Phytother. Res.* **17**: 661–664

Enciso-Rodríguez, R. (1997) Micropropagation of *Valeriana edulis* ssp. *procera*. *Planta Med.* **63**: 274–275

Fernandez, S., Wasowski, C., Paladini, A. C., Marder, M. (2004) Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. *Pharmacol. Biochem. Behav.* **77**: 399–404

Francis, A. J., Dempster, R. J. (2002) Effect of valerian, *Valeriana edulis*, on sleep difficulties in children with intellectual deficits: randomised trial. *Phytomedicine* **9**: 273–279

Fugh-Berman, A., Cott, J. M. (1999) Dietary supplements and natural products as psychotherapeutic agents. *Psychosom. Med.* **61**: 712–728

Fugh-Berman, A., Ernst, E. (2001) Herb-drug interactions: review and assessment of report reliability. *Br. J. Clin. Pharmacol.* **52**: 587–595

Hadley, S., Petry, J. J. (2003) Valerian. *Am. Fam. Physician* **67**: 1755–1758

Herrera-Arellano, A., Luna, V. G., Cuevas-Uriostegui, M. L., Alvarez, L., Vargas-Pineda, G., Zamilpa-Alvarez, A., Tortoriello, J. (2001) Polysomnographic evaluation of the hypnotic effect of *Valeriana edulis* standardized extract in patients suffering from insomnia. *Planta Med.* **67**: 695–699

Hiller, K., Zetler, G. (1996) Neuropharmacological studies on ethanol extracts of *Valeriana officinalis* L.: behavioral and anticonvulsant properties. *Phytother. Res.* 10: 145–151

Houghton, P. J. (1999) The scientific basis for the reputed activity of valerian. *J. Pharm. Pharmacol.* **51**: 505–512

Huang, S. M., Lesko, L. J. (2004) Drug-drug, drug-dietary supplement, and drug-citrus fruit and other food interactions: what have we learned? *J. Clin. Pharmacol.* **44**: 559–569

List, P. H., Schmidt, P. C. (1989) *Phytopharmaceutical technology*. CRC Press, Boca Raton, USA

Martínez, M. (1990) *Las Plantas medicinales de México*. 6th edn, Editorial Botas, México

Miranda, H., Sierralta, F., Pinardi, G. (2001) An isobolographic analysis of the adrenergic modulation of diclofenac antinociception. *Anesth. Analg.* **93**: 430–435

Oliva, I., González-Trujano, M. E., Arrieta, J., Enciso-Rodriguez, R., Navarrete, A. (2004) Neuropharmacological profile of hydroalcoholic extract of *Valeriana edulis* ssp. *procera* roots in mice. *Phytother. Res.* **18**: 290–296

Ortíz, J. G., Nieves-Natal, J., Chavez, P. (1999) Effects of *Valeriana officinalis* extracts on [^{3}H] flunitrazepam binding, synaptosomal [^{3}H] GABA uptake, and hippocampal [^{3}H] GABA release. *Neurochem. Res.* **24**: 1373–1378

Plushner, S. (2000) Valerian: *Valeriana officinalis*. *Am. J. Health Syst. Pharm.* **57**: 328–333

Rolland, A., Fleurentin, J., Lanhers, M. C., Younos, C., Misslin, R., Mortier, F., Pelt, J. M. (1991) Behavioral effects of the American traditional plant *Eschscholzia californica*: sedative and anxiolytic properties. *Planta Med.* **57**: 212–216

Rotblatt, M., Ziment, I. (2002) *Evidence-based herbal medicine*. Hanley & Belfus, Inc. USA

Sánchez, S. F. (1980) *La flora del Valle de México*. 6th edn, Edit. Herrero. México

Santos, M., Ferreira, F., Faro, C., Pires, E., Carvalho, A., Cunha, A., Macedo, T. (1994) The amount of GABA present in aqueous extracts of Valerian is sufficient to account for [^{3}H]GABA release in synaptosomes. *Planta Med.* **60**: 475–476

Schulz, V., Hänsel, R., Tyler, V. E. (1998) *Rational phototherapy: a physician's guide to herbal medicine*. 4th edn, Springer, New York, USA

Schumacher, B., Scholle, S., Holzl, J., Khudeir, N., Hess, S., Muller, C. (2002) Lignans isolated from Valerian: identification and characterization of a new olivil derivative with partial agonist activity at A_1 adenosine receptors. *J. Nat. Prod.* **65**: 1479–1485

Tallarida, R. (1992) Statistical analysis of drug combinations for synergism. *Pain* **49**: 93–97

Tallarida, R. (2000) *Drug synergism and dose-effect data analysis*. Chapman & Hall/CRC, USA

Tallarida, R., Porreca, F., Cowan, A. (1989) A statistical analysis of drug-drug and site–site interaction with isobolograms. *Life Sci.* **45**: 947–961

Tallarida, R., Kimmel, H. L., Holtzman, S. G. (1997a) Theory and statistics of detect synergism between two active drugs: cocaine and buprenorphine. *Psychopharmacology* **133**: 378–382

Tallarida, R., Stones, D. J., Raffa, R. B. (1997b) Efficient designs for studying synergistic drug combinations. *Life Sci* **61**: PL417–PL425

Wagner, H., Hörhammer, L., Hölz, J., Schaette, R. (1970) Zur bestimung der baldrian-droga undihrer zubereitungen. *Arzneimittelforschung* **20**:1149–1152

Wasowski, C., Marder, M., Viola, H., Medina, J. H., Paladini, A. C. (2002) Isolation and identification of 6-methylapigenin, a competitive ligand for the brain $GABA_A$ receptors, from *Valeriana wallichii*. *Planta Med.* **68**: 934–936

Yuan, C. S., Mehendale, S., Xiao, Y., Aung, H. H., Xie, J. T., Ang-Lee, M. K. (2004) The gamma-aminobutyric acidergic effects of valerian and valerenic acid on rat brainstem neuronal activity. *Anesth. Analg.* **98**: 353–358