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A Comparative Study of the Effects of Sparteine, Lupanine and Lupin Extract on the Central Nervous System of the Mouse

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Abstract

Lupin is toxic because of its alkaloid content, sparteine and lupanine in particular. Although the pharmacological properties of sparteine are well known those of lupanine have not been much studied. This paper reports procedures for extraction, purification and crystallization of lupanine, and methods for the preparation of an extract for injection of *Lupinus mutabilis* Sweet, and for the determination of the acute toxicity and maximum non-lethal dose (DL0) of lupanine, sparteine and lupin extract in the mouse.

The three substances were tested on the central nervous system (CNS) for locomotor activity, for interaction with specific drugs used for treatment of the CNS (the stimulant drugs amphetamine and pentetrazol and the depressant drugs pentobarbital and chlorpromazine) and for analgesic activity.

The results indicate that lupanine and lupin extract are less toxic than sparteine and that at the doses studied the three products have a weak sedative effect on the CNS.

Lupin, an interesting source of alimentary proteins, could be suitable for animal feed except that this member of the *Fabaceae* is toxic because of its alkaloid content, sparteine and lupanine in particular. Although the pharmacological and toxicological properties of sparteine are well known (Duke 1987; Bruneton 1993) those of lupanine have not been much studied because the compound is not commercially available. Sparteine was first extracted from *Cytisus scoparius* (L.) Link., but also occurs widely in the genus Lupin (Kinghorn & Balandrin 1984).

Sparteine has numerous pharmacological properties: cardiovascular, antihypertensive (Faucon & Ollagnier 1978; Schmitt 1980; Cohen 1981; Carraz & Carraz 1985; Piéri & Kirkiacharian 1986); effects on the autonomic nervous system including ganglion blocking and antimuscarinic effects (Lechat 1978; Schmitt 1980; Cohen 1981; Piéri & Kirkiacharian 1986); and effects on the central nervous system (CNS) including depressant (Schmitt 1980; Piéri & Kirkiacharian 1986), oxytocic (Lechat 1978; Cohen 1981; Piéri et al 1986), diuretic (Schmitt 1980; Cohen 1981) and local anaesthetic (Schmitt 1980; Cohen 1981) effects.

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Pharmacology books and other publications contain little information about lupanine. It is less toxic than sparteine; at high dose it stops the heart in diastole and at low dose it reduces coronary flow, contraction amplitude and heart rate (Duke 1987; Bruneton 1993). It is spasmolytic, oxytocic and ganglion blocking (Schmitt 1980). In the mouse, non-toxic intraperitoneal doses (LD0) of these two compounds are $30{\cdot}7\,mg\,kg^{-1}$ and $150\,mg\,kg^{-1}$, respectively for sparteine and lupanine; intraperitoneal LD100 values (i.e. the doses killing all the animals tested) are $150\,mg\,kg^{-1}$ (sparteine) and $225\,mg\,kg^{-1}$ (lupanine) (Yovo 1982). LD0 values established by other authors are $30\,mg\,kg^{-1}$ intravenous and $100\,mg\,kg^{-1}$ subcutaneous in the rabbit (Flury & Zernik 1928), $120\,mg\,kg^{-1}$ subcutaneous (Zipf & Triller 1943) and $75\,mg\,kg^{-1}$ subcutaneous in the mouse (Gordon & Henderson 1951). The LD50 (i.e. the doses killing half the animals tested) of sparteine and lupanine in the mouse are, respectively, 36 and $175\,mg\,kg^{-1}$ for intraperitoneal administration and 220 and $410\,mg\,kg^{-1}$ for oral administration (Yovo et al 1984). In the rat the intraperitoneal LD50 of lupanine was found to be $177\,mg\,kg^{-1}$ (Petterson et al 1987); LD50 values measured after oral administration are $1664\,mg\,kg^{-1}$ (Petterson et al 1987) and $1440\,mg\,kg^{-1}$ (Shani et al 1974). Intraperitoneal

LD50 values for lupanine are, therefore, similar for the mouse and rat but rats are more resistant after oral administration.

In this paper, we compare the effects of lupanine and extract of lupin (*L. mutabilis* Sweet) on the CNS with those of sparteine under the same experimental conditions. After determination of acute toxicity, the orientation test of Irwin (1962) as modified by Moreau (1975) and Foussard-Blanpin (1980) was used to evaluate the effects on animal behaviour in different situations. A series of pharmacological tests (Boissier & Simon 1962; Koster et al 1959) was then used to explore the action of sparteine, lupanine and lupin extract on the CNS.

Because lupanine is not commercially available we perfected an effective and simple method for isolation of lupin alkaloids and then optimized the conditions for obtaining pure lupanine, which was assayed by scanning densitometry after thin-layer chromatography.

Materials and Methods

Plant material

The seeds of *Lupinus mutabilis* Sweet were provided by the Institut National de la Recherche Agronomique (INRA) F-86600 Lusignan, France.

Preparation of products for injection

Lupin extract. Lupin seeds were ground and the powder was defatted with hexane in a Soxhlet extractor. The defatted powder was made alkaline with aqueous ammonia (15%) and extracted with dichloromethane. The crude extract was concentrated by evaporation under reduced pressure and purified by successive transfer to the aqueous phase (HCl 0·1 M) and to organic phases (dichloromethane, 15% ammonia).

The extract was prepared at a concentration of 100 mg/25 mL by addition of HCl (0·1 M) to pH 7 and adjusting with water to the correct concentration.

Extraction, purification and preparation of lupanine and sparteine. Lupanine was obtained in purified form from a lupin extract. The extract containing the alkaloids was subjected to column chromatography on silica gel 60 (70–230 mesh; Merck #7734) which was eluted with 100 : 5 (v/v) $CHCl_3$–CH_3OH. Successive fractions were monitored simultaneously with a test sample of lupanine by thin layer chromatography (TLC) on silica gel $60F_{254}$ (Merck #5715) (Dormeau 1992; Pothier 1995) with $CHCl_3$–acetone–28% NH_4OH, 25 : 24 : 1 (v/v) as mobile phase. After development alkaloids were detected by spraying the plates with Dragendorff's reagent (Munier & Macheboeuf 1949). The fractions containing lupanine (hR_F 66) were concentrated under reduced pressure and monitored by GC–MS (lupanine, m/z (%), 70 eV: 248 (37) (M^+), 247 (25), 150 (32), 149 (59), 136 (100), 98 (32), 97 (26), 94 (18), 69 (18), 55 (59); Kinghorn & Balandrin (1984)).

It is not easy to obtain crystalline lupanine hydrochloride because the dihydrochloride gives deliquescent prisms and the hydrochloride dihydrate is hygroscopic.

For sparteine we proceeded in the same manner with a standard of sparteine sulphate pentahydrate (Aldrich).

Densitometry

Densitometry was performed according to the TLC method of Pothier (1995). Lupin extract was spotted by means of a Linomat IV (Camag) and 2, 6, 10, 12 and 16 μg of lupanine in methanolic solution was spotted as a reference. After elution the plates were sprayed with Dragendorff's reagent as modified by Munier & Macheboeuf (1949), then read with a Camag densitometer 76510 TLC/HPTLC Scanner coupled with a Merck 2500 integrator.

Pharmacological experiments

Preparation of solutions for injection. The three basic products were acidified by adding HCl (0·1 M) to physiological pH.

Animals. EOPS male Swiss mice, 20 ± 2 g, were supplied by CERJ (53680-Le Genêt, France). For each test mice were allotted at random to several groups of five animals and kept under observation for 8 days.

Determination of acute toxicity. Increasing doses (50, 100, 125, 250 or 500 mg kg^{-1}) of sparteine, lupanine and lupin extract were administered intraperitoneally (0·5 mL/20 g) to groups of five mice. Animals were observed for eight days.

Effect on psychomotor activity. Psychomotor activity was evaluated using mice (three per group) housed in small Plexiglas cages under temperature-(20 ± 2°C) and noise-controlled conditions. The mice were submitted to various tests according to the technique described by Foussard-Blanpin (1980) and Picq et al (1991). Assays were performed before and after treatment (30 min, 1, 2, 3 h) to establish behaviour codification.

Exploration test. A naive mouse was placed on an automated hole board (16 holes Apelex) with

automatic counting of the animal's movements across the board (photoelectric cell). The assays were performed 30 min after administration of the three compounds (five mice per dose) and both locomotor activity and exploratory behaviour recorded every minute for 5 min.

Interaction with drugs acting on the CNS. The effects of stimulant drugs (amphetamine, pentetrazol) and depressant drugs (pentobarbital, chlorpromazine) dissolved in distilled water were compared in controls and in animals treated 30 min previously with the substances under study. Tests were performed on four groups of five mice; three groups were treated with the substances under study at the doses indicated in Tables 1 and 2 (five control animals were injected with an equal volume of normal saline solution). Animals were placed in transparent cages and symptoms were observed for a period of 1 h after injection and lethality after 24 h was recorded.

Antagonism of amphetamine-induced effects. Amphetamine ($70\,mg\,kg^{-1}$) injected intraperitoneally elicited an increase in agitation, aggressive behaviour, and sweat and saliva hypersecretion. A behaviour rating (or cotation index) from 0 to 5 was used, a score of 5 representing the maximum reaction of the control in the presence of amphetamine.

Antagonism of pentetrazol-induced convulsions and death. A modification of Hester's method (Hester et al 1971) was used. Pentetrazol ($125\,mg\,kg^{-1}$) was injected subcutaneously and survival time and lethality were noted after 24 h.

Antagonism of pentobarbital-induced hypnotic effect. Pentobarbital ($60\,mg\,kg^{-1}$) was injected intraperitoneally and the time necessary to induce sleep in the animals and the sleeping time were noted.

Antagonism of chlorpromazine-induced cataleptic effect. Chlorpromazine ($35\,mg\,kg^{-1}$) was injected intraperitoneally and the induced catalepsy was codified: 0, impossible to cross limbs; 10, possible to place together forelimbs and hindlimbs on the same side; 20, possible to cross forelimbs and hindlimbs on the same side; 25, possible to join forelimbs and hindlimbs on the same side and to cross them on the other side; 30, possible to cross forelimbs and hindlimbs on both sides simultaneously.

Analgesic effect. The effects of the substances were studied using four groups of five mice. The abdominal constriction test was performed by the method of Koster et al (1959)—sparteine, lupanine and lupin extract were injected intraperitoneally into three groups of mice 30 min before administration of 0·4% acetic acid (0·40 mL/20 g). Control animals (1 group) received normal saline solution (0·5 mL) under the same experimental conditions. Immediately after injection of the acetic acid each animal was isolated in an individual cage ($24 \times 11 \times 10\,cm^3$) and observed for 20 min. The number of abdominal constrictions and the amount of stretching were recorded.

Statistics

Results are expressed as means ± s.e.m.; statistical comparisons were made by use of Student's *t*-test with $P < 0.05$ being regarded as indicative of a significant difference.

Results

Determination of acute toxicity

The maximum non-lethal intraperitoneal doses (LD0) were $25\,mg\,kg^{-1}$ for sparteine and $64\,mg\,kg^{-1}$ for lupanine and lupin extract. Lethal intraperitoneal doses (LD100) were $100\,mg\,kg^{-1}$ for sparteine and $250\,mg\,kg^{-1}$ for lupanine and lupin extract. The clinical signs of poisoning: shaking, excitation and convulsions, were the same for the three products.

Table 1. Influence of intraperitoneal sparteine, lupanine and lupin extract on the effects of intraperitoneal amphetamine ($70\,mg\,kg^{-1}$) and subcutaneous pentetrazol ($125\,mg\,kg^{-1}$).

Product	Dose ($mg\,kg^{-1}$)	Amphetamine Lethality (%)	Pentetrazol		
			Latency period (s)	Survival period (s)	Lethality (%)
Control	–	80	1 ± 0	120 ± 4	100
Sparteine	13	100	129 ± 55*	628 ± 132**	100
Lupanine	32	80	101 ± 10*	398 ± 127*	100
Lupin extract	32	80	315 ± 162*	603 ± 198*	100

Values are means ± s.e.m. *$P < 0.05$, **$P < 0.01$, significantly different from control result.

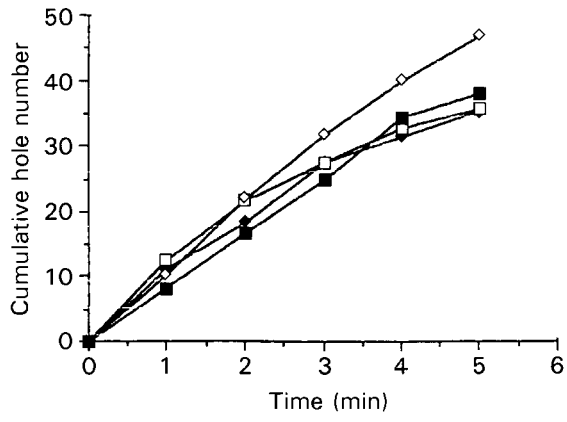

Figure 1. Cumulative number of hole pokes (exploring behaviour) in the hole-board: □, control; ◆, sparteine; ■, lupanine; ◇, lupin extract.

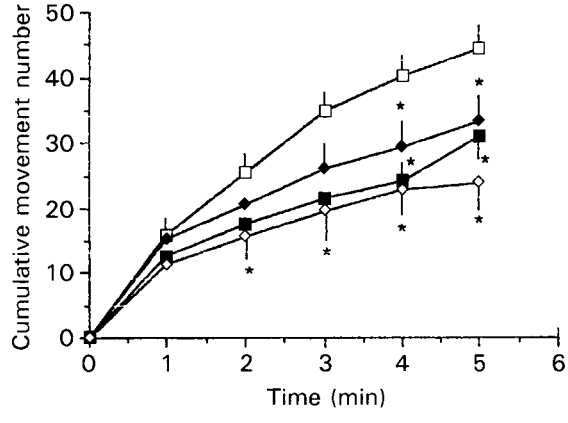

Figure 2. Cumulative number of movements (locomotor activity) in the hole-board test during 5 min observation: □, control; ◆, sparteine; ■, lupanine; ◇, lupin extract.

Effect on psychomotor activity

At the LD0 the compounds elicited a weak decrease in spontaneous activity. The tactile index and the algogenic stimulations reactivity were not modified. The behaviour indices of sedation (Boissier & Simon 1962) taking into account experimental observations were −34, −27 and −20 for sparteine, lupanine and lupin extract, respectively (Figure 1).

Exploration test

As can be seen from Figure 2, at the LD0 the three products weakly reduced locomotor activity. The number of holes explored by mice increased slightly, but not significantly, after administration of lupin extract.

Interaction with drugs acting on the CNS

Effect on stimulant drugs. The results presented in Table 1 show that the three products did not induce an antagonist effect towards amphetamine-induced stereotypy, aggression, agitation and secretion

Table 2. Influence of intraperitoneal sparteine, lupanine and lupin extract on the hypnotic effect induced by intraperitoneal pentobarbital (50 mg kg^{-1}).

Product	Dose (mg kg^{-1})	Pentobarbital	
		Sleep latency time	Sleeping time
Control	–	216±40	101±30
Sparteine	13	372±164	103±46
Lupanine	32	420±225	83±7
Lupin extract	32	246±23	85±19

Table 3. Influence of intraperitoneal sparteine, lupanine and lupin extract on the cataleptic effect of intraperitoneal chlorpromazine (40 mg kg^{-1}).

Product	Dose (mg kg^{-1})	Chlorpromazine			
		30 min	1 h	1·5 h	2 h
Control	–	10±6	12±4	16±6	27±4
Sparteine	13	11±2	16±6	19±4	15±6
Lupanine	32	19±4	19±4	18±7	23±3
Lupin extract	32	15±3	23±3	23±3	19±4

Table 4. Effect of intraperitoneal sparteine, lupanine and lupin extract on the abdominal constrictions induced by intraperitoneal administration of 0·4% acetic acid (80 mg kg^{-1}).

Product	Dose (mg kg^{-1})	Number of constrictions	Variation (%)
Control	–	30±10	–
Sparteine	13	15±6	50
Lupanine	32	12±5	60
Lupin extract	32	13±4	56

neither did they protect mice against mortality induced by pentetrazol. They did, however, delay the onset of convulsions and increase the survival time of the animals.

Effect on depressant drugs. The results presented in Table 2 show that the three substances had no effect either on the latency time or on the duration of pentobarbital-induced sleep. The cataleptic effect of chlorpromazine was not modified by sparteine, lupanine or lupin extract (Table 3).

Analgesic effect

As is apparent from Table 4 the number of abdominal constrictions and the amount of stretching induced by 0·4% acetic acid solution were slightly but not significantly reduced by the three substances.

Discussion

Several solvents have been used for extraction of lupin alkaloids. To find optimum conditions for extraction we tested two general methods: sequential extraction by maceration at room temperature and continuous extraction in a Soxhlet apparatus. Five extraction solvents were compared; from the results obtained (Table 5) it is apparent that diethyl ether–chloroform, 2 : 1 (v/v) is the most efficient both at room temperature and in a Soxhlet apparatus. Lower yields were obtained with chloroform, diethyl ether and methanol. Yields with individual solvent were not affected by whether the extraction was performed at room temperature or in a Soxhlet apparatus.

The study shows that sparteine is more toxic than lupanine. These results confirm those of Yovo (1982) and Mazur et al (1966) but not those of Couch (1926) who found lupanine to be more toxic than sparteine.

The general results obtained show that sparteine, lupanine and lupin extract have two effects on the CNS. At high doses near the LD100 (lethal dose for 100% of animals) the effects are nicotinic-like (Schmitt 1980) whereas at lower doses (< LD0) the three products have slight sedative action on the CNS, as shown by the effect on behaviour (−34, −27 and −20) (Figure 1). This last result confirms the effects reported by Piéri and Kirkiacharian (1986) for sparteine and are probably a consequence of ganglion blocking action (Schmitt 1980).

A weak depressant effect has been observed on the movement curves (Figure 2) but not on psychomotor activity. The three products modify the action of pentetrazol by increasing the latency time of convulsive attacks and by prolonging the survival time of animals (Table 1). Depressant actions were not modified (Tables 2 and 3) but the preparations were slightly but not significantly analgesic (Table 4).

In conclusion, at the doses studied sparteine, lupanine and lupin extract have a slight action on the CNS. At the lethal doses the clinical signs of intoxication are excitation (trembling and tonico-clonic convulsions). At half the LD0 the sedative effects are noticeable.

These results confirm those obtained by Nucifora & Malone (1971) who observed no specific depressive action on the CNS of rats except, at intraperitoneal doses from 55·5 mg kg^{-1}, for a reduction of motor activity in respiratory rhythm and hypothermia. Yovo (1982) used a dose equal to 0·2 × the LD50 and observed no significant effect of sparteine and lupanine on CNS activity.

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Table 5. Comparison of yield (%) of alkaloids obtained by maceration and by extraction in a Soxhlet apparatus.

Solvent	Compound	Maceration				Soxhlet			
		1	2	3	Average	1	2	3	Average
Chloroform	Sparteine	0·58	0·58	0·62	0·59	0·33	0·47	0·46	0·42
	13-Hydroxylupanine	0·37	0·40	0·39	0·39	0·31	0·39	0·34	0·35
	Lupanine	1·29	1·33	1·09	1·23	1·13	0·88	0·88	0·96
	Total	2·24	2·31	2·33	2·21	1·95	1·74	1·68	1·73
Diethyl ether	Sparteine	0·37	0·34	0·35	0·35	0·28	0·40	0·36	0·35
	13-Hydroxylupanine	0·39	0·36	0·37	0·37	0·27	0·35	0·34	0·32
	Lupanine	1·51	1·29	1·29	1·36	0·98	1·01	0·88	0·96
	Total	2·27	1·99	2·01	2·08	1·53	1·76	1·58	1·63
Diethyl ether–chloroform 2 : 1	Sparteine	0·59	0·49	0·59	0·56	0·22	0·33	0·37	0·31
	13-Hydroxylupanine	0·60	0·64	0·50	0·58	0·34	0·30	0·32	0·32
	Lupanine	1·72	1·51	1·66	1·66	1·09	1·12	1·09	1·10
	Total	2·91	2·64	2·75	2·80	1·65	1·75	1·78	1·73
Ethanol–2% acetic acid	Sparteine	0·05	0·06	0·06	0·05	0·21	0·15	0·20	0·19
	13-Hydroxylupanine	0·03	0·03	0·03	0·03	0·30	0·13	0·20	0·21
	Lupanine	0·18	0·21	0·20	0·20	1·15	0·52	0·92	0·86
	Total	0·26	0·27	0·29	0·28	1·66	0·80	1·32	1·26
Methanol	Sparteine	0·19	0·19	0·16	0·18	0·33	0·29	0·25	0·29
	13-Hydroxylupanine	0·09	0·08	0·07	0·08	0·33	0·15	0·13	0·20
	Lupanine	0·54	0·51	0·40	0·48	1·10	0·62	0·50	0·74
	Total	0·82	0·78	0·63	0·74	1·76	1·06	0·88	1·23

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