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Neuropharmacological Effects of Extracts from *Sickingia williamsii*

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Abstract

Sickingia williamsii Standl. (Rubiaceae) is used in Peruvian folk medicine for its analgesic and anti-inflammatory activity. In this study we have examined the pharmacological profiles of petroleum ether, chloroform, chloroform-methanol (9:1) and methanol extracts of the tree.

Both chloroform-methanol (250, 500 or 750 mg kg^{-1}, p.o.) and methanol (125, 250 or 500 mg kg^{-1}, p.o.) extracts significantly reduced both the locomotor activity and motor co-ordination of mice; they also prolonged sleep induced by pentobarbital, although no significant cataleptic response was observed. These extracts did not have a significant impact on the nociceptive threshold in the hot-plate and tail-flick tests. Petroleum ether and chloroform extracts did not, furthermore, induce any significant pharmacological effects.

The results of the study suggest that these extracts possess neurosedative-like properties.

Although the bark of *Sickingia williamsii* Standl., a typical South American small tree belonging to the family Rubiaceae, is empirically used for its analgesic and anti-inflammatory activity in Peruvian folk medicine (Soukup 1987), there are no data in the literature on the possible pharmacological effects of the plant. We recently reported the isolation and purification of several glucoindole and β-carboline alkaloids and iridoids from the above plant (Aquino et al 1994). All the alkaloids isolated from *S. williamsii* were characterized by the presence of an indole nucleus. Alkaloids with the β-carboline structure are present in plants known to affect the central nervous system (CNS) including *Banisteriopsis* species, *Ailanthus altissima* (Crespi Perellino et al 1988) and *Passiflora incarnata* (Lutomski et al 1981; Speroni & Minghetti 1988); the pharmacological activity of these plants is related to their indole alkaloid content. Administration of crude alkaloid extracts of various species of *Banisteriopsis* has, for example, resulted in various CNS effects including sedation, passivity of movements, prolongation of hexobarbital sleeping time, antagonism of reserpine-induced hypothermia and analgesia (Stull et al 1971). In a recent study, we showed that extracts from *S. williamsii* inhibited the electrical contractions of guinea pig ileum (Aquino et al 1996); this paper reports studies on the pharmacological effect of extracts from *S. williamsii* on the CNS.

Materials and Methods

Plant material

The bark of *S. williamsii* Standl. was collected in October 1990 from Cachiya de Huaquisha, Dist. Tochache Nuevo, Prov. Mariscal Càceres, Dep. to San Martin, Peru and was identified by Dr O. Lock de Ugaz, Pontificia Universidad Catolica del Peru. A voucher sample is deposited at the Departamento de Quimica of this University.

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Extraction procedure

The dried and powdered bark (450 g) was successively extracted with petroleum ether, chloroform, chloroform-methanol (9:1) and methanol in a Soxhlet apparatus to give 2·8, 1·4, 12·0 and 20·1 g of residues, respectively.

Animals

Male Swiss mice, 20–25 g, were supplied by Charles River (Italy). The animals were housed in colony cage (ten mice per cage) under standard conditions of lighting (light on from 0700 h to 1900 h), temperature (22 ± 1°C) and room humidity (60 ± 10%) for at least a week before the experimental sessions. Food and water were freely available.

Locomotor activity

The animals were placed in the activity cage (Basile, Milan, Catalogue No. 7400) for at least 30 min for acclimatization before receiving an injection of the extract of *S. williamsii* Standl. Temperature, sound and light conditions were maintained uniform during the course of the experiments. Mouse locomotor activity was recorded for 120 min in the cage. Measurements were performed at 10-min intervals and cumulative counts were recorded automatically (Capasso et al 1991).

Motor co-ordination

Motor co-ordination of the mice was evaluated by use of a rotarod apparatus (Ugo Basile, Italy) consisting of a bar (3·0 cm diam.) subdivided into five compartments by a disk 24 cm in diameter. The bar rotated at a constant speed of 16 rev min^{-1} (Pieretti et al 1994). The integrity of motor co-ordination was assessed on the basis of the amount of time the animals spent on the rotating rod. One day before the test the animals were trained twice. On the day of the test only mice able to stay balanced on the rotating rod for between 70 and 120 s (cut-off time) were selected. The performance time was measured before treatment and at various times (20, 40, 80 and 120 min) afterwards (Malcangio et al 1991).

Pentobarbital-induced sleep

One hour after administration of *S. williamsii* extract mice were given an intraperitoneal dose of pentobarbital (50 mg kg^{-1}). The time between loss and subsequent recovery of the righting reflex was taken as the sleeping time and was recorded for animals pretreated either with saline (0.9% NaCl) or drug (Pieretti et al 1992).

Stereotyped behaviour

Rearing (RE), grooming (GR), social response test (SRT), crossing (CR), smelling (SM), washing face (WF), scratching (SC) and bar holding (BH) were monitored as previously reported (Hecht & Schiorring 1979). The frequency of all behaviour was recorded manually by one observer who was blind to the drug treatment. Animals were tested for a period of 120 min after injection of the extracts from *S. williamsii*, with each mouse being observed during time intervals 10–20, 40–50, 70–80 and 100–120 min post-injection.

To check the social responsiveness of the treated mouse, each session contained a social response test. An untreated mouse was placed in the experimental cage with the drug-treated mouse for 5 min. The presence or absence of common social behaviour (sniffing, play or aggression) was recorded.

Catalepsy

The presence of a state of catalepsy was detected using an abnormal posture test (Fog 1972). In mice, catalepsy was quantitatively estimated by placing the forepaws of the animals on a horizontal rod which was mounted 3 cm above the floor of the experimental box. The test was regarded as positive if the animal remained in this position for at least 45 s. Latency to step down was recorded before and at various times (20, 40, 80 and 120 min) after drug treatment. A maximum cut-off time of 45 s was used. Cataleptic responses were calculated as a percentage of the maximum possible response (%MPR) defined as $(R - B)/(45 - B) \times 100\%$, where B is the mean baseline latency, R the post-treatment response latency and 45 the cut-off time (Kiritsy-Roy et al 1989).

Nociceptive assays

The nociceptive assays performed were the hot-plate and the tail-flick tests. The hot-plate test was performed as previously described (Pieretti et al 1991). Briefly, the hot plate (Socrel Mod. DS-37, Ugo Basile, Italy, 25 × 25 cm) was set at a temperature of 55 ± 0·5°C to give a response latency of 17–20 s in control animals. The time of hind paw licking was recorded, and measurement was terminated if the licking exceeded the cut-off time (60 s). The tail-flick test was performed as previously described (Capasso et al 1992). Briefly, the tail-flick latency was obtained using a tail-flick unit (Socrel Mod. DS-20, Ugo Basile, Italy), in which the animals were gently immobilized by use of a glove and radiant heat was focused on a blackened spot 1–2 cm from the tip of the tail. Beam intensity was adjusted to give a tail-flick latency of 2–3 s in control animals. To avoid tissue damage, measurement was terminated if the latency exceeded the cut-off time (10 s). In all the experiments mice were tested 60 and 30 min before drug administration to determine a baseline latency and again 30 min after drug administration.

Experimental procedure

On the day of testing both chloroform-methanol and methanol extracts of *S. williamsii* used in the experimental sessions were dissolved in saline solution for administration, whereas petroleum ether and chloroform extracts were dissolved in a solution of carboxymethylcellulose (0.1%). Drugs were injected in a volume of 10 mL kg^{-1} orally. The chloroform-methanol (250, 500 and 750 mg kg^{-1}, p.o.), methanol (125, 250 and 500 mg kg^{-1}, p.o.), petroleum ether (250, 500 and 750 mg kg^{-1}, p.o.) and chloroform (250, 500 and 750 mg kg^{-1}, p.o.) extracts of *S. williamsii* were administered 1 h before the beginning of the tests.

Statistical analysis

All data (expressed as mean ± s.e.m.) were analysed using analysis of variance and Dunnett's procedure for multiple comparisons with a single control group. When the analysis was restricted to two means, a Student's *t*-test (two-tailed) was used. The Fisher exact test was used to analyse the rotarod data. Significance was assumed at a 5% level.

Results

Locomotor activity

The activity of the control (saline-treated) animals and those treated with chloroform-methanol and methanol extracts from *S. williamsii* are shown in Fig. 1. Both extracts significantly reduced the locomotor activity of the mice; the extent of the effect was dose-dependent. The extract-induced reduction in activity was significant 10–20 min after the beginning of the test and lasted beyond the recording period (120 min) for all the mice. No changes in locomotor activity were induced by the petroleum ether or chloroform extracts (data not shown).

Motor co-ordination

The motor co-ordination of the control (saline) mice and the mice treated with chloroform-methanol and methanol extracts is shown in Fig. 2. At the doses used both extracts induced a significant and dose-dependent reduction of the motor co-ordination of mice on the rotarod bar, in comparison both with the saline-treated mice and with the respective pre-drug performance (Fig. 2). The reduction induced by these extracts was significant 10–20 min after the beginning of the test and lasted for the entire recording period (120 min). No effects on the motor co-ordination of mice were induced by the petroleum ether and chloroform extracts (data not shown).

Pentobarbital-induced sleep

As shown in Table 1, both chloroform-methanol and methanol extracts of *S. williamsii* significantly lengthened the sleep induced by pentobarbital. No effects on pentobarbital-induced sleep were induced by the petroleum ether and chloroform extracts (data not shown).

Stereotyped behaviour

The stereotyped behaviour of the control (saline) mice and mice treated with *S. williamsii* are reported in Table 2. The chloroform-methanol and methanol extracts both significantly reduced all the behaviour elements of the mice considered in our study. The reduction was significant 10–20 min after the beginning of the test and lasted for the entire recording period (120 min).

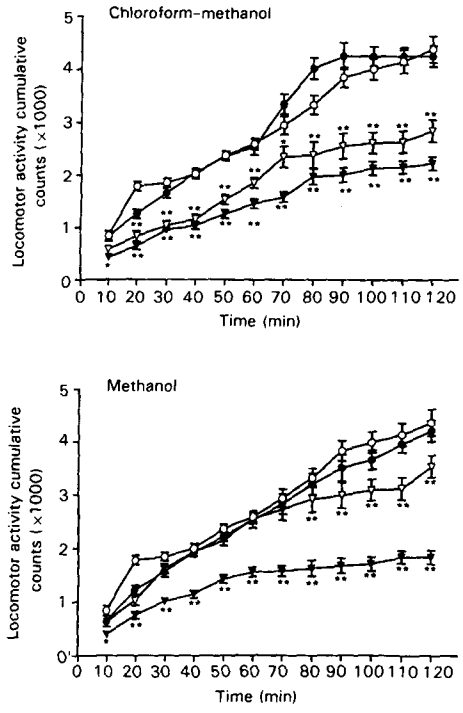

FIG. 1. Time- and dose-effect curves of the chloroform-methanol and methanol extracts of *Sickingia williamsii* on locomotor activity in mice. Saline ○; chloroform-methanol 250 ●, 500 ▽, 750 mg ▼; methanol 125 ●, 250 ▽, 500 mg ▼. Results are ± s.e.m. (n = 6). $*P < 0.05$, $**P < 0.01$.

Catalepsy

None of the *S. williamsii* extracts induced a significant cataleptic effect in mice during the 120 min observation period (data not shown).

Nociceptive assays

None of the *S. williamsii* extracts induced significant changes in the nociceptive threshold measured using either the hot-plate or tail-flick tests (data not shown). The reaction times registered after the injection of the extracts from *S. williamsii* were similar to those seen for animals treated with saline.

Discussion

The results of this study indicate that the chloroform-methanol and methanol extracts of *S. williamsii* Standl. induce a significant reduction in locomotor activity, motor co-ordination, and stereotyped behaviour in the mouse. An increase in pentobarbital-induced sleep was also observed, indicating that *S. williamsii* Standl. extracts induce important depressant effects on the CNS. The extracts of *S. williamsii* did not, however, induce analgesia and catalepsy.

There is no data in the literature on the pharmacological effects exerted by extracts of *S. williamsii*, whereas much data has been reported on *Banisteriopsis*, *Ailanthus altissima* and *Passiflora incarnata* species which are known to contain indole alkaloids (Stull et al 1971; Lutomski et al 1981; Crespi Perellino et al 1988; Speroni & Minghetti 1988). In a previous paper the bark of *S. williamsii* was also shown to contain a number of indole alkaloids, some of which possess a β-carboline nucleus (Aquino et al 1994). The β-carboline nucleus is also contained in alkaloids from some *Banisteriopsis* species,

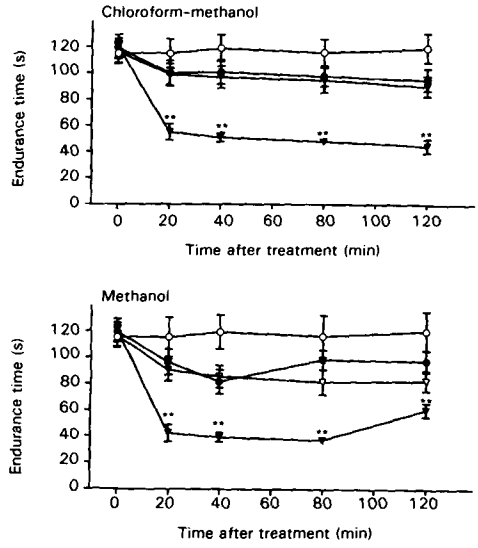

FIG. 2. Time- and dose-effect curves of the chloroform-methanol and methanol extracts of *Sickingia williamsii* on motor co-ordination in mice. Saline ○; chloroform-methanol 250 ●, 500 ▽, 750 mg ▼; methanol 125 ●, 250 ▽, 500 mg ▼. The cluster of symbols related to time 0 on the abscissa indicates the endurance time before treatment. Results are mean ± s.e.m. (n = 6). $**P < 0.01$.

Table 1. Effect of the chloroform-methanol and methanol extracts of *Sickingia williamsii* extracts on pentobarbital-induced sleep in mice after intraperitoneal injection. Values indicate the minutes elapsed between loss and recovery of righting reflex.

Extract	Sleeping time (min)
Saline	110 ± 10
Chloroform-methanol 250 mg	100 ± 2
Chloroform-methanol 500 mg	130 ± 8
Chloroform-methanol 750 mg	175 ± 9*
Methanol 125 mg	110 ± 10
Methanol 250 mg	160 ± 8
Methanol 500 mg	200 ± 11**

Results are mean ± s.e.m. (n = 6). $*P < 0.05$, $**P < 0.05$

A. altissima and *P. incarnata* (Hashimoto & Kawanisha 1975, 1976; Lutomski et al 1981; Crespi Perellino et al 1988). Alkaloids with a β-carboline structure are reported to affect CNS activity (Stull et al 1971). In this study the extracts of *S. williamsii* seem to possess pharmacological properties very similar to those of *Banisteriopsis* species as they induce a significant reduction of the neuronal activity of mice.

If the reduction of motor co-ordination, stereotyped behaviour and locomotor activity which were observed are considered together with the increase in pentobarbital-induced sleep, they support the hypothesis that *S. williamsii* might act as a mild neurosedative drug. It is, in fact, already known that many neurosedative drugs tend to increase sleeping time and reduce locomotor activity (Baldessarini 1990).

The activity of the extracts is probably related to their alkaloid content, and the lack of effect of the other two extracts from *S. williamsii* (petroleum ether and chloroform) may further confirm this hypothesis as they do not contain alkaloids with a β-carboline structure.

We cannot, on the other hand, exclude the possibility that because of the high doses of both the chloroform-methanol and methanol extracts, the sedative effects produced by these compounds could be non-selective; further studies are in

Table 2. Effect of saline and chloroform–methanol (250, 500 or 750 mg kg^{-1}, p.o.) and methanol (125, 250 or 500 mg kg^{-1}, p.o.) extracts of *Sickingia williamsii* Standl. on the stereotyped behaviour of mice.

	Rearing	Grooming	Social response test	Crossing	Smelling	Washing face	Scratching	Bar holding
10–20 min post-injection								
Saline	91	87	79	84	94	85	77	81
Chloroform-methanol 250 mg	97	79	95	88	92	86	89	77
Chloroform-methanol 500 mg	66*	57*	44*	56*	63*	66*	47*	55*
Chloroform-methanol 750 mg	42**	37**	39**	27**	36**	45**	33**	41**
Methanol 125 mg	95	81	86	84	91	80	79	85
Methanol 250 mg	72	76	69	75	84	77	88	73
Methanol 500 mg	42**	37**	41**	39**	36**	44**	32**	42**
40–50 min post-injection								
Saline	92	86	93	88	91	79	97	89
Chloroform-methanol 250 mg	88	76	79	81	84	83	88	87
Chloroform-methanol 500 mg	64*	59*	57*	62*	67*	55*	62*	54*
Chloroform-methanol 750 mg	34**	42**	37**	33**	43**	35**	44**	39**
Methanol 125 mg	96	89	87	79	93	81	91	80
Methanol 250 mg	69*	75*	77*	68*	71*	63*	66*	68*
Methanol 500 mg	47**	41**	33**	42**	38**	37**	31**	44*
70–80 min post-injection								
Saline	93	95	85	84	85	91	94	87
Chloroform-methanol 250 mg	87	89	79	76	80	85	91	83
Chloroform-methanol 500 mg	82	79	75	71	76	69*	84	80
Chloroform-methanol 750 mg	37**	43**	48**	36**	39**	44**	31**	47*
Methanol 125 mg	86	79	78	81	78	86	75	82
Methanol 250 mg	69*	71*	66*	59*	58*	77*	65*	75
Methanol 500 mg	52**	46**	34**	37**	41**	43**	44**	41**
100–120 min post-injection								
Saline	91	87	84	86	92	88	84	93
Chloroform-methanol 250 mg	87	82	79	77	83	91	88	85
Chloroform-methanol 500 mg	63*	76	64*	58*	61*	56*	61*	70
Chloroform-methanol 750 mg	34**	38**	42**	32**	38**	37**	42**	39**
Methanol 125 mg	85	88	76	82	87	91	86	87
Methanol 250 mg	61*	59*	62*	59*	63*	65*	67*	57*
Methanol 500 mg	42**	39**	37**	40**	38**	39**	43**	41**

Stereotyped behaviour is significantly reduced in a dose-dependent manner by *Sickingia williamsii*. $*P < 0.05$, $**P < 0.01$ compared with saline.

progress to verify the underlying mechanism responsible of the neurosedative activity of the extracts of *S. williamsii*.

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