

Pharmacological activity of the essential oil of *Satureja viminea* (Lamiaceae)

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Received 15-II-2001. Corrected 04-VI-2001. Accepted 04-IX-2001.

Abstract: The aqueous extract and the essential oil of *Satureja viminea* (Lamiaceae) were tested. General physiologic effects were assessed through the Hippocratic screening test. Non fasted female Sprague Dawley rats were utilized and 250, 500, 750 and 1000 mg/kg doses were used. Two animals were used for each dosage level and for the vehicle alone. Exploratory behavior and curiosity were measured using a hole board apparatus and placing non-trained mice on the board and recording the number of holes explored in a 5 minute period. The Boissier chimney test was used to evaluate motor coordination. Muscle strength was assessed through a grasping test where mice were hung by their fore-limbs 40 cm above the base on a horizontal metal stainless bar. In all these tests, 3 groups of 6 albino mice, were treated with 1000 mg/kg of each the essential oil of *S. viminea*, the vehicle and diazepam (1 mg/kg) as a positive control. Analgesic activity was explored in Sprague-Dawley rats. The tail flick method described by D'Amour and Smith (1941) modified by CYTED was implemented on three groups (6 rats each) of animals treated with, each the essential oil of *S. viminea* (1000 mg/kg), the vehicle and indomethacine. The test was carried out just before and 30, 60 and 120 min after oral treatment. Peristaltic activity was measured in albino mice, three groups of 6 animals each, treated orally with each the essential oil of *S. viminea* (1000 mg/kg), the aqueous extract (1000 mg/kg), and the vehicle. The marker used was activated carbon. Animals were sacrificed 30 min after the marker was given and the percent of total small intestine traversed by it was calculated. Also a lethal dose 50 (LD 50) was determined with the Spearman-Kärber method. A dose-related spontaneous motor activity reduction was observed. Exploratory behavior and curiosity were diminished. The grasping strength of mice was reduced. A very clear and significant analgesic effect was observed with the oral administration of the essential oil of *S. viminea* (1000 mg/kg). This effect is compared to that of indomethacine. Intestinal transit and gastric emptying were inhibited by the essential oil. The LD50 of the essential oil of *S. viminea* is 556.8 mg/kg.

Key words: *Satureja viminea*, Lamiaceae, traditional medicine, Hippocratic screening, analgesic activity, intestinal transit.

Aromatic herbs are commonly utilized worldwide in folk remedies against a variety of complaints (Navarro *et al.* 1989). An example of this is the widespread use of infusions of dry peppermint leaves (*Mentha piperita* L., Lamiaceae) for their known antispasmodic, carminative and sedative effects (Della Loggia *et al.* 1990). In Costa Rica a plant that has foliage with a strong peppermint scent is consumed in the same way and for the same pur-

poses even though it belongs to a different genus. The leaves of *Satureja viminea* L., Lamiaceae, are used in an infusion and the plant is popularly known as "menta" (mint) (Ocampo 1987). In Jamaica it has also been used as a tea mixed with ginger for the treatment of colic (cited in Tucker *et al.* 2000).

Satureja viminea (L.) is a scrambling shrub that grows easily in shaded areas of gardens in the central valley of Costa Rica. Its

flowers are white with a faint pinkish tinge and they are also used in infusions specially for the treatment of insomnia (Ocampo 1987).

Different *Satureja* species have been used in traditional medicine as antimicrobial, spasmolytic, analgesic, cicatrizing and diuretic agents since antiquity. The antibacterial properties of several essential oils of *S. montana* (Melegari *et al.* 1985) and *S. thymbra* (Capone *et al.* 1989) have been studied. The essential oils of *S. obovata* (Navarro *et al.* 1989, Cruz *et al.* 1990), *S. cuneifolia* (Tümen *et al.* 1998) and *S. hortensis* (Hajhashemi *et al.* 2000) have been evaluated as spasmolytic agents. Stanic and Samarzija (1993) studied the diuretic activity of the oil from *S. montana* ssp. *montana* from Croatia.

The composition of the essential oil from the leaves (Vila *et al.* 2000) and vegetative tops (Tucker *et al.* 2000) of *Satureja viminea* has been recently described. Forty compounds corresponding to ca. 94% of the oil were identified (Vila *et al.* 2000). The main components were *p*-menth-3-en-8-ol (40.0%), pulegone (35.3%) and *p*-mentha-3,8-diene (5.2%) with minor amounts of β -caryophyllene (3.6%), α -humulene (1.3%), limonene (1.0%) and terpinolene (0.9%). To the best of our knowledge nothing has been reported concerning biological activity of the oil of *Satureja viminea*.

The purpose of this research was to study the biological effects of the essential oil and aqueous extract of *Satureja viminea*. General effects of the extracts were determined through Hippocratic screening. The influence of the extracts on the nervous system was assessed studying spontaneous motor activity, exploratory behavior, curiosity and motor coordination. The analgesic activity, its effects on intestinal transit and the toxicity of the oil were also explored. All this information will contribute towards a rational use of the plant.

MATERIALS AND METHODS

Plant material: The aerial parts of the plant were collected in a garden within the lim-

its of Montes de Oca (San José, Costa Rica) at an elevation of 1250 m. The identity of the species was confirmed in the Herbarium of the University of Costa Rica at the School of Biology. A voucher specimen was deposited under the number USJ 67660.

Preparation of extracts: Extracts were made using both fresh and air-dried leaves of *Satureja viminea*. The aqueous extract was made as an infusion placing 180 g of the fresh leaves with 2 liters of distilled water at 95°C during 15 minutes. The extract was filtered and concentrated under vacuum at 40-45° C and then lyophilized to obtain 16.9 g of powder (9.4 % yield). The essential oil was obtained through hydrodistillation of 100 g of the fresh aerial parts of the plant with 3 liters of water during 3 hours using a modified Clevenger type apparatus. The light yellow-green oil obtained was dried over anhydrous sodium sulfate to give a 2 % yield.

The aqueous extract was dissolved in distilled water for oral administration to the animals. The essential oil was suspended in 2 % Tween 80 in distilled water.

Hippocratic screening: Non-fasted female Sprague-Dawley rats weighing 160-180 g were utilized. The aqueous extract and the essential oil were administered orally to the animals using a gastric cannula. The initial test was performed with a dosis of 500 mg/kg and depending on observations it was repeated with lower or higher doses (250, 750, 1000 mg/kg). Two animals were used for each dosage level of the extracts and the vehicle. Signs due to the administration of the treatment were monitored 5, 10, 15, 30, 60, 120, 240 min later. Documentation of signs observed was made according to the method of Malone and Robichaud (1962) modified by Sandberg (1967). All the tests were carried out at the same time of the day to avoid variability induced by circadian rhythms. An autopsy was performed on all the animals.

Exploratory behavior and curiosity: A hole-board apparatus measuring 40 cm x 40 cm with 16 equidistant 3 cm holes and 50 cm from the base was used. The test consists in

placing a non-trained mouse on the center of the board and recording the number of holes explored over a 5 min period. Three groups, of six N:GP(S) albino mice each, were treated orally with each essential oil (1000 mg/kg), vehicle and diazepam (1 mg/kg i.p.) as a positive control. The test is performed on each mouse before and 30 minutes after the oral administration of the treatments.

Motor coordination: The Boissier's "chimney" test modified by CYTED (Anonymous 1995) was used. In this test the ability of the mice to climb backwards in a vertical 25 cm long tube is observed. Animals that take more than 30 s to cover 20 cm of the tube are considered as uncoordinated. The test was performed before and 30 minutes after oral administration of the essential oil of *Satureja viminea* (1000 mg/kg), the vehicle or diazepam as a positive control (1 mg/kg). Three groups of 6 N:GP(S) albino mice each were used.

Grasping test: Mice are hung with their fore-limbs 40 cm above the base on a horizontal stainless bar, 1.5 mm in diameter. The animal will quickly, in less than 5 s, place its hind-limbs on the bar. Drugs with a muscle relaxant activity decrease grip strength and the animal will fall from the bar or hang without placing the rear limbs on the bar. Three groups of 6 N:GP(S) albino mice each were treated with the essential oil of *Satureja viminea* (1000 mg/kg), the vehicle or diazepam (1 mg/kg). The test was performed before and 30 min after oral administration of the treatments.

Analgesic activity: Analgesic activity of the essential oil of *S. viminea* was tested by the tail-flick method described by Dámour and Smith (1941) modified by CYTED (1995). This test is based on the measurement of the latency of the avoidance response elicited by radiant heat applied on the base of its tail. The heat source is an electrical resistance made of nicrome and the rats are placed in a restrainer that holds them in position. Three experimental groups, each of six Sprague-Dawley rats weighing 200-240 g, were used. The animals were tested before and 30, 60 and 120 min after treatment by orogastric gavage with the

essential oil of *Satureja viminea* (1000 mg/kg), the vehicle or indomethacine (10 mg/kg) as a positive control.

Intestinal transit: The test described by CYTED was used (Anonymous 1995). 18 male N:GP(S) albino mice weighing an average of 30 g were placed in three groups of six mice each. After six hours of fasting, the mice were treated by orogastric gavage with the essential oil of *S. viminea* (1000 mg/kg), the aqueous extracts (1000 mg/kg) or the vehicle, all in a fixed volume of 0.3 ml. The pretreated animals were given 0.3 ml of a charcoal marker (10% charcoal in 1.5% agar saline) with an oral cannula. Mice were killed after 30 min and the length of intestine traversed by charcoal marker in relation to total length of small intestine was calculated as a percentage.

Lethal dose 50 (LD₅₀): The LD₅₀ was determined using the method described by Spearman-Kärber (Gené 1987). Thirty N:GP(S) albino mice weighing 26-30 g were placed in 5 groups of 6 mice each. The doses utilized were 384, 450, 528, 619 and 726 mg/kg of the essential oil of *S. viminea* given i.p. in a fixed volume of 0.3 ml. The animals were observed 6, 12, 24 and 48 hours after treatment and the number of dead animals was quantified.

Statistical analysis: Data are expressed as mean \pm SEM. The significance of results was assessed using analysis of variance (ANOVA) and Student's t test. A P value of less than 0.05 was considered significant.

RESULTS

Hippocratic screening: The oral administration of the aqueous extract of *S. viminea* in 500 and 1000 mg/kg doses only produced a very slight decrease in motor activity of the rats, which started 30 min after the treatment, and an increased frequency of micturitions. A lower dose of 250 mg/kg was ineffective. The essential oil had more biological activity and its effects were dose related. Doses of 500 and 750 mg/kg produced a decrease in motor

activity and in the alarm reaction of the rats. An analgesic effect was also observed. All the animals presented important lacrimation, micturition and diarrhea. The tests performed with the vehicle were negative. None of the animals died as a result of the treatments. In an autopsy performed 7 days after, there were no macroscopic pathological signs.

Based on these general effects observed with this pharmacological screening method, the aqueous extract was not tested further and the rest of the experiments were carried on only with the essential oil which apparently has the active biological principles.

Exploratory behavior and curiosity:

The essential oil of *Satureja viminea* (1000 mg/kg) diminished in a significant way the number of holes explored by the mice 30 min after treatment (from 60 ± 2.6 to 2.2 ± 1.1 , $p < 0.001$). This result was significantly different ($p < 0.01$) from the one observed with the vehicle (from 61 ± 4.2 to 43 ± 7.5) and did not differ from the positive control with diazepam (from 60.5 ± 5.9 to 9.33 ± 1.37).

Motor coordination: Motor coordination was not altered by the administration of the essential oil of *S. viminea* (1000 mg/kg). Most of the mice treated with diazepam were uncoordinated.

Grasping test: The grasping strength of the mice was diminished and they were incapable of placing their hind-limbs on the bar or fell from it. With diazepam a similar response was observed. The vehicle did not alter the muscle strength of the animals.

Analgesic activity: A significant ($p < 0.05$) analgesic activity was observed 30 min after the oral administration of the essential oil of *S. viminea* (1000 mg/kg) which was not different from that seen with indomethacine. This was evidenced by an increase in the latency of the avoidance response from 20.3 ± 1.6 to 34 ± 2.5 s (Fig. 1).

Intestinal transit: The percentage of small intestine traversed by the charcoal marker was significantly less after the oral administration of the essential oil and the aqueous extract of *S. viminea* compared to the control

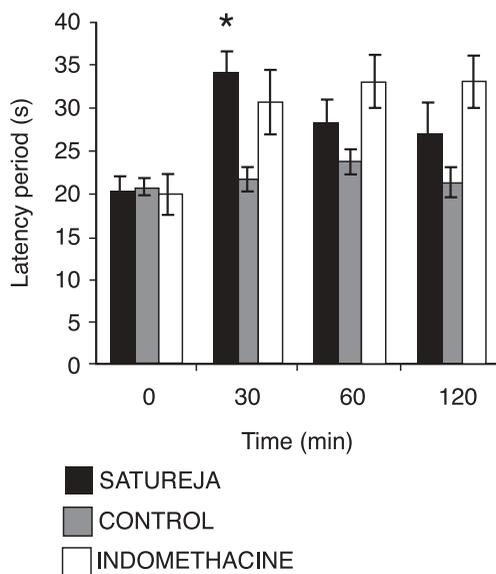


Fig. 1. Latency period of the avoidance response to a nociceptive stimulus after treatment with the essential oil of *S. viminea*, indomethacine and the vehicle. Each bar represents the mean of six rats. * $p < 0.05$.

group ($0\% \pm 0$, $54\% \pm 11.82$ and $74\% \pm 4.34$ respectively). Intestinal transit was significantly decreased by both the aqueous and the essential oil of *S. viminea*. The essential oil inhibited gastric emptying since all the charcoal marker remained in the stomach after 30 min of the treatment.

Lethal dose 50 (LD 50): the LD₅₀ (95% confidence limits) of the essential oil of *S. viminea* is 556.8 mg/kg (496.42-624.48).

DISCUSSION

Through the Hippocratic screening test one can make a general evaluation of the physiological effects of a substance. It was evident that the essential oil of *S. viminea* caused a dose related spontaneous activity reduction. This was confirmed through a more specific test which uses a hole board apparatus and where an evident reduction of the exploratory and curiosity activity of mice was observed. This sedative effect of the oil could be due to

its high contents of pulegone (35.3%) which has been described to have a sedating activity (Ortiz 1989) in mice.

An important analgesic activity was observed with the administration of the essential oil of *S. viminea* to rats. This activity was compared to that of indomethacine, a known analgesic and anti-inflammatory drug. The greatest analgesic activity was observed 30 minutes after the administration of the oil, which indicates a very rapid absorption from the gastrointestinal tract. Since the analgesic activity is accompanied with a sedating activity, it can be theorized that the analgesic activity could be explained by the same mechanism which causes the sedative effect, that is, a central nervous system effect. This possibility must be explored by comparing it to known analgesics of central action.

The decrease in intestinal transit time and in gastric emptying could be due to an effect of the essential oil of *S. viminea* on the autonomous nervous system. It could be attributed to an inhibition of the parasympathetic system (PS) or to a direct effect over gastrointestinal smooth muscle. However, the increased frequency of micturitions and the diarrhea observed during the Hippocratic screening do not confirm this inhibitory effect over PS. The sedating activity and the inhibitory effect over intestinal transit time may, rather, be related and confirm an inhibitory effect of the essential oil of *S. viminea* over the excitability of certain neurons and intestinal smooth muscle fibers.

In conclusion, it is confirmed that the essential oil of *S. viminea* besides having a sedative and an analgesic effect, it also diminishes intestinal motility. All these effects would justify its use in the treatment of insomnia and for abdominal colics. Additional toxicity tests must be carried out since pulegone, a mayor component of the essential oil, has been reported to have hepatotoxic activity (Thorup 1983).

ACKNOWLEDGMENTS

We thank J. Gómez-Laurito (University of Costa Rica) for the botanical classification of the plant and N. R. Farnsworth (University of Illinois at Chicago) for his help with the NAPRALERT database. This work was supported by the grant from the Universidad de Costa Rica N° 422-98-348.

RESUMEN

Se estudiaron el extracto acuoso y el aceite esencial de *Satureja viminea*. Los efectos fisiológicos generales se comprobaron por medio del ensayo hipocrático o tamizaje farmacológico en ratas Sprague Dawley. En ratones albinos se midieron la actividad exploratoria y la curiosidad por medio del ensayo de la placa perforada. Se utilizó el ensayo de la chimenea de Boissier para evaluar la coordinación motora y la fuerza muscular se comprobó con el ensayo del alambre. La actividad analgésica se exploró en ratas Sprague Dawley por medio del ensayo de calor sobre la cola (tail flick) descrito por D'Amour y Smith (1941) y modificado por CYTED. La actividad sobre la motilidad intestinal se investigó con el ensayo del tránsito intestinal sobre ratones albinos. También se determinó la dosis letal 50 (DL 50) con el método de Spearman-Kärber. Se observó una disminución de la actividad motora espontánea la cual fue relacionada a la dosis. La actividad exploratoria y la curiosidad disminuyeron. La fuerza muscular de los ratones disminuyó. Se observó un claro y significativo efecto analgésico con la administración oral del aceite esencial de *S. viminea* (1000 mg/kg) comparado al observado con indometacina. El aceite esencial inhibió el tránsito intestinal y el vaciamiento gástrico. La DL 50 del aceite esencial de *S. viminea* es de 556.8 mg /kg.

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