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Effect of leaf essential oil from *Piper solmsianum* C.DC. in mice behaviour

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ABSTRACT

The essential oil from *Piper solmsianum* leaves and its major compound (sarisan) were tested to verify their influences upon mice behaviour. The essential oil was obtained by hydrodistillation in a modified Clevenger extractor and analysed by GC/ MS. This analysis revealed in the oil the presence of monoterpenes, sesquiterpenes and of arylpropanoids. The compound sarisan, a myristicin analogue, was isolated from the oil to perform the pharmacological tests. Emulsions of the oil and of sarisan (5.0 and 10.0% v/v) were used in the tests. Pentobarbital (30 mg/ kg s.c.) or diazepam (2.5 mg/ kg s.c.) were tested as standard drugs to verify depressant or anxiolytic effects, respectively. Both essential oil and sarisan showed to have exciting and depressant effects in the tested animals.

Key words: Piperaceae, *Piper solmsianum*, essential oil, sarisan, psychopharmacologic effect.

INTRODUCTION

The family Piperaceae comprises about 10 genera and 1400 species (Yunker 1972). Some of these species are used in folk medicine to treat many diseases (Duke 1985). Chemical investigation of Piperaceae essential oils has shown the presence of monoterpenes, sesquiterpenes and arylpropanoids (Bessiere et al. 1994, Jantan et al. 1994; Martins et al. 1998, Moreira et al. 1997). It has been frequent the isolation and chemical characterisation of arylpropanoids (Parmar et al. 1997) from crude extracts and essential oils of Piperaceae species (safrole, myristicin, eugenol, dillapiol, apiol, asarone). Arylpropanoids and related compounds have shown interesting biological properties including psychotropic, antimicrobial, antioxidant and cytotoxic effects (Shulgin & Sargent 1967, Masuda et al. 1991, Priyadarsini et al. 1998, Atsumi et al. 2000). *Piper solmsianum* C.DC. (syn. *Piper leucathum* C.DC. and *P. santosanum* C.DC.) is a shrub measuring 1 to 3 meters high, commonly found in Southeast Brazil. There are not medicinal uses reported to this species. However Brazilian people usually employ plants of the family Piperaceae as a pepper like condiment (Corrêa 1984). This usage may promote toxicological side effects on humans

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since very little is known about the chemistry of Brazilian Piperaceae.

Phytochemical investigation of *P. solmsianum* showed the presence of eupomatenoid-6 in the hexane extract and acaciin in the methanolic extract among other compounds (Moreira et al. 1995). Previous essential oil analyses from leaves of *P. solmsianum* (Moreira et al. 1998) revealed the presence of monoterpenes, sesquiterpenes and as major compound an arylpropanoid derivative (sarisan - 39.23%). Toxicological effects, including weak pulse, hypothermia, delirium, vertigo and nausea, associated to ingestion of about 5-15 g of nutmeg (*Myristica fragrans*), rich in the arylpropanoid derivative myristicin, has been reported (Shulgin 1966, Hallstrom & Thuvander 1997). The structure similarities of myristicin and of sarisan, the main constituent of *P. solmsianum* essential oil, encouraged us to perform some pharmacological studies on mice behaviour.

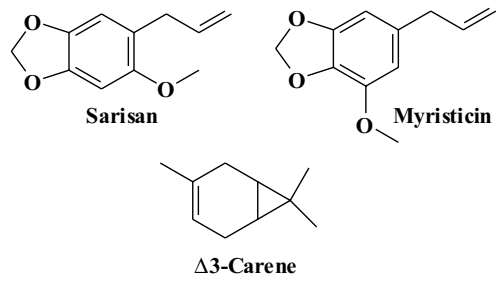
MATERIALS AND METHODS

Plant Material and Extraction of the Essential Oil

P. solmsianum was collected near Teresopolis, Brazil, in September 1996. The plant was identified by the botanist Prof. E.F.Guimarães and a botanical sample is found at the Herbarium of the Botanical Garden of Rio de Janeiro (RB 306115), Rio de Janeiro, Brazil. Fresh leaves of *P. solmsianum* (1.5 kg) has undergone hydrodistillation for one hour in a modified Clevenger extractor, yielding a colourless oil (10ml) with a pleasant odour. A sample of this oil was dissolved in dichloromethane for GC and GC/MS analysis (Moreira et al. 1997).

Isolation of the Main constituent of the Essential Oil

The essential oil (10ml) obtained from leaves of *P. solmsianum* was fractionated in a silica gel column using mixtures of hexane and ethyl acetate as eluent. The 1-allyl-2-methoxy-4,5-methylenedioxybenzene (sarisan, 3.0 ml) and Δ3-carene (0.02 ml) were isolated from the volatile mixture (Moreira et al. 1998).



Analysis of the Essential Oil

GC – It was performed using a Varian Star 3400 CX gas chromatograph, fused silica capillary column (DB5, 30m x 0.20mm), hydrogen as carrier gas and temperature programming from 60°C to 240°C (3°C/ min.).

GC/MS – It was performed using a HP5890 SII gas chromatograph coupled to a VG Autospect Mass Spectrometer at 70 eV, fused silica capillary column (DB-1, 30m x 0.20 mm), helium as carrier gas and temperature programming from 60°C to 240°C (3°C/ min). Identification of the substances was carried out by comparison of their retention indices (RI) with literature values and their mass spectral data with those from the NBS mass spectral database (Adams 1995, McLafferty & Stanffer 1989).

Retention Indices – Retention indices (RI) were calculated using GC data of a homologous series of saturated aliphatic hydrocarbons within C_8 to C_{22}, performed in the same column and conditions as used in the GC analysis for the essential oils.

NMR Analysis – It was recorded on a Varian Gemini (200 MHz – ^1H; 50.2 MHz – ^{13}C) – NMR instrument. The chemical shifts were determined in $CDCl_3$, using TMS as internal standard. NMR data of pure compounds were previously published (Moreira et al. 1998).

PHARMACOLOGICAL TESTS

Groups of five adult albino mice of both sexes, weighting about 20 g, were used in each experiment. In the tests with pentobarbital and diazepam, two groups of the animals were used. Emulsions (water/ oil) of the essential oil (EO) with 5.0% and 10.0% (v/v) were prepared. In preliminary tests, the EO was given i.p. 0.5ml/ animal (10.0% emulsion - sarisan = 20μl) and 0.1ml/ animal (5.0% emulsion- sarisan = 2μl). For the experiments using pentobarbital (30mg/ kg s.c.), the EO was given i.p. 0.1ml/ animal (5.0% emulsion - sarisan = 2μl) 30 minutes before the administration of the drug. The EO was administered (5.0% emulsion - 0.1ml/ animal, - sarisan = 2μl) 30 minutes before diazepam (2.5mg/ kg s.c.). Sarisan was given i.p. at a dose of 0.5ml/ animal (5.0% emulsion - real conc. of sarisan = 25μl/ animal). In the experiments using sarisan and pentobarbital, three groups of five adult albino mice each were used. Group A received pentobarbital (30mg/ kg s.c.) 30 minutes after receiving sarisan (0.1ml/ animal- 5.0% emulsion- real conc. of sarisan = 25μl/ animal) and Group B 60 minutes later. Group C received only sarisan in the same dose as A and B groups. Sarisan concentration in the essential oils emulsions was estimated in relation to the percentual ratio given by the GC analysis (Table I).

TABLE I

Identified Compounds in the Essential Oil from *P. solmsianum*.

Compound	RI lit*	RI	Percentage
α-pinene	939	935	1.00
Myrcene	991	989	0.79
α-phellandrene	1005	1003	2.18
Δ3-carene	1011	1005	23.29
o-cymene	1022	1020	< 0.01
p-cymene	1026	1025	< 0.01
β-phellendrene	1031	1030	0.44
Limonene	1031	1034	0.86
γ-terpinene	1062	1055	0.39
Isoterpinolene	1086	1090	1.28
Methyl eugenol	1401	1395	1.10
Z-caryophyllene	1404	1400	2.11
Sarisan	—	1450	39.23
9-*epi*-caryophyllene	1467	1460	< 0.01
γ-muurolene	1477	1465	< 0.01
Germacrene D	1480	1477	< 0.01
α-farnesene	1508	1505	0.10
Germacrene B	1556	1545	3.48
Spathulenol	1576	1570	2.89
Carotol	1594	1597	1.22
Guaiol	1595	1600	0.12

* (Adams 1995).

RESULTS AND DISCUSSION

A strong sedative effect was observed when the essential oil was given i.p. at dose of 0.5ml/ animal in 10.0% emulsion (sarisan = 20μl/ animal). Few minutes after the drug administration it was noted an exciting behaviour, characterised by fast movements of the animals in the cage. Five minutes later the animals showed ataxia and 30 minutes afterward the animals slept. In approximately 14 hours ($RSD = \pm 1$) all mice died. Mydriasis was observed with no changes in the cardiac and the respiratory frequencies during the sleeping time. A weaker depressant behaviour was noted when the above dose was reduced ten times (0.1ml/ animal of a 5.0% emulsion; sarisan = 2μl/ animal). The tested animals showed ataxia after 5 minutes of drug injection and death were not observed. This dose of the essential oil (sarisan equivalent = 2μl/ animal) was taken to perform the pharmacological tests employing pentobarbital or diazepam.

After administration of sarisan emulsion (5.0%) at dose of 0.5ml/ animal (sarisan = 25μl/ animal) it was observed a tail stiffening and piloerection, signals that characterise a medullary excitation. Increase of external stimulation response was observed during this phase. It was noted a depressant effect 30 minutes later, characterised by ataxia.

The sleeping time promoted by administration

of pentobarbital (30mg/ kg s.c.) 30 minutes later than essential oil (0.1ml/ animal- 5.0%; sarisan = 2μl/ animal), was increased 60% ($RSD = \pm 5\%$).

All animals that received pentobarbital (30mg/ kg s.c.) 30 minutes later than sarisan (5μl/ animal) died due to respiratory depression. However, only 50% ($RSD = \pm 10\%$) of the animals died due to pentobarbital given one hour later than sarisan at the same concentration. Sarisan increased 70% ($RSD = \pm 3\%$) the sleeping time promoted by pentobarbital in the survived animals.

No changes in behaviour was observed when the animals received diazepam (2.5mg/ kg) and the essential oil (0.1ml/ animal- 5.0%, sarisan = 2μl/ animal).

These results are in agreement to those psychopharmalogical effects published for myristicin, an analogue of sarisan (Shulgin 1966, Hallstrom & Thuvander 1997). It has been demonstrated that myristicin promotes CNS depressant effect besides an induction of the cytochrome P-450 system (Hallstrom & Thuvander 1997). Previous investigation with eugenol and safrole, arylpropanoid derivatives, showed that these compounds intervene in the cytochrome P-450 functions (Nagababu & Lakshmaiah 1994, Janiaud et al. 1997). The exciting action of the essential oil in SNC was demonstrated by an increase of the sensitivity induced by external stimulation. This exciting effect was confirmed by administration of pure sarisan that originated medullary responses. The depressant effects of the essential oil and of sarisan was sustained since the sleeping time promoted by co-administration of pentobarbital was increased 60% and 70%, respectively. This observed depressant effect may arise from a cytochrome P-450 system induction that contributes to increase the effects of pentobarbital.

The preliminary psycopharmacological results obtained for the essential oil from *P. solmsianum* leaves and for its major compound sarisan recommend to avoid the use of this plant as a pepper-like condiment. Since diversified biological effects have been shown by arylpropanoids, species of Piperaceae rich in these compounds should be better investigated before their usage as condiment or in the treatment of human diseases.

RESUMO

O óleo essencial das folhas de *Piper solmsianum* e seu constituinte majoritário (sarisan) foram testados para avaliar suas influências no comportamento de camundongos. O óleo essencial foi obtido por hidrodestilação em aparelho do tipo Clevenger modificado e analisado por CG/ EM. Essa análise mostrou a presença de monoterpenos, sesquiterpenos e arilpropanóides. Sarisan, um análogo da myristicina, foi isolado do óleo para se efetuar os testes farmacológicos. Emulsões do óleo essencial e do sarisan a 5,0% e 10,0% (v/v) foram utilizadas nos testes. Pentobarbital (30 mg/ kg s.c.) ou diazepam (2,5 mg/ kg s.c.) foram testados como padrões na verificação de efeitos depressores e ansiolíticos, respectivamente. O óleo essencial e seu componente majoritário apresentaram efeitos excitantes e depressores nos animais testados.

Palavras-chave: Piperaceae, *Piper solmsianum*, óleo essencial, sarisan, psicofarmacologia.

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