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Antidepressant-like effect of the extract from leaves of *Schinus molle* L. in mice: Evidence for the involvement of the monoaminergic system

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Abstract

Schinus molle L. (Anacardiaceae), among other uses, is popularly employed for the treatment of depression. In this study, the antidepressant-like effect of the hexanic extract from leaves of *S. molle* was investigated in the mouse tail suspension test (TST), a predictive model of depression. The immobility time in the TST was significantly reduced by the extract (dose range 30–600 mg/kg, p.o.), without accompanying changes in ambulation when assessed in an open-field test. The efficacy of extract was found to be comparable to that of fluoxetine (10 mg/kg, p.o.). The anti-immobility effect of the extract (100 mg/kg, p.o.) was prevented by pretreatment of mice with *p*-chlorophenylalanine methyl ester (PCPA, 100 mg/kg, i.p., an inhibitor of serotonin synthesis, for four consecutive days), NAN-190 (0.5 mg/kg, i.p., a 5-HT_{1A} receptor antagonist), WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist), ketanserin (5 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist), MDL72222 (0.1 mg/kg, i.p., a 5-HT_3 receptor antagonist), prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, s.c., a D_1 receptor antagonist) or sulpiride (50 mg/kg, i.p., a D_2 receptor antagonist). It may be concluded that the hexanic extract of *S. molle* produces an antidepressant-like effect that seems to be dependent on its interaction with the serotonergic, noradrenergic and dopaminergic systems. These results provide evidence that the extract from *S. molle* shares with established antidepressants some pharmacological effects, at least at a preclinical level.

Keywords: Depression; Dopamine; Noradrenaline; *Schinus molle*, Serotonin; Tail suspension test

1. Introduction

Schinus molle L. is a pepper tree belonging to the family Anacardiaceae. It originates from South America, but has been introduced to most of the tropical and subtropical areas of the world (Taylor, 2005).

Pharmacological studies carried out with extracts from *S. molle* show that this plant exerts several biological effects, such as: hypotensive (Bello et al., 1996), antitumoral (Ruffa et al., 2002), antifungal (Quiroga et al., 2001; Schmourlo et al., 2005), antispasmodic (Bello et al., 1998), anti-inflammatory (Yueqin et al., 2003), and analgesic (Barrachina et al., 1997). Other properties/actions of *S. molle* suggested by traditional use are: antihemorrhagic, antiseptic, aperient (mild laxative), astringent,

Abbreviations: ANOVA, analysis of variance; DMSO, dimethylsulfoxide; 5-HT, serotonin, MAOi, monoamine oxidase inhibitor; MDL72222, tropanyl 3, 5-dichlorobenzoate; NAN-190, 1-(2-methoxyphenyl)-4[-(2-phthalimido)butyl] piperazine); PCPA, p-chlorophenylalanine methyl ester; SCH23390, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; SKF 38393, (1-phenyl-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine) hydrochloride; *S. molle*, *Schinus molle*; SSRI, selective serotonin reuptake inhibitor; TST, tail suspension test; WAY100635, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide.

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cardiotonic, digestive stimulant, diuretic, menstrual stimulant, stimulant, tonic, and antidepressant (Taylor, 2005).

Depression is a common disorder associated with high rates of chronicity, relapse, and recurrence; psychosocial and physical impairment; and a high suicide rate. Currently available therapy for depression treatment is often associated with several undesirable side effects, and it is effective only in a certain portion of the population (Wong and Licinio, 2001; Nestler et al., 2002). Therefore, the identification of alternative therapeutic tools for the treatment of depression is still needed. Herbal therapies may be effective alternatives in the treatment of depression, as in the case of St John's wort (Whiskey et al., 2001; Bilia et al., 2002; Linde and Knüppel, 2005), and the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses, including depression, has progressed significantly in the past decade (Zhang, 2004). It is interesting to note that most of the novel treatments for depression (including St. John's wort) seem to act through a mechanism which does not differ significantly with respect to that of "classical" antidepressants.

In spite of the popular use of *S. molle* to treat depression (Taylor, 2005) there is no scientific evidence about potential effects of this plant in animal models of depression. Thus, this study aims, firstly, to examine the antidepressant-like action of the hexanic extract from leaves of *S. molle* in the mouse tail suspension test (TST), a model predictive of antidepressant activity (Steru et al., 1985; Cryan et al., 2005) and, secondly, to investigate by the use of pharmacological procedures the possible participation of the monoaminergic system in its antidepressant-like action.

2. Methods

2.1. Plant material and preparation of the hexanic extract

Stems and leaves of *Schinus molle* L. (Anacardiaceae) were collected in Florianópolis, Santa Catarina, and identified by Dr. Daniel Falkenberg, Department of Botany, Federal University of Santa Catarina. A voucher specimen (FLOR 34411) was deposited in the Herbarium of the Department of Botany, Federal University of Santa Catarina, Santa Catarina, Brazil. Botanical material (390 g) were dried under air circulation and minced. Dried sample was extracted with hexane at room temperature (25±2 °C) for 15 days. Thereafter, the extract was filtered and then concentrated under reduced pressure (at approximately 60°). The maceration was repeated three times. The evaporation of solvent yielded a residue of 17.8 g of dried extract (4.6% w/w yield). The remaining residue was kept in a refrigerator and dissolved in saline with 10% Tween 80 before the behavioral tests. The preliminary chemical composition of the hexanic extract from *S. molle* was demonstrated by thin layer chromatography (TLC) and gas chromatography (GC) analysis that revealed the presence of a high content of triterpenes, but not of flavonoids or tannins. Thus, further investigations are needed to determine the structure of the triterpenes and also to identify the active principles present in hexanic extract of *S. molle*.

2.2. Animals

Male Swiss mice (35–45 g) were maintained at constant room temperature (22–25 °C) with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). All experiments were carried out between 11:00 and 16:00 h, with each animal used only once (N=5–12 animals per group). The procedures in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Institution. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.3. Drugs and treatment

The following drugs were used: ketanserin tartarate, 1-(2-methoxyphenyl)-4[-(2-phthalimido)butyl]piperazine) (NAN-190), p-chlorophenylalanine methyl ester (PCPA), N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexanecarboxamide (WAY100635), tropanyl 3, 5-dichlorobenzoate (MDL72222), sulpiride, prazosin, yohimbine, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), fluoxetine (all from Sigma Chemical Company, St. Louis, MO, U.S.A.). All drugs were administered by intraperitoneal (i.p.) route in a constant volume of 10 ml/kg body weight except SCH 23390 and WAY100635 that were administered by subcutaneous (s.c.) route (10 ml/kg body weight). Drugs were dissolved in saline except NAN-190 and MDL72222, that were diluted in saline with 1% Tween 80 and sulpiride that was diluted in saline with 5% dimethylsulfoxide (DMSO). Control animals received appropriate vehicle.

The extract of *S. molle* or vehicle was administered by oral route (p.o.) 60 min before the TST or open-field test. Fluoxetine (10 mg/kg, p.o., a classical antidepressant) was used as a positive control. To address some of the mechanisms by which the extract of *S. molle* causes antidepressant-like action in the TST, animals were treated with different drugs. The doses of the drugs used were selected on the basis of literature data and on previous results from our laboratory (O'Neill and Conway, 2001; Redrobe and Bourin, 1997; Rodrigues et al., 2002; Yamada et al., 2004; Kaster et al., 2005).

In order to investigate a possible contribution of the serotonergic system to the effect of the extract of *S. molle* in reducing the immobility time in the TST, animals were pretreated with PCPA (100 mg/kg, an inhibitor of serotonin synthesis) or vehicle, once a day, for 4 consecutive days (Rodrigues et al., 2002; Gavioli et al., 2004; Kaster et al., 2005). Then, 24 h after the last PCPA or saline injection, animals were treated with the extract of *S. molle* (100 mg/kg, p.o.), or vehicle and were tested in the TST 60 min later.

In a separate series of experiments, the involvement of the serotonin (5-HT) receptor subtypes in the effect of the extract of *S. molle* in the TST was studied. In order to investigate the possible involvement of the serotonergic system in the antidepressant-like effect of the extract, mice were pretreated with NAN-190 (0.5 mg/kg, i.p. a 5-HT_{1A} receptor antagonist),

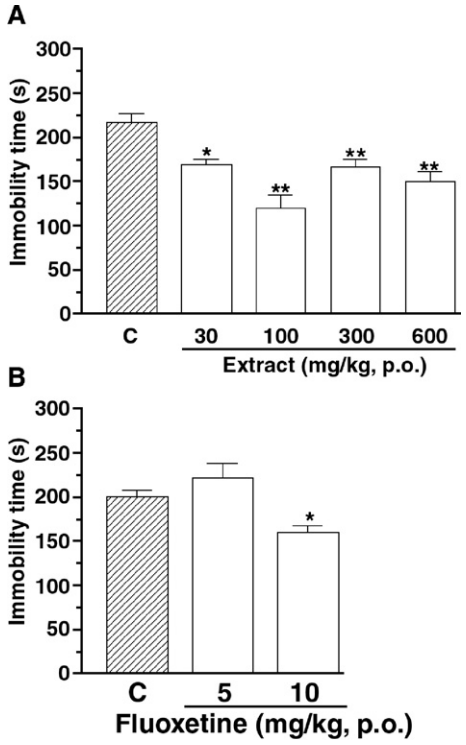

Fig. 1. Effect of treatment of mice with the hexanic extract of *S. molle* or fluoxetine given orally on the immobility time in the TST. Each column represents the mean+S.E. of 6–7 animals. *$P<0.05$; **$P<0.01$ compared with the vehicle-treated control (C).

WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist), ketanserin (5 mg/kg, a preferential 5-HT_{2A} receptor antagonist), MDL72222 (0.1 mg/kg, i.p., a selective 5-HT_3 receptor antagonist) or vehicle and after 30 min they received the extract of *S. molle* (100 mg/kg, p.o.) or vehicle injection before being tested in the TST 60 min later.

To assess the possible involvement of the noradrenergic and the dopaminergic systems on the antidepressant-like effect of the extract in the TST, animals were pretreated with prazosin (1 mg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, s.c., a D_1 receptor antagonist) or sulpiride (50 mg/kg, i.p., a D_2 receptor antagonist), and after 30 min they received the extract of *S. molle* (100 mg/kg, p.o.) or vehicle and were tested in the TST 60 min later.

The administration schedule was chosen on the basis of experiments previously performed in our laboratory and literature data confirm the efficacy of the above-mentioned protocol (Viana et al., 2005).

2.4. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period (Rodrigues et al., 2002; Mantovani et al., 2003).

2.5. Open-field behavior

To assess the possible effects of the extract of *S. molle* on locomotor activity, mice were evaluated in the open-field paradigm as previously described (Rodrigues et al., 2002). Mice were individually placed in a wooden box (40×60×50 cm) with the floor divided into 12 squares. The number of squares crossed with the four paws was registered during a period of 6 min. Animals were treated with the extract of *S. molle* (100, 300 and 600 mg/kg) or with vehicle given by the oral route 1 h before the experiments.

2.6. Statistical analysis

Comparisons between experimental and control groups were performed by one or two-way ANOVA followed by Tukey's HSD test when appropriate. A value of $P<0.05$ was considered to be significant.

3. Results

3.1. Effect of the extract of S. molle on the immobility time in the TST

The effects of oral administration of the extract of *S. molle* and fluoxetine on the immobility time in the TST were shown in Fig. 1A and B, respectively. As depicted in Fig 1 A, the extract given by oral route at doses of 30, 100, 300 and 600 mg/kg significantly decreased the immobility time as compared to the control group. The one-way ANOVA revealed a significant effect of treatment [$F(4,25)=11.14$, $P<0.01$]. As a positive control, we show that the antidepressant fluoxetine (10 mg/kg, p.o.) also produced a significant reduction in the immobility time in the TST (Fig. 1B). The one-way ANOVA revealed a main effect of treatment [$F(2,18)=8.15$, $P<0.01$].

3.2. Effect of the extract of S. molle on the open-field test

As shown in Fig. 2 the extract of *S. molle* (dose range 100–600 mg/kg, p.o.) did not significantly alter the locomotor activity of mice in the open-field test as compared to control

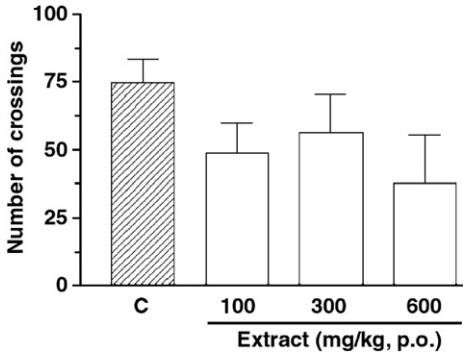

Fig. 2. Effect of treatment of mice with the hexanic extract of *S. molle* given orally on the number of crossings in the open-field test. Each column represents the mean+S.E. of 5–6 animals.

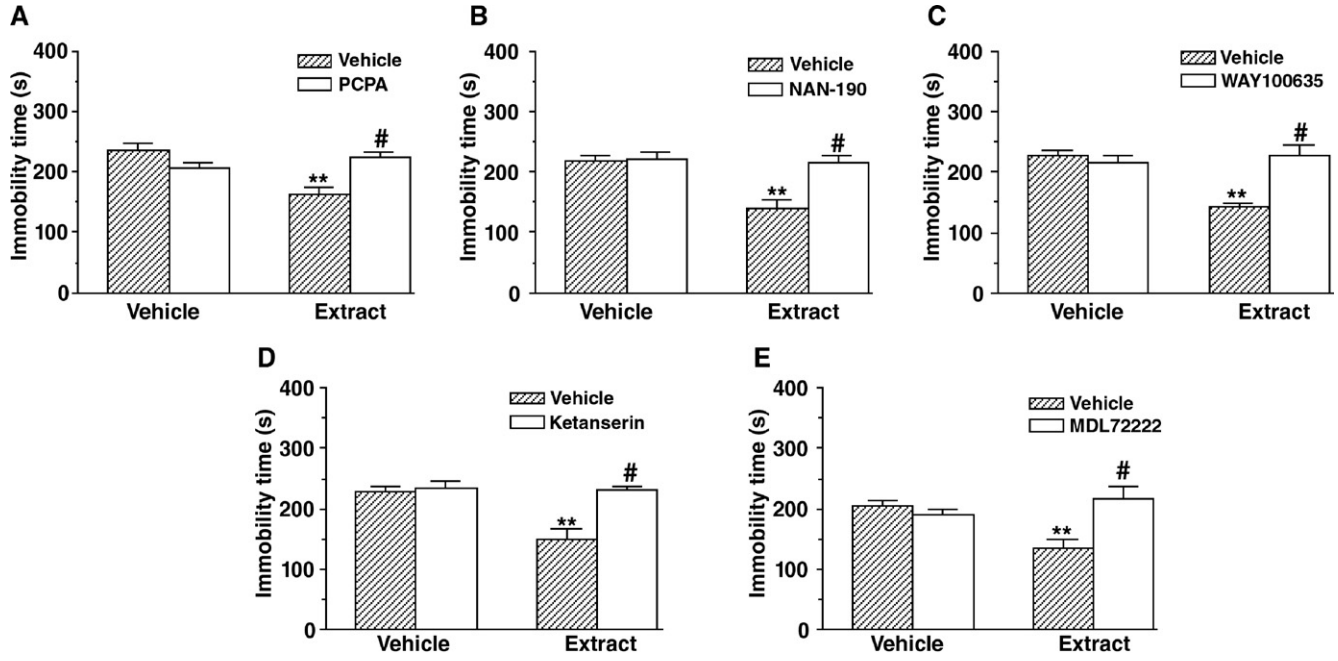

Fig. 3. Effect of pretreatment of mice with PCPA (100 mg/kg, i.p. once a day for 4 consecutive days, panel A), NAN-190 (0.5 mg/kg, i.p., panel B), WAY100635 (0.1 mg/kg, s.c., panel C), ketanserin (5 mg/kg, i.p., panel D), or with MDL72222 (0.1 mg/kg, i.p., panel E) on the hexanic extract of *S. molle* (100 mg/kg, p.o.)-induced reduction in immobility time in the TST. Each column represents the mean+S.E. of 6–9 animals. **$P<0.01$ compared with the vehicle-treated control. # $P<0.01$ as compared with the same group pretreated with vehicle.

group, as revealed by one-way ANOVA [$F(3,18)=1.38$, $P=0.27$].

3.3. Investigation of some possible mechanisms underlying the antidepressant-like effect of the extract of S. molle in the TST

3.3.1. Involvement of the serotonergic system

Fig. 3A shows that the pretreatment of mice with the inhibitor of serotonin synthesis PCPA (100 mg/kg, i.p., once a day for 4 consecutive days) significantly prevented the decrease in the immobility time elicited by the extract (100 mg/kg, p.o.). The results obtained in this experiment were analyzed by a two-way ANOVA. There was a significant effect of treatment [$F(1,26)=7.47$, $P<0.01$], treatment X pretreatment interaction [$F(1,26)=19.55$, $P<0.01$], but not of pretreatment [$F(1,26)=2.39$, $P=0.13$]. Moreover, the pretreatment of mice with NAN-190 (0.5 mg/kg, i.p.) also prevented the antidepressant-like effect elicited by the extract. A two-way ANOVA showed significant differences for treatment [$F(1,20)=14.56$, $P<0.01$], pretreatment [$F(1,20)=11.67$, $P<0.01$] and treatment X pretreatment interaction [$F(1,20)=10.57$, $P<0.01$], Fig. 3B. In addition, the results depicted in Fig. 3C show that the pretreatment of animals with WAY100635 (0.1 mg/kg, s.c.) prevented the effect of the extract of *S. molle* in the TST. A two-way ANOVA showed significant differences for treatment [$F(1,23)=9.49$, $P<0.01$], pretreatment [$F(1,23)=10.24$, $P<0.01$] and treatment X pretreatment interaction [$F(1,23)=16.20$, $P<0.01$]. Fig. 3D shows that the pretreatment of mice with ketanserin (5 mg/kg, i.p.) also prevented the action of the extract in the TST. The two-way ANOVA revealed a main effect of the treatment [$F(1,19)=10.65$, $P<0.01$], pretreatment [$F(1,19)=12.58$, $P<0.01$] and treatment X pretreatment interaction [$F(1,19)=9.35$, $P<0.01$]. Fig. 3E shows that MDL72222 (0.1 mg/kg, i.p.) completely blocked the anti-immobility effect

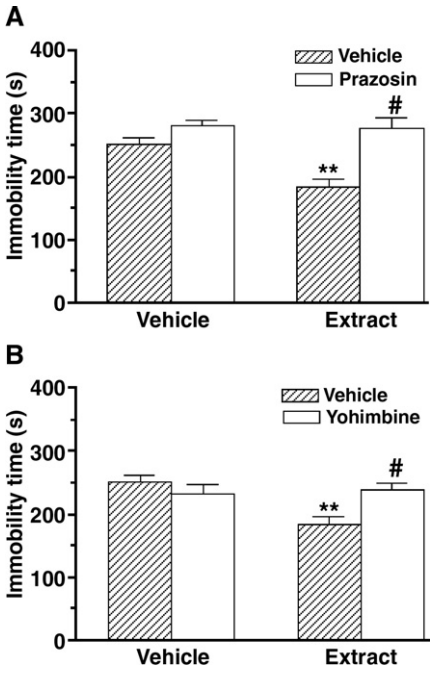

Fig. 4. Effect of pretreatment of mice with prazosin (1 mg/kg, i.p., panel A) or with yohimbine (1 mg/kg, i.p., panel B) on the hexanic extract of *S. molle* (100 mg/kg, p.o.)-induced reduction in immobility time in the TST. Each column represents the mean+S.E. of 6–8 animals. **$P<0.01$ compared with the vehicle-treated control. # $P<0.01$ as compared with the same group pretreated with vehicle.

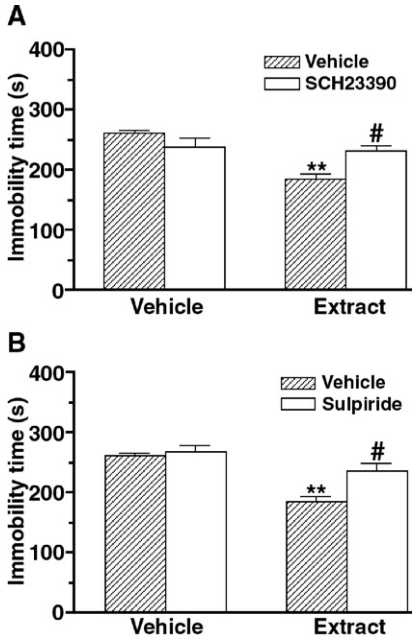

Fig. 5. Effect of pretreatment of mice with SCH23390 (0.05 mg/kg, s.c., panel A) or with sulpiride (50 mg/kg, i.p., panel B) on the hexanic extract of *S. molle* (100 mg/kg, p.o.)-induced reduction in immobility time in the TST. Each column represents the mean+S.E. of 9–12 animals. $^{**}P<0.01$ compared with the vehicle-treated control. # $P<0.01$ as compared with the same group pretreated with vehicle.

of the extract of *S. molle* in the TST. The results were analyzed by a two-way ANOVA that showed a significant effect of pretreatment [$F(1,30)=6.09$, $P<0.01$], treatment X pretreatment interaction [$F(1,30)=12.02$, $P<0.01$], but not of treatment ($F(1,30)=2.52$, $P = 0.12$).

3.3.2. Involvement of the noradrenergic system

The results depicted in Fig. 4A show that pretreatment of mice with prazosin (1 mg/kg, i.p) was able to reverse the antidepressant-like effect the extract of *S. molle* (100 mg/kg, p.o.) in the TST. The two-way ANOVA revealed a main effect of the treatment [$F(1,22)=7.09$, $P<0.05$], pretreatment [$F(1,22)=20.94$, $P<0.01$] and treatment X pretreatment interaction [$F(1,22)=5.75$, $P<0.05$]. Fig 4B shows that the pretreatment of mice with yohimbine (1 mg/kg, i.p.) was also able to prevent the anti-immobility effect the extract of *S. molle* (100 mg/kg, p.o.) in the TST. The two-way ANOVA revealed a main effect of the treatment [$F(1,23)=5.85$, $P<0.05$], treatment X pretreatment interaction [$F(1,23)=8.87$, $P<0.01$], but not of the pretreatment [$F(1,23)=1.97$, $P=0.17$].

3.3.3. Involvement of the dopaminergic system

The anti-immobility effect of the extract of *S. molle* (100 mg/kg, p.o.) was significantly prevented by pretreatment of mice with SCH23390 (0.05 mg/kg, s.c., Fig 5A). The two-way ANOVA revealed a main effect of the treatment [$F(1,36)=22.36$, $P<0.01$], treatment X pretreatment interaction [$F(1,36)=15.32$, $P<0.01$], but not of pretreatment [$F(1,36)=1.88$, $P=0.17$]. Fig 5B shows that sulpiride (50 mg/kg, i.p., 5B) was also able to prevent the anti-immobility effect of the extract of *S. molle* in the TST. The two-way ANOVA revealed a main effect of the treatment [$F(1,37)=32.59$, $P<0.01$], pretreatment [$F(1,37)=8.75$, $P<0.01$] and of treatment X pretreatment interaction [$F(1,37)=5.01$, $P<0.05$].

4. Discussion

The TST is a well characterized behavioral model predictive of antidepressant activity that is sensitive to antidepressants from different pharmacological classes (Steru et al., 1985; Cryan et al., 2005). In this study we provide convincing evidence that the extract of *S. molle* administered by oral route produces a specific antidepressant-like effect in this test, since the reduction of immobility time elicited by its administration cannot be attributable to any psychostimulant effect. Furthermore, the effect of the extract of *S. molle* in the TST was similar to the effect produced by the oral administration of fluoxetine, used as a positive control.

This study also analyzes some of the possible mechanisms related to the antidepressant-like effects observed for the extract of *S. molle*. As monoaminergic system is one of the most important targets in the pathophysiology and treatment of depression (Elhwuegi, 2004; Millan, 2004), we investigated the involvement of the serotonergic, noradrenergic and dopaminergic systems in its anti-immobility effect in the TST. Thus, we have assessed herein the effects of several pharmacological antagonists on the anti-immobility action of the extract of *S. molle* (100 mg/kg, p.o., 1 h before) in mice.

Depressive disorder has long been associated with disturbances of brain 5-HT activity and data concerning 5-HT variations in depression have probably been the most widely studied. Moreover, the serotonergic system plays a major role in the action of antidepressants (Millan, 2004). The involvement of the serotonergic system in the antidepressant-like effect of the extract of *S. molle* is indicated by the results showing that its effect in the TST was completely prevented by pretreament of mice with the neuronal serotonin store depletor, PCPA as well as with the 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 antagonists WAY100635, ketanserin and MDL72222, respectively.

PCPA is an inhibitor of tryptophan hydroxylase and its administration, for four consecutive days, depletes the endogenous stores of 5-HT by about 60% in mice (Redrobe et al., 1998b). PCPA treatment used in the present study produces partial but highly significant reductions on brain 5-HT levels while noradrenaline and dopamine levels are not affected (Redrobe et al., 1998a,b). Moreover, the same treatment with PCPA was previously shown by our group to completely prevent the antidepressant-like effect of fluoxetine, leaving the antidepressant action caused by imipramine in the TST unaffected (Rodrigues et al., 2002).

Studies have demonstrated the involvement of 5-HT_{1A} receptors in the mechanism of action of several classes of antidepressant drugs, including tricyclics, SSRIs (selective serotonin reuptake inhibitors) and MAOi (monoamine oxidase inhibitors) (Hensler, 2002). An evidence of the involvement of 5-HT_{1A} receptors in the antidepressant-like effect of the extract of *S. molle* was given by the finding that the selective 5-HT_{1A}

receptor antagonist WAY100635 was able to prevent its anti-immobility effect in the TST.

A role for 5-HT_2 receptors in the action of some antidepressants has been shown. Many established antidepressants are 5-HT_2 receptor antagonists and share the ability to decrease 5-HT_2 receptor binding after repeated administration. The down-regulation of 5-HT_{2A} receptors is proposed to mediate the long-term actions of antidepressants (Deakin, 1988). In the present work, the pretreatment of animals with ketanserin was able to reverse the anti-immobility effect of the extract of *S. molle* in the TST, providing evidence of the participation of 5-HT_2 receptors in its effect in the TST. A similar result was reported in previous studies from our group in which ketanserin was able to reverse the antidepressant-like effect of agmatine (Zomkowski et al., 2004) and of the lectin from *Canavalia brasiliensis* (Barauna et al., 2006) in the mouse forced swimming test. In addition, the preferential 5-HT_{2A} receptor agonist DOI was reported to enhance the antidepressant-like effect of some compounds (Zomkowski et al., 2004; Khisti and Chopde, 2000). Thus, the present study indicates that the antidepressant-like effect of the extract of *S. molle* appears to be mediated by stimulation of 5-HT_{2A} receptors.

We also investigate the participation of 5-HT_3 receptors in the antidepressant-like effect of the extract of *S. molle*. The pretreatment of animals with MDL72222 was able to prevent the anti-immobility effect of the extract in the TST, demonstrating that this effect is mediated, at least in part, by an interaction with this serotonergic receptor subtype. The involvement of 5-HT_3 receptors in the pathophysiology of depression is much less studied than 5-HT_{1A} and 5-HT_2. However, literature data demonstrate that different classes of antidepressants act as functional antagonists at the 5-HT_3 receptors, indicating that the suppression of 5-HT_3 receptor activity may contribute to the action of antidepressants (Eisensamer et al., 2003). Moreover, a recent study has shown that MDL72222 administered at a higher dose (3 mg/kg, i.p.) in mice produced an antidepressant-like effect in the TST (Kos et al., 2006). Although the apparent inconsistency with previous data, the reversal of the antidepressant-like effect of the extract of *S. molle* by MDL72222 suggests that its antidepressant-like effect is mediated by an activation of 5-HT_3 receptors. In fact, in line with this hypothesis is the finding that eletroconvulsive therapy, which is clinically used to treat drug resistent depression, was able to potentiate the function of 5-HT_3 receptors in the hippocampus (Ishihara and Sasa, 2001).

The role of noradrenaline in the pathophysiology of depression has been also extensively studied, since some antidepressant drugs increase the synaptic concentration of NA and some of these drugs were found to act directly at noradrenergic receptor (Elhwuegi, 2004). In addition, it was recently demonstrated that NA-deficient mice lack responses to antidepressant drugs, including SSRIs (Cryan et al., 2004). In our study both prazosin (an α_1-adrenoceptor antagonist) and yohimbine (an α_2-adrenoceptor antagonist) were able to reverse the antidepressant-like effect of the extract of *S. molle*. This result indicates that the extract may exert its effect in the TST by interacting with both α_1 and α_2-adrenoceptors. Accordingly, there is compelling evidence for a role of α_1 and α_2-adrenoceptors in the actions of antidepressant agents (Millan, 2004).

The dopaminergic system is also strongly implicated in regulation of mood (Dailly et al., 2004). Some biochemical evidence derives from clinical studies showing that the plasma levels of homovanillic acid and 3,4-dihydroxyphenylacetic acid, two dopamine metabolites, were significantly lower in the depressed patients, indicating a diminished dopamine turnover (Mitani et al., 2006; Sher et al., 2006). It has been considered that the potentiation of dopaminergic neurotransmission induced by chronic antidepressant treatments might contribute to their therapeutic effect (D'Aquila et al., 2000) and there is also a considerable amount of pharmacological evidence regarding the efficacy of antidepressants with dopaminergic effects in the treatment of depression (Papakostas, 2006). As shown in the results, the selective dopamine D_1 receptor antagonist SCH 23390 and the dopamine D_2 receptor antagonist, sulpiride significantly antagonized the anti-immobility effects of the extract of *S. molle* in the TST. Our results are in accordance with literature data indicating that both dopamine D_1 and D_2 receptors might play a role in depression. Indeed Yamada et al. (2004) suggested that dopamine D_1 and D_2 receptors play a role in the effects of dopamine reuptake inhibitors on forced swimming test, another animal model predictive of antidepressant activity. In addition, it was reported that the dopamine D_1 receptor agonist SKF 38393 enhances anti-immobility effects of SSRIs, suggesting that the dopamine D_1 receptor may participate in the antidepressant effects of SSRIs (Renard et al., 2001). It was also reported that the anti-immobility effects of the trycliclic antidepressant imipramine were reduced by antisense dopamine D_2 receptor (Dziedzicka-Wasylewska et al., 2000). Indeed, clinical studies reported that dopamine D_2 receptor agonists are effective for treating depressive patients (Waehrens and Gerlach, 1981).

In summary, our data indicate an antidepressant-like effect of the hexanic extract of *S. molle*, which is not due to any psychostimulant effect and that seems to be mediated by an interaction with the monoaminergic system. It is interesting to note that a low toxicity was reported for the extract obtained from the leaves of *S. molle* (Barrachina et al., 1997). Phytochemical studies have identified active components from *S. molle* such as tannins, triterpenoids, flavonoids and saponins (Pozzo-Balbi et al., 1978; Olafsson et al., 1997; Yueqin et al., 2003; Taylor, 2005). However, a preliminary characterization of the hexanic extract used in the present study did not show the presence of flavonoids and tannins. This extract contains triterpenoids as major compounds. Further chemical analysis of the extract will be conducted to isolate and characterize the active principles responsible for the observed effects.

5. Conclusion

In conclusion, the present study provides the first evidence indicating that the hexanic extract of *S. molle* produces a specific antidepressant-like effect in an animal model predictive of antidepressant properties, the TST, similar to the result

produced by the classical antidepressant fluoxetine. In addition, we have shown that its antidepressant-like effect is dependent on its interaction with the serotonergic (5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors), noradrenergic (α_1 and α_2-receptors) and dopaminergic (D_1 and D_2 receptors) systems. Thus, our results suggest that the extract from *S. molle* shares with established antidepressants some pharmacological effects, at least at a preclinical level.

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References

Barauna SC, Kaster MP, Heckert BT, Nascimento KS, Rossi FM, Teixeira EH, et al. Antidepressant-like effect of lectin from *Canavalia brasiliensis* (ConBr) administered centrally in mice. Pharmacol Biochem Behav 2006;85:160–9.

Barrachina MD, Bello R, Martínez-Cuesta MA, Primo-Yúfera E, Espluges J. Analgesic and central depressor effects of the dichloromethanol extract from *Schinus molle* L. Phytother Res 1997;11:317–9.

Bello R, Barrachina MD, Moreno L, Primo-Yúfera E, Espluges J. Effects on arterial blood pressure of the methanol and dichloromethanol extracts from *Schinus molle* L. in rats. Phytother Res 1996;10:634–5.

Bello R, Beltrán B, Moreno L, Calatayud S, Primo-Yúfera E, Espluges J. In vitro pharmacological evaluation of the dichloromethanol extract from *Schinus molle* L. Phytother Res 1998;12:523–5.

Bilia AR, Gallori S, Vincieri FF. St. John's wort and depression. Efficacy, safety and tolerability—an update. Life Sci 2002;70:3077–96.

Cryan JF, O'Leary OF, Jin S, Friedland JC, Ouyang M, Hirsch BR, et al. Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. Proc Natl Acad Sci U S A 2004;101:8186–91.

Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev 2005;29:571–625.

Dailly E, Chenu F, Renard CE, Bourin M. Dopamine, depression and antidepressants. Fundam Clin Pharmacol 2004;18:601–7.

D'Aquila PS, Collu M, Gessa GL, Serra G. The role of dopamine in the mechanism of action of antidepressants drugs. Eur J Pharmacol 2000;405:365–73.

Deakin JF. $5HT_2$ receptors, depression and anxiety. Pharmacol Biochem Behav 1988;29:819–20.

Dziedzicka-Wasylewska M, Kolasiewicz W, Rogoz Z, Margas W, Maj J. The role of dopamine D_2 receptor in the behavioral effects of imipramine-study with the use of antisense oligonucleotides. J Physiol Pharmacol 2000;51:401–9.

Eisensamer B, Rammes G, Gimpl G, Shapa M, Ferrari U, Hapfelmeier G, et al. Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol Psychiatry 2003;12:994-1007.

Elhwuegi AS. Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:435–51.

Gavioli EC, Vaughan CW, Marzola G, Guerrini R, Mitchell VA, Zucchini S, et al. Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. Naunyn Schmiedebergs Arch Pharmacol 2004;369:547–53.

Hensler JG. Differential regulation of 5-HT_{1A} receptors–G protein interactions in brain following chronic antidepressant administration. Neuropsychopharmacology 2002;26:565–73.

Ishihara K, Sasa M. Potentiation of 5-HT_3 receptor functions in the hippocampal CA1 region of rats following repeated electroconvulsive shock treatments. Neurosci Lett 2001;307:37–40.

Kaster MP, Santos ARS, Rodrigues ALS. Involvement of 5-HT_{1A} receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test. Brain Res Bull 2005;67:53–61.

Khisti RT, Chopde CT. Serotonergic agents modulate antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice. Brain Res 2000;865:291–300.

Kos T, Popik P, Pietraszek M, Schäfer D, Danysz W, Dravolina O, et al. Effect of 5-HT_3 receptor antagonist MDL 72222 on behaviors induced by ketamine in rats and mice. Eur Neuropsychopharmacol 2006;16:297–310.

Linde K, Knüppel L. Large-scale observational studies of hypericum extracts in patients with depressive disorders—a systematic review. Phytomedicine 2005;12:148–57.

Mantovani M, Pértile R, Calixto JB, Santos ARS, Rodrigues ALS. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. Neurosci Lett 2003;343:1–4.

Millan MJ. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 2004;500:371–84.

Mitani H, Shirayama Y, Yamada T, Kawahara R. Plasma levels of homovanillic acid, 5-hydroxyindoleacetic acid and cortisol, and serotonin turnover in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:531–4.

Nestler EJ, Barrot M, DiLeonem RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002;34:13–25.

O'Neill MF, Conway MW. Role of 5-HT_{1A} 5-HT_{1B} receptors in the mediation of behavior in the forced swim test in mice. Neuropsychopharmacology 2001;24:391–8.

Olafsson K, Jaroszewski JW, Smitt UW, Nyman U. Isolation of angiotensin converting enzyme (ACE) inhibiting triterpenes from *Schinus molle*. Planta Medica 1997;63:352–5.

Papakostas GI. Dopaminergic-based pharmacotherapies for depression. Eur Neuropsychopharmacol 2006;16:391–402.

Pozzo-Balbi T, Nobile L, Scapini G, Cini M. The triterpenoid acids of *Schinus molle*. Phytochemistry 1978;17:2107–10.

Quiroga EN, Sampietro AR, Vattuone MA. Screening antifungal activities of selected medicinal plants. J Ethnopharmacol 2001;74:89–96.

Redrobe JP, Bourin M. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur J Pharmacol 1997;325:129–35.

Redrobe JP, Bourin M, Colombel MC, Baker GB. Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. Psychopharmacology 1998a;138:1–8.

Redrobe JP, Bourin M, Colombel MC, Baker GB. Psychopharmacological profile of the selective serotonin reuptake inhibitor, paroxetine: implication of noradrenergic and serotonergic mechanisms. J Psychopharmacol 1998b;12:348–55.

Renard CE, Fiocco AJ, Clenet F, Hascoët M, Bourin MS. Is dopamine implicated in the antidepressant-like effects of selective serotonin reuptake inhibitors in the mouse forced swimming test? Eur Neuropsychopharmacol 2001;11:208–9.

Rodrigues ALS, Silva GL, Matteussi AS, Fernandes E, Miguel O, Yunes RA, et al. Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. Life Sci 2002;70:1347–58.

Ruffa MJ, Ferraro G, Wgner ML, Calcagno ML, Campos RH, Cavallaro L. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J Ethnopharmacol 2002;79:335–9.

Schmourlo G, Mendonça-Filho RR, Alviano CS, Costa SS. Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. J Ethnopharmacol 2005;96:563–8.

Sher L, Mann JJ, Traskman-Bendz L, Winchel R, Huang YY, Fertuck E, et al. Lower cerebrospinal fluid homovanillic acid levels in depressed suicide attempters. J Affect Disord 2006;90:83–9.

Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985;85:367–70.

Taylor L. The healing power of rainforest herbs. A Guide to Understanding and Using Herbal Medicinals. New York: Square One Publishers; 2005.

Viana A, Rego J, Poser G, Ferraz A, Heckler AP, Costentin J, et al. The antidepressant-like effect of *Hypericum caprifoliatum* Cham & Schlecht (Guttiferae) on forced swimming test results from an inhibition of neuronal monoamine uptake. Neuropharmacology 2005;49:1042–52.

Waehrens J, Gerlach J. Bromocriptine and imipramine in endogenous depression. A double-blind controlled trial in out-patients. J Affect Disord 1981;3:193–202.

Wong M, Licinio J. Research and treatment approaches to depression. Nat Rev Neurosci 2001;2:343–51.

Whiskey E, Werneke U, Taylor D. A systematic review and meta-analysis of *Hypericum perforatum* in depression: a comprehensive clinical. Int Clin Psychopharmacol 2001;16:239–52.

Yamada J, Sugimoto Y, Yamada S. Involvement of dopamine receptors in the anti-immobility effects of dopamine re-uptake inhibitors in the forced swimming test. Eur J Pharmacol 2004;504:207–11.

Yueqin Z, Recio MC, Mánez S, Giner RM, Cerdá-Nicolás M, Ríos J. Isolation of two triterpenoids and a biflavanone with anti-inflammatory activity from *Schinus molle* fruits. Planta Med 2003;69:893–8.

Zhang Z. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci 2004;75:1659–99.

Zomkowsk ADE, Rosa AO, Lin J, Santos ARS, Calixto JB, Rodrigues ALS. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant-like effect in the mouse forced swimming test. Brain Res 2004:253–63.