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Antispasmodic, antidepressant and anxiolytic effects of extracts from *Schinus lentiscifolius* Marchand leaves

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ABSTRACT

Schinus lentiscifolius (Anacardiaceae) is widely used in folk medicine for treating gastrointestinal and emotional complaints but there are no scientific studies that support these uses. This work aims at evaluating the antispasmodic and central effects of *S. lentiscifolius* as well as the flavonoids presence in the tincture (SchT) and the composition of the essential oil (SchO). SchT inhibited the concentration-response curves (CRC) of carbachol and calcium in a non-competitive way in isolated rat intestine, bladder and uterus. SchT also non-competitively inhibited the CRC of histamine in guinea-pig intestine and the CRCs of serotonin and oxytocin in rat uterus. Isoquercetin and rutin were identified in SchT. The behavioral effects of SchT, SchO and infusion of *S. lentiscifolius* leaves (SchW) were tested in mice. These extracts showed an anxiolytic-like effect in the novelty-suppressed feeding test, which was reversed by flumazenil except in SchO-treated mice. Only SchO reduced the spontaneous locomotor function in the open field test. Also, SchT and SchW decreased immobility time in both, the tail suspension (TST) and forced swimming tests, while SchO produced the same effect in the TST. D-limonene and α-santalol were the main components found in SchO. The results demonstrated that extracts obtained from *S. lentiscifolius* leaves were effective as intestinal, urinary and uterine antispasmodics. SchT and SchW exhibited anxiolytic and antidepressant properties without sedation, whereas SchO showed also sedative properties. Therefore, the present study gives preclinical support to the traditional use of this plant for gastrointestinal and depressive or emotional symptoms.

1. Introduction

Schinus lentiscifolius (Anacardiaceae), a native species of South American popularly known as 'ashen *molle*', is an aromatic, ornamental and flowering small tree. This plant is widely used in folk medicine to treat several diseases incluiding gastrointestinal and central disorders.[28]

There are some reports about antispasmodic and central antidepressant activities of other species of *Schinus*, such as *S. terebinthifolius* Raddi or *S. molle* Hort.[31,41] Often consumers confuse them; however, despite being morphologically different, they have similar traditional medicinal applications.[28] Similarly, there is only sufficient evidence about the antifungal, antimicrobial and antioxidant properties of *S. lentiscifolius*,[20] but there are no scientific studies that support other uses as antispasmodic and emotional symptoms.

Phytochemical studies of *S. lentiscifolius* have resulted in the isolation of natural mono- and sesquiterpenes, triterpenes, phenolic compounds, essential oil and flavonoids such as

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List of abbreviations

CRC	concentration-response curve
CCh	carbachol
His	histamine
Oxt	oxytocin
5-HT	serotonin
Ca^{2+}	ionic calcium
SchT	tincture of Schinus lentiscifolius
SchW	infusion of Schinus lentiscifolius
SchO	essential oil of Schinus lentiscifolius
IC50	50 % inhibitory concentration
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
GC-FID-MS	gas chromatography with flame ionization and mass spectrometry detectors
OFT	open field test
NFT	novelty-suppressed feeding test
TST	tail suspension test
FST	forced swimming test
FL	feed latency
IT	immobility time

quercetin.[20] Flavonoids have a wide variety of biological activities not only on the central nervous system but also on smooth muscle.[21,24] Preliminary flavonoid characterization suggested the presence of rutin and isoquercetin in the tincture of *S. lentiscifolius*.[44] Both of them have been demonstrated to be sedatives while rutin also showed antidepressant action[24] Besides, their spasmolytic activity has been reported in isolated intestine.[22] These reports allow us to hypothesize that *S. lentiscifolius* could exert antispasmodic, antidepressant and anxiolytic-like effects, at least in part due to the presence of flavonoids in the extracts.

On the other hand, the composition of the essential oil of Uruguayan and Brazilian specimens of *S. lentiscifolius* has been described previously, finding some differences between them.[33] As it happens with other plants, it seems that the variability in the main components of the essential oil is closely related to the geographical location of the tested specimen.[6] Therefore, the essential oil of the Argentine *S. lentiscifolius* may have a different composition, which would confer other biological activities and it was considered necessary to evaluate it, since there are no bibliographic data.

Due to the lack of information on the pharmacological potential and chemical components of *S. lentiscifolius*, this work evaluates the antispasmodic and central effects of this plant extracts, as well as the presence of rutin and isoquercetin in the tincture. Moreover, the composition of the essential oil obtained from *S. lentiscifolius* leaves was analyzed, and it was evaluated in models of anxiety and depression in mice for comparing with the ethanolic and aqueous extracts.

2. Materials and methods

2.1. Plant material and extracts preparation

Fresh leaves of *S. lentiscifolius* were collected from the "Carlos Spegazzini" Botanical Garden and Arboretum, School of Agricultural and Forestry Sciences of the National University of La Plata in spring. Plant material was identified by Marta Colares, MSc.

The *S. lentiscifolius* tincture (SchT) was prepared by 20 % w/v maceration of leaves in 70 % v/v ethanol from which dilutions were made (yield of 10.0 % w/w). The infusion of *S. lentiscifolius* leaves (SchW) was prepared at 20 % w/v. The essential oil of leaves of *S. lentiscifolius* (SchO) was obtained by hydrodistillation (density of 0.9221 g/ml) according to the Argentine National Pharmacopoeia (National Formulary method, XIV, 1975). For the biological studies, it was diluted in dimethyl sulfoxide (DMSO).

Concentration and doses of SChT, SchW and SchO were expressed as mass of dry leaves by ml or kg, respectively.

2.2. Chromatographic analysis

SchT was analyzed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) using a Dionex UltiMate 3000 UHPLC (Thermo Scientific, Sunnyvale, CA, USA) configured with a dual ternary gradient pump (DGP-3000) and a DAD-3000 diode array detector (DAD). The stationary phase was a C18 column (250 × 4.6 mm, LiChrospher 5 μm, Merck, USA) and the mobile phase was a 85:10:5 mixture of water:tetrahydrofuran:methanol adjusted to pH = 2.5 with phosphoric acid with a flow of 1 ml/min. Detection was performed at 330 nm. The extracts were diluted 1:5 with mobile phase. Reference standard solutions of isoquercetin and rutin were diluted in methanol and then prepared in mobile phase at 20 and 10 mg/ml, respectively. Chromatography peaks were confirmed by comparing their retention time and DAD spectra (200–400 nm) with those of the standard sample.

2.3. Analysis of the essential oil

The essential oils of *S. lentiscifolius* leaves was carried out on a GC-FID-MS system (PerkinElmer Clarus 500) with one injector (split ratio 1:100) connected by a flow splitter to two capillary columns (60 m × 0.25 mm id and 25 μm stationary phase thickness): (a) polyethylene glycol MW ~20,000 (DB-Wax, J&W Scientific) and (b) 5 % phenyl-95 % methyl silicone. The polar column was connected to a flame ionization detector (FID), while the nonpolar column was connected to an FID and a quadrupole mass detector (70 eV) by a vent system (MSVent®). Helium was used as mobile phase at a constant flow of 1.87 ml/min (split ratio 1:100). The temperature was programmed according to the following gradient: 90–225 °C at 3 °C/min, and then isothermal for 15 min. The injector and FID were set at 255 °C and 275 °C, respectively. The temperatures of the transference line and the ion source were 180 °C and 150 °C, respectively. The range of masses analyzed was 40–300 Da (10 scan/s). Identification of the compounds in the SchO was performed by comparison of the linear retention indices (relative to a homologous series of alkanes [C8–C20]) on both columns with those of reference compounds. Additionally, each mass spectrum obtained was compared with those from widely used mass spectral databases[39] and mass spectra obtained from reference compounds. The relative percentage contribution of the compounds was calculated from the FID responses by computerized integration, without considering corrections for response factors. The lowest response obtained from both columns for each component was considered.

2.4. Pharmacological studies

2.4.1. Animals

In order to study the pharmacological activities of *S. lentiscifolius*, male and female Wistar rats (200–250 g), male Swiss mice (25–30 g) and female guinea pigs (280–300 g) were used. The animals were cared for according to international standards established by US guidelines (NIH publication #85-23, revised in 1996) and the principles in the Declaration of Helsinki. A local ethical committee of the School of Exact Sciences of the

National University of La Plata approved the protocols (015-05-15 and D-01-15-16). Rats and guinea pigs were deprived of food but allowed water *ad libitum* 12 h before the *ex vivo* experiments (isolated tissues), while the mice were only kept fasting for 24 h for the novelty-suppressed feeding test (NFT).

2.4.2. Isolation of smooth muscles preparations and contractile measurements

Wistar rats (200–250 g) were anesthetized with urethane (doses of 1.5 g/kg) after 12 h of fasting. Portions of the rat or guinea pig intestine and the rat urinary bladder were excised and mounted in organ baths containing constantly oxygenated Tyrode's solution (20 ml, pH 8.2) at 37 °C.[19,35] The uteri of rats were isolated and immersed in de Jalon's solution at 32 °C, bubbled with air.[19] All tissues were equilibrated for 35 min at 1 g of preload. Intestinal muscle and bladder strips were connected to isometric transducers (WPI, USA) and the contractile signals were amplified and recorded with Eagle software (USA). The contractility of the uterus was detected by PanLab MLT0210/A isometric transducers and recorded with a PowerLab 2/26 system with LabChart software (ADInstruments, Australia).

2.4.3. Concentration-response curves (CRCs) with carbachol, serotonin, histamine or oxytocin

CRCs of agonist carbachol (CCh-CRC) were performed on rat duodenum or ileum, bladder and uterus by cumulatively adding CCh to the bath within a range of concentrations from 0.01 to 10 μg/ml up to a maximal contraction. Similarly, a CRC of histamine (His-CRC; range 1–200 μg/ml) was performed on isolated guinea-pig ileum, whereas CRCs of oxytocin (Oxt-CRC; range 1–200 μg/ml) and serotonin (5-HT-CRC; range 0.01–3 μg/ml) were performed on rat uterus. First, control curves were obtained in the absence (control condition) and in the presence of vehicle (control vehicle, 70 % v/v ethanol) for comparison with the respective SchT concentration. Curves made in the presence of vehicle did not show differences in the contractile effect compared to control conditions. SchT (range 0.1–20 mg/ml) were added individually to the medium 5 min before the corresponding CRC. The contractile effects were expressed as percentage of the maximal tissue contraction (% Emax), obtained with the control vehicle CRC.

2.4.4. Concentration-response curves with $CaCl_2$ (Ca^{2+}-CRC)

Ca^{2+}-CRCs were performed on intestinal, bladder and uterine smooth muscle by cumulatively adding $CaCl_2$ (range 0.0195–17.5 mmol/L). After the stabilization period, Ca^{2+} was removed from the medium and then the tissues were depolarized with 40 mmol/L of KCl. There were no differences in the contractile effect in the presence or absence of vehicle. Each SchT concentration was added to the bath 5 min before the Ca^{2+}-CRC. The % Emax was measured with the same approach previously described.

2.4.5. Pharmacological parameters of CRC

From the CRCs, the pEC_{50} of the agonist were calculated as −log EC_{50}, in mol/L. For the tincture, the 50 % inhibitory concentration (IC_{50}) was obtained by extrapolation at 50 % effect of the individual inhibitory curves at the maximal effect of the agonist.[19] IC_{50} values of SchT were expressed as mg/ml.

2.4.6. Behavior studies in mice

All drugs were administered by intraperitoneal (i.p.) injections to mice in a volume of 0.1 ml per 30 g of body weight. The SchT doses used in the behavioral tests (30 and 100 mg/kg) were selected according to the concentrations that were effective as antispasmodics in the CRCs, as scaled from plasmatic level. By other side, higher doses of SchW (200 and 400 mg/kg) were chosen because the content of flavonoids should be lower in the aqueous than in the ethanolic extracts.[14] In both cases, ranges of similar doses showed to be effective in behavior studies in other medicinal plants.[17,38] Moreover, the SchO doses (10 and 30 μg/kg) used are in the range of those reported as active for *S. terebinthifolius* in memory deficits studies.[42]

2.4.6.1. Open field test (OFT). Spontaneous locomotor, exploratory and emotionality activities were evaluated by using the OFT, which consisted of a white enclosure of 30 × 50 cm divided into 15 squares of 10 cm^2 by black lines surrounded by walls that were 27 cm high. Adequate environmental conditions were maintained.[11] Prior to treatment, each animal was placed in the same corner of the open field, allowing free exploration for 5 min. The test was performed 30 min after the injection of extracts or vehicle, with diazepam (1 mg/kg), a known benzodiazepine, as reference compound. During a 5-min period, the number of crossed lines, rearings, grooming and other signs were measured. The same protocol was repeated at 60, 90 and 120 min after administration.

2.4.6.2. Novelty-suppressed feeding test (NFT). Mice were placed in individual cages, being allowed to remain for 30 min to habituate. After 1 h of extract i.p. administration, each animal was placed in the same corner of a 40 × 40 × 30 cm wooden box containing a white circular filter paper with a diameter of 11 cm and a single food pellet in the center. The latency time to start eating was recorded. Immediately after the first bite, each mouse was removed and placed in its individual cage with a previously weighed pellet; after 5 min the amount of food consumed was determined. Finally, the mice were removed to their home cage with water and food *ad libitum*.[25] Diazepam (Dzp, 0.3 mg/kg) and flumazenil (Fzl, 0.5 mg/kg), a benzodiazepine antagonist, were used as reference compounds.

2.4.6.3. Tail suspension test (TST). TST protocol was previously described.[40] In order to test the *Schinus* extracts, a structure 50 cm high, 12 cm deep and 60 cm wide was used. The mice were suspended 50 cm above the ground with adhesive tape placed 2 cm from the base of their tails in identical individual compartments. The measurement was performed 1 h after i.p. administration. Immobility was defined as the permanence in a vertical position and the lack of any attempt at movement of the hind legs during suspension and the immobility time (IT, sec) was video recorded for 6 min. Clomipramine (1.25 mg/kg) a known antidepressant, was used as a reference compound.

2.4.6.4. Forced swimming test (FST). FST protocol was previously described.[45] Swiss mice were injected via i.p. An hour later, they were introduced into a transparent glass cylinder (diameter: 24 cm; height: 22 cm) with 10 cm of water at 22–25 °C for 6 min and the IT was recorded during the final 4 min. It was considered immobility when the mouse floated without movement of the hind legs or made only the movements necessary to keep its head above the water. At the end of the test, the mice were removed to a dry, heated place. The bath water was changed after every session to avoid influence on the next mouse. Clomipramine (1.25 mg/kg) was used as a reference compound.

2.4.7. Drugs and solution

Composition medium and solution used for CRC and the drugs employed in the biological tests are described in Appendix A.

2.4.8. Statistical analysis

Results are expressed as the mean ± SEM (standard deviation of the mean), n (number of experiments). Multiple comparisons were

done by two-way ANOVA test curve and by one-way ANOVA test as appropriate. *A posteriori* Tukey's test was used. Statistical analyses were carried out with Graph Pad Prism 8.0 software. $P < 0.05$ was considered significant in all tests performed.

3. Results

3.1. Identification of components in S. lentiscifolius tincture

Fig. 1a shows the chromatograms of SchT by HPLC. We found two peaks with retention times of 45 and 55 min, corresponding to the reference substances rutin (peak #1) and isoquercetin (peak #2), respectively. The UV spectra (Fig. 1b) matched the typical flavonoid spectra.[43]

3.2. Composition of the essential oil of S. lentiscifolius

Table 1 shows the relative percentage composition of SchO, whose yield was 1.2 % v/w (ml of essence/100 gr of dried leaves).

3.3. Antispasmodic effects of S. lentiscifolius tincture on smooth muscle

In the intestinal muscle, SchT reduced significantly the maximum contractile effect of the CCh-CRC ($pEC_{50} = 6.17 \pm 0.16$) in a concentration-dependent way (Fig. 2a). This effect showed a typical non-competitive antagonism on the cholinergic contraction with an IC_{50} of 6.32 ± 1.2 mg SchT/ml. In bladder and uterus muscle, SchT also inhibited the CCh-CRC ($pEC_{50} = 6.47 \pm 0.08$ and $pEC_{50} = 5.88 \pm 0.10$, respectively) with a non-competitive pattern (Fig. 2c and e). The IC_{50} was 2.63 ± 0.12 mg of SchT/ml in bladder and 4.05 ± 1.03 mg of SchT/ml in uterus.

Table 1
Relative percentage composition of the essential oils obtained from leaves of *Schinus lentiscifolius*.

Components	%	Components	%
cis-3-hexenol	tr	α -humulene	0.9
α -pinene	2.4	alloaromadendrene	2.5
Camphene	tr	*trans*-cadina-1 (6),4-diene	1.7
methyl heptenone	tr	λ-muurolene	0.2
α -terpinene	0.1	germacrene d	0.1
p-cymen	tr	*epi*-bicyclosesquiphellandrene	1.7
Sabinene	1.4	cadina-3,5-diene	1.7
D-limonene	12.9	bicyclogermacrene	1.4
1,8-cineole	0.1	α -muurolene	2.6
λ -terpinene	0.1	δ -cadinene	21
Terpinolene	tr	zonarene	0.9
Linalool	tr	*trans*-cadina-1,4-diene	1.4
terpinen-4-ol	0.3	α -calacorene	0.2
α -terpineol	0.3	β -calacorene	0.3
Bicycloelemene	0.1	spathulenol	4.1
α -cubebene	1.2	Oxy- caryophyllene	0.9
α- longipinene	0.1	copaborneol	1
Cyclosativene	0.2	1-*epi*-cubenol	4.7
α -copaene	4.4	globulol	0.2
β -bourbonene	0.1	ledol	tr
β -cubebene	0.4	ϯ-muurolol	0.6
α-gurjunene	0.1	α-cadinol	0.2
β -caryophyllene	2.7	mustakona	0.4
λ -maaliene	tr	cetyl alcohol	0.1
guaia-6,9-diene	0.1	isocalamendiol	0.2
β -copaene	0.1	trans phytol	0.1
Aromadendrene	0.2	hydrocarbon 25	0.1
		TOTAL	**76.5**

To evaluate whether the antispasmodic effects of SchT were due to inhibition of calcium influx, these effects were assessed on Ca^{2+}-

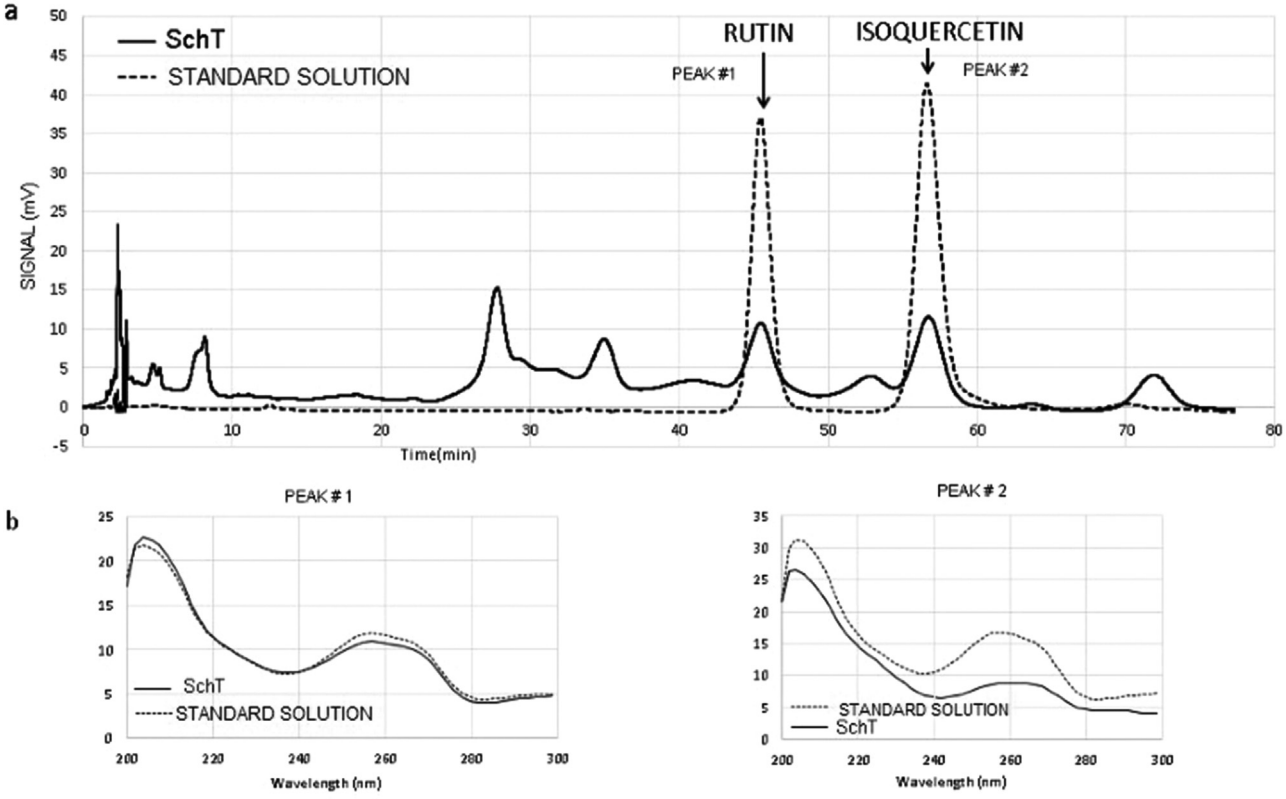

Fig. 1. High-performance liquid chromatography. Profiles of SchT and standard Solution (a); UV spectra of the identified peaks (b).

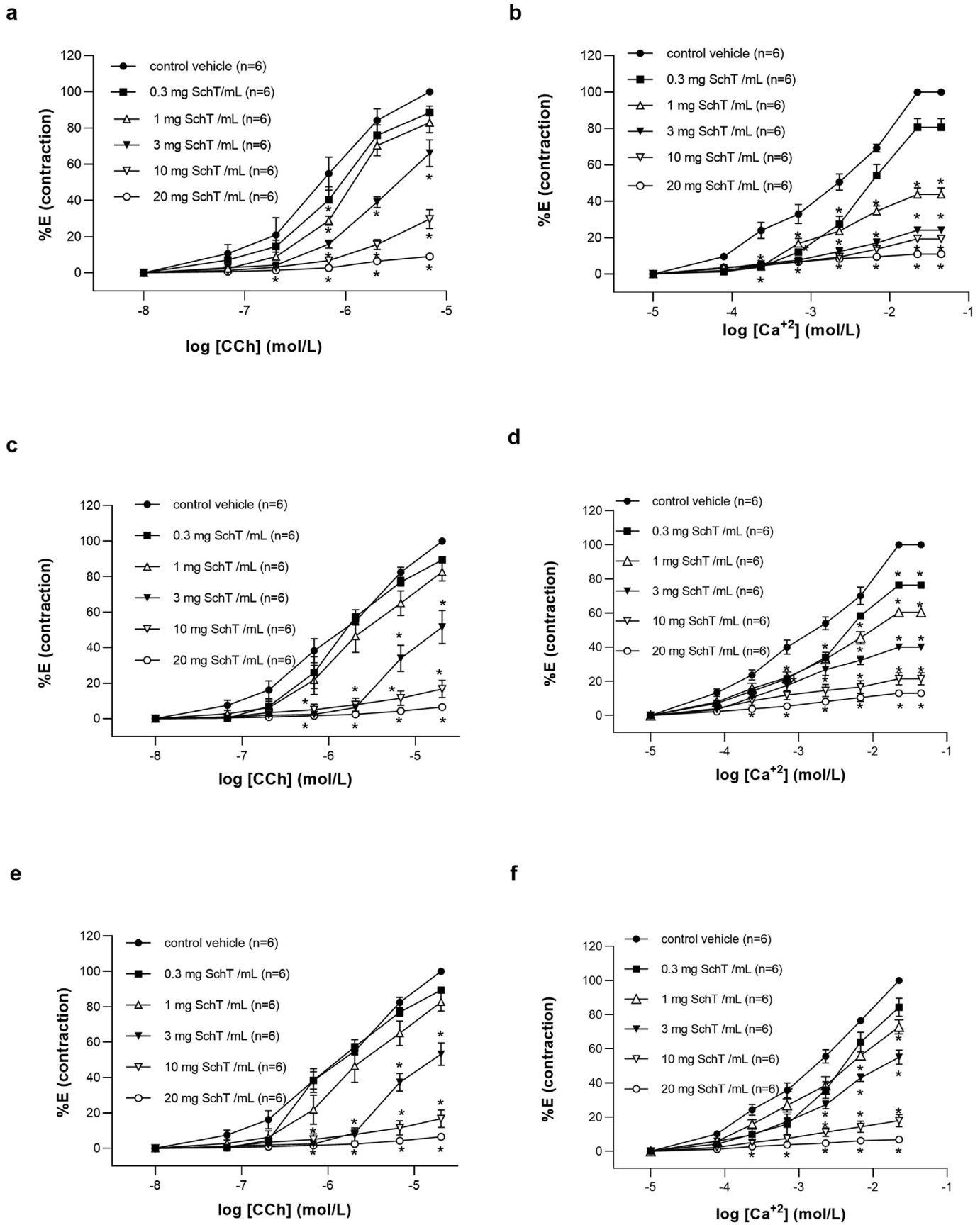

Fig. 2. Effects of SchT on the concentration-response curves (CRCs) of carbachol and $CaCl_2$ in isolated rat intestine (a, b), bladder (c, d) and uterus (e, f). Two-way ANOVA: $P < 0.001$ (Table A3) for both treatment and log [agonist] factors. *A posteriori* Tukey's test: *$p < 0.05$ vs control vehicle. ($n = 6$ in all groups).

CRC. In all smooth muscles, SchT was a non-competitive antagonist of Ca^{2+} influx (Fig. 2b, d and f in intestinal, bladder and uterine muscle, respectively). In the intestinal muscle Ca^{2+}-CRC's (pEC_{50} of 2.64 ± 0.12) SchT exhibited an IC_{50} of 1.90 ± 0.64 mg SchT/ml. In the bladder and uterine muscles Ca^{2+}-CRC's (pEC_{50} of 2.70 ± 0.06 and 2.76 ± 0.08, respectively), SchT showed IC_{50} of 2.14 ± 0.10 and 4.20 ± 0.56 mg SchT/ml, respectively.

In addition, SchT was evaluated in the His-CRC in guinea-pig ileum SchT also inhibited the His-CRC (pEC_{50} of 5.79 ± 0.18) as a non-competitive antagonist (Fig. 3a), with an IC_{50} value of 5.91 ± 0.36 mg SchT/ml.

Moreover, in rat uterus SchT was evaluated in the 5-HT-CRC (pEC_{50} of 5.79 ± 0.18) and Oxt-CRC (pEC_{50} of 5.52 ± 0.06) (Fig. 3b and c). SchT showed a non-competitive antagonism on both, 5-HT-CRC and Oxt-CRC, with IC_{50} of 5.55 ± 0.28 and 2.10 ± 0.09 mg SchT/ml, respectively.

3.4. Anxiolytic and sedative effects of extracts of S. lentiscifolius on mice

Three different extracts of *S. lentiscifolius* (SchT, SchW and SchO) were tested in the OFT to evaluate their effects. Neither SchT nor SchW markedly affected the spontaneous locomotion of mice measured as the number of crossed lines in 5 min (LC) compared to their respective vehicles. In contrast, both doses of SchO (10 and 30 μg/kg) strongly decreased LC with respect to the vehicle and in a similar way to diazepam (Dzp, Table 2). Simultaneously, the exploratory behavior was measured as the number of rearings in 5 min (RE). All extracts decreased RE similarly to Dzp (Table A1). There were no significant differences in the number of grooming bouts (Table A2).

In order to evaluate whether *S. lentiscifolius* induced anxiolytic effect, the extracts were tested by the NFT. Doses of 30 mg/kg of SchT or 200 mg/kg of SchW did not change feeding latency (FL) in NFT compared to their respective vehicles. In contrast, the administration of 100 mg/kg of SchT or 400 mg/kg of SchW significantly reduced FL, similarly to Dzp. The decrease in FL induced by higher doses of SchW and SchT was reversed with flumazenil (Fzl, Fig. 4a and b). The flavonoid rutin also decreased FL (Fig. 4a). SchO 30 μg/kg also significantly decreased FL but its effects were not reversed with Fzl (Fig. 4c). Moreover, only in mice treated with SchO did the home-cage food consumption decrease (Fig. A2).

3.5. Antidepressant effects of extracts of S. lentiscifolius on mice

The antidepressant effects of *Schinus* extracts were evaluated using TST and FST. In TST, the IT was only significantly reduced in the mice treated with higher doses of each extract with a similar effect to clomipramine (Fig. 5). Based on these results, SchT (100 mg/kg), SchW (400 mg/kg) and SchO (30 μg/kg) were tested in mice exposed to FST. Similarly, IT of mice treated with 400 mg/kg of SchW in FST was significantly lower than in saline group and similar to clomipramine results. On the other hand, 100 mg/kg of SchT decreased the IT compared to the ethanolic vehicle, but it was higher than the positive control (Fig. 6). Mice treated with SchO required rescue in the FST. The shorter IT in both tests suggests antidepressant effects.

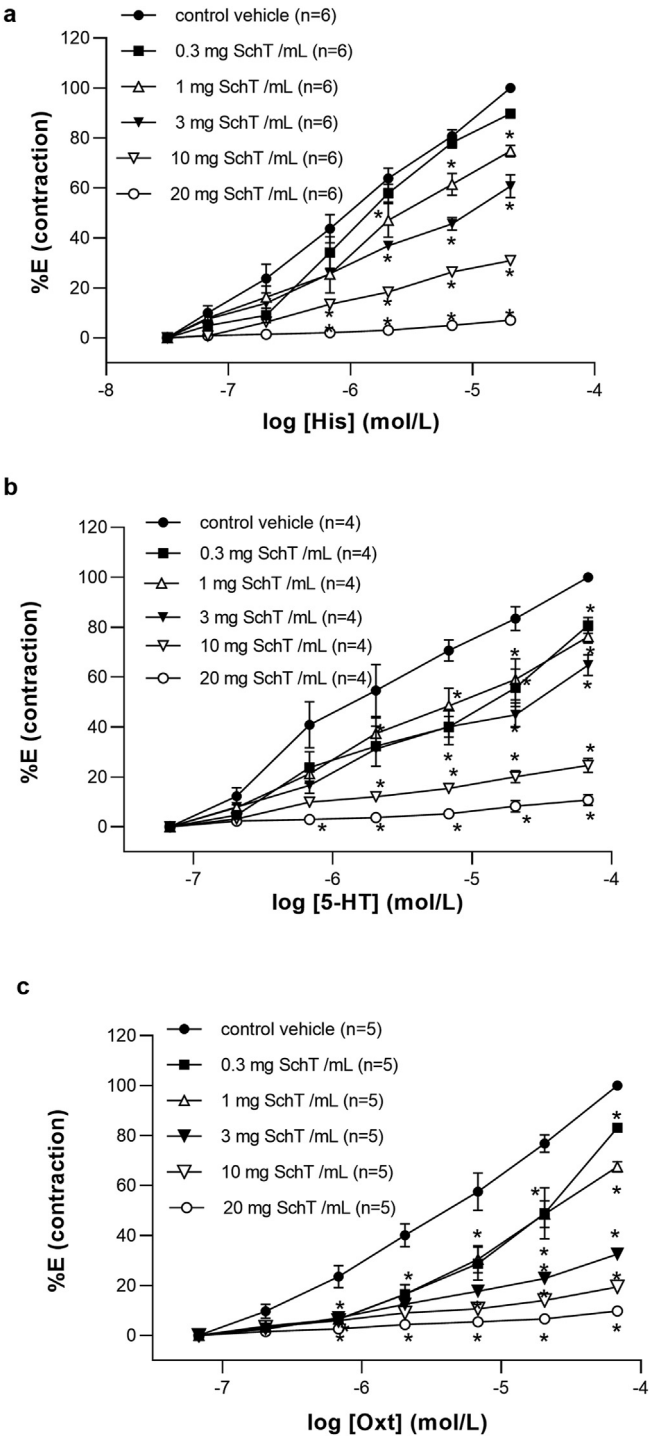

Fig. 3. Effects of SchT on the concentration-response curves (CRCs) of histamine (His) in isolated guinea-pig ileum (a; n = 6), serotonin (5-HT) (b; n = 4) and oxytocin (Oxt) in isolated rat uterus (c; n = 5). Two-way ANOVA: $P < 0.001$ (Table A4) for both treatment and log [agonist] factors. *A posteriori* Tukey's test: *p < 0.05 vs control vehicle.

4. Discussion

This is the first time a study provides scientific evidence of the antispasmodic, anxiolytic and antidepressant effects of extracts obtained from the leaves of *S. lentiscifolius*. Several species of the genus *Schinus* are used popularly in pathologies such as intestinal spasms.[5] However, there are only reports on the antispasmodic properties of *S. terebinthifolius*.[31] Our experimental results showed that SchT has antispasmodic effect given that it inhibited in a non-competitive way the cholinergic contraction and the blockade of calcium influx via calcium channels in rat intestine, bladder and uterus. Likewise, many reports showed similar results for other medicinal plants.[15,35] In the intestinal muscle alone, the IC_{50} on

Table 2
Spontaneous locomotion of mice measured as the number of crossed lines in 5 min produced by *Schinus lentiscifolius* extracts.

Treatment	0 min	30 min	60 min	90 min	120 min
Vehicle (saline) (n = 8)	141.88 ± 9.10	105.12 ± 12.73	87.25 ± 8.73	77.87 ± 11.03	65.25 ± 11.58
Diazepam 1 mg/kg (n = 9)	126.22 ± 11.02	61.00 ± 12.26	35.89 ± 6.70*	26.44 ± 4.03*	22.44 ± 4.88
SchW 200 mg/kg (n = 6)	152.33 ± 9.29	128.00 ± 13.74#	85.50 ± 11.66	67.17 ± 9.55	59.83 ± 10.68
SchW 400 mg/kg (n = 6)	154.50 ± 9.67	128.00 ± 7.75#	100.17 ± 12.02#	88.50 ± 10.01#	79.33 ± 9.16#
Vehicle (ethanol 70 % v/v) (n = 9)	146.66 ± 14.63	72.22 ± 22.21	37.78 ± 10.21*	21.67 ± 8.96*	19.78 ± 8.32
SchT 30 mg/kg (n = 6)	113.17 ± 8.36	69.89 ± 11.11	58.33 ± 9.00*	52.00 ± 5.72	53.55 ± 7.40
SchT 100 mg/kg (n = 15)	154.5 ± 9.67	128.00 ± 7.75	100.17 ± 12.02	88.50 ± 10.01	79.33 ± 9.16
Vehicle (DMSO) (n = 6)	118.17 ± 2.02	84.47 ± 2.99	87.83 ± 2.99#	85.67 ± 2.90#	103.50 ± 1.87#
SchO 10 μg/kg (n = 6)	120.88 ± 6.96	27.0 ± 13.05* a	12.50 ± 3.42*a	14.33 ± 2.23*a	11.00 ± 3.25*a
SchO 30 μg/kg (n = 6)	122.20 ± 14.04	5.60 ± 1.47*#a	7.40 ± 3.01*a	12.60 ± 5.02*a	10.60 ± 1.83a
Two way ANOVA	**By treatment**	**F = 24.13**	**P < 0.0001**	**DFn = 9**	
	By time	**F = 96.32**	**P < 0.0001**	**DFn = 4**	
	By interaction	**F = 1.945**	**P = 0.0014**	**DFn = 36**	

*p < 0.05 vs vehicle (saline); #p < 0.05 vs diazepam and [a] vs respective vehicle by *a posteriori* Tukey's tests.

CCR-CCh was significantly higher than the IC_{50} on CCR-Ca^{2+} (*p < 0.05; *t*-test: t = 3.250, df = 10), suggesting that the effect of SchT on this muscle involves a more sensitive pathway to affect the calcium influx than that derived from the muscarinic receptor activation. Since the spasmolytic effect of *S. terebinthifolius* could be associated with an antihistaminic effect,[31] we evaluated whether SchT has the same pattern. However, SchT inhibited the histamine-induced contraction in a non-competitive way on guinea-pig ileum. Also, we show that SchT does not block the serotonin and oxytocin receptors in the uterus because it reduced non-competitively the contraction produced by these agonists.

The presence of isoquercetin and rutin was demonstrated in SchT by HPLC-UV. While rutin had no significant effect on contracture of isolated rat ileum, isoquercetin decreased the phasic and tonic contractions in the same tissue.[22] Therefore, isoquercetin could be one of the components responsible for the effect of SchT on the intestine. Regarding the presence of flavonoids in SchT, there are several articles about its relaxant effects, not only in rat intestine[35] but also in uterus[37] and bladder.[18] In fact, it has been found that flavonoids effect involves multiple pathways, mainly the non-competitive muscarinic antagonism and competitive inhibition of Ca^{2+} channels.[12] Also, SchT exhibited in the Ca^{2+}-CRC of the intestine a pattern similar to verapamil, a calcium channels blocker.[7] In addition, verapamil also showed a non-competitive effect on cholinergic and oxytocin contractions in isolated rat uterus,[2] as well as SchT does. Therefore, the inhibition of calcium influx could be behind the antispasmodic effect of SchT in rat isolated bladder, intestine and uterus, and even in other smooth muscles. Besides, this effect is in agreement with the traditional use of *S. molle* or *S. terebinthifolius*for gastrointestinal disorders and uterine inflammation, respectively.[1,41]

Psychiatric disorders such as anxiety, insomnia and depression have a high population prevalence. Despite efforts to treat these disorders, the drugs commonly used have several side effects and their effectiveness is discussed. Therefore, the research on medicinal plants with sedative, anxiolytic or antidepressant effects is a promising field. In this regard, *S. molle* produces an antidepressant-like effect on various systems, for instance on the dopaminergic, noradrenergic and serotonergic pathways.[29] In this case, we evaluated the effect on behavior of *S. lentiscifolius* extracts. As this plant is popularly consumed as tea, we studied the central effects not only of SchT, but also of leaf infusion (SchW). SchT and SchW did not change either the spontaneous mobility or the grooming, but they decreased rearing in the OFT. Our results discard a sedative effect of SchT or SchW. By other side, the anxiolytic effect of both extracts were evaluated using NFT. This test evaluate the anxiety-related state in mice under basal conditions and following exposure to an acute stressor.[30] A shorter FL in a food-deprived animal caused by exposure to a potentially anxiogenic novel environment is related with an anxiolytic effect.[30] High doses of SchT and SchW dropped the FL without changing home-cage consumption in NFT. This effect of both extracts was reversed by flumazenil, which suggests that a benzodiazepine-like effect is in fact involved in this activity. Regarding the presence of rutin in SchT, we found that this flavonoid also induced a decrease FL in NFT. Since rutin exerts its anxiolytic effect by a mechanism that involves GABAergic transmission but this effect was not reversed by flumazenil,[23] the effect of SchT in NFT should not only be due to the presence of rutin. Thus, SchT and SchW showed to be effective in an anxiolytic model without producing sedative effect, which is the main side effect of classical anxiolytic drugs. This property agrees with that of selective drugs linked to the α2 subunit of $GABA_A$ receptors, which display a similar pattern.[10]

Because NFT test does not provide information on putative effect on depressive-like behavior in acute stress,[30] it was evaluated by using the TST and FST tests. Both of them widely used to screen new antidepressant drugs. These models are based on the application of acute stress to the animal by restriction space.[8,45] High doses of SchT and SchW decreased the IT in TST and FST. The effect of SchT in FST was less than the one found in TST when comparing to clomipramine effect (see Figs. 5 and 6). A comparative study provides supporting evidence on the difference in sensitivity of both tests, probably due to the dissimilarities between pathophysiological mechanisms.[4] It has been previously reported that drugs that stimulate locomotor activity can throw false-positives results in both TST and FST.[8] However, neither SchT nor SchW increased spontaneous mobility in OFT, therefore these findings in TST and FST support the antidepressant use of these extracts.

Essential oils exhibit several biological properties for the treatment of central nervous system disorders.[9] They are complex mixtures of components including phenylpropanoids and volatile terpenes.[5] Moreover, the variability of their chemical composition depends on the nature of the geographical distribution and it defines the variety of biological effects.[6] The SchO native of Argentina was tested by GC-FID-MS and showed among its main components a high proportion of D-limonene, α-santalol, β-caryophyllene and α-pinene. D-limonene and α-santalol have been reported to have effects on behavior.[16,36] SchO caused a decrease in spontaneous mobility and rearing, similarly to diazepam in OFT. Also, it led to a decrease in FL and home-cage consumption in NFT; these effects were not reversed by flumazenil. These results confirm the sedative and anxiolytic effects of SchO by a mechanism unrelated to that of benzodiazepines. There are studies that provide evidence of the anxiolytic-like effect of D-limonene; however, the mechanism

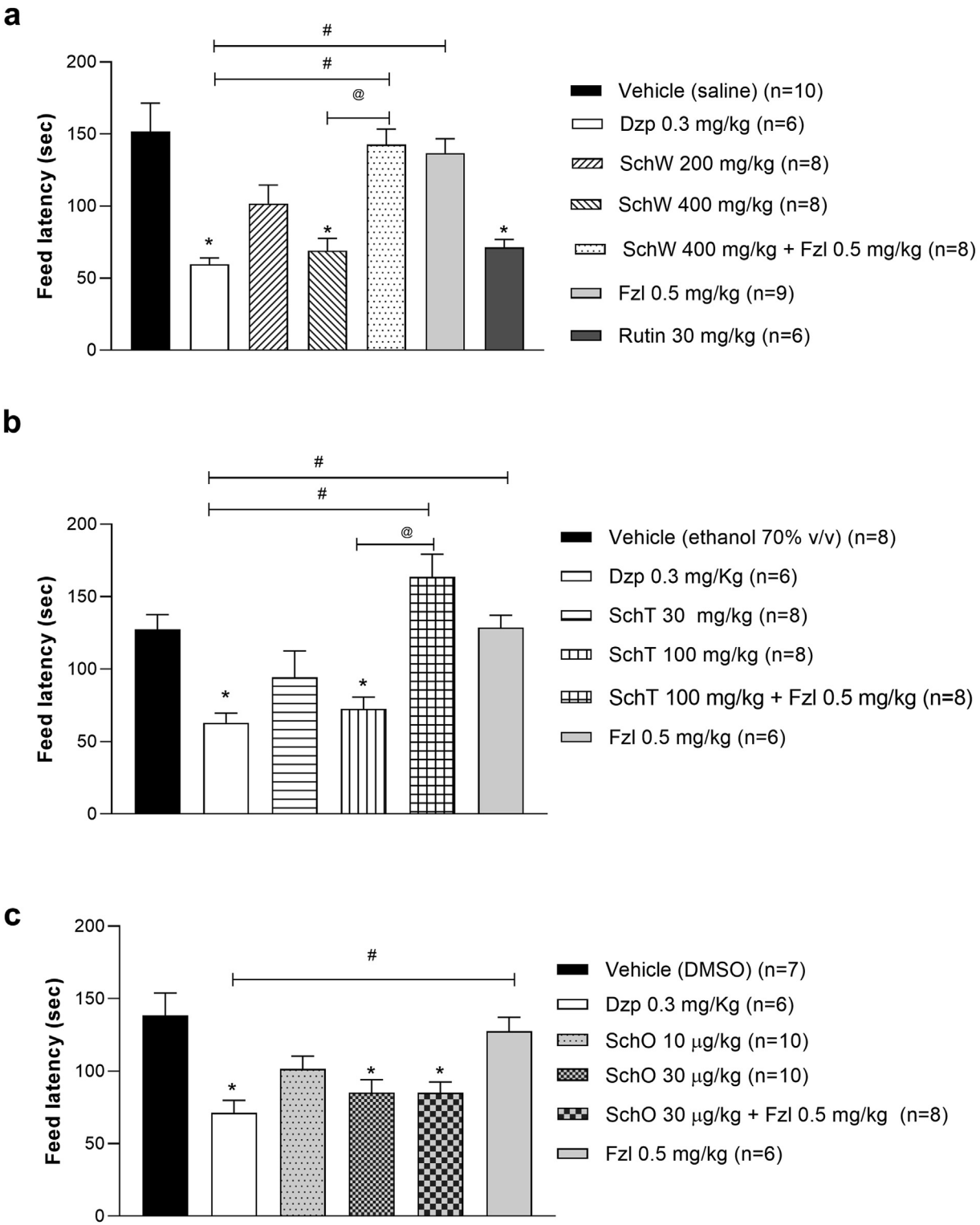

Fig. 4. Effects of *Schinus lentiscifolius* extracts (SchW, SchT and SchO) in comparison with the respective vehicle and diazepam (in a, b and c), rutin (a), and flumazenil (a, b, c) on feed latency in novelty-suppressed feed test. One-way ANOVA: $P < 0.001$ (Table A5). A posteriori Tukey's test: *$p < 0.05$ vs vehicle, #$p < 0.05$ vs diazepam, $^{@}p < 0.05$ vs extract + flumazenil (Fzl) ($n = 6–10$ as indicated in each group).

involved could be different depending on the route of administration used.[13,27] Regarding the presence of α-santalol in SchO, there are several reports about its sedative effect.[32,36] Thus, the components identified in SchO may exert synergistic actions on the behavior of mice. SchO also decreased the IT in TST, suggesting an antidepressant effect. Similar effects have also been reported for the essential oil of *S. terebinthifolius* fruits,[34] whereas components present in SchO, such as D-limonene, β-caryophyllene and α-pinene, have been reported for their antidepressant action.[26,29] In addition, since β-caryophyllene has shown a similar effect in TST

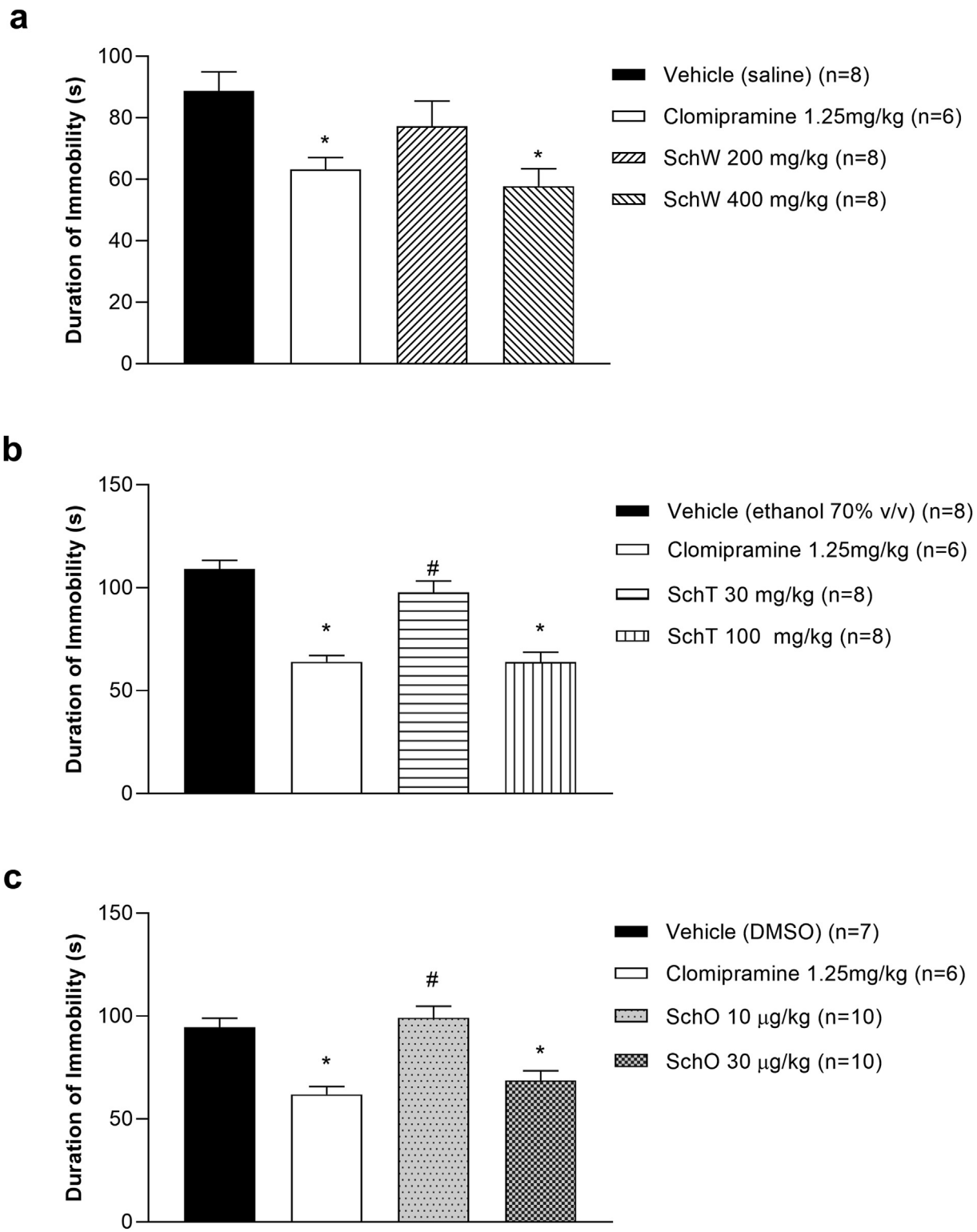

Fig. 5. Effects of *Schinus lentiscifolius* extracts (SchW in a, SchT in b and SchO in c) on duration of immobility tail suspension test. One-way ANOVA: $P < 0.001$ (Table A6). *A posteriori* Tukey's test: $^{*}p < 0.05$ vs vehicle, $^{\#}p < 0.05$ vs clomipramine ($n = 6–10$, as indicated in each group).

and FST linked to action on the cannabinoid CB2 receptor,[3] its presence in SchO could contribute to its anxiolytic and antidepressant-like effects. However additional research will be necessary to probe this hypothesis and deep the mechanism underlying.

5. Conclusions

Our results demonstrate that the tincture obtained from *S. lentiscifolius* leaves is effective as an intestinal, urinary and uterine antispasmodic. These findings appear to be associated with

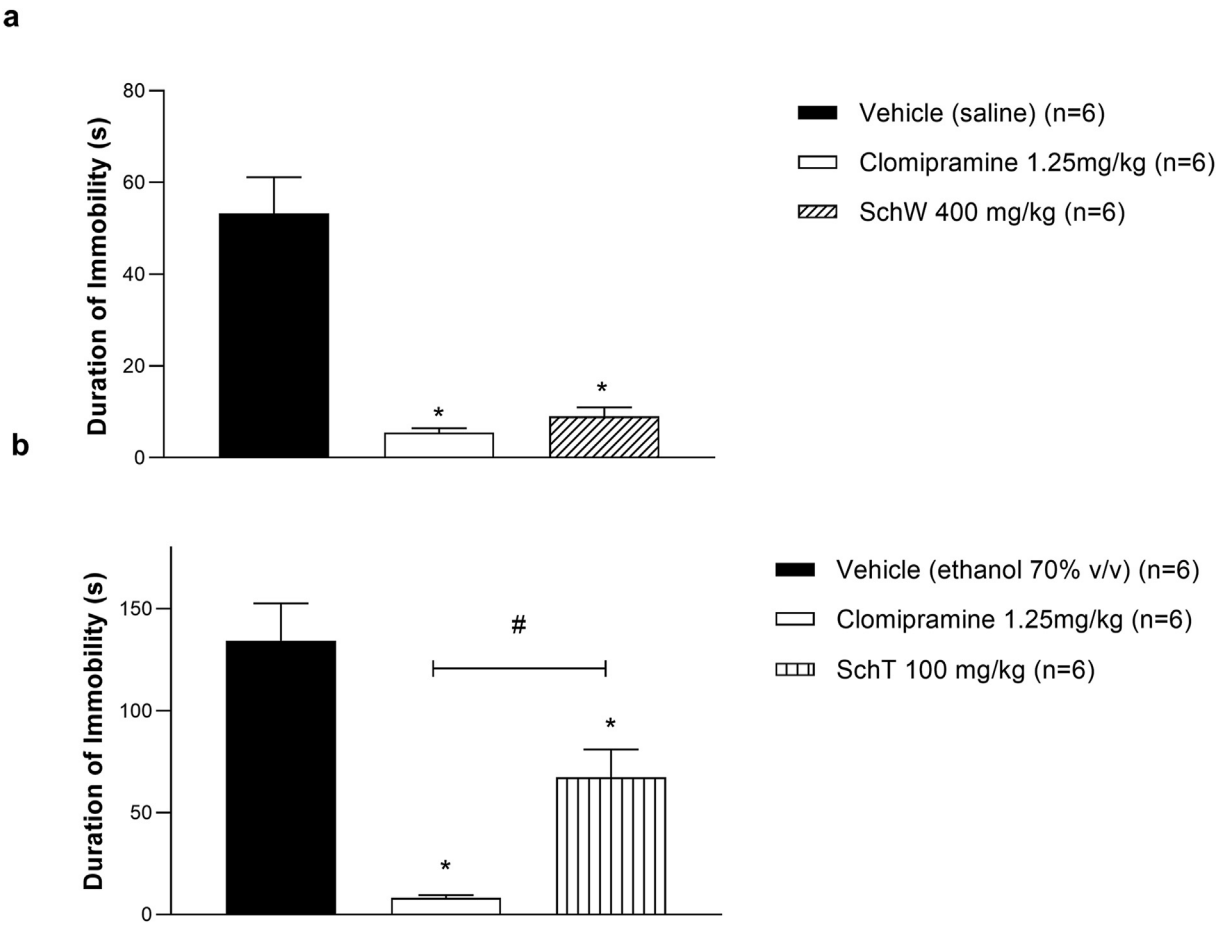

Fig. 6. Effects of *Schinus lentiscifolius* extracts (SchW in a, SchT in b) on duration of immobility in forced swimming test. One-way ANOVA: $P < 0.001$ (Table A7). *A posteriori* Tukey's test: $^{*}p < 0.05$ vs vehicle, $^{\#}p < 0.05$ vs clomipramine. ($n = 6$ for all treatments).

the presence of flavonoids, such as rutin and isoquercetin, both identified in SchT. Moreover, its antispasmodic effect is mediated by a mechanism that involves several pathways, mainly the non-competitive antagonism of agonists and the non-competitive inhibition of Ca^{2+} channels with a pattern similar to verapamil.

Our results suggest that the tincture and infusion of *S. lentiscifolius* have an anxiolytic effect similar to that of benzodiazepines, as well as an antidepressant-like effect, and that the presence of rutin could be partly responsible for these. The essential oil showed similar effects and significantly reduced locomotor activity in mice. D-limonene, α-santalol, β-caryophyllene and α-pinene, its main components, may be involved in these actions. However, to improve the knowledge of the mechanisms involved, further research is required.

Thus, the present study contributes to the new knowledge on the antispasmodic effects of *S. lentiscifolius* in urinary and uterine tissues, as well as it reinforces the traditional use of this plant for gastrointestinal and central depressive symptoms.

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Declaration of competing interest

We have no known conflicts of interest in the publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2021.07.004.

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