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ORIGINAL ARTICLE

Toxic rather than neuropharmacological effect of *Ternstroemia sylvatica* fruits and identification of 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol as a new compound with toxic effects in mice*

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Abstract

Context: Fruits of *Ternstroemia sylvatica* Schltdl. and Cham. (Theaceae) are used in Mexican traditional medicine to alleviate anxiety, sleep disorders and seizures; however, the active principles have not been identified.

Objective: To identify the neuroactive principles of *T. sylvatica* fruits using neuropharmacological tests on mice.

Materials and methods: The methanol and aqueous extracts of pericarp or seeds of *T. sylvatica* fruits were intraperitoneally administered (1–562 mg/kg, single doses) to mice. The exploratory cylinder, hole board, open field, Rota-rod and sodium pentobarbital-induced hypnosis tests were used to evaluate the CNS depressant effect after 30 min single administration of extracts. From aqueous seeds extract, triterpene glycoside 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol was isolated an active compound.

Results: Crude extracts of *T. sylvatica* fruits, separated from seed and pericarp, showed sedative effect in mice. The aqueous (ED₅₀ = 4.9 ± 0.8 mg/kg) seed extracts is the most active among them. This extract also decrease locomotor activity and disrupt motor coordination of mice. This extract was also the most toxic extract (LD₅₀ = 5.0 ± 1.4 mg/kg; *i.p.*). The triterpene glycoside 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol was identified in this extract as one of the active sedative compounds (ED₅₀ = 0.12 ± 0.01 mg/kg) also with toxic effect (LD₅₀ = 1.11 ± 0.23 mg/kg).

Conclusion: The results suggest that *T. sylvatica* fruits has toxic activity rather than CNS depressant activity in mice and that this effect might be related to the presence of 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol, one of the active principles of *T. sylvatica* fruits with sedative and toxic effect.

Introduction

Ternstroemia is the largest genus in the Theaceae family with an estimated 130 species worldwide. It has been reported that 12 *Ternstroemia* species occur in México (Boom, 1989). Fruits of several species from *Ternstroemia* are commonly known in México as ‘Flor de Tila’, and they are used in Mexican traditional medicine to alleviate anxiety, sleep disorders and seizures (Aguilar-Santamaría & Tortoriello, 1996; Molina et al., 1999; Tortoriello & Romero, 1992).

Ternstroemia pringlei (Rose) Standl. (synonym *Ternstroemia lineata* DC) and *Ternstroemia sylvatica* Schltdl. and Cham. are the two major *Ternstroemia* species used as decoctions in Mexican traditional medicine to treat insomnia and fear (Molina et al., 1999). The *T. sylvatica* fruits contain 40% of seeds and 60% of pericarp. Traditionally, the decoctions of these plants are prepared from their whole ground fruits. Pharmacological studies have shown that fruits of *T. pringlei* (Bartholomew & McVaugh, 1997; Kobuski, 1942) produce sedative and anticonvulsive effects in rats (Aguilar-Santamaría & Tortoriello, 1996), and interact in a complex form with CNS depressant drugs (Balderas et al., 2008). Fruits of *T. sylvatica* have sedative effects more than anxiolytic effects in rats (Aguilar-Santamaría & Tortoriello, 1996; Molina et al., 1999; Tortoriello & Romero, 1992). Recently, the isolation of jacaranone as a sedative substance from *T. pringlei* was reported (Lozada-Lechuga et al., 2010). Phytochemical studies of *Ternstroemia* species have reported the isolation of oleanane- and ursane-type

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triterpenoids, triterpenoid glycosides, triterpenoid saponins, carotenoids, monoterpenoids, tannins and other aromatic compounds (Balderas et al., 2008; Ikuta et al., 2003; Jo et al., 2005; Kikuchi & Yamaguchi, 1974; Shin et al., 2003; Tori et al., 2005). However, there are no additional reports concerning neuropharmacological effects of *T. sylvatica* fruits. Therefore, this study was undertaken to evaluate the neuropharmacological effects of *T. sylvatica* fruits using several experimental models to identify the active principle(s) of this medicinal plant. However, the experimentation led to the identification of a toxic effect greater than the neuropharmacological effect of this plant and to the identification of a new glycoside triterpenoid as one of the toxic compounds from *T. sylvatica*.

Materials and methods

Drugs and reagents

Tween 80 was purchased from Sigma Co. (St. Louis, MO), diazepam (Valium™) was purchased from Roche S.A. (México). The extracts were dissolved in saline solution (0.9%) and the drugs were dissolved in 0.5% Tween 80 in saline solution. All solutions were freshly prepared each time and administered intraperitoneal or orally in a volume of 0.1 mL/10 g body weight in single administration. Control animals received the same volume of vehicle (0.5% Tween 80 in saline solution or saline solution only).

Experimental animals

All experiments were performed on adult male ICR mice (25–34 g; Centro UNAM-Harlan, Harlan México, S. A. de C. V.). The experimental groups consisted of six animals. The animals were maintained at constant room temperature ($22 \pm 2^\circ\text{C}$) and subjected to a 12 h light/dark cycle with free access to food and water.

To optimize the number of laboratory animals used, exploratory cylinder and hole board tests and open field and sodium pentobarbital-induced hypnosis tests were performed consecutively.

Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) adopted in our laboratory, and in compliance with international rules on care and use of laboratory animals. Furthermore, clearance for conducting the studies was obtained from the Ethics Committee for the Use of Animals in Pharmacological and Toxicological Testing (CICUAL/020/11, 2011), Facultad de Química, UNAM. Behavioral experiments were carried out between 10:00 and 14:00 h.

Plant material

The fruits of *T. sylvatica* Schltdl. and Cham. were collected at Pahuatlán, Puebla, Mexico, in June 2007. The authenticity of the plant materials was certified by M.E. Flores-Villafranco, botanist of the FES Iztacala UNAM Herbarium. Samples were deposited in this herbarium with the voucher number IZTA42169. The fruits of *T. sylvatica* have an average weight of 543 ± 123 mg ($n = 10$); 40% of them correspond to

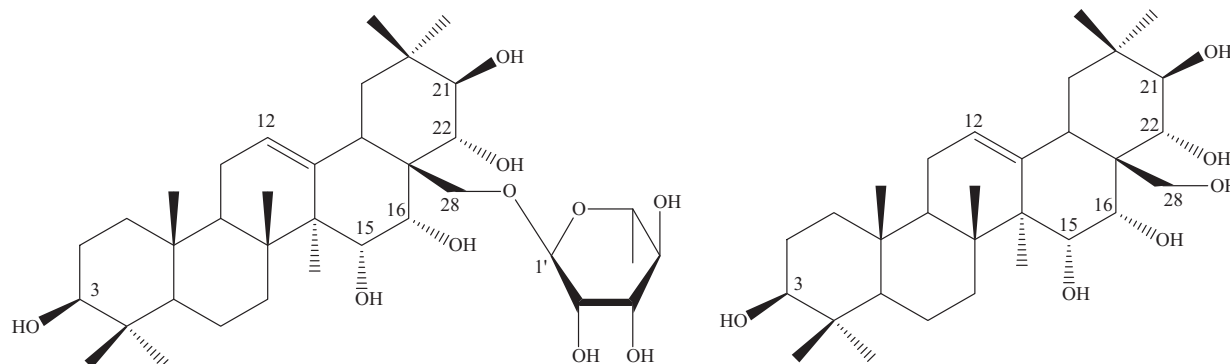
seeds (221 ± 81 mg). Therefore, we decided to test the pericarp and seeds separately.

Preparation of extracts

The seeds and pericarp from dried fruits of *T. sylvatica* were manually separated. After grinding using a manual miller, 374 g of pericarp and 56.2 g of seeds were extracted separately at room temperature with hexane (3×2 L, 24 h each), then with dichloromethane (3×2 L, 24 h each) and, finally, with methanol (3×2 L, 24 h each); evaporation of the solvents in vacuum gave 1.44 g of hexane (0.39% yield), 2.01 g of dichloromethane (0.54% yield) and 50.17 g of methanol (13.41% yield) extracts as syrupy residues from pericarp, and 17.59 g of hexane (31.30% yield), 5.51 g of dichloromethane (9.81% yield) and 3.80 g of methanol (6.76% yield) extracts from seeds. Aqueous extracts were prepared with 200 g of dried and powdered seeds or 200 g of pericarp by boiling them in 1.8 L of distilled water for 10 min. Afterwards, the extracts were filtered by gravity and concentrated through air current at room temperature ($22 \pm 2^\circ\text{C}$) obtaining 27.9 g (13.9% yield) of a breakable reddish solid for pericarp and 28.4 g (14.2% yield) of a pale beige solid for seeds.

Isolation of 28-O- $[\beta\text{-L-6-rhamnopyranosyl}]\text{-R}_1\text{-barrigenol}$

The active aqueous seeds extract of *T. sylvatica* (20 g) was partitioned with *n*-BuOH (2 L). Both aqueous and *n*-butanol fractions were concentrated under reduced pressure (40°C at 72 mbar for water and 40°C at 25 mbar for *n*-BuOH) to obtaining 9.3 g and 9.0 g of aqueous and *n*-BuOH fractions, respectively. A sample of the *n*-butanol soluble fraction (1.0 g) was chromatographed using a Sephadex LH-20 column ($400\text{ mm} \times 40\text{ mm i.d.}$) and eluted with MeOH to give 20 fractions of 50 mL each. Fractions 3–5 (200 mg) were pooled and subjected to an additional medium pressure RP-C₁₈ silica gel column chromatography ($450\text{ mm} \times 25\text{ mm i.d.}$) eluted with MeOH:H₂O (9:1, 8:2, 7:3 and 5:5, each 30 mL) to afford 78.60 mg of white powder. A part of this powder (50 mg) was applied to a medium pressure C₁₈ column ($450\text{ mm} \times 25\text{ mm i.d.}$) and eluted with MeOH to afford 30 mg of white amorphous powder. A posterior purification by preparative RP-TLC eluted with H₂O:MeOH:CH₃CN (2:1:1) yielded a pure white amorphous powder (20 mg) with a melting point (m.p.) $288\text{--}290^\circ\text{C}$. This compound was identified as 28-O- $[\beta\text{-L-6-rhamnopyranosyl}]\text{-R}_1\text{-barrigenol}$ (**1**, Figure 1) by mass spectrometry and nuclear magnetic resonance (Table 1). A portion of this powder (15 mg) was refluxed with 2 N HCl for 3 h. After neutralization with Na₂CO₃ (10% w/v in water), the reaction mixture was extracted with CH₂Cl₂ (5 mL $\times$ 3). The aqueous phase was dried under vacuum and then treated with 40 μL of Sigma-Sil-A reagent at 60°C by 10 min and analyzed by GC/MS for sugars. The organic phase was washed with H₂O (2 mL $\times$ 2) and evaporated to yield a pale yellow solid (7 mg). From this, reaction mixture was separated by preparative silica gel TLC (eluting with CH₂Cl₂:MeOH 9:1) a white solid with a m.p. $>300^\circ\text{C}$. The MS and proton NMR (400 MHz, Pyridine-*d*₅) data corresponded to data previously reported (Fu et al., 2005) for R₁-barrigenol (**1a**).

Figure 1. Structure of compounds **1** and **1a**.Table 1. ¹H NMR and ¹³C NMR spectral data for compounds **1** and **1a**.

Position	1		1a
	δ (¹³ C) ^a	δ (¹ H) ^b	δ (¹ H) ^b
1	47.92	1.48 dd (<i>J</i> = 4, 12 Hz)	
2	41.57	2.82 m	
3	78.13	3.06 t (<i>J</i> = 12 Hz)	3.19 m
4	39.41	—	—
5	55.72	0.97 m	
6	19.22	1.66 m	
7	36.90	2.17 m	
8	42.11	—	—
9	39.36	1.89 m	
10	37.53	—	—
11	24.14	1.98 m	
12	124.58	5.55 t (<i>J</i> = 4 Hz)	5.58 m
13	144.86	—	—
14	54.58	—	—
15	67.51	4.46 m	3.88 m
16	72.48	4.98 m	4.57 m
17	48.21	—	—
18	47.51	1.84 m	
19	47.58	—	—
20	36.52	—	—
21	78.45	4.85 d (<i>J</i> = 12 Hz)	3.92 d (<i>J</i> = 6 Hz)
22	77.20	4.63 d (<i>J</i> = 12 Hz)	4.27 dd (<i>J</i> = 9, 6 Hz)
23	16.74	1.88 s	1.20 s
24	17.72	1.08 s	1.60 s
25	16.06	1.01 s	1.01 s
26	28.82	1.25 s	1.14 s
27	21.19	1.14 s	1.55 s
28	72.28	4.51 m	4.10 m
29	30.71	1.37 s	1.48 ws
30	19.54	1.41 s	—
1'	102.79	5.12 d (<i>J</i> = 4 Hz)	—
2'	72.87	4.44 dd (<i>J</i> = 4, 8 Hz)	—
3'	73.96	4.26 t (<i>J</i> = 8 Hz)	—
4'	67.78	4.13 m	—
5'	69.69	3.63 m	—
6'	18.71	1.64 d (<i>J</i> = 8 Hz)	—

^a100 MHz, Pyridine-*d*₅; ^b400 MHz, Pyridine-*d*₅.

Pharmacological studies

Sedative activity in the exploratory cylinder test

The apparatus consisted of a glass cylinder (30 cm in height, 11 cm in diameter, with wall of 3 mm). The cylinder was placed on a filter paper in a room with constant lighting and isolated from external noise. An individual naïve mouse was put on the filter paper-covered floor of the glass cylinder; the number of rears performed over a 5 min period

was recorded. The inner side of the apparatus and floor were cleaned with ethanol solution (10% v/v) and the filter paper was changed between each animal test session. The extracts, compounds or drugs were administered 30 min before testing in different doses. During observation, the experimenter stood next to the apparatus always at the same place. The observations were made without prior knowledge of the experimental conditions applied to the animal. Reduced exploratory rearing showed by naïve mice after placement in an unfamiliar environment reveals a sedative effect (Hiller & Zetler, 1996; Oliva et al., 2004; Rolland et al., 1991; Ugalde et al., 2005).

Open field test

The locomotor behavior was assessed on open field test. The open field was made with an acrylic cage of 20 cm in height. The floor of the open field, 26 cm in length and 18 cm in width, was divided into 12 squares (4 cm × 4 cm) with 2 cm strips. Thirty minutes after the administration of extracts or compounds, each mouse was placed individually at the center of the apparatus and observed for 2 min to record the locomotion as number of segments crossed with the four paws (Walsh & Cummins, 1976).

Hole board test

Thirty minutes after the administration of extracts or compounds, each mouse was placed into the hole board apparatus (Ugo Basile 6650 Hole Board). The number of holes explored was recorded automatically during 3 min. This test was used to evaluate emotionality, anxiety and/or response to stress in animals. A decrease in the number of head-dips reveals the anxiolytic behavior (Boissier & Simon, 1962).

Sodium pentobarbital-induced hypnosis.

Sodium pentobarbital at a dose of 42 mg/kg was intraperitoneally administered 33 min after the intraperitoneal administration of extracts. Each mouse was observed for the loss of righting reflex (hypnosis) and duration of sleep. The time between loss and recovery of the righting reflex was recorded as sleeping time (González-Trujano et al., 1998).

Effect on motor coordination on rota-rod test.

Mice which remained on the rod (Rota-rod Treadmills for mice, constant speed model 7600, Ugo Basile; 4 cm diameter, 16 rpm) for at least 3 min were selected and allocated to

Table 2. Sedative effect in the exploratory cylinder test of *Ternstroemia sylvatica* extracts after intraperitoneal administration in mice.

Plant/Drug	Extract	ED ₅₀ ± S.E.M. (mg/kg)
<i>Ternstroemia sylvatica</i> (pericarp)	Methanol	61.9 ± 6.4
	Aqueous	41.5 ± 19.9
<i>Ternstroemia sylvatica</i> (seeds)	Methanol	13.8 ± 1.7
	Aqueous	4.9 ± 0.8
Diazepam	–	3.04 ± 1.14

groups of six animals each. Immediately after administration extracts, diazepam or vehicle by intraperitoneal route, mice were placed on the rod. The time each animal remained on the rod (“time-on-rod”) was recorded every 10 min for a total recording of 120 min. Effect on motor coordination was considered when mice remained on the rod for less than 2 min (González-Trujano et al., 1998).

Acute toxicity study (LD₅₀)

The acute toxicity of extracts was estimated by intraperitoneal administration. Mice were kept under observation for the following 14 d after single administration of extracts (1–316 mg/kg single doses) at constant room temperature (22 ± 2 °C) and submitted to a 12 h light/dark cycle with free access to food and water. Their weights were registered daily, and at the end of the study a macroscopic tissue evaluation was done. The acute toxicity of 28-*O*-[β-L-6-rhamnopyranosyl]-R₁-barrigenol was estimated by a single intraperitoneal administration (0.1–10.0 mg/kg) and observation for the following 14 d in the same conditions described above.

Statistical analysis

Results are expressed as mean ± S.E.M. of six repetitions. Statistical differences between experimental and control groups were performed by one-way analysis of variance (ANOVA), followed by Dunnett’s test. A value of $p < 0.05$ was considered to be significant.

Results

The methanol and aqueous extracts of *T. sylvatica* seeds showed potent sedative effect in exploratory cylinder test after intraperitoneal administration in mice (Table 2). The aqueous seeds extract showed a sedative effect (ED₅₀ = 4.9 ± 0.8 mg/kg) like diazepam (ED₅₀ = 3.04 ± 1.14 mg/kg), which was used as reference a sedative drug (Table 2). However, single or repeated intragastric administration of aqueous extracts did not show sedative effect (Table 3). In addition, the sodium pentobarbital-induced hypnosis time was prolonged by the intraperitoneal administration of methanol or aqueous extracts of *T. sylvatica* seeds (Figure 2). However, higher doses of methanol (32 and 100 mg/kg) or aqueous (56 and 100 mg/kg) extracts of *T. sylvatica* seeds induced death of the animals during sodium pentobarbital-induced hypnosis.

Aqueous extract of *T. sylvatica* seeds significantly decreased the motor coordination of the animals in a dose-dependent manner (ED₅₀ = 52.21 ± 4.45 mg/kg) without recuperation in the rota-rod test (Figure 3). However at 100 mg/kg this extract induced the death of all animals 70 min after

Table 3. Sedative effect of *Ternstroemia sylvatica* aqueous extracts in the exploratory cylinder test by acute and repeated intragastric administration.

Dose (mg/kg)	Single dose		Repeat dose by 5 d	
	Rearings/ 5 min ± S.E.M.	Mortality	Rearings/ 5 min ± S.E.M.	Mortality
Vehicle				
–	55.2 ± 3.9	0/24	52.7 ± 3.7	0/17
<i>Ternstroemia sylvatica</i> (pericarp)				
100	49.3 ± 3.6	0/6	55.7 ± 3.8	0/6
316	40.2 ± 5.2	0/6	53.3 ± 7.3	0/6
1000	50.5 ± 4.4	0/6	55.3 ± 6.5	
<i>Ternstroemia sylvatica</i> (seeds)				
5.6	32.0 ± 5.7*	0/6	63.0 ± 3.5	0/6
10	38.0 ± 2.9	0/6	60.2 ± 6.8	0/6
100	40.2 ± 6.3	0/6	55.5 ± 7.7	0/6
316	35.8 ± 7.8	0/6	39.7 ± 2.9	3/6
1000	37.3 ± 5.6	0/6	30 ± 0	5/6

Data represent mean ± S.E.M. $n = 6$. * $p < 0.05$ significantly different from vehicle; ANOVA followed by Dunnett’s test.

its administration. Methanol extract did not change the motor coordination of the mice.

From the aqueous seeds extract, it was isolated as a white amorphous powder (Compound **1**) with a m.p. 288–290 °C (recrystallized from MeOH). This compound was identified as 28-*O*-[β-L-6-rhamnopyranosyl]-R₁-barrigenol by mass spectrometry and nuclear magnetic resonance (Table 1). This pure compound showed potent sedative activity in the exploratory cylinder test (ED₅₀ = 0.12 ± 0.01 mg/kg, Figure 4A) and significantly reduced the nose-poking activity in the hole board test (ED₅₀ = 1.02 ± 0.0 mg/kg, Figure 4B). Whereas the aqueous extract of seeds of *T. sylvatica* was less active (ED₅₀ = 8.81 ± 2.83 mg/kg) than compound **1**. Diazepam showed an ED₅₀ = 2.56 ± 1.35 mg/kg in this test.

Compound **1** and aqueous extract of seeds from *T. sylvatica* significantly decreased, in a dose-dependent manner, the locomotor activity (ED₅₀ = 0.35 ± 0.11 and 26.64 ± 2.37 mg/kg, respectively) in the open field test (Figure 5).

T. sylvatica seed aqueous extract was the most toxic extract by the intraperitoneal route (LD₅₀ = 5.0 ± 1.4 mg/kg). *T. sylvatica* aqueous extract produced a weight loss of 15% of the animals during observation period (14 d). Autopsy of mice showed peritoneal extravasations and bleedings in stomach and gut. The intragastric administration of the aqueous extract up to 1000 mg/kg neither induced death nor weight loss of the animals during the observation period (14 d). But repeated intragastric administration of 316 mg/kg of *T. sylvatica* seeds aqueous extract every 24 h for 5 d induced 50% mortality, while the repeated intragastric administration of 1000 mg/kg of *T. sylvatica* seeds aqueous extract every 24 h for 5 d induced 83% mortality (Table 3).

The 28-*O*-[β-L-6-rhamnopyranosyl]-R₁-barrigenol showed also high toxicity in mice (LD₅₀ = 1.11 ± 0.23 mg/kg).

Identification of 28-*O*-[β-L-6-rhamnopyranosyl]-R₁-barrigenol

In the negative ion FAB-MS of compound **1**, quasi-molecular ion peak was observed at m/z 651 (M – H)[–] and high-resolution negative ion FAB-MS revealed the molecular

Figure 2. Effect of methanol (A) and aqueous (B) extracts of *Ternstroemia sylvatica* on latency and duration of pentobarbital-induced hypnosis in mice. Bars represent mean $\pm$ S.E.M. $n = 6$. * $p < 0.05$ significantly different from vehicle; ANOVA followed by Dunnett's test.

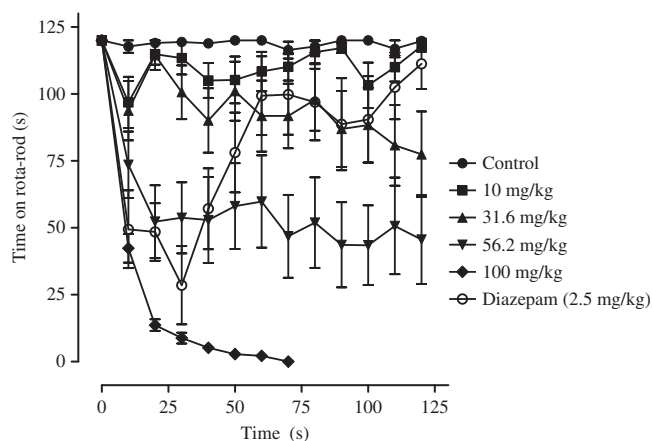
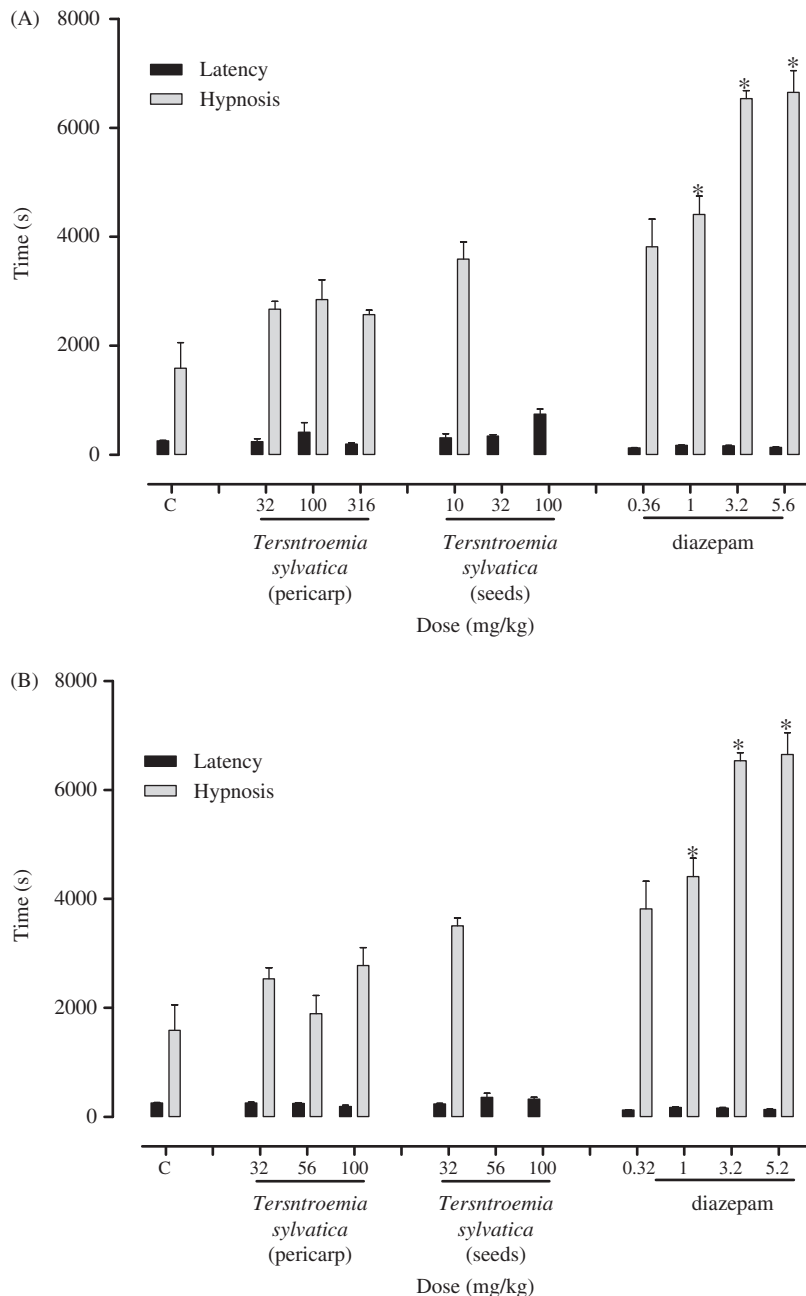


Figure 3. Effect of aqueous extract of *Ternstroemia sylvatica* seeds on motor coordination in the rota-rod test. Symbols represent mean $\pm$ S.E.M. $n = 6$.

formula to be $C_{36}H_{60}O_{10}$. Another significant fragment ion peak was observed at m/z 505 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety. Full assignment of the 1H and ^{13}C signals of **1** were accomplished through analysis of the 1H - 1H COSY, TOCSY, HSQC, HMBC (Figure 6) and NOESY (Figure 7) spectra. The 1H NMR spectrum of **1** showed signals for seven angular methyl groups as singlets [1.88 (H-23), 1.08 (H-24), 1.01 (H-25), 1.25 (H-26), 1.14 (H-27), 1.37 (H-29), 1.41 (H-30)], one olefinic proton at δ H 5.55 (1H, t, $J = 4$ Hz, H-12), five oxygen-bearing methyne protons at 3.06 (1H, t, $J = 12$ Hz, H-3), 4.46 (1H, m, H-15), 4.98 (1H, m, H-16), 4.85 (1H, d, $J = 12$ Hz, H-21) and 4.63 (1H, d, $J = 12$ Hz, H-22), and one primary alcoholic function at 4.51 (2H, m, H-28). In the NOESY experiment, cross-peaks between δ H 4.46 (H-15) and δ H 4.98 (H-16), and between δ H 4.98 (H-16) and δ H 4.51 (H-28), allowed the location of two secondary

Figure 4. Effect of 28-*O*-[β -L-6-rhamnopyranosyl]-R₁-barrigenol in Exploratory Cylinder test (A) and hole board test (B) in mice. Symbols represent mean $\pm$ S.E.M. $n = 6$. * $p < 0.05$ significantly different from vehicle; ANOVA followed by Dunnett's test.

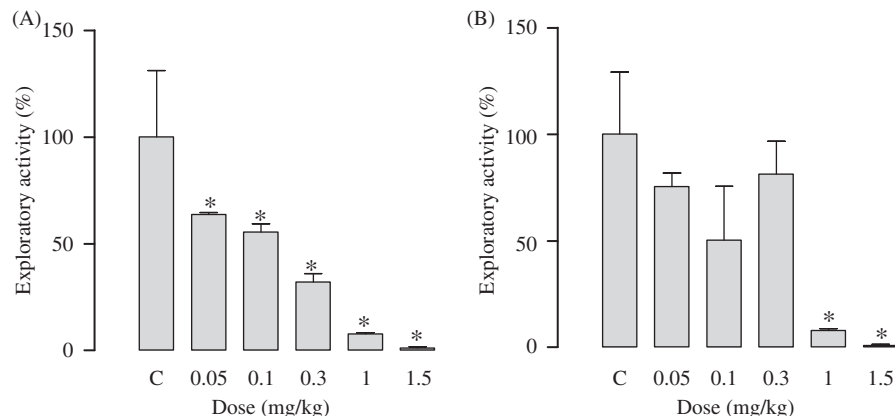


Figure 5. Effect of 28-*O*-[β -L-6-rhamnopyranosyl]-R₁-barrigenol (A) and aqueous extract of seeds from *Ternstroemia sylvatica* (B) in open field test in mice. Symbols represent mean $\pm$ S.E.M. $n = 6$. * $p < 0.05$ significantly different from vehicle; ANOVA followed by Dunnett's test.

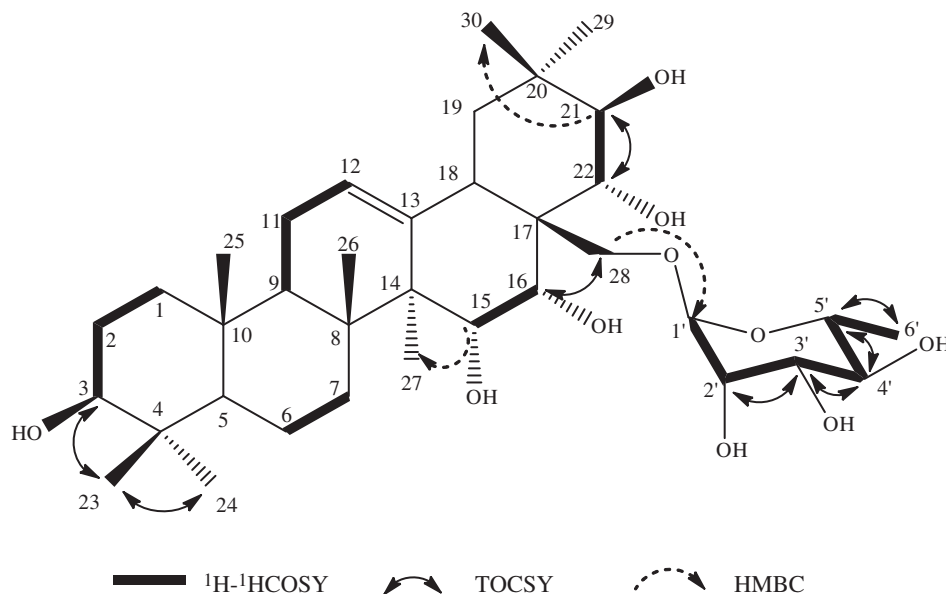
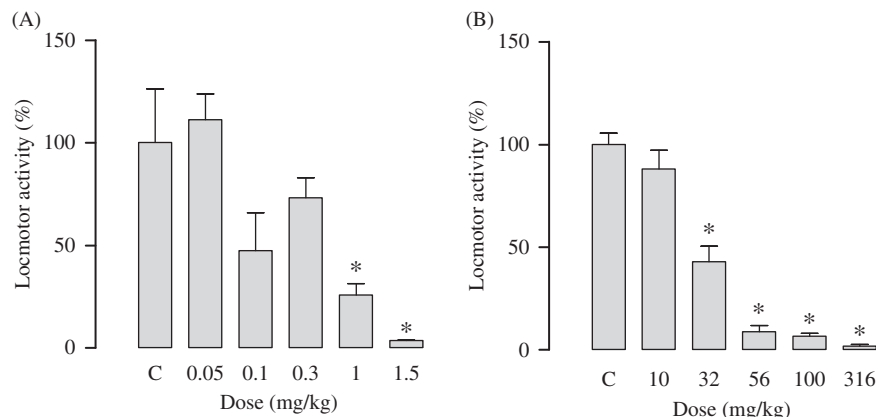


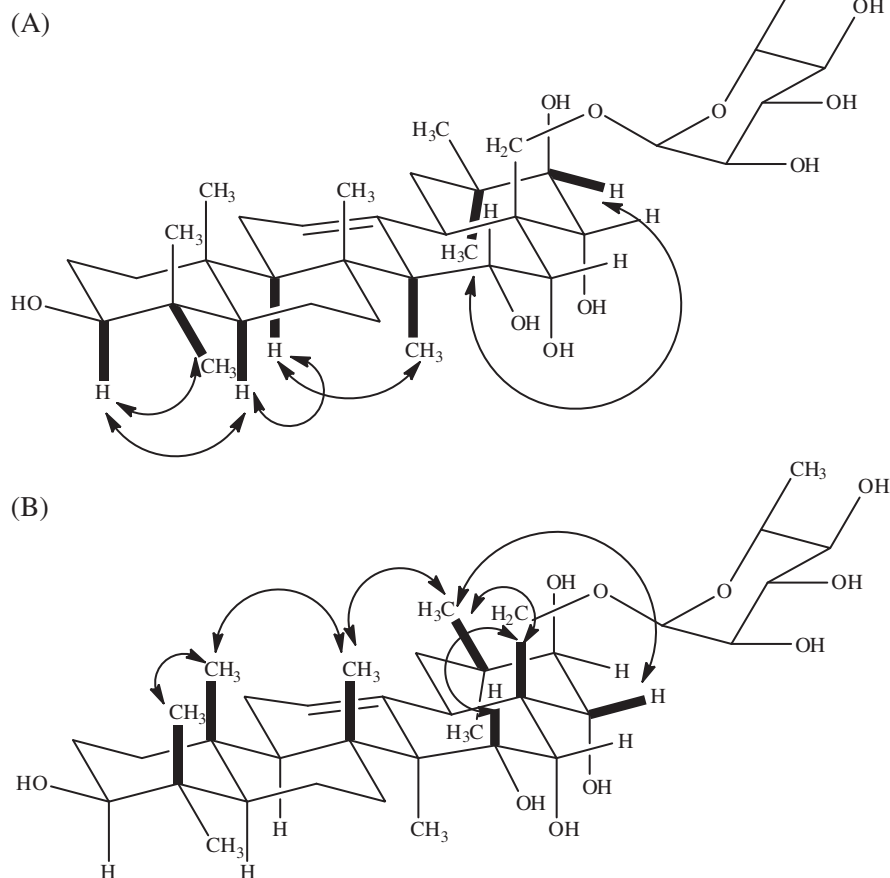
Figure 6. Selected key correlations in ^1H - ^1H COSY, TOCSY and HMBC experiments of compound 1.

alcoholic functions at C-15 and C-16. The hydroxyl group at C-15 was confirmed by a correlation between δC 67.51 (C-15) and δH 1.14 (s) (H-27) in the HMBC experiment. In this spectrum, it was also observed the correlation between C-21 at δC 78.45 with the two methyl group signals at δH 1.37 (s, H-29) and 1.41 (s, H-30). A correlation in the HMBC experiment between H-28 at δH 4.51 and the anomeric signal at δC 102.8 suggested that a sugar was attached to C-28. The

sugar was identified as L-rhamnose with the help of ^1H - ^1H COSY and TOCSY spectra. The characteristic double signal for C-6' of L-rhamnose appeared at δH 1.64 ($J = 8$ Hz, Table 1).

The relative configurations of C-3, C-15, C-16, C-21 and C-22 of the R₁-barrigenol unit were determined by the multiplicity and the coupling constants of carbinol protons, supported by the connectivity observed in the NOESY

Figure 7. Selected key correlations in NOESY experiment of compound **1**.



spectrum: H-3, H-5 and H-24; H-15, H-16, H-26 and H-28; H-22 and H-30; and H-21 and H-29. On the basis of the above results, the structure of **1** was elucidated as 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol, a new glycoside triterpene. Acid hydrolysis of **1** with 2 N HCl yielded **1a** as an aglycone, together with L-rhamnose identified by GC/MS analysis of their trimethylsilyl derivative (Kirmizigül et al., 1995). The structure of the aglycone of **1** was thus recognized to be the triterpene R₁ barrigenol and it was in agreement with the literature data (Fu et al., 2005).

Discussion

According to obtained results on the neuropharmacological evaluation of *T. sylvatica* fruits, it is not possible to consider that this plant possesses a convincing CNS depressant effect. Despite the aqueous and methanol extracts showed sedative effect in two very well-known sedative models, and these results apparently are in agreement with a previous work (Molina et al., 1999). The aqueous extract obtained from *T. sylvatica* seeds was the most active extract, but it was also the most toxic extract for mice. The neurotoxicity was also evidenced for this extract because it decreased the locomotor activity, disturbed the motor coordination and decreased the exploratory behavior with signs of discomfort for the animals. These results suggest that sedative effect for this plant might be caused more by a toxic effect than by an effect on the CNS. The fruits of *T. sylvatica* are used to prepare the decoctions used as sedative traditional remedies. These fruits are constituted by 40% of seeds and 60% of pericarp. We were

interested in knowing if seeds or pericarp showed the same activity; therefore, we studied separately seeds and pericarp, finding that seeds are more active than pericarp.

From the aqueous seeds extract, the glycoside triterpenoid 28-O-[β -L-6-rhamnopyranosyl]-R₁-barrigenol was isolated as a new natural product. This substance showed also CNS depressant and toxic effect ($LD_{50} = 1.11 \pm 0.23$ mg/kg). Therefore, these results suggested that this substance is responsible for the sedative and toxic effects of *T. sylvatica* seeds. A preliminary HPLC analysis of the extracts of *T. sylvatica* showed that this glycoside triterpenoid is present in methanol and aqueous extracts of *T. sylvatica* seeds but it was not detected in the extracts of pericarp. Therefore, the activity of this plant may be related to the presence of this glycoside.

Previous pharmacological work did not report any toxic effect for this species, probably since the evaluation was performed in an acute single doses regimen and the experiments were performed in a time less than 3 h (Balderas et al., 2008). On the other hand, the repeated oral administration for 5 d of aqueous extract was less active and less toxic than the single intraperitoneal administration. These results suggest this compound is poorly absorbed from the gastrointestinal tract.

On the other hand, jacaranone was identified as the sedative principle in *T. pringlei* (Lozada-Lechuga et al., 2010). However, Xu and coworkers reported neurotoxic effects for this natural quinone that lead to death of mice in 30–90 min, with a LD_{50} of 150–200 mg/kg (Xu et al., 2003). Here, we are reporting the triterpene glycoside 28-O-[β -L-6-

rhampnopyranosyl]-R₁-barrigenol as a new natural product and as the second one sedative and toxic principle in *Ternstroemia* genus. Toxic effect seems to be the major effect of fruits of these medicinal plants, and the neuropharmacological effects observed here and those previously reported might be signs of this toxic effect. These findings highlight the need to consider not only the pharmacological effect to support the traditional use of the medicinal plants but also to take in count their toxicity.

In conclusion, we demonstrated that the aqueous extract of *T. sylvatica* seeds, which represent 40% of the fruits, has toxic activity rather than CNS depressant activity in mice and that this effect might be related to the presence of 28-*O*-[β-L-6-rhampnopyranosyl]-R₁-barrigenol, a new triterpenoid glycoside.

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Declaration of interest

There are no conflicts interest related with the present investigation.

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