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Diuretic activity and neuropharmacological effects of an ethanol extract from *Senna septemtrionalis* (Viv.) H.S. Irwin & Barneby (Fabaceae)

Angel Josabad Alonso-Castro[a,*], Clara Alba-Betancourt[a], Eunice Yáñez-Barrientos[a], Cynthia Luna-Rocha[a], Astrid Selene Páramo-Castillo[a], Othoniel H. Aragón-Martínez[b], Juan Ramón Zapata-Morales[a], Gustavo Cruz-Jiménez[a], Deisy Gasca-Martínez[c], Alan Alexander González-Ibarra[a], Daniel Antonio Álvarez-Camacho[a], Martha Alicia Devezé-Álvarez[a,**]

[a] *División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Noria Alta, 36050, Guanajuato, Gto, Mexico*
[b] *Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico*
[c] *Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, Mexico*

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clonazepam (PubChem CID: 2802)
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ABSTRACT

Ethnopharmacological relevance *Senna septemtrionalis* (Viv.) H.S. Irwin & Barneby (Fabaceae) is a shrub empirically used as diuretic, and for the treatment of neurological disorders. These pharmacological effects have not been previously evaluated.

Aim of the study: To evaluate the diuretic and CNS effects of a standardized ethanol extract of *Senna septemtrionalis* aerial parts (SSE).

Materials and methods: Gas chromatography mass spectrometry was used to perform a chemical analysis with SSE. In all tests, SSE was evaluated from 10 to 100 mg/kg p.o.

The diuretic activity of SSE was assessed in mice individually placed in metabolic cages. After 6 h, the urine volume and the electrolyte excretion (Na and K) were measured. The role of prostaglandins and nitric oxide was assessed administrating mice with indomethacin and N(ω)-nitro-L-arginine methyl ester (L-NAME), prior the administration of 100 mg/kg SSE. The sedative effects of SSE were analyzed with the pentobarbital-induced sleeping time test. The effects of SSE on motor coordination in mice were evaluated with the rotarod test. The antidepressant-like activity of SSE was analyzed with the forced swimming test (FST) and the tail suspension test (TST). The role of 5-HT2 receptor, α1-and α2-adrenoceptors, or muscarinic receptors was assessed administrating mice with cyproheptadine, prazosin, yohimbine, and atropine, respectively, prior the administration of 100 mg/kg SSE in the FST. The anxiolytic-like activity of SSE (10–100 mg/kg p.o.) was assessed using the light-dark test (LDB), the elevated plus maze test (EPM), the cylinder exploratory test, and the open field test (OFT). The anticonvulsant effect of SSE (1–100 mg/kg) was evaluated in mice administered with different convulsant agents: strychnine, pentylenetetrazol (PTZ), isoniazid (INH) or yohimbine.

Results: The main compound found in SSE was D-pinitol (42.2%). SSE (100 mg/kg) increased the urinary volume (2.67-fold), as well as the excretion of Na (5.60-fold) and K (7.2-fold). The co-administration of SSE with L-NAME or indomethacin reverted the diuretic activity shown by SSE alone. SSE lacked sedative effects and did not affect motor coordination in mice. SSE (100 mg/kg) showed higher and similar antidepressant-like effect, compared to 20 mg/kg fluoxetine, in the FST and TST, respectively. The co-administration of SSE with yohimbine reverted the antidepressant-like activity shown by SSE alone. SSE (100 mg/kg) showed anxiolytic-like activity in the four models of anxiety, with similar activity with 1.5 mg/kg clonazepam. The seizure-protective effect of SSE was ED_{50} = 73.9 ± 8.4 mg/kg (INH) and 40.4 ± 5.2 mg/kg (yohimbine).

Conclusion: The diuretic effects of SSE involve the possible contribution of prostaglandins and nitric oxide. SSE

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; CNZ, clonazepam; CNS, central nervous system; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; FST, forced swimming test; FUR, furosemide; GC-MS, gas chromatography–mass spectrometry; INH, isoniazid; LDB, light-dark test; L-NAME, N(ω)-nitro-L-arginine methyl ester; OFT, open field test; PTZ, pentylenetetrazol; SSE, ethanol extract of *Senna septemtrionalis* aerial parts; TST, tail suspension test

* Corresponding author. Noria Alta S/N, C.P. 36050, Guanajuato, Gto, Mexico.
** Corresponding author. Noria Alta S/N, C.P. 36050, Guanajuato, Gto, Mexico.
E-mail addresses: angeljosabad@ugto.mx (A.J. Alonso-Castro), devezem@ugto.mx (M.A. Devezé-Álvarez).

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showed moderate anxiolytic and anticonvulsant effects, whereas the participation of α2-adrenoceptors is probably associated in the antidepressant-like effects of SSE.

1. Introduction

Senna genus compromises nearly 350 plant species, from which 200 are native to America. Plants from Senna genus exert antioxidant, antimicrobial, and anti-inflammatory effects and anthraquinones are the main metabolites found in this genus (Neves et al., 2017). *Senna septemtrionalis* (Viv.) H.S. Irwin & Barneby (Fabaceae) (synonym *Cassia laevigata*) is a shrub, with height of 1–5 m, global distributed, but native to tropical America (Bye and Linares, 2013). In Mexico, *Senna septemtrionalis* has been used since pre-Hispanic times for the treatment of headache, wounds, flu, and stomachache (Bye and Linares, 2013). Nowadays, *Senna septemtrionalis* is used as diuretic, anti-inflammatory, laxative, vermifuge, expectorant, and fungicide agent, as well as for the folk treatment of snakebites, fever, burns, cholera, hemorrhoids, pain, gastroenteritis. *Senna septemtrionalis* is also a remedy for neurological disorders including epilepsy and anxiety, among others (Guzman, 1976; Jones et al., 2000; Karhagomba et al., 2013; Tabuti et al., 2003; González-Elizondo et al., 2004; Lulekal et al., 2008; Maryo et al., 2015; Randriamiharisoa et al., 2015; personal communication).

Many medicinal plants native to Mexico remain to be studied for their pharmacological, chemical, and toxicological effects. This scientific data might provide findings that corroborate the ethnomedicinal information used for centuries in Mexico. In our knowledge, no pharmacological effects of *Senna septemtrionalis* have been previously reported. The aim of this work was to evaluate the antioxidant, diuretic, anxiolytic-like, antidepressant-like, and anticonvulsant effects of an ethanol extract of *Senna septemtrionalis* (SSE).

2. Materials and methods

2.1. Reagents

Pentobarbital sodium was acquired from Pisa Farmaceutica (Mexico City, Mexico) and clonazepam was obtained from Tecnofarma (Queretaro, Mexico). All other reagents, including furosemide, fluoxetine, indomethacin, L-NAME, among others were obtained from Sigma Aldrich (St Louis, MO, USA).

2.2. Plant material

Samples of *Senna septemtrionalis* were gathered at the following coordinates: 21° 8′ north latitude and 101° 42’ west longitude, in the city of Leon, State of Guanajuato (Mexico). A voucher specimen (18198) was deposited at the herbarium of Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autonoma de Mexico (FEZA).

2.3. Preparation of ethanol extract from Senna septemtrionalis aerial parts (SSE)

Aerial parts (stem and leaves), powdered and dried, of *Senna septemtrionalis* (240 g) were macerated with ethanol (1500 mL) for 10 days. The extract was concentrated *in vacuo*.

2.4. Gas chromatography mass spectrometry analysis

For derivatization of samples, 10 mg of dried extract was weighed and transferred to silanized vial (in triplicate). The extract was resuspended in 1 mL of pyridine and 100 μL of *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA kit of SUPELCO) was added. The mixture was heated at 50 °C for 30 min. Then, 1 μL of detivatized sample or alkanes series (C7-C40, SUPELCO certified reference material, 1000 μg/mL) was injected by an autosampler into a PerkinElmer Clarus 580 Gas Chromatograph equipped whit a column Elite-5 MS (30 m × 0.32 mm, i.d. and 0.25 μm film thickness of coated material). The injector temperature was set at 270 °C. The injection operated at a split ratio 5:1, from 0 to 5 min and then operated in splitless mode. Helium was used as carrier gas at flow rate 1 mL min^{-1} through the column. The oven column temperature programed was initially kept to 80 °C for 2 min and then increased to 300 °C at 12 °C min^{-1}, where it was held for 6 min. The column effluent was into the ion source of a PerkinElmer Clarus SQ 8 S Mass Spectrometer. The transference line temperature was set at 250 °C and the ion source temperature at 250 °C. Mass were acquired from *m/z* 50 to 620 and the filament was turned on after solvent delay of 5 min.

The analysis of data files was performed used the AMDIS software in Use Retention Index Data mode (Automated Mass Spectral Deconvolution and Identification System; http://www.amdis.net/). The AMDIS settings for deconvolution was as follows: Width, 2; Adjacent peak substractions, 0ne; Resolution, Medium; Sensitivity, Medium; Shape requirements, Medium. The identification of compounds was made through the NIST MS Search 2.0 software when comparing the deconvoluted spectra whit the spectra in the mainlib library.

2.5. Antioxidant activity

2.5.1. Ferric reducing antioxidant power (FRAP) assay

The method was carried out following the standardized method described by Gonzalez-Rivera et al. (2018). Briefly, 100 μL of test sample (SSE at 1000–10 000 μg/mL or Trolox at 10–500 μg/mL) were added into a tube containing 250 μL of 300 mM CH_3COONa (pH 3.6), 25 μL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine prepared in 40 mM HCl, and 25 μL of 20 mM $Cl_3FeH_{12}O_6$. The mixture was incubated at 37 °C for 4 min and then, maintained at room temperature for 1 min. Finally, 200 μL of the mixture were placed in a well of a 96-well plate for single spectrophotometric analysis at 596 nm. The identity of assays was confirmed with spectra records from 550 to 650 nm. In all antioxidant assays, inhibitory concentration IC_{50} values were calculated by linear regression of log concentration and the antioxidant response.

2.5.2. ABTS assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay was carried out as described by Arnao et al. (2001), with some modifications. In brief, 3 μL of the test samples (SSE at 1000–5000 μg/mL or Trolox at 1–300 μg/mL) were added into a tube which contained 300 μL of a water-diluted ABTS radical cation solution. The mixture was incubated at 30 °C for 4 min. After incubation, the mixture was maintained at room temperature for 1 min. For the single spectrophotometric analysis at 730 nm, 200 μL of each mixture was placed in a well of a 96-well plate. The identity of assays was confirmed with spectra records from 450 to 850 nm.

2.5.3. DPPH assay

The method of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) was followed as proposed by Lee et al. (2017). Briefly, 50 μL of the test sample (SSE at 3000–15 000 μg/mL or Trolox at 10–1000 μg/mL) were added to 200 μL of a methanolic 900 μM DPPH radical solution. The mixture was incubated at 30 °C for 20 min. Then, 200 μL of the mixture were placed in a well of a 96-well plate for the single spectrophotometric analysis at 517 nm. The identity of assays was confirmed with spectra records from

400 to 700 nm.

2.6. Animals

Male Balb/c mice and CD-1 mice, weighing 25–30 g, 6–9 weeks old, were acquired from the Universidad of Guanajuato animal facility and the bioterium of the Institute of Neurobiology (UNAM), respectively. The animals were maintained under standard laboratory conditions. A local research ethic committee (Dixpertia) approved the protocol (CEID-008A-2017) to carry out experiments with animals.

2.7. Acute toxicity test

Groups of mice (n = 5, each dose) were orally or intraperitoneally treated with doses of SSE (250–2000 mg/kg). Mice were supervised for any signs of toxicity for 14 days (OECD), 2008.

2.8. Pharmacological treatment

Sixty min preceding each experiment, mice were separated (n = 8 per group) and orally dispensed with saline solution (vehicle), 10 mg/kg furosemide (FUR; in diuresis experiment), 1.5 mg/kg clonazepam (CNZ, in anxiety and convulsant experiments), 20 mg/kg fluoxetine (in depressant-like experiments), or 10–100 mg/kg SSE. The doses of SSE were selected based on the results obtained with the acute toxicity test and on preliminary experiments carried out in our laboratory.

2.9. Diuretic activity and mechanism of action

The experiment was carried out following the protocol of Lahlou et al. (2007), with some modifications. Two days-prior the experiment, animals were placed in individual metabolic cages, with access to water and food, for their acclimatization. Eighteen hour-prior the experiment, the animals were fasted overnight, with free access to water. In additional experiments, N(ω)-nitro-L-arginine methyl ester (L-NAME p.o.) (60 mg/kg), a nitric oxide synthesis inhibitor, or indomethacin (5 mg/kg p.o.), a prostaglandin synthesis inhibitor, were co-administered, each, 15 min prior the administration with 100 mg/kg SSE. Then, animals received 0.3 mL/kg physiological saline (0.9% NaCl) and were individually placed in a metabolic cage. After 6 h, urine was collected in a graduated cylinder and measured, filtered, and finally stored at −20 °C for further analyses. Electrolyte content (Na and K) in urine samples was determined by spectrophotometric methods using Spinreact kits (Girona, Spain). The results were expressed as mmol of electrolyte/L/6 h.

2.10. Neuropharmacological effects

2.10.1. Pentobarbital-induced sleeping time

Sodium pentobarbital (30 mg/kg i.p.) was injected to mice. Sleep onset was registered as the time spent from pentobarbital injection up to the failure of righting reflex. The extent of sleep was stated as the time between loss and recuperation of righting reflex (Darias et al., 1998).

2.10.2. Rotarod test

Mice able to keep walking on the rotarod (Panlab, Barcelona, Spain), set at 4 rpm, for 30 s were elected for the experiment. Time spent (sec) on rotarod was recorded at 60 and 120 min after treatment administration (Shiotsuki et al., 2010).

2.10.3. Tail suspension test (TST)

Mice were acoustically isolated and suspended by the tail, using adhesive tape, 50 cm above the floor. Immobility time (sec) was documented for 6 min (Ripoll et al., 2003).

2.10.4. Forced swimming test (FST)

Mice were placed individually in acrylic cylinder (45 cm in height, 20 cm in diameter), filled with water at 25 °C to a height of 30 cm (Porsolt et al., 1977). Immobility time (sec) was recorded with a camera for 6 min.

2.10.5. Mechanism of action of the antidepressant-like activity

In additional experiments, groups of mice were intraperitoneally treated with: i) 3 mg/kg cyproheptadine, a 5-HT2 receptor antagonist, ii) 0.06 mg/kg prazosin, an α1-adrenoceptor antagonist, iii) 1 mg/kg yohimbine, an α2-adrenoceptor antagonist, or iv) 1 mg/kg atropine, a muscarinic antagonist. Fifteen min later, mice were orally treated with 100 mg/kg SSE and the FST was carried out as described above.

2.10.6. Light-dark box (LDB)

The LDB had the following dimensions 46 cm length × 27 cm width × 30 cm height, one third of the box corresponded to the dark compartment and two third corresponded to the light compartment with light intensity of 400 lux. After 1 h of treatment, each mouse was introduced into the light compartment, and the transition between the light and the dark box and time consumed in the light box were documented for 5 min (Crawley and Goodwin, 1980).

2.10.7. Elevated plus maze (EPM)

The EPM consisted of two open arms (35 cm × 6 cm) and two closed arms (35 cm × 6 cm) with 15 cm walls, and 41 cm above the floor. Mice were individually placed at the center of the maze and allowed to explore the maze for 5 min (Walf and Frye, 2007). Time spent on the opened arms and the number of entries in the open arms were recorded with a video camera. The analysis was analyzed with the smart video tracking software (Panlab, Barcelona, Spain).

2.10.8. Exploratory cylinder test

Each mouse was placed in the cylinder (45 cm in height, 20 cm in diameter, with wall of 3 mm) and the number of rearings were recorded for 5 min (Aguirre-Hernández et al., 2007).

2.10.9. Open field test (OFT)

The OFT had the following dimensions: 42 cm length × 42 cm width × 30 cm height. A digital video camera was installed above the OFT. Total distance traveled, ambulatory velocity, as well as the time and the distance in the center of the apparatus were recorded within 5 min (Archer, 1973), using the software Fusion v5.3 SuperFlex activity monitoring cage system (Omnitech Electronics, Columbus, OH, USA).

2.10.10. Anticonvulsant activity

Each convulsant agent was intraperitoneally administered in independent experiments: 4 mg/kg strychnine (Porter et al., 1984), 90 mg/kg pentylenetetrazol (PTZ) (Swinyard et al., 1952), 250 mg/kg isoniazid (INH) (Bernasconi et al., 1985), or 45 mg/kg yohimbine (Dunn and Fielding, 1987). The onset of convulsion, the duration of convulsions, the protection against seizures, and the number of deaths were documented for 60 min.

2.11. Statistical analysis

All data are expressed as mean ± standard error of the mean. One-way analyses of variance followed by Dunnett's test were performed to calculate differences between control and treated groups. The level of $p \leq 0.05$ was designed significant. All the calculations were done with the software system JMP V.8 (Sas Institute).

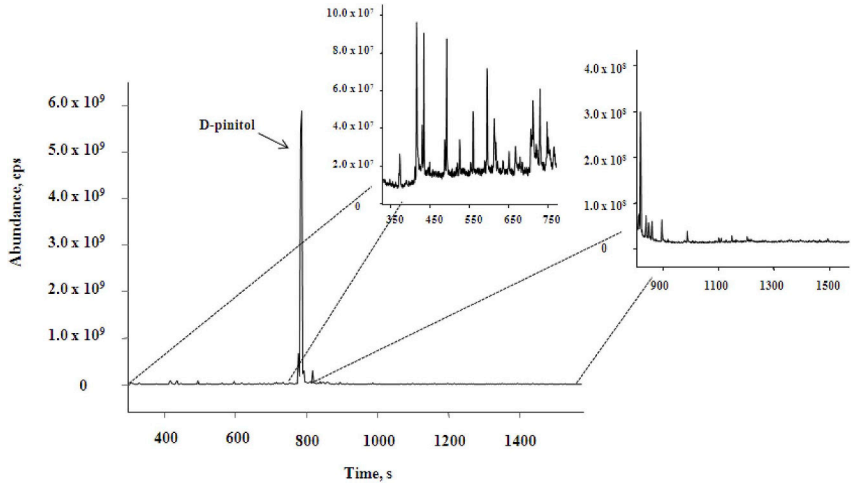

Fig. 1. Experimental chromatogram of extract derivatized with BSTFA obtained by GC-MS corresponding to Total Ion Chromatogram (TIC).

3. Results

3.1. Chemical composition

The ratio of the herbal substance to the native herbal drug preparation (DER native) was 20:1. The main compound found in SSE was D-pinitol (42.2%).

The chemical composition of SSE resulted in fatty acids (oleic acid, octadecanoic acid, hexadecenoic acid, and linoleic acid), carboxylic acids (benzoic acid, oxalic acid, malic acid, and succinic acid) among others. However, there were many unidentified compounds (34% of relative abundance) in SSE (Fig. 1). Chemical studies are being carried out in our laboratory to identify these compounds.

Compound	Experimental Retention Index	Retention Time	Relative abundance (%)
Oxalic acid	1124	5.31	0.38 ± 0.01
Benzoic acid	1249	6.92	0.16 ± 0.00
Glycerol	1274	7.23	3.45 ± 0.17
Malic acid	1387		0.45 ± 0.02
exo-2-oxyisoxazolidino[1,2-b]-1,3-dioxacyclopentane	1398	8.76	0.09 ± 0.05
α-[3'-(Trifluoromethyl)benzyl]-γ-b-utyrolactone	1508	9.94	0.11 ± 0.00
Succinic acid	1665	10.97	0.63 ± 0.03
2,2-Dimethyl-2,3a,4-tetrahydroin-deno[1,2-d]pyrrol-3a-ol 1-oxi-de	1706	11.93	0.08 ± 0.04
D-pinitol	1828	13.07	42.2 ± 2.11
Galactose	1970	13.42	3.56 ± 0.16
L-sorbose	1982	13.62	1.19 ± 0.05
β,D-Glucopyranose,	2037	14.61	0.15 ± 0.03
1-Ethyl-2-(hexen2-yl)benzene	2043	14.89	0.05 ± 0.01
Palmitic acid	2046	14.92	2.31 ± 0.12
Caffeic acid	2143	15.62	0.12 ± 0.00
Inositol	2194	15.99	5.13 ± 0.25
Linoleic acid	2208	16.10	1.13 ± 0.06
Oleic acid	2212	16.15	2.54 ± 0.13
Stearic acid	2240	16.43	2.36 ± 0.16

3.2. Antioxidant activity

The antioxidant activity of SSE is described as follows: IC_{50} = 5592.9 ± 161.4 μg/mL (DPPH assay), 1925.9 ± 18.9 μg/mL (FRAP assay), and 14 107 ± 633 μg/mL (ABTS assay). The antioxidant activity for Trolox was: IC_{50} = 206.4 ± 1.8 μg/mL (DPPH assay), 67.1 ± 0.8 μg/mL (FRAP assay), and 526.8 ± 10.4 μg/mL (ABTS assay).

3.3. Acute toxicity test

The LD_{50} calculated for SSE was higher than 2000 mg/kg (p.o. or i.p.).

3.4. Diuretic activity

FUR (10 mg/kg) increased ($p < 0.05$) the urinary volume (1.97-fold), as well as the excretion of Na (1.92-fold) and K (3.2-fold), compared to the vehicle group. SSE (100 mg/kg) increased ($p < 0.05$) the urinary volume (2.67-fold), as well as the excretion of Na (5.60-fold) and K (7.2-fold) (Fig. 2A–C). The ED_{50} of SSE was 56.4 mg/kg.

The coadministration of 100 mg/kg SSE with indomethacin (5 mg/kg) or L-NAME (60 mg/kg), each, decreased ($p < 0.05$) the excretion of urine volume, compared to the treatment with 100 mg/kg SSE alone (Fig. 2D).

3.5. Sedative effect

SSE (1–100 mg/kg p.o.) did not affect the onset or duration of sleep in the pentobarbital-induced sleeping time, compared to the vehicle group. CNZ (1.5 mg/kg) reduced ($p < 0.05$) the onset of sleep by 54% but increased the duration of sleep by 176%.

3.6. Effects on motor coordination

SSE (10–100 mg/kg p.o.) did not affect the motor coordination of mice in the rotarod test, compared to the vehicle group. CNZ (1.5 mg/kg) decreased ($p < 0.05$) the time spent on the rotarod by 47% (30 min) and 54% (60 min).

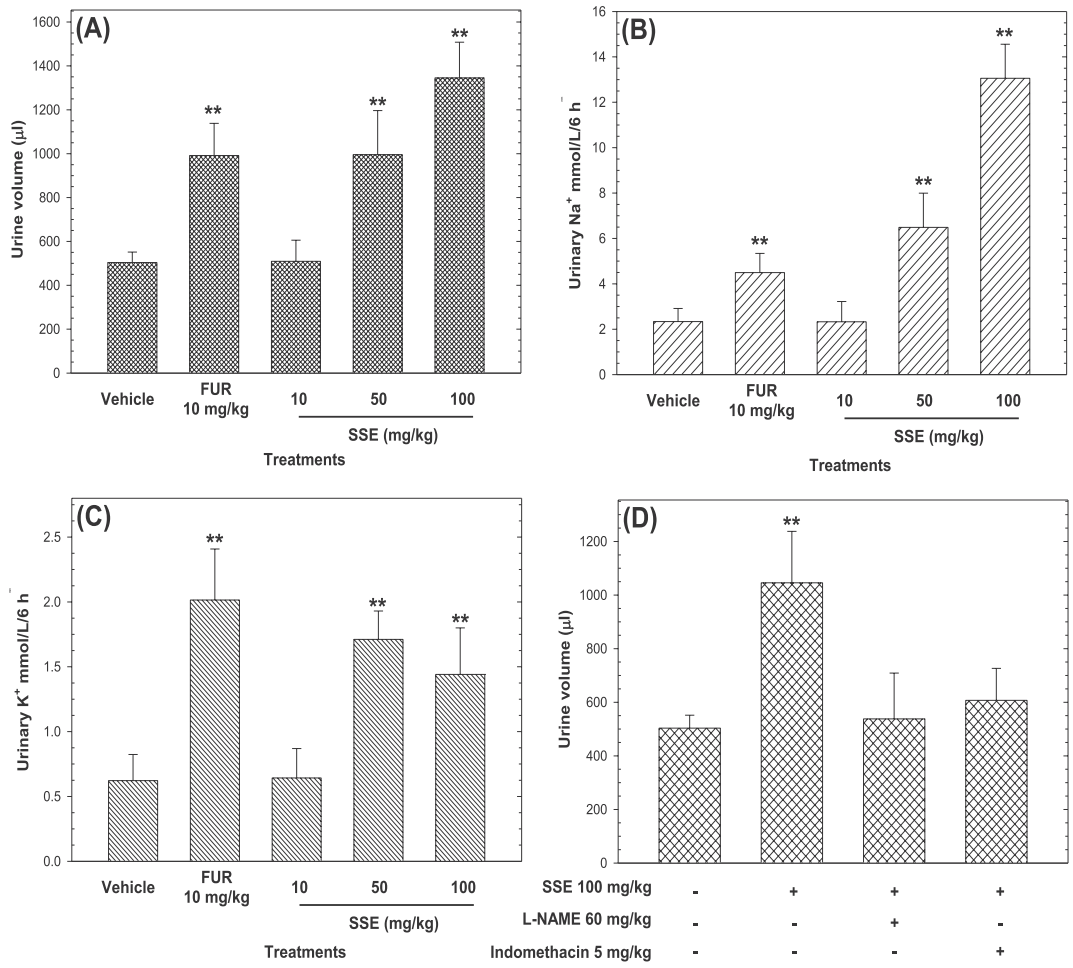

Fig. 2. Diuretic activity of SSE. The diuretic activity of SSE (10–100 mg/kg) was assessed with the urine volume (A), and the urinary levels of Na (B) and K (C). The diuretic activity of SSE was evaluated with the co-administration of L-NAME and indomethacin (D). Other groups of mice received 10 mg/kg furosemide (FUR) as the positive control or the vehicle (saline solution). Data are representative of two independent experiments (n = 8). Results represent the mean ± standard error of the mean (SEM). ** denotes $p \leq 0.05$ compared to the vehicle group using ANOVA followed by the Dunnett's test.

3.7. Antidepressant-like activity and mechanism of action

In the TST, SSE (50 and 100 mg/kg) decreased ($p < 0.05$) immobility time, compared to the vehicle group, by 26% and 20% respectively. FLX (20 mg/kg p.o.) decreased immobility time by 60% (Fig. 3A).

In the FST, only 100 mg/kg SSE significantly ($p < 0.05$) decreased immobility by 80%, with higher activity compared to 20 mg/kg FLX (59% of antidepressant-like effect) (Fig. 3B). The ED_{50} value calculated for this experimental test was 76.7 mg/kg.

The pre-treatment with yohimbine abolished the antidepressant-like effect shown by SSE alone, whereas the administration with cyproheptadine, prazosin, or atropine did not affect the antidepressant-like activity of SSE (Fig. 3C).

3.8. Anxiolytic-like activity

In the light-dark test, SSE (10–100 mg/kg) significantly enlarged ($p < 0.05$) the time in the light compartment and the latency light/dark, with similar activity compared to CNZ (1.5 mg/kg) (Fig. 4A–B). In the EPM, SSE enlarged the time consumed on the opened arms and the amount of entries in the open arms (Fig. 4C–D). Only 100 mg/kg SSE diminished the number of rearings in the cylinder exploratory test, and 1.5 mg/kg CNZ declined the number of rearings by 94% (Fig. 4E). In the OFT, only 100 mg/kg SSE enlarged ($p < 0.05$) the time in central squares and the distance walked in the central squares (Table 1). In addition, SSE (10–100 mg/kg) did not affect motor coordination (shown by ambulatory velocity and distance moved) in mice (Table 1).

3.9. Anticonvulsant activity

The reference anticonvulsant drug CNZ (1.5 mg/kg) completely abolished the mortality, as well as the onset, and the duration of seizures in three out of the four models (PTZ, INH, and yohimbine) (Table 2). SSE decreased ($p < 0.05$) the duration of convulsions in a dose-dependent manner in two out of the four models (strychnine and yohimbine) (Table 1). SSE delayed ($p < 0.05$) the onset of convulsions in a dose-dependent fashion in two out of the four models (PTZ and yohimbine) (Table 2). SSE decreased the mortality in mice in a dose dependent manner in two out of the four models (INH and yohimbine) (Table 2). The seizure-protective effect of SSE was $ED_{50} = 73.9 \pm 8.4$ mg/kg (INH) and 40.4 ± 5.2 mg/kg (yohimbine).

4. Discussion

The findings indicated that the major component of SSE was D-pinitol, which represented 42% of the plant extract. Up to now, no studies regarding the diuretic and SNC effects of D-pinitol have been carried out. Currently, we are conducting these experiments in our laboratory.

Drugs that reduce K levels in urine are considered as potassium-sparing diuretic, whereas thiazides like furosemide augment diuresis and urinary elimination of sodium by the inhibition of Na + /K + /2Cl − symporter (co-transporter system) in the thick ascending limb of the loop of Henley (Jackson, 1996). SSE (50 mg/kg) and SSE (100 mg/kg) showed similar and higher diuretic activity compared to FUR (10 mg/kg), respectively, with a similar form in the elimination of Na and K. The participation of prostaglandins and nitric oxide was estimated with inhibitors (indomethacin and L-NAME, respectively). The results denoted a reduction of the diuretic effect of SSE when co-administered with indomethacin and L-NAME, respectively, which suggest that prostaglandins and nitric oxide participate in the diuretic effect of SSE. The compounds in SSE responsible of the diuretic effect in this extract remain to be studied.

Pentobarbital inhibits GABA-mediated neurotransmission, which is reflected in sedative-hypnotic effects. Drugs that potentiate the sedation induced by pentobarbital are considered as sedative agents (Darias et al., 1998). Rotarod test estimates motor coordination ability in rodents. Central depressant drugs induce muscle relaxant activity which generate motor dysfunction (Nishino et al., 2008). Unlike benzodiazepines (BZDs), the results showed that SSE did not affect motor coordination or induce sedation in mice (shown in the rotarod test, the pentobarbital-induced sleeping test, and the OFT).

Antidepressant-like effects of SSE were evaluated in the stress-induced depression models: FST and TST, established on the immobility of mice when placed in inescapable stressful situations imitating behavioral despair (Porsolt et al., 1977; Ripoll et al., 2003). SSE (100 mg/kg) exerted higher and moderate antidepressant-like activity in the FST and the TST, respectively, compared to 20 mg/kg FLX. The participation of α2-adrenoceptors receptors seems to be involved in the antidepressant-like activity shown by SSE.

The anxiolytic-like activity of SSE was evaluated using the following models: the LDB, the EPM, the exploratory cylinder test, and the OFT. The LDB is established on the aversion of rodents to unfamiliar environments and brightly illuminated areas (Crawley and Goodwin, 1980), whereas the EPM indicates fear to height and open spaces, and the exploratory cylinder test reveals anxiety of mice to unfamiliar environment evidenced by rearing (Aguirre-Hernández et al., 2007). OFT is based on the aversion of mice for the exploration of new environment and the avoidance to open spaces (Archer, 1973). SSE showed anxiolytic-like properties evidenced by increasing the time in the light compartment and increasing the latency light/dark (the LDB test), increasing the time and the entries in the open arms (the EPM test), decreasing the rearing (exploratory cylinder test), as well as by increasing the time and distance in the central squares (OFT). Bernal-Morales et al. (2017) reported the anxiolytic-like effects, mediated by $GABA_A$ receptors, of a mixture containing oleic acid, palmitic acid, stearic acid, and linoleic acid. It is possible that the anxiolytic-like and antidepressant-like activities shown by SSE might be, in part, due to these fatty acids, which compose 8.3% of SSE.

On the other hand, INH, yohimbine, strychnine, and PTZ induce, each, the depletion of GABA by the inhibitory neurotransmission mediated by GABA receptors (Swinyard et al., 1952; Porter et al., 1984; Bernasconi et al., 1985; Dunn and Fielding, 1987). This study showed that SSE protected mice against convulsions in two models of epilepsy (yohimbine and INH). The antagonism shown by SSE in yohimbine and INH-induced seizures could suggest the effect of SSE on the neurotransmission of GABA.

5. Conclusion

This study validates the traditional use of *Senna septemtrionalis* as diuretic, anxiolytic-like, antidepressant-like, and anticonvulsant agent. The diuretic effects of SSE involve the possible contribution of prostaglandins and nitric oxide. SSE showed moderate anxiolytic and anticonvulsant effects, whereas the participation of α2-adrenoceptors is

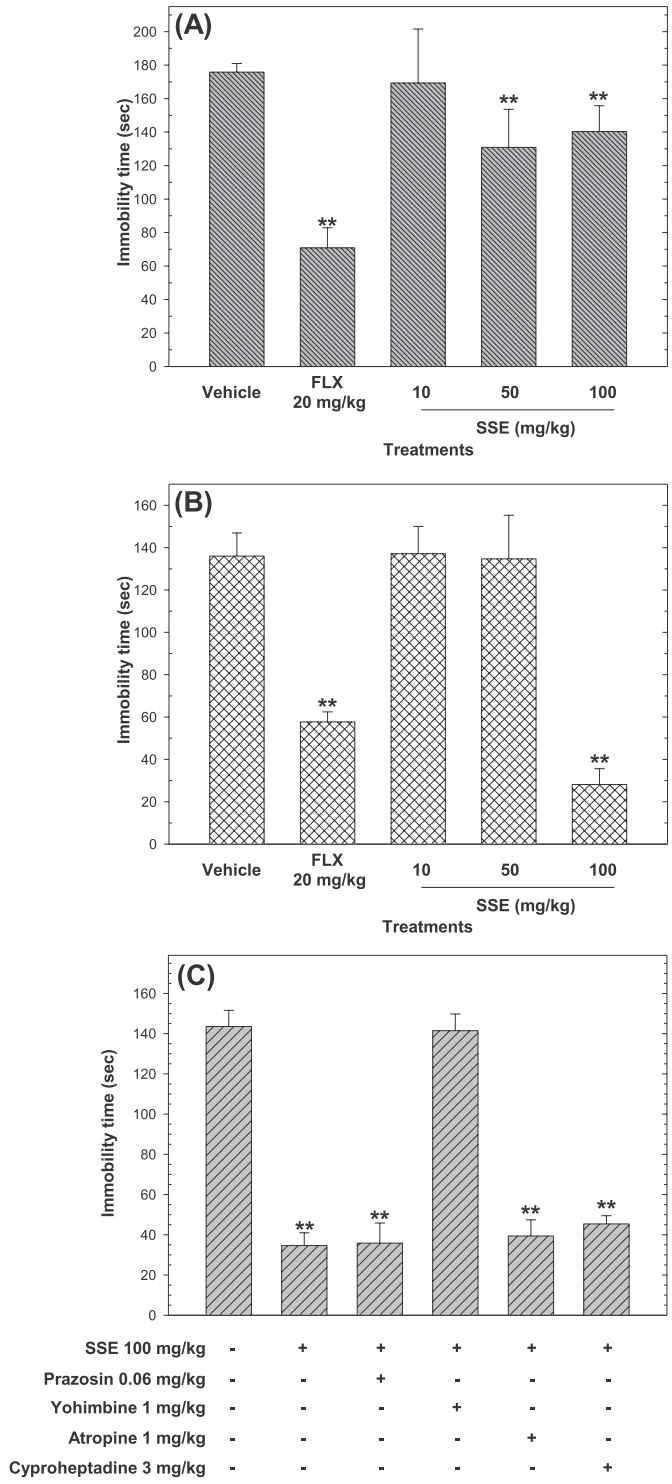

Fig. 3. The antidepressant-like activity of SSE (10-100 mg/kg) was assessed using the forced swimming test (A) and the tail suspension test (B), recording the immobility time. The anti-depressant-like activity of SSE (100 mg/kg) was evaluated with the co-administration of cyproheptadine, prazosin, yohimbine, or atropine (C). Other groups of mice received 20 mg/kg fluoxetine (FLX) as the positive control or the vehicle (saline solution). Data are representative of two independent experiments (n = 8). Results represent the mean ± standard error of the mean (SEM). ** denotes $p \leq 0.05$ compared to the vehicle group using ANOVA followed by the Dunnett's test.

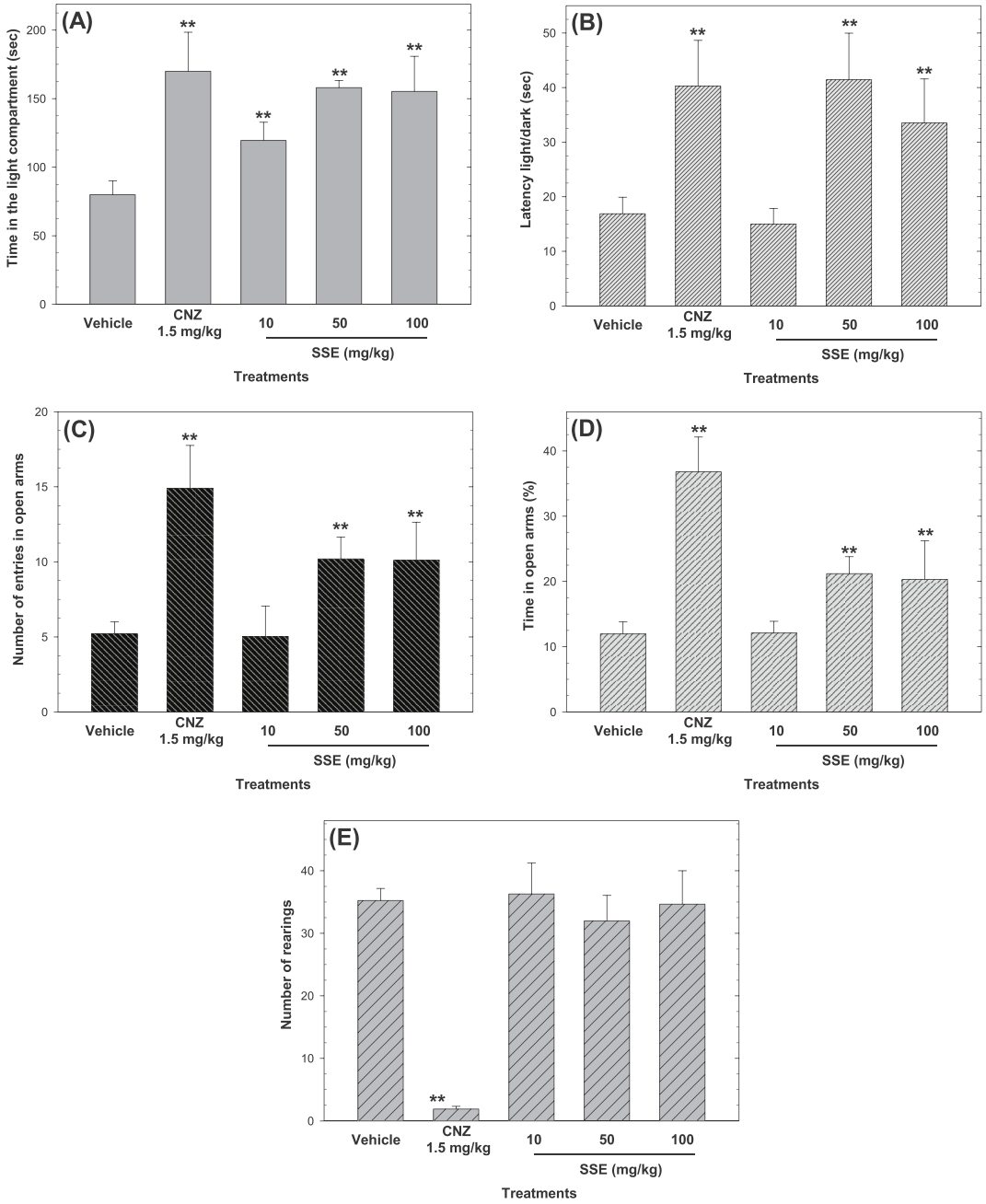

Fig. 4. Anxiolytic-like effects of SSE. The anxiolytic-like effects of SSE (10–100 mg/kg p.o.) were evaluated using the LDB evaluating the time in the light compartment (A) and the latency light/dark (B), the EPM assessing recording the number of entries in open arms (C) and the time in open arms (D), and the exploratory cylinder test counting the number or rearings (E). Other groups of mice received 1.5 mg/kg of clonazepam (CNZ) as the positive control or the vehicle (saline solution). Data are representative of two independent experiments (n = 8). Results represent the mean ± standard error of the mean (SEM). ** denotes $p \leq 0.05$ compared to the vehicle group using ANOVA followed by the Dunnett's test.

Table 1
Effects of SSE on the OFT.

Treatment	Total distance (cm)	Ambulatory velocity (cm/s)	Time in center squares (sec)	Distance in center squares (cm)
Vehicle	1078.3 ± 147.2	12.2 ± 1.3	20.4 ± 3.2	202.2 ± 45.8
CNZ 1.5 mg/kg	545.3 ± 62.4 **	5.5 ± 2.4 **	61.2 ± 7.8 **	508.3 ± 72.3 **
SSE 10 mg/kg	1168.8 ± 175.2	10.1 ± 0.9	17.6 ± 5.3	191.7 ± 66.7
SSE 50 mg/kg	1033.7 ± 118.1	12.1 ± 1.2	21.3 ± 6.1	212.2 ± 39.9
SSE 100 mg/kg	1253.8 ± 126.1	12.8 ± 1.9	34.4 ± 5.1 **	397.7 ± 50.7 **

The data were expressed as mean ± S.E.M., n = 8. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. **$p < 0.05$ compared to the vehicle group.

Table 2
Anticonvulsant activity of SSE in four different models.

Convulsant/treatment	Onset of convulsion (sec)	Duration of convulsion (sec)	Mortality (%)	Protection against seizures (%)
Strychnine 4 mg/kg				
Vehicle	102.9 ± 7.3	34.8 ± 2.3	100	0
CNZ 1.5 mg/kg	185.8 ± 10.7 **	24.5 ± 2.4 **	100	0
SSE 10 mg/kg	106.1 ± 2.3	28.4 ± 3.1	100	0
SSE 50 mg/kg	124.4 ± 10.1 **	29.7 ± 2.3	100	0
SSE 100 mg/kg	167.3 ± 14.6 **	29.3 ± 1.9	100	0
PTZ 90 mg/kg				
Vehicle	71.1 ± 9.8	157.8 ± 25.2	78	0
CNZ 1.5 mg/kg	0 **	0 **	0	100
SSE 10 mg/kg	79.2 ± 7.3	107.6 ± 19.6 **	78	0
SSE 50 mg/kg	76.8 ± 5.8	87.3 ± 16.6 **	78	22
SSE 100 mg/kg	80.1 ± 5.2	76.4 ± 10.8 **	78	34
INH 250 mg/kg				
Vehicle	30.5 ± 2.4 min	72.1 ± 6.9	66	0
CNZ 1.5 mg/kg	0 **	0 **	0	100
SSE 1 mg/kg	32.5 ± 2.3 min	73.4 ± 5.6	66	22
SSE 10 mg/kg	29.5 ± 1.2 min	69.2 ± 13.6	66	34
SSE 50 mg/kg	33.6 ± 3.1 min	66.9 ± 7.2	55	44
SSE 100 mg/kg	33.7 ± 1.8 min	66.2 ± 4.6	44	56
Yohimbine 45 mg/kg				
Vehicle	473.9 ± 22.8	191.9 ± 21.5	78	0
CNZ 1.5 mg/kg	0 **	0 **	0	100
SSE 1 mg/kg	440.2 ± 32.3	184.5 ± 12.4	66	22
SSE 10 mg/kg	504.2 ± 76.1	103.2 ± 20.3 **	66	34
SSE 50 mg/kg	798.2 ± 102.3 **	76.4 ± 14.5 **	44	56
SSE 100 mg/kg	1088.1 ± 116.7 **	41.4 ± 11.3 **	44	56

The data were expressed as mean ± S.E.M., n = 9. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. **$p < 0.05$ compared to the vehicle group.

probably associated in the antidepressant-like effects of SSE.

Author contribution

CAB, CLR, ASPC, JRZM, DGM, DAAC and MADA carried out the pharmacological experiments. EYB, AAGI, and GCJ performed the GC-MS analysis. OHAM performed the antioxidant study. AJAC and MADA conceived the study. AJAC wrote the manuscript and coordinated the study.

Conflicts of interest

The authors state that there are no conflicts of interest.

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