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Original article

# Sauroxine reduces memory retention in rats and impairs hippocampal long-term potentiation generation

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ABSTRACT

In the present paper it was investigated the role of sauroxine, an alkaloid of *Phlegmariurus saurur*us, as a modulator of some types of learning and memory, considering the potential nootropic properties previously reported for the alkaloid extract and the main alkaloid sauroine. Sauroxine was isolated by means of an alkaline extraction, purified by several chromatographic techniques, and assayed in electrophysiological experiments on rat hippocampus slices, tending towards the elicitation of the long-term potentiation (LTP) phenomena. It was also studied the effects of intrahippocampal administration of sauroxine on memory retention *in vivo* using a Step-down test. Being the bio distribution of a drug an important parameter to be considered, the concentration of sauroxine in rat brain was determined by GLC–MS. Sauroxine blocked LTP generation at both doses used, 3.65 and $3.610^{-2}$ μM. In the behavioral test, the animals injected with this alkaloid ($3.65\,10^{-3}$ nmol) exhibited a significant decrease on memory retention compared with control animals. It was also showed that sauroxine reached the brain (3.435 μg/g tissue), after an intraperitoneal injection, displaying its ability to cross the blood-brain barrier. Thus, sauroxine demonstrated to exert an inhibition on these mnemonic phenomena. The effect here established for **1** is defeated by other constituents according to the excellent results obtained for *P. saurur*us alkaloid extract as well as for the isolated alkaloid sauroine.



## 1. Introduction

*Phlegmariurus saurur*us (Lam.) B. Øllg. [(=*Huperzia saurur*us (Lam.) Trevis.; *Lycopodium saurur*us Lam.; *H. sanctae-barbarae* (Rolleri) Rolleri & Deferrari; Lycopodiaceae)] is a Lycophyta that can be found in South America, from Peru to northern and central region of Argentina, growing at high altitudes [1,2]. Its commercial importance has jeopardized its natural survival, becoming a species at risk of extinction [3]. Belonging to the Lycopodiaceae family, it has an extensive ethnomedical use, mainly because of its aphrodisiac properties [4], and the literature also reported its use in folk medicine for memory improvement [5]. We have previously demonstrated that Lycopodium alkaloids are the bioactive components in this species [6–8], being sauroine [(8*R*,15*S*)-7,8-dihydroxy-15-methyllycopodan-5-one], sauroxine [(4a*S*,5*S*,12*R*)-1,12-dimethyl-2,3,4,4a,5,6,9,10-octahydro-1*H*-5,10b-propano-1,7-phenanthrolin-8(7*H*)-one, **1**] and 6-hydroxylycopodine [(6α,15*R*)-6-hydroxy-15-methyllycopodan-5-one] the main alkaloids of the purified *P. saurur*us alkaloid extract (PSAE). In turn, PSAE presented marked inhibition of acetylcholinesterase (AChE) [6].

Hippocampus is a brain structure implicated in spatial and context-dependent learning. Long-term potentiation (LTP) is a form of synaptic plasticity in the hippocampus characterized by an enduring increase in the efficacy of glutamatergic synaptic transmission. This phenomenon is accepted as a molecular mechanism for learning and memory in the brain, in which contextual cues are relevant [9,10]. Acetylcholine (ACh) modulates this phenomenon and also participates in learning and memory processes [11]. *In vitro* studies from our laboratories, demonstrated that the PSAE increased synaptic transmission in the hippocampus

*Abbreviations:* ACh, acetylcholine; AChE, acetylcholinesterase; ACSF, artificial cerebro-spinal fluid; BBB, blood-brain barrier; CA1, cornus ammonis 1; fEPSP, field excitatory post synaptic potentials; GLC–MS, gas liquid chromatography–mass spectrometry; HFS, high-frequency stimulation; i.p., intraperitoneal; LTP, Long-term potentiation; NMR, nuclear magnetic resonance; PSAE, *Phlegmariurus saurur*us alkaloid extract; PP, perforant path.

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[12], and enhanced memory retention in rats, when it was administered into the hippocampus [13]. The main PSAE constituent sauroine also presented these effects [14]. Thus, sauroine showed to be one of the contributing alkaloids for the PSAE *in vitro* effects upon hippocampal synaptic plasticity [12], and in *in vivo* memory improvement [13,14]. These results explain the effects claimed by the ethnomedicine, even when it did not exert inhibitory effect on AChE [15], stating the potential use of sauroine as a nootropic agent.

On the other hand, the alkaloid **1** is the second main component of PSAE, and its inhibitory action on AChE was even validated by docking and molecular dynamics studies. Nevertheless, the inhibition level of **1** was lower compared to PSAE effect [15].

As it is already known, the miscellaneous nature of plant extracts gives as result a potential chemical diversity. So, the bioactivities of each individual agent could be spread in a wide range of effects, affording synergism, antagonism or no-interaction phenomena, summarizing the total effect of the extract. Usually, the active isolated components are expected to exert stronger activity than the extracts, but sometimes their effects are lacking, weaker or totally opposite to that of the extract. An example can be taken from *Cannabis sativa* components, cannabidiol would tend to reduce some of the acute and subchronic effects of $\Delta^9$-tetrahydrocannabinol [16].

Considering all the diverse evidences presented above, the aim of the present investigation was to evaluate if the effects of **1** upon hippocampal synaptic transmission and memory were similar to PSAE and sauroine, especially considering its inhibitory action on AChE. Additionally, we examined the blood-brain barrier (BBB) crossing of **1** when it was administered intraperitoneally (i.p.) in rats, in order to determine the alkaloid level in the target tissue.

## 2. Material and methods

### 2.1. Plant material

Aerial parts of *P. saururus* were collected in Pampa de Achala, San Alberto Department, Province of Córdoba, Argentina, in October 2012 (Spring in Argentina) at 2300 m in high, and they were identified by Dr. Gloria Barboza, Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba. A voucher specimen is deposited at the herbarium of the Museo Botánico de Córdoba (CORD) as CORD 684.

### 2.2. Extraction, isolation and identification

Aerial parts of *P. saururus* (2.0 kg) were dried, ground, and then alkalinized with NaOH reduced to a powder (160 g). This mixture was hydrated with distilled water until pH 12, and extracted with $CHCl_3$ using a Soxhlet extractor. The organic solvent was evaporated under reduced pressure until dryness. This crude total extract (59 g) was dissolved in 0.01 N HCl to pH 2, filtered and partitioned twice with $CHCl_3$. The acidic aqueous extracts were combined and then alkalinized with 0.1 N NaOH to pH 12 and

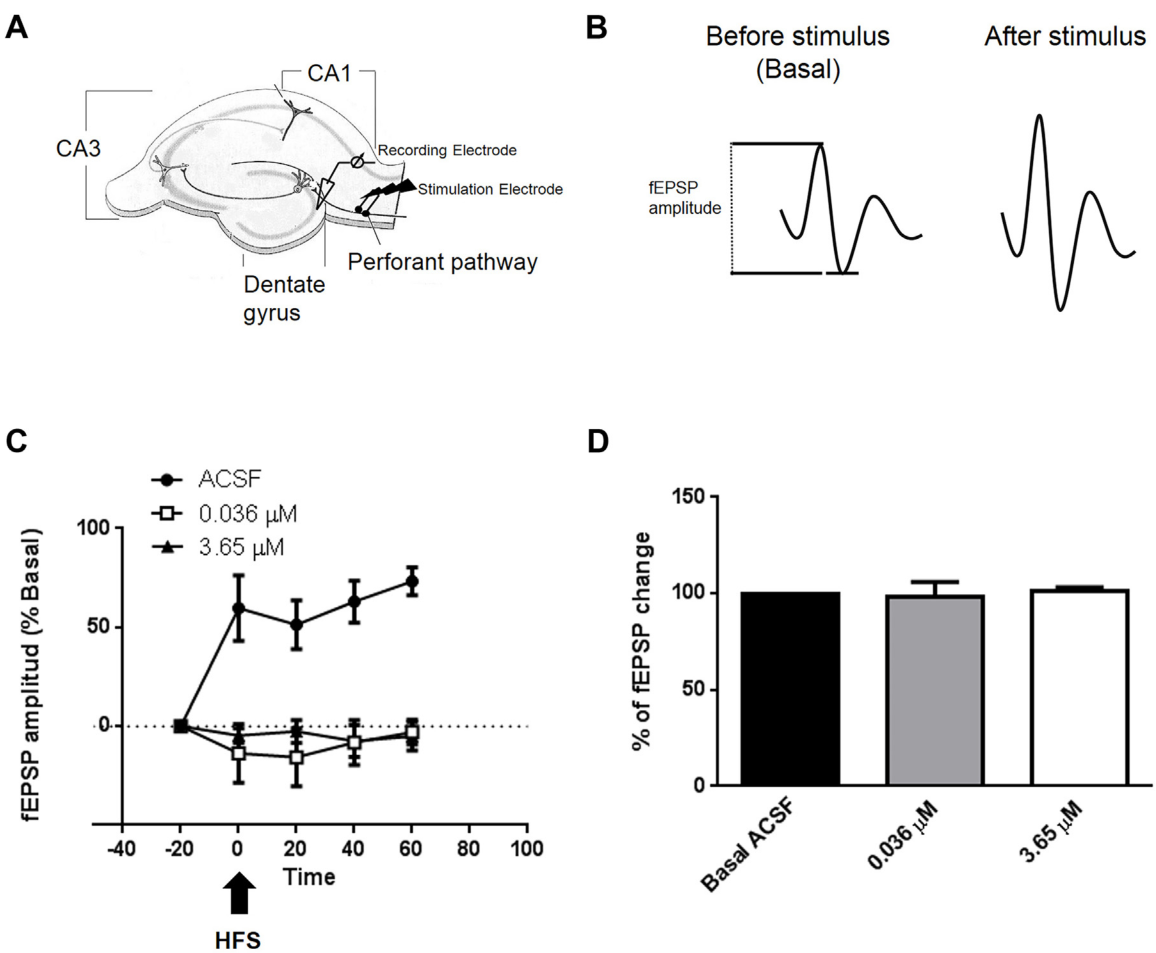


**Fig. 1.** Effect of **1** on LTP generation. A. Recording and stimulation electrodes positions in rat hippocampal slice. B. Typical average fields potentials for control and **1**, before and after effective tetanus. C. Time course of the LTP after perfusion with 3.65 μM (open squares) and 0.0365 μM (solid squares) of **1** during 60 min and LTP induced by tetanus under control condition (solid circles). D. Fast synaptic transmission expressed as % of fEPSP change.