

ACETYLCHOLINESTERASE INHIBITORY PROPERTY OF *DATURA METEL* L. WITHANOLIDES

GARGI NAG AND BRATATI DE*

Pharmacognosy Research Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700019, India.
Email: bratatide@hotmail.com

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ABSTRACT

Objective: Many therapeutic drugs used for the treatment of Alzheimer's disease are acetylcholinesterase inhibitors. This work was carried out to find out the acetylcholinesterase inhibitory properties of the withanolides of *Datura metel*.

Method: We used modified in vitro assay method of Ellman using acetylcholinesterase from *Electropus electricus* (electric eel). Methanolic extract of *D. metel* particularly the withanolide rich fraction of the extract inhibited acetylcholinesterase in a dose dependent manner.

Results: Four withanolides e.g. 12-deoxywithastramonolide [IC_{50} value = 4.6 ± 0.27 ($9.775 \mu M$)], lycium substance B [IC_{50} value = 13.61 ± 0.18 ($29.978 \mu M$)], withametelin [IC_{50} value = 21.03 ± 0.51 ($48.234 \mu M$)], secowithametelin [IC_{50} value = 22.97 ± 0.06 ($45.039 \mu M$)], had acetylcholinesterase inhibitory property.

Conclusion: The results suggest that the acetylcholinesterase inhibitory property of *D. metel* is due to the presence of these withanolides.

Keywords: *Datura metel*, Acetylcholinesterase inhibitor, Withametelin, Secowithametelin, Lycium substance B, 12-deoxywithastramonolide.

INTRODUCTION

The leaves of *Datura metel* L. are used to cure ophthalmia, lumbago, sciatica, neuralgia, painful swellings, epilepsy, cephalalgia, rheumatic pain, asthma [1]. *D. metel* is reported to contain a number of withanolides e.g. 12-deoxywithastramonolide [2,3], secowithametelin [4], withametelin and isowithametelin [5], lycium substance B, withametelin C, withametelin D, withametelin E [6], daturametelin H-J, daturaturin A, 7,27-dihydroxy-1-oxowitha-2,5,24-trienolide [7], withametelin Q, 12 α -hydroxydaturametelin B, daturametelin B [8]. All of the current therapeutic drugs for treatment of Alzheimer's disease are acetylcholinesterase (AChE) inhibitors [9]. Herbal medicines are good sources for the search for potential AChE inhibitors [10]. In continuation to our endeavour to search for AChE inhibitors from plant [11, 12] we studied such property of *D. metel* leaf extract, withanolide fraction and withanolides of *D. metel* in vitro.

MATERIALS AND METHODS

Plant Materials

D. metel leaves (Voucher No. Bot 332 D1) were collected from the experimental Garden, Department of Botany, University of Calcutta, Kolkata.

Chemicals

5,5' dithiobis (2 nitrobenzoic acid), acetylthiocholine iodide were obtained from Sisco Research Laboratories PVT. Ltd., India. Acetylcholinesterase from *Electropus electricus* (electric eel) was purchased from Sigma. All other reagents were of analytical grade. Withametelin, secowithametelin, 12-deoxywithastramonolide and lycium Substance B, withametelin C and D mixture were gifted by Dr. A.B. Ray, Banaras Hindu University, India.

Preparation of extracts

The dried ground leaves were extracted by refluxing with 100% methanol for 5 hours. The extract was then evaporated to dryness to obtain ME. The ME was dissolved in distilled water and extracted three times with diethyl ether to obtain the withanolide rich fraction (WF).

Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibitory property was measured modifying the Ellman method [13]. AChE from electric eel was used for assay. Different concentrations of methanolic solutions of plant extract (0.01ml) were added to 0.02 ml AChE (19.93 unit/ml buffer, pH 8) and 1ml of buffer. The reaction was started by adding 0.01 ml 5, 5' dithiobis (2 nitrobenzoic acid) (DTNB) (0.5 mM) and 0.02 ml acetylthiocholine iodide solution (0.6 mM). The reaction mixture was incubated at 37°C for 20 min. The optical density was measured at 412 nm immediately. The percentage inhibition of AChE activity by plant extract / withanolide was calculated.

Thin layer Chromatography

The dried extracts (ME and WF) were dissolved in methanol and the aliquots were chromatographed along with the withanolides on HPTLC plates (10 x 20 cm) precoated with silica gel 60 F₂₅₄ (0.25 mm thickness). The mobile phase was Ethyl acetate: Hexane :: 3: 1. Presences of the withanolides were identified by comparison of the R_f values and the colour reaction with Liebermann-Burchard reagent.

Statistical analysis

The results were statistically analyzed by ANOVA (one way) and by paired t-test to show significant differences in activity. P values < 0.05 were regarded as significant. Correlation coefficients to determine the relationship between two variables (concentrations and % inhibition activity) were calculated using MS Excel software. Each experiment was repeated three to five times. Regression equations were prepared from the concentrations of the extracts and percentage inhibition due to activity. IC₅₀ values (concentration of sample required for 50% enzyme inhibitory activity) were calculated from these regression equations. IC₅₀ value is inversely related to the activity.

RESULTS AND DISCUSSION

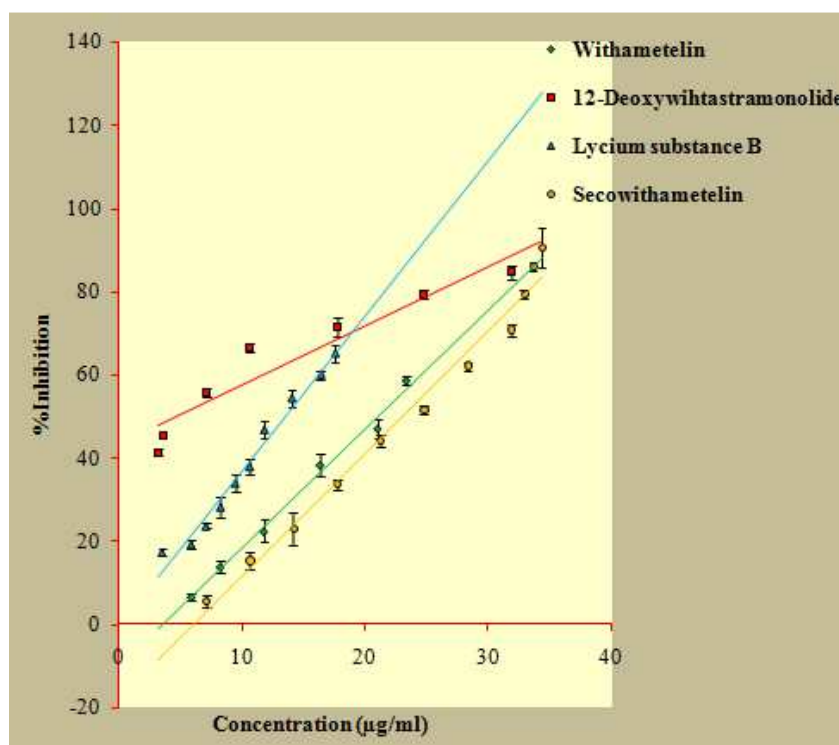
During the present study, it was observed that the methanolic extract of *D. metel* leaves (ME) and the withanolide rich fraction (WF) significantly inhibited AChE in a dose dependent manner. IC₅₀ values are shown in (Table 1).

Table 1: Acetylcholinesterase inhibitory property of *D. metel* extracts and the withanolides

S. No.	Plant materials	IC ₅₀ values µg/ml ± sd (µM)
1.	ME	125.42 ± 0.92
2.	WF	50.98 ± 0.58
3.	Withametelin (48.234 µM)	21.03 ± 0.51
4.	Secowithametelin	22.97 ± 0.06 (45.039 µM)
5.	Lycium substance B	13.61 ± 0.18 (29.978 µM)
6.	12-Deoxywithastramonolide	4.6 ± 0.27 (9.775 µM)
7.	Physostigmine	0.391 ± 0.006 (1.42 µM)

WF was found to have significantly higher activity than that of ME. So with the aim to identify withanolides with potential to inhibit AChE, we measured the activity of 5 withanolides isolated previously from *D. metel* leaves by Ray and his group [3-6]. These were withametelin, secowithametelin, 12-deoxywithastramonolide, lycium substance B, withametelin C and D mixture. Presence of these compounds in WF was also confirmed by the TLC properties. Four withanolides inhibited AChE in a dose dependant manner (Figure 1).

12-Deoxywithastramonolide showed highest activity [IC₅₀ value = 9.78 µM]. The activity of this compound was close to but less than that of physostigmine an alkaloid having AChE inhibitory property. The activities of the other withanolides are in the order secowithametelin [(IC₅₀ value = 45.04 µM)], withametelin [IC₅₀ value = 48.234 µM], lycium substance B [IC₅₀ value = 29.978 µM]. Withametelin C and D mixture did not show any activity up to 50 µg/ml concentration.

Fig. 1: AChE inhibitory activity of the withanolides of *Datura metel*

Withanolides are reported to have COX-2 inhibitory activity [14, 15], leishmanicidal activity [16], trypanocidal activity [17], cytotoxic [8] and anticancer activity [18-21], tumour cell proliferation inhibitory activity [15], nitric oxide production inhibitory activity [22]. Withanolides induced cell apoptosis [23-26], regulated prosurvival factors heat shock factor 1 (HSF1) and breast cancer susceptibility gene 1 (BRCA1) [27]. Some withanolides of *D. metel* are cytotoxic [28]. Withanolides are a new class of cholinesterase inhibitors [29]. But all the withanolides do not possess AChE inhibitory property. Out of the six withanolides isolated from *W. somnifera* (L.) Dunal only three withanolides (withaferin-A, 2,3-dihydrowithaferin-A, 5beta,6beta-epoxy-4beta-hydroxy-1-oxowitha-2,14,24-trienolide) were found to be active against AChE [30]. Three withanolides (bracteosin A, bracteosin B, bracteosin C) of *Ajuga bracteosa* Wall ex. Benth [31] exhibited inhibitory potential against AChE. During this study we first report that the withanolide fraction of *D. metel* and some withanolides e.g. 12-deoxywithastramonolide, lycium

substance B, withametelin, secowithametelin showed acetylcholinesterase inhibitory property. Further in vivo study is required to be carried out to prove their efficacy.

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CONFLICT OF INTEREST

None

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