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Cyperus rotundus extract inhibits acetylcholinesterase activity from animal and plants as well as inhibits germination and seedling growth in wheat and tomato

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Abstract

Cyperus rotundus (nutgrass) is the world's worst invasive weed through tubers. Its success in dominating natural habitats depends on its ability to prevent herbivory, and to kill or suppress other plants growing in its vicinity. The present study was done to investigate whether chemicals in nutgrass target neuronal and non-neuronal acetylcholinesterases to affect surrounding animals and plants respectively. Methanolic extract of tubers of nutgrass strongly inhibited activity of AChE from electric eel, wheat and tomato. It also inhibited seed germination and seedling growth in wheat and tomato. Our results suggest that inhibitor of AChE in nutgrass possibly acts as agent of plant's war against (a) herbivore animals, and (b) other plants trying to grow in the same habitat. An antiAChE from nutgrass has been purified by employing chromatography and crystallization. The structural determination of the purified inhibitor is in progress.

Keywords: Acetylcholinesterase; Allelopathy; Anticholinesterases; *Cyperus rotundus*; Nutgrass; Tomato; Wheat

Introduction

Acetylcholine (ACh) is a universal cell molecule in all plants and animals and is used in neuronal as well as non-neuronal signaling (Wessler et al., 2001). Plants have developed a repertoire of toxic compounds that inhibit the cholinergic signal transduction (Roshchina, 2001). This has helped the plants to survive on earth during evolution even in the overwhelming presence of herbivores. Plants also compete with other plant species in their habitat by releasing allelochemicals that are harmful to the growth of other plants, a phenomenon known as allelopathy (Inderjit and Duke, 2003). The biochemical mechanism of some plant allelochemicals as a factor in invasiveness of weeds has been studied (Weston and Duke, 2003; Bais et al., 2003). However, it is surprising that ecological success and invasiveness of weeds has not been probed for: (a) the possible involvement of ubiquitous signally molecule ACh and (b) effect of plant cholinergic chemicals on animals. To the best of our knowledge, this paper presents the first ever investigation into the involvement of phytochemicals, as well as neuronal and non-neuronal acetylcholinesterase (AChE) in mechanism of invasive success of a plant. We have probed *Cyperus rotundus* (nutgrass), a weed in over 90 countries and considered to be world's worst weed through tubers (Holm et al., 1977).

Materials and methods

Plant material

C. rotundus Linn. plants were collected from the botanical gardens of the Department of Botany, University of Delhi, and its tubers were air-dried in shade for 10 days. Seeds of *Lycopersicon esculentum* Mill. (tomato) and *Triticum aestivum* Linn. (wheat) were grown in Petri plates lined with wet filter paper (Whatman No. 1).

Preparation of plant extract

Tubers of *C. rotundus* were pulverised by grinding in liquid nitrogen. Powdered tubers were extracted in methanol (1:5 w/v)

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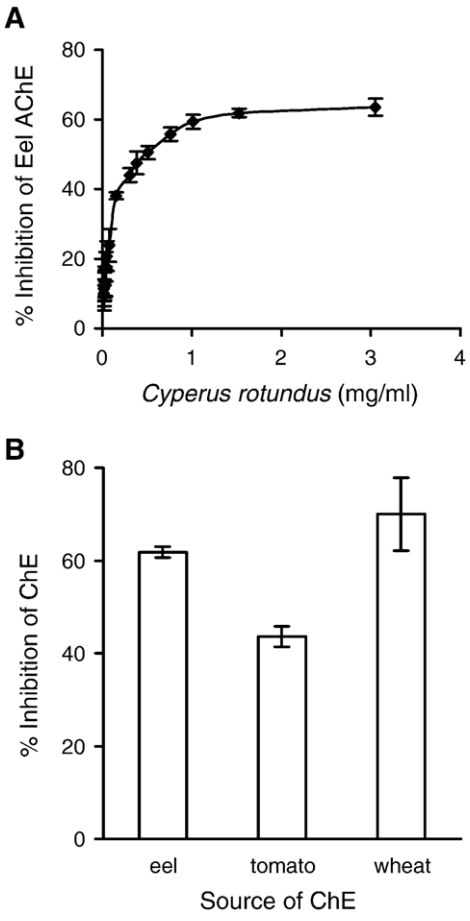

Fig. 1. A. Effect of methanolic extract of *Cyperus rotundus* on electric eel AChE. B. Comparison of inhibition by *Cyperus* extract of AChE from eel, tomato and wheat. *Cyperus* extract 3 mg/ml was used for each assay. The data represents averages of three experiments and the bars represent ± S.E.M.

and the extract was kept overnight before filtering through Whatman No. 1 filter paper.

Seed germination

Tomato and wheat seeds were placed in Petri plates (25× 150 mm and 15×90 mm for wheat and tomato respectively) lined with filter paper (Whatman No. 1) soaked in different concentration of *Cyperus* extract or solvents. One hundred seeds per Petri plate were placed almost equidistantly.

Acetylcholinesterase assay

AntiAChE activity on electric eel AChE (EC 3.1.1.7) was measured by employing a minor modification of Ellman's spectrophotometric assay (Ellman et al., 1961). The reaction medium (4 ml) contained 0.1 M potassium phosphate buffer (pH 8), 0.1 mM dithiobis, 2-nitrobenzoate, 1 mM acetylthiocholne iodide, AChE from electric eel (Sigma–Aldrich, St. Louis, USA) and 200 µl of different concentrations of the plant extract. Concentration of AChE was adjusted to give an absorbance change of 0.8–1.0 in controls. Each assay was done in triplicate. Experiments were carried out at 30 °C. The enzyme activity was stopped after 30 min by addition of neostigmine bromide to final concentration of 0.1 mM. Leaves of tomato (8-d-old) and shoots of wheat (5-d-old) were used as source of plant AChE. Finely chopped (2 mm pieces) of plant material were used for in vivo assay of AChE (Gupta and Gupta, 1997).

Purification of anticholinesterase from C. rotundus

The crude methanolic extract of tubers of *C. rotundus* was concentrated in vacuum to obtain a gummy mass. It was diluted in methanol and loaded on preparative thin layer chromatography plates (TLC). The TLC plates (20×15 cm) coated with Silica Gel G (0.5 mm thickness) were developed in $ChCl_3$–MeOH (1:5 v/v). All portions of the TLC plates were tested for antiAChE activity; the active portion was eluted in MeOH and the procedure was repeated twice. The active band was eluted in methanol and crystallized.

Results

Methanolic extract of *C. rotundus* strongly inhibited AChE from electric eel in a concentration-dependent manner (Fig. 1A). A solution containing 0.5 mg *Cyperus* per ml caused 50% of the maximum inhibition. The *Cyperus* extract also inhibited AChE in leaves of wheat and tomato. The AChE in wheat was inhibited most followed by the enzyme from eel and tomato respectively (Fig. 1B). The inhibitory properties were intact even on heating the extract up to 60 °C in water bath for 45 min. The active compound has been crystallized and its chemical characterization is in progress. The nutgrass extract inhibited seed germination, as well as growth of root and shoot in wheat and tomato (Fig. 2A–F).

Discussion

Plants and animals have co-evolved over millions of years and possess large a number of common biochemical mechanisms. Ecological success of any plant species in nature depends on its ability to inhibit one or more crucial biochemical pathways in other organisms so as to prevent being eaten by animals, and to prevent nutritional competition from other plants in the same habitat. Several chemical compounds have been isolated from world's worst weed *C. rotundus* (Jeong et al., 2000; Sonwa and Konig, 2001) and some of these chemicals possess medicinal properties (Singh et al., 1970; Gupta et al., 1971; Weenen et al., 1990; Hamada, 1993; Thebtaranonth et al., 1995; Makino et al., 2003). However, this paper reports for the first time the presence of antiAChE properties in *C. rotundus*. Naturally, the presence of antiAChE in a plant would be a strong survival tool because it would jeopardize herbivores' vital body functions of voluntary muscles as well as of autonomic nervous system. It is interesting to note that various preparations of *C. rotundus* have been used for centuries in perfumes, spices and traditional medicines in India, China, Arab and Africa. *C. rotundus* is also an important ingredient of anti-aging Ayurvedic neutraceutical Chyavanprash.

The presence of AChE in wheat and tomato is already reported (Tretyn et al., 1986; Fletcher et al., 2004). Our results that