available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Effects of the extract of *Anemopaegma mirandum* (Catuaba) on Rotenone-induced apoptosis in human neuroblastomas SH-SY5Y cells

Deyse Valverde G. De Andrade[a], *Diêgo Madureira de Oliveria*[a], *George Barreto*[a], *Laura-Aon Bertolino*[b], *Ezequiel Saraceno*[b], *Francisco Capani*[b], *Lisandro Diego Giraldez*[a,*]

[a]*Laboratório de Neuroquímica e Biologia Celular, Universidade Federal da Bahia (UFBA), Salvador, Bahia, Brazil*
[b]*Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires (UBA). Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Accepted 3 January 2008
Available online 14 January 2008

Keywords:
Parkinson disease
SH-SY5Y cells
Catuaba
Cytoprotective
Rotenone

ABSTRACT

Parkinson's disease (PD) is one of the most important neurodegenerative worldwide disorders. It is characterized by a selective and progressive degeneration of dopaminergic neurons, causing a series of symptoms which might ultimately induce programmed cell death. The potential cytoprotective effects of one of the commercial extracts of *Anemopaegma mirandum* (Catuaba), a Brazilian tree, on Rotenone-induced apoptosis in human neuroblastomas SH-SY5Y cells was demonstrated. The cell viability, analysis of cellular morphology, nuclei morphology and ultra structural research were done by MTT-tetrazole (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, phase contrast microscopy, stained with Hoechst 33258 and electron microscopy transmission, respectively. Three different concentrations of Catuaba extract were used (0.312, 0.625 and 1.250 mg/mL). These extracts promoted an increase of 22.3±3.6%, 22.0±2.1% and 15.8±0.7% on the cell viability. Notable changes in the cellular morphology, condensation of the cell body, nuclear fragmentation and condensation into discrete dense chromatin clumps were observed when the cells were treated with 300 nM Rotenone for 48 h. These effects were partially altered when the extract of *A. mirandum* was added to the Rotenone treatment. Ultra structural analysis by electron microscopy demonstrated that citoplasmatic membranes and mitochondria membrane were also clearly preserved in the group treated with the extract. Therefore, in this study, our findings indicated that extracts of *A. mirandum* have cytoprotective effects on Rotenone-induced apoptosis in human neuroblastomas SH-SY5Y cells.

1. Introduction

After Alzheimer's disease which affects 1–2% of the population around 65 years old (Eberhardt and Schulz, 2003), Parkinson's disease (PD) is one of the most common neurodegenerative disorders. PD is characterized by a selective and progressive degeneration of dopaminergic neurons and the presence of Lewy bodies in the neurons of the Substantia Nigra pars compacta

* *Corresponding author*. Desembargador Ezequiel Pondé 130., CEP. 40155050, Salvador, Bahia, Brazil.
E-mail address: lisgiral@yahoo.com.br (L.D. Giraldez).
Abbreviations: PD, Parkinson's disease; MTT, MTT-tetrazole (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); ROS, oxygen free radicals; EM, electron microscopy

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.01.006

(Bradshaw et al., 2004). Systems of imaging studies have shown that 70% of the Substantia Nigra's dopaminergic neurons loss are the result of a scarcity in the catecholamine neurotransmitter dopamine, generating the characteristic motor symptoms of Parkinson's disease (Greenamyre et al., 2001; Fagan et al., 2006). The cause of dopaminergic cell death in PD remains unknown, but is associated with a number of insults that may trigger programmed cell death: calcium influx, oxygen free radicals species (ROS) and mitochondrial complex I inhibition (Li et al., 2002; Sipos et al., 2003a; Molina-Jimenez et al., 2003; Dale et al., 2006).

One of the models to study PD is the administration of the plant-derived pesticide Rotenone, a specific inhibitor of mitochondrial complex I. This is done by inducing apoptosis and generating in this way mitochondrial reactive oxygen species, in PC12, HT1080, HEK-293 and SH-SY5Y cell lines. This model has caused effects in rats which closely resemble PD (Seaton et al., 1997; Betarbet et al., 2002; Greenamyre et al., 2001; Li et al., 2002; Sipos et al., 2003b). Several studies consistent with this model, have suggested that ROS play a crucial role in a complex interplay of different mechanisms, in aging and neurodegenerative diseases (Beal, 1995).

Therapeutic efforts aimed at the removal of ROS or prevention of their formation may be beneficial in PD. In this regard, natural products are attractive sources of chemical structures that exhibit potent biological activities with desirable pharmacological profiles. Several reports have suggested that flavanoids and alkaloids could be useful to protect cells from Rotenone toxicity (Seaton et al., 1997; Hori et al., 2000; Molina-Jimenez et al., 2003; Cumming and Zhong, 2006). Studies with *Anemopaegma mirandum* (Catuaba) have showed efficacy in protecting normal human epidermis keratinocytes [NHEK(B)] against the cytotoxicity caused by squalene monohydroperoxide (SQOOH) (Uchino et al., 2004).

It is worthwhile mentioning that the main constituents of Catuaba are the tropane alkaloids (catuabines A, B and C), tannins, resins, aromatic and fatty substances (Graf and Lude, 1977, 1978; Charam, 1987; Coimbra, 1994; Zanolari et al., 2005;

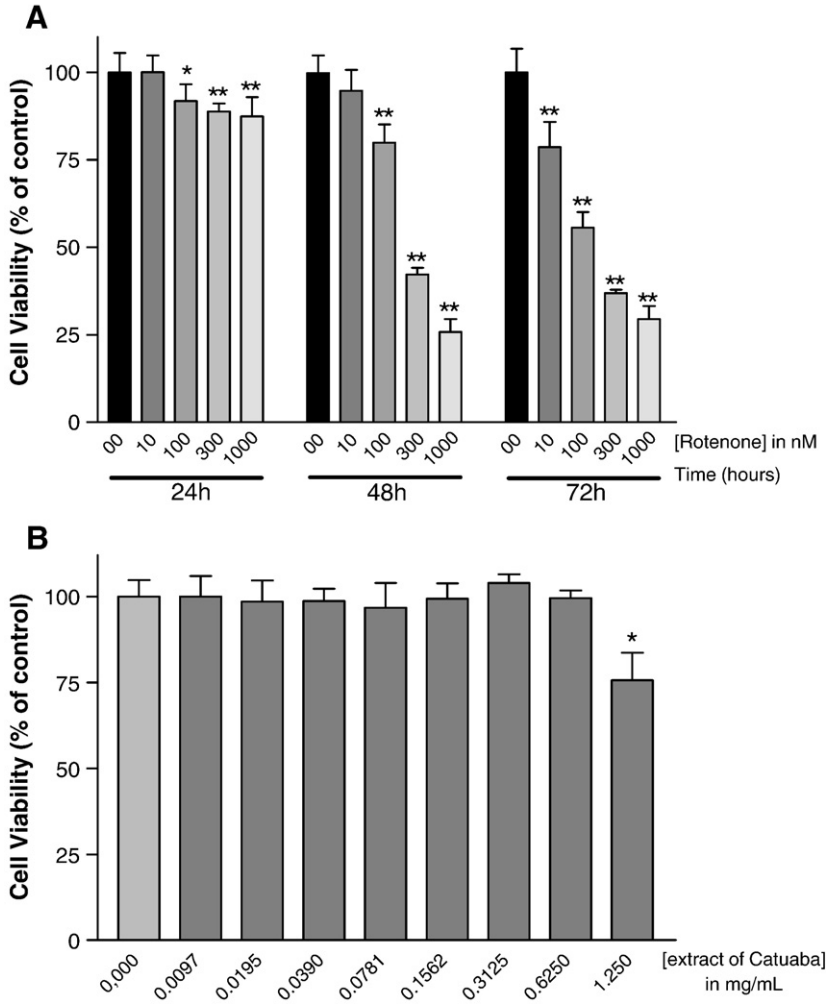

Fig. 1 – A. Toxicity of Rotenone at different times and concentrations measured by MTT assay. 100, 300 and 1000 nM Rotenone treatments caused, at 24 and 48 h, a significant drop in cell viability. Though, 100 nM Rotenone was also more toxic at 72 h than at 24 or 48 h, but the toxicity of 300 and 1000 nM Rotenone were similar at 72, 24 or 48 h (*$p<0.05$; **$p<0.001$). B. Effects of extract of Catuaba against human neuroblastoma SH-SY5Y cells. The cell culture was treated with the extract at different concentrations and viability was measured by MTT assay. With the aim of obtaining statistical evaluations one-way ANOVA was used followed by Dunnett's Test. The only difference found was at 1.250 mg/mL (24.4±8.1) (*$p<0.05$).