CHEMISTRY, ANTIOXIDANT AND ANTICHOLINESTERASIC ACTIVITIES OF LYCOPODIACEAE SPECIES

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disease, which causes loss of memory, cognition disturbances, considered to be the most common form of dementia among the elderly population[1]. This chronic disease is characterized by early cholinergic neuronal loss, and therefore the inhibition of acetylcholinesterase (AChE) is an important approach for the treatment of AD, since the use of inhibitors may increase the cholinergic transmission by blocking the degradation of acetylcholine. The use of antioxidants may also be applied as neuroprotective for AD, reason for the search of multiple target agents, mostly from natural sources[2]. The *Lycopodium* alkaloids, a structurally related class of compounds found in plants from the family Lycopodiaceae, are studied as potent inhibitors of AChE, since the discovery of huperzines A and B in *Huperzia serrata*, traditionally used in China for mental disorders[3].

Objectives: In the present work, we investigate the chemistry of species of *Huperzia*, *Lycopodium* and *Lycopodiella* (Lycopodiaceae) plants growing in the province of Rio Grande do Sul, Brazil, together with their anti-AChE and antioxidant activities through *in vitro* and *in vivo* assays. The anti-AChE will be performed also for isolated products.

Materials and Methods: Alkaloidal extracts were obtained by the classical methodology to obtain metabolites in their basic form, and the isolation of the alkaloids present in the extracts was conducted using chromatographic techniques such as column chromatography and thin layer chromatography. The evaluation of the acetyl and butyrylcholinesterase (BuChE) activities was achieved by some minor modifications of the method introduced by Ellman[4]. All reagents and solvents used were of the highest purity available.

Results and Discussion: All alkaloids extracts (AE) were analyzed by means of GC-MS techniques and the individual alkaloids identifications were made by comparison with literature data. *Lycopodium clavatum* and *Lycopodium thyoides* alkaloids extracts possess similar chemical profile, with lycopodine and acetyldihydrolycopodine as the major compounds found. In *L. clavatum*, the presence of *N*-methyl lycodine was detected, an alkaloid not previously described for this species. The AE of both species was submitted to column chromatography techniques, which allowed the isolation of the known alkaloids lycopodine and acetyldihydrolycopodine, submitted to enzymatic inhibition assays. From the alkaloidal extract of *Lycopodiella cernua* two known compounds (cernuine and lycocernuine), were isolated and also assayed. AE from *H. reflexa*, *H. acerosa*, *H. quadrifariata* and *H. heterocarpon* were also analyzed by GC-MS, and the presence of some known alkaloids was already confirmed, together with others whose structures still remain to be elucidated. In *H. reflexa* we detected the presence of lycopodine, anhydrolycodoline, lycodine, lycodoline, α-obscurine and an alkaloid with a mass peak of 245. From the AE of *H. quadrifariata* it was found lycopodine, 6-hydroxylycopodine, clavolonine, acetylclavolonine, sauroine and 2 alkaloids of peak 272 whose isolation procedures are in progress. In *H. acerosa,* lycopodine, lycodine and flabelline were detected, together with a compound of peak 261, with a profile of rupture for a *Lycopodium* alkaloid, and the extract for *H. heterocarpon* had 6 alkaloids whose fragmentation did not coincide with any alkaloid described yet. The *in vitro* anticholinesterasic activity for these extracts was already performed using human blood as an enzymatic source, and the order of inhibition for AChE was: *H. reflexa* > *H. quadrifariata*> *H. acerosa* > *H. heterocarpon* > *L. cernua* >*L. thyoides* > *L. clavatum.* For BuChE, only *H. reflexa, H. quadrifariata* and *H. heterocarpon* possessed a potent inhibition, which allowed the estimation of the IC_{50} values for them. Moreover, we also estimated the time-inhibition curves and the type of inhibition of AChE for the AE of *L. clavatum* and *L. thyoides*. For that, rat brain homogenates from cortex, hippocampus and striatum samples were used as sources of enzyme, and we found out an interesting activity,

not previously described, but less potent when compared to physostigmine. The type of enzymatic inhibition was evaluated for both extracts through the Michaelis-Menten curves. Antioxidant activities for both extracts were also assayed through *in vitro* experiments, such as DPPH discoloration, 2-deoxyribose degradation, nitric oxide radical and TRAP. The *ex vivo* antioxidant activity was undertaken using Wistar rats aging 10 months after an i.p. administration of a single dose of 25 and 10 mg/kg of *L. clavatum* and *L. thyoides* extracts. This way, we evaluated the enzymatic effects on catalase and superoxide dismutase, as well as TBA-RS. Also, *in vivo* experiments using CF1 mice were performed, by intraperitoneal administration of the AE for *H. reflexa*, *H. quadrifariata*, *L. clavatum* and *L. thyoides*, using huperzine A as standard inhibitor. The activity of AChE was inhibited in cortex and hippocampus mostly by *H. reflexa*, followed by *H. quadrifariata*. Both *Lycopodium* species displayed similar inhibitions. The isolation and elucidation of the major alkaloids from the most active extracts are being executed, as well as the determination of the anticholinesterasic (AChE and BuChE) activities for the pure isolated compounds. Assays on synaptic activity in the hippocampus are still to be performed with the most active extracts, in order to verify the effects on long-term potentiation (LTP).

Conclusion: The chemical profile and the anticholinesterasic activity for the alkaloidal extracts of the Lycopodiaceae plants studied could be achieved, and some of them with no scientific reports so far.

References

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