ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Botany and Ecology

Candidate: **Mgr. Marcela Šafratová**

Supervisor: **Doc. Ing. Lucie Cahlíková PhD.**

Title of Doctoral Thesis: **Study of inhibition (toxicity) activity of alkaloids from selected plant species of Amaryllidaceae family on human enzyme systems (*in vitro* study) III**

Key words: *Narcissus poeticus* cv. Pink Parasol, Amaryllidaceae, alkaloids, acetylcholinesterase, butyrylcholinesterase, prolyl oligopeptidase, cytotoxicity

Bulbs of *Narcissus poeticus.* cv. Pink Parasol were selected as a source of Amaryllidaceae alkaloids for study of their biological activity. Concentrated alkaloid extract has been prepared were prepared by standard extraction and fractionated in aluminium oxide column chromatography column using step gradient elution with petrol, chloroform and ethanol. Column chromatography, vacuum liquid column chromatography, preparative TLC and crystallizations resulted in the isolation of 15 alkaloids, 2 of them were identified as new structures. The chemical structures of isolated compounds were determined on the basis of spectrometric techniques (NMR, MS, optical rotation) and by comparison with literature. Alkaloids isolated in sufficient amounts were tested on their inhibitory ability of human erythrocyte AChE and serum BuChE, POP (IC_{50} was ascertained), cytotoxicity, inhibition AKR3C1 and antimicrobial activity.

The cholinesterase inhibitory activity was determined *in vitro* by modified spectrophotometric Ellman's method. Within testing the inhibitory activity of AChE all the tested alkaloids proved negative activity except of galanthamine. The most potent inhibitor of BuChE was narcipavline with IC_{50} value of 24,4 ± 1,2 µM. Other isolated alkaloids were considered to be inactive in these terms given their high butyrylcholinesterase activity (IC_{50} > 40 µM).

The POP inhibition activity was determined spectrophotometric method using *Z*-Gly-Pro-*p*-nitroanilid as substrate. The strongest inhibition activity was shown by norlycoramine (IC_{50} = 0,21 ± 0,01 mM) was comparable to the standard berberine

(IC_{50} = 0,14 ± 0,21 mM). Norlycoramine demonstrated strongest POP activity from till the date tested Amaryllidaceae alkaloids. Other isolated alkaloids were considered to be inactive (IC_{50} > 20 mM).

Haemanthamine was chosen as most potent structure from the isolated alkaloids that were tested on their cytotoxicity, because its selectivity on cancer cell lines. Tested cell lines were Caco-2 (IC_{50} = 0,99 ± 0,14 µM), HT-29 (IC_{50} = 0,59 ± 0,0,01 µM) and healthy cell line FHs 74 (IC_{50} = 19,5 ± 8,9 µM).

Some of the isolated alkaloids were tested on their inhibition activity of AKR1C3 enzyme and were considered as inactive.

Antimicrobial and antifungal studies carried out on chosen alkaloids did not prove any significant inhibitory effect on growth of selected bacterial strains and yeast cells.