Abstract

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Title of Doctoral Thesis: **Study of the inhibitory (toxic) effect of the alkaloids from chosen plants of Amaryllidaceae family on some human enzymatic systems (in vitro study) II.**

Key words: *Nerine bowdenii*, Amaryllidaceae, alkaloids, biological activity, acetylcholinesterase, butyrylcholinesterase, prolyl oligopeptidase, cytotoxicity

An alkaloid extract prepared from the fresh bulbs of *Nerine bowdenii* Watson was chosen as a source of a variety of Amaryllidaceae alkaloids with a potential for interesting biological activity. The mixture of alkaloids, prepared by standard extraction techniques, was fractionated by column chromatography on aluminium oxide using step gradient elution with petrol, chloroform and ethanol. The isolation of 21 alkaloids was a result of column chromatography, preparative TLC and crystallizations. Two of these alkaloids were isolated from this plant for the first time. The chemical structures of isolated compounds were determined on the basis of spectrometric techniques (NMR, MS, optical rotation) and by comparison with literature data. If the amount of the isolated alkaloid were sufficient, it was tested for its biological activity, in particular inhibitory activity against human cholinesterases and prolyl oligopeptidase, cytotoxicity, inhibition of GSK-3β.

The inhibitory activity against human cholinesterases was determined *in vitro* by a modified spectrophotometric Ellman's method. Undulatine (NB-2) and powelline (NB-21) were the most potent inhibitors of AChE with the IC_{50} values of 23.5 ± 1.2 μM for undulatine and 29.1 ± 1.6 μM for powelline. In the context of inhibitory activity against BuChE, most of the isolated alkaloids proved inactive (IC_{50} >100 μM). The only exception was 4'-*O*-demethylbelladine with the value of IC_{50} 30.7 ± 4.0 μM.

The POP inhibition activity was determined using a spectrophotometric method with *Z*-Gly-Pro-*p*-nitroanilid as a substrate. The IC_{50} values of acetylcaranine, buphandrine, 4'-*O*-demethylbelladine, 6-*O*-demethylbelladine, 1-*O*-acetyllycorine, crinamidine, powelline were

under 1 μM. The activity closest to that of the standard berberine was shown by buphandrine (IC_{50} 0.37 ± 0.04 μM) and 4'-*O*-demethylbelladine (IC_{50} 0.37 ± 0.03 μM).

From all of the isolated alkaloids that were tested for their cytotoxicity, only haemanthamine and buphanisine demonstrated significant activity. The tested cell lines were Caco-2, HT-29 and healthy cell lines FHs 74 Int. The IC_{50} values for the mentioned alkaloids and cell lines were: haemanthamine – Caco-2 IC_{50} 0.99 ± 0.14 μM; HT-29 IC_{50} 0.6 ± 0.01 μM; FHs 74 Int IC_{50} 19.5 ± 8.9 μM and buphanisine – Caco-2 IC_{50} 8.6 ± 0.2 μM; HT-29 IC_{50} 5.3 ± 1.7 μM; FHs 74 Int IC_{50} 22.8 ± 2.6 μM.