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IN VITRO STUDY ON THE INHIBITORY PROPERTIES AGAINST PROLYL OLIGOPEPTIDASE OF GALLOTANNINS FROM THE LEAVES OF COTINUS COGGYGRIA SCOP.

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Abstract

Prolyl oligopeptidase (POP, EC 3.4.21.26) is a widely distributed protease belonging to the small group of postproline specific peptidases. A number of studies show that the enzyme is involved in pathogenesis of neurodegenerative and tumor diseases (reviewed in Dunaevsky et al., 2020). The specific inhibition of POP results in a suppression of the tumor growth *in vivo* (Jackson et al., 2015). Our experiments *in vitro* showed that the leaves extract of *C. coggygria*, obtained by ethyl acetate/water system inhibits tumour cells growth with the highest selectivity index (SI) for HeLa cells (cervical carcinoma; SI > 10) and HepG2 cells (hepatocellular carcinoma; SI = 2.7) (Iliev et al., 2021). Main components of the extract (analysed using high performance liquid chromatography - mass spectrometry (HPLC-MS)) proved to be oligogalloyl glucoses with different numbers of gallic acid residues (from 5 to 10) (gallotannins), as well as small quantities of quercetin and myricetin glycosides.

The aim of the present study is to evaluate *in vitro* the inhibitory properties against POP of the ethyl acetate extract from *C. coggygria* leaves in HeLa and HepG2 cell lines.

For the purpose, the cell lines were cultured and three concentrations of the extract, corresponding to IC_{20} , IC_{35} and IC_{50} , were applied for 48 hours. Then, the cells were homogenized and POP activity was evaluated using the fluorogenic substrate benzyloxycarbonyl-glycyl-prolyl-4-methylcoumarin-7-amide in phosphate buffer (pH = 7.4). The results for POP activity were compared to those in non-treated cells. According to the results, the effect on POP was concentration dependent in both cell lines. In HepG2 cells, the enzyme activity decreased with the increase of the extract concentration. However, in HeLa cells, the enzyme was activated and the degree of activation decreased with the increase of the extract concentration.

Gallotannins are known to have favourable effects on health by *per os* application, mainly due to gallic acid. The most studied of those compounds is pentagalloyl glucose (PGG) for which anticancer, anti-inflammatory, antidiabetic and other properties have been described (reviewed in Torres-Leon et al., 2017). Pro-apoptotic activity of the compound is also documented (Kantapan et al., 2020). Additionally, PGG have been shown to inhibit protein phosphatase 1 (EC 3.1.3.16), which suppression is in parallel to the cytotoxic effect of PGG on HeLa cells (Kiss et al., 2013). Finally, our own studies have shown that gallotannins from *C. coggygria* inhibit fibroblast activation protein alfa (FAP, EC 3.4.21.B28) (Iliev et al., 2020; Iliev et al., 2021), as well. The inhibition of other enzymes involved in the development of cervical carcinoma can be the cause of activation of POP in HeLa cell by small amounts of gallotannins. In any case, this interesting result will be a subject of our future studies.

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